Biochemistry and genetics of carotenoid composition in potato tubers

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Potato cultivars exhibit a wide variation in skin and flesh colour due to the presence of pigments. This study established that potato cultivars differ greatly with respect to types and concentrations of carotenoids in tubers. A total of 46 cultivars were evaluated for quantitative and qualitative carotenoid composition in different growing seasons, locations, storage conditions and disease symptoms. Factors controlling carotenoid accumulation were also tested by developing an in vitro minituber system as a new high-throughput model system for carotenogenesis in potato tubers. Tuber flesh colour was found to correlate with total carotenoid content in potato cultivars grown in both New Zealand and Netherlands. The main carotenoids identified in 32 potato cultivars in New Zealand were lutein, neoxanthin, violaxanthin and β-carotene. The ratio of these carotenoids varies between cultivars. Neoxanthin was detected in only 13 cultivars (10.59 to 69.21µg/g DW); violaxanthin was found only in 1 cultivar (32.76 µg/g DW). Whereas lutein and β-carotene were found in most of the cultivars but the concentration varied from (0.00 to 160.63 µg/g DW) and (0.00 to 13.62 µg/g DW) respectively. The main carotenoids identified in 12 cultivars grown in the Netherlands were neoxanthin, violaxanthin and lutein, whereas zeaxanthin was not found in any of the cultivars analysed. Marked differences were observed between the same potato cultivars grown in New Zealand and the Netherlands. Therefore cultivars were analysed over

a second growing season to assess stability in carotenoids composition. The carotenoid profiles of the potato tubers grown for two different seasons showed highly significant differences between the cultivars, the seasons, the carotenoid pigments, and all combinations of interactions, indicating the complex nature of factors influencing carotenoid composition. Reflectance colorimeter measurement of yellow hue component in this study confirmed that the higher the total carotenoid content, the greater the yellow intensity colour. Eight cultivars were grown at three locations in New Zealand and Agria and Desiree were grown at eight locations in the Netherlands to further investigate the stability of carotenoid composition. Highly significant differences were observed between the cultivars, the locations, the carotenoid pigments, and all combinations of interactions, which emphasises that changes in carotenoid composition are complex and the responses are not consistent across cultivars. Reflectance colorimeter measurement of yellow hue component confirmed the relationship between the yellow colour intensity of tuber flesh, as well as confirming the interaction between colour and locations. Disease and post harvest storage conditions markedly influenced the levels of total carotenoid, neoxanthin, violaxanthin, zeaxanthin, lutein and β-carotene in potatoes. The magnitude of these effects depends on the cultivar, time of storage, and the intensity of powdery scab symptoms. Results showed that long term storage resulted in the accumulation of neoxanthin, violaxanthin and zeaxanthin with a concomitant decreased of lutein, β-carotene and total carotenoid content. Genotypes infected with disease (lower and higher scab score) resulted in accumulation of violaxanthin, β-carotene and total carotenoid with a concomitant decreased in neoxanthin and lutein. A high-throughput model system for investigating carotenoid biogenesis in potato tubers was developed. This involved in vitro potato minitubers and was validated by assessing the effects of environmental variables, such

as drought stress, light intensity and nutrient availability on carotenoid accumulation. Light influenced the presence of zeaxanthin, whereas water stress and nutrient strength influenced the accumulation of neoxanthin and violaxanthin. Although these factors had an effect on the carotenoid content and profile, the most influential factor appeared to be cultivar selection.

Keywords:
Carotenoids, potato tuber, neoxanthin, violaxanthin, zeaxanthin, lutein, β-carotene, total carotenoid, potato minituber, genotype, growing season, location, storage condition and disease symptom.

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ABBREVIATIONS AND SYMBOLS

a^*	bluish-green (-)/red-purple (+) hue component
Anova	analysis of variance
b^*	yellow (+)/blue (-) hue component
C^*	chroma
DMAPP	Dimethylallyl diphosphate
DW	dry weight
FW	fresh weight
g	gram
h	hour
h^o	hue angle
HPLC	High performance liquid chromatography
IPP	Isopentenyl diphosphate
L	litre
L^*	lightness
mg	milligram
min	minute
ml	millilitre
mM	millimolar
mm	millimetre
MVA	Mevalonic acid

ºC	Degree Celcius
s	second
SE	standard error
sp	species
spp.	species (plural)
x *g*	gravity; measured in metres per second
β	beta
µg	microgram
µl	Microlitre

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CHAPTER 1

Biochemistry and genetics of carotenoid composition in potato tubers

1.1 Micronutrient deficiencies in year 2020

In 1990, the World Health Organization (WHO), United Nations Children's Fund (UNICEF), and the World Summit for Children endorsed the elimination of micronutrient malnutrition in developing countries by the year 2000, specifically deficiencies of vitamin A, and two trace elements iodine and iron. In the Third Report of the World Nutrition Situation (United Nations ACC/SCN, 1997), a third trace element, zinc, was added to this list (Gibson and Hotz, 2001). Consumption of diets which lack in both macro and micronutrients is a widespread problem worldwide, with 250 million children at risk from vitamin A deficiency, of which 250,000 – 500,000 will suffer irreversible blindness every year. Furthermore, two billion people (33% of the world's population) are at risk for iron deficiency (infants, children and women of reproductive age are particularly vulnerable), and 1.5 billion people are at risk for iodine deficiency (FAO, 1997). Deficiencies of micronutrients occur from inadequate intakes, impaired absorption or utilization, excessive losses and are worsened during times of infancy, pregnancy and lactation combining to affect health, mental and physical function (Gibson and Hotz, 2001). Most of these situations exist in cities of the developing world where micronutrient deficiencies, morbidity and mortality are highest (Miflin, 2000). Even though the potential of food fortification to reduce micronutrient deficiencies in developing countries was recognised over 30 years ago (Levinson, 1972; Nestel, 1993) and the number of such programs has increased exponentially over the past decade, it is regrettable that such programs still are not available to most of the poorest people in the world (Ross, 2002).

In developed countries food fortification has proven an effective and low cost way to increase the micronutrient supply and reduce the consequences of micronutrient deficiencies (Dary and Mora, 2002). However in most developing countries, where rural diets are based primarily on cereals, legumes, starchy roots and tubers that are poor sources of vitamin A, consumption of meats or dairy products which are rich sources of iron, zinc and preformed vitamin A, is often small. Many people survive largely on plant-based diets or monotonous consumption which can lead to deficiencies of essential vitamins and minerals. Problems associated with politics, distribution, economics, poverty, cultural and religious constraints also contribute to food shortages and malnutrition (Gibson, 1994; Gibson and Hotz, 2001; Zimmermann and Hurrell, 2002). The world's population which reached 6 billion in late 1999 has increased steadily through the centuries and is forecast to reach 8.3 billion in 2020 (Zimmermann and Hurrell, 2002). From well known facts on population growth and economics it is estimated that 800 million people are malnourished today. About 1.3 billion people live on less than US$1 per day and another 3 billion on less than US$2 per day (Watson, 1999).

These are all concerns for several countries, the World Health Organization (WHO) and the World Bank. Vitamin A deficiency, which is an important public health problem (WHO/UNICEF, 1994), is another global theme for urgent consideration to meet basic human needs (Watson, 1999). To meet the macronutrient and micronutrient needs of over 8 billion people by the end of the coming quarter century, it is likely that new approaches using both conventional crop technology and biotechnology will be needed (Zimmermann and Hurrell, 2002; Gibson and Hotz, 2001).

1.2 Micronutrients

Micronutrients are present in limited quantities in foods or diets worldwide (Khachatourians et al., 2002). Plant leaves, fruits, seeds, tubers and roots are valuable sources of nutrients and medicinal compounds which make up approximately 40 micronutrients (the vitamins, essential minerals and other compounds required in small amounts for normal metabolism) necessary in the human diet. These groups of micronutrient can profoundly affect our well-being or risk of disease throughout our lives of pregnancy to lactation, childhood, adolescence and old age (Saltman et al., 1993; Bhatia, 1991).

Depending on their biochemical properties, vitamins have been classified into fat and water soluble vitamins. The group of fat soluble vitamins made by plants consist of provitamin A (also known as β-carotene), vitamin D (calciol), E (tocopherols, tocotrienols) and K1 (phylloquinone). The class of water soluble vitamins includes the following molecules: vitamin B1 (thiamine), the B2 complex (riboflavin, nicotinamide, folate, pantothenate), B6 (pyridoxal), B12 (cobalamine), C (ascorbate) and H (biotin). Water soluble vitamins are more susceptible to losses due to leaching during washing or blanching, whereas fat soluble vitamins are more sensitive to oxidation during processing or storage. Vitamins, on the other hand, are often more stable to oxygen and heat. Vitamin C and thiamin are the vitamins that are most heat sensitive. There is limited knowledge of the biosynthesis and regulation of most vitamins in plants. As a consequence little work has been done to engineer transgenic plants with increased vitamin content (Herbers, 2003).

Vitamins were the first substances employed as nutraceuticals in foods, beverages, dietary supplements and specialized nutritional preparations. The worldwide market for these substances in global food and feed was estimated at $2.65 billion in 1999 and was expected to reach $2.74 billion in 2005 (Business Communication Co., study RGA-096R, Press Release, 3/6/2000). To reach these production levels requires chemical synthesis or fermentative processes in microorganisms or natural product processing. The natural production as compared to synthetic chemistry has several advantages (Herbers, 2003; Dary and Mora, 2002): (i) the relevant biopotent stereoisomers are produced exclusively; (ii) vitamins made in and derived from plants are considered natural and therefore socially acceptable; (iii) a plant-based production system may be cheaper; (iv) it requires minimal changes in food habits; and (v) its delivery system is already in place and it can become sustainable.

So far, the recent application of plant biotechnology to improve the nutritional content of staple food crops has perhaps provided the greatest potential to benefit global health. Because poverty limits food access for much of the developing world's population, it is important that affordable staple foods be as nutritious as possible (Graham et al., 1999; Frossard et al., 2000; Ye et al., 2000; Lucca et al., 2001). Yet, plant systems bear one of the greatest potentials compared to other production systems as factories of vitamin production for the future. Thus far, current results in metabolic engineering already show that plants can be tailored to be used as functional foods with increased levels of provitamin A, vitamin C and E. Provitamin A in transgenic rice plants is the first example in this respect (Herbers, 2003).

1.3 Vitamin A

Humans normally obtain preformed vitamin A (esters of retinols) from animal tissues in the diet and provitamin A carotenoids (mainly β-carotene) from fruits, vegetables and oils (Allen, 2002). The animal-based substance, known as retinol, is ready for use by the human body. It is derived from the break down of β-carotene which can be synthesized naturally by plants and microorganisms (Guilliano et al., 2000). Vitamin A is accepted widely as a broad-based preventive medicine agent (Mayne, 1996) and an essential component of the human diet. It quenches free radicals and prevents cellular oxidative damage, supporting the human immune system and normal development, and has anti-cancer activity (Bendich, 1989, 1993, 1994; Ross, 1992; West et al., 1989; Bartley and Scolnik, 1995). The Recommended Dietary Allowance (RDA) for vitamin A is 1000 retinol equivalents, equal to 6 mg β-carotene, per day (Herbers, 2003).

Insufficient provitamin A in the diet leads to severe clinical symptoms. Vitamin A deficiency causes symptoms ranging from night blindness to those of xerophthalmia, keratomalacia leading to total blindness (Sommer and West, 1996) and in some cases premature death (Mayne, 1996). In Southeast Asia, it is estimated that 5 million children suffer the eye disease xerophthalmia each year, of which 0.25 million eventually go blind (Sommer, 1988). Vitamin A deficiency has been reported as the most common dietary problem affecting 124 million children worldwide with some 1.2 million deaths annually among children aged 1-4 years (Humphrey et al., 1992). It is also strongly correlated with an increased susceptibility to potentially fatal afflictions including diarrhea, respiratory diseases and childhood diseases such as measles (Grant, 1991).

In the developing world vitamin A deficiency is not only a restricted to preschool-aged child but it also affects pregnant and lactating women and sometimes school-aged children and adolescents (Dary and Mora, 2002). It is common for infants and children under 5 years of age to be provided with a single, high dose retinyl palmitate supplement (17.5-30 mg), however this does not reduce rates of morbidity (WHO/CHD, 1998). The new recommendation by WHO for infants aged 0-5 months and children less than 5 years of age is between 15-60 mg (Allen, 2002). WHO also advises that supplements during pregnancy not exceed 10 000 IU (3000 μg of retinol equivalents) of vitamin A per day or 25 000 IU (7500 RE) per week (WHO, 1988). According to UNICEF, improved vitamin A nutrition could prevent 1 to 2 million deaths of children aged 1 to 4 years each year and an additional 0.25 to 0.5 million deaths during later childhood (West et al., 1989; Humphrey et al., 1992).

Vitamin A supplementation has been extensively researched both in terms of its health and nutritional impact (Sommer and West, 1996). It is also has been widely implemented because it is relatively simple, cheap and highly cost effective (World Bank, 1993). It would be desirable to meet the daily requirements for vitamin A by raising the carotenoid levels within staple foods through genetic manipulation. Currently there is considerable interest of manipulating carotenoid content and composition in plants to improve the agronomic and nutritional value for human and animal consumption (Cunningham and Gantt, 1998). Therefore, improving nutritional quality of food crops and its ingredients for human consumption is one of the urgent health issues and high priority areas of research worldwide (Hui and Khacatourians, 1995; DellaPenna, 1999).

1.4 Carotenoids

Plant carotenoids are red, orange and yellow lipid soluble pigments that protect photosynthetic organisms against photooxidation by quenching oxygen radical species. They are efficient antioxidants which protect cells from oxidative damage and also essential as photosynthetic antenna and reaction centre complexes or as structural determinants in plastid pigment-protein complexes (Sun et al., 1996; Van den Berg et al., 2000). These pigments are of important agronomic value in many crop and ornamental plants (Cunningham and Gantt, 1998) and are found embedded in the membranes of chloroplasts and chromoplasts. In photosynthetic tissues the color of carotenoids is masked by chlorophyll (Bartley and Scolnik, 1995). Some of these compounds such as epoxy xanthophylls are precursors of abscisic acid (ABA), a phytohormone that modulates developmental and stress processes (Koorneef, 1986; Pfander and Packer, 1992).

In photosynthetic tissues, their most important function is protection from excessive light energy by quenching triplet chlorophylls, superoxide anion radicals and singlet oxygen (Niyogi, 1999). In non-photosynthetic tissues carotenoids act as accessory pigments for light harvesting that determine or contribute to the colour of flowers and fruits where they serve as visual attractants of insects and animals to facilitate pollination and seed dispersal (Bartley and Scolnik, 1995). These two principal functions involve interactions with chlorophylls but in different directions. Photoprotection involves channelling energy away from chlorophyll whereas light collection requires passing energy on to chlorophylls (Demmig-Adams et al., 1996).

Carotenoids are antioxidants with pharmaceutical potential. More than 600 carotenoid structures are known, but source material for their extraction is limited (Johnson and Schroeder, 1995). The major carotenoids important to humans are α-carotene, β-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin (Olmedilla et al., 1994; Khachik et al., 1997). Up to now, the well established function of carotenoids in the human diet is the provitamin A activity associated with β-carotene. α-carotene and β-cryptoxanthin also possess some provitamin A activity, but less than β-carotene (Van den Berg et al., 2000; Van Vliet et al., 1996; Parker, 1996). Some of the most important biological functions and actions of carotenoids are listed in Table 1.1. The health benefits of dietary carotenoids, especially β-carotene which is the most potent dietary precursor of vitamin A, are becoming increasingly apparent in human and animal nutrition (Van den Berg et al., 2000; Bramley, 2002). Intake of carotenoids both pro- and non-provitamin A is known to reduce the risk of a number of health problems (Khachatourians et al., 2002). In a poorly nourished population from China, cancer mortality was significantly decreased by a supplementation of vitamin E, selenium and β-carotene (Blot, 1997), whereas supplementation of the diet with a mixture of the antioxidant vitamins C, E and β-carotene significantly lowered oxidative DNA damage in lymphocytes of both smokers and non-smokers (Duthie et al., 1996).

Table 1.1: Functions of carotenoids

Function	Carotenoids	Reference:
Provitamin A activity	β-carotene, α-carotene, β-cryptoxanthin	Van Vliet et al., 1996
Antioxidant		Palozza and Krinsky, 1992
Cell communication (Morphogenesis and cell differentiation)	all carotenoids β-carotene, canthaxanthin, cryptoxanthin	Stahl and Siess, 1998
Immune function enhancers		Solomons and Bulux, 1997 Lindley, 1998
UV skin protectant	β-carotene	Seddon et al., 1994
Macula protection	β-carotene, lycopene lutein, zeaxanthin	

Antioxidants such as β-carotene and lycopene, glutathione, lipoic acid, and selenium, zinc and copper containing proteins (Ames, 1998), are not strictly required in the diet, but they promote good health, longevity and vitality (Bhatia, 1991). Van den Berg et al. (2000) reported that positive effects of β-carotene were found in many cardiovascular disease (CVD) studies, whereas Machlin (1995), Van Poppel and Goldbohm (1995) and Giovanucci (1999) found that lycopene and β-carotene could reduce cancer risk especially prostate cancer. Cataract risk was reported to be increased in patients with low plasma β-carotene levels (Knekt et al., 1992). Low levels of carotenoids or lycopene were reported to be associated with age related macular degeneration (AMD) (Seddon et al., 1994; Mares-Perlman, 1995). Lutein and zeaxanthin are the principal components of the macular pigment. Epidemiological studies have shown that antioxidant or carotenoid intake could reduce cataract and AMD risk (Van den Berg et al., 2000). Recently, evidence has been shown that different carotenoids have different beneficial effects, although the mechanisms of action remain unclear. For example, lycopene appears to have a protective effect against prostate cancer (Gann and Khachik, 2003; Hadley et al., 2002) and is effective in reducing the amount of DNA damage in white blood cells and prostate tissues of prostate cancer victims (Chen et al., 2001). Lutein and zeaxanthin offer protection against age-related macular degeneration (Krinsky et al., 2003). Age related macular degeneration is the major cause of blindness in the elderly. High dietary intake of zeaxanthin and lutein can protect against this disease (Seddon et al., 1994). The major dietary sources or lutein are dark green leafy vegetables whereas zeaxanthin is found in significant levels in some maize cultivars (Quackenbush et al., 1963), as well as yellow and orange pepper cultivars (Minguez-Mosquera and Horneo-Mendez, 1994)

Plants only synthesize provitamin A carotenoids which are used as substrates for retinol synthesis by humans (DellaPenna, 1999). Consuming natural sources of vitamin A rarely results in toxicity. There is no report of high β-carotene intake from foods ever having caused vitamin A toxicity (Allen, 2002). Only preformed retinol and other retinoids can cause acute toxicity. β-carotene and other provitamin A carotenoids from foods are not toxic, because the absorption falls as carotenoid intake increases (Brubacher and Weiser, 1985). The upper safe intake level for β-carotene is 20 times that of retinol or 100 times the RDA for vitamin A. On this basis, it is ideal to manipulate provitamin A carotenoid synthesis in plants and as such this has become the target for human health rather than retinol synthesis (Khachatourians et al., 2002).

1.5 Functions of carotenoids

Carotenoids are one of the largest classes of natural pigments synthesized in all photosynthetic organisms (plants, algae and cyanobacterial) and in some non-photosynthetic organisms such as bacteria and fungi (Burkhardt et al., 1997). Mammals including humans cannot synthesize carotenoids even though they are essential source of retinoids and vitamin A (Botella-Pavia et al., 2004) and are responsible for the colour of familiar animals such as lobster, flamingo and fish (Klaui and Bauernfeind, 1981). In plants the carotenoid pigments are synthesized in the plastids. They accumulate primarily in the chloroplasts of the photosynthetic membranes and senescing leaves or in the chromoplasts of ripening fruits, flower petals or other tissues such as carrot root (Cunningham and Gantt, 1998; Bartley and Scolnik, 1995). In some cases, carotenoids also can be formed in the amyloplasts of plant storage tissues such as maize and potato (Burkhardt et al., 1997). Most of the carotenoids important in photosynthetic organisms are

xanthophylls or oxygenated carotenoids (Goodwin, 1980). The sesqui- and triterpenoids are produced in the cytoplasm whereas mono-, di- and tetraterpenoids are produced in the plastids (Kleinig, 1989). The dihydroxy carotenoid zeaxanthin is thought to play a central role in the nonradiative dissipation of light energy. Zeaxanthin is formed from β-carotene by hydroxylation serves as the substrate for biosynthesis of many other important xanthophylls (Demmig-Adams et al., 1996). Lutein, violaxanthin and neoxanthin are the essential components of the light-harvesting antennae where they absorb photons and transfer the energy to chlorophyll as well as assisting in the harvesting of light in the range of 450-570 nm (Van den Berg et al., 2000).

At present, more than 600 different carotenoid structures have been identified with β-carotene is the most prominent number in this group (Pfander, 1987). The typical carotenoids found in plant chloroplasts are lutein, zeaxanthin, antheroxanthin, violaxanthin and neoxanthin and in chromoplasts are capsanthin, capsorubin, bixin, crocetin and citraurin (Van den Berg et al., 2000). In this context, the natural biological functions and actions of carotenoids are based on the physical and chemical properties of the molecules to ensure its fits into cellular and subcellular structures in the correct location and orientation to allow its function efficiently and to determine the photochemical properties and chemical reactivity that form the basis of these functions (Britton, 1995).

1.6 Carotenoid biosynthesis

Carotenoids are biosynthesized by the well known isoprenoids pathway of mevalonic acid (Figure 1.1) and often commences with the formation of phytoene (Figure 1.2) from condensation of two GGPP molecules (Taylor and Ramsay, 2005).

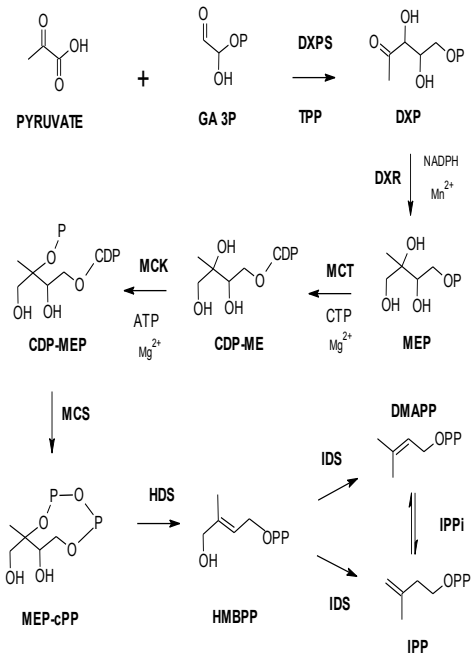


Figure 1.1: Early stage and formation of isopentenyl diphosphate by the MVA-independent pathway. Abbreviations: GA3P, glyceraldehyde-3-phosphate; TPP, thiamine pyrophosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; CDP-ME, 4-(cytidine 5´-diphospho)-2-C-methyl-D-erythritol; CDP-MEP, 2-phospho-4-(cytidine 5´-diphospho)-2-C-methyl-D-erythritol; MEP-cPP, 2-C-methyl-D-erythritol-2,4-cyclodiphosphate; HMBPP, 1-hydroxy-2-methyl-2-(E)-butenyl 4-phosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; DXPS, 1-deoxy-D-xylulose 5-phosphate synthase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; MCT, 2-C-methyl-D-erythritol 4-phosphate cytidyl transferase; CMK, 4-(cytidine 5´-diphospho)-2-C-methyl-D-erythritol kinase; MCS, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; HDS, 1-hydroxy-2-methyl-2-(E)-butenyl 4-phosphate synthase and IPPi, isopentenyl diphosphate isomerase.

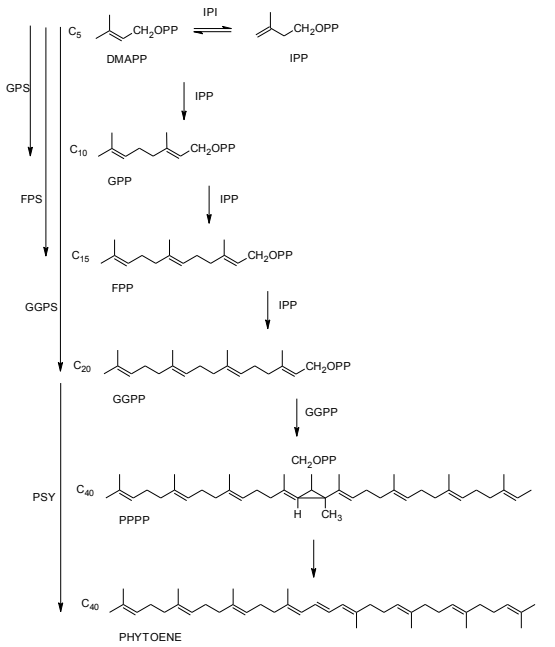


Figure 1.2: Condensation or phytoene synthesis from IPP and DMAPP. Abbreviations: IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; PPPP, prephytoene diphosphate; GPS, geranyl diphosphate synthase; GGPS, geranylgeranyl diphosphate synthase and PSY, phytoene synthase.

Four desaturation forms (Figure 1.3), sequentially, phytofluene, ζ-carotene, neurosporene and finally red coloured lycopene are converted and derived from colourless phytoene. In the final stage of this reaction, one of two alternative hydrogen atoms is lost, stereospecifically, and this determines whether the product is trans (all-E) or 15-cis (15Z) phytoene (Britton, 1989). The cyclization of lycopene (Figure 1.4) with lycopene cyclases, β-(LCYB) and ε-(LCYE), is a significant branch-point in carotenoid biosynthesis. These rings are formed by separate pathways. On one branch a single enzyme LCYB can catalyses the introduction of two β rings into lycopene to form β-carotene and in the other branch of the pathway LCYE can only incorporate one ε-ring forming δ-carotene. In order to form α-carotene both LCYE and LCYB must act.

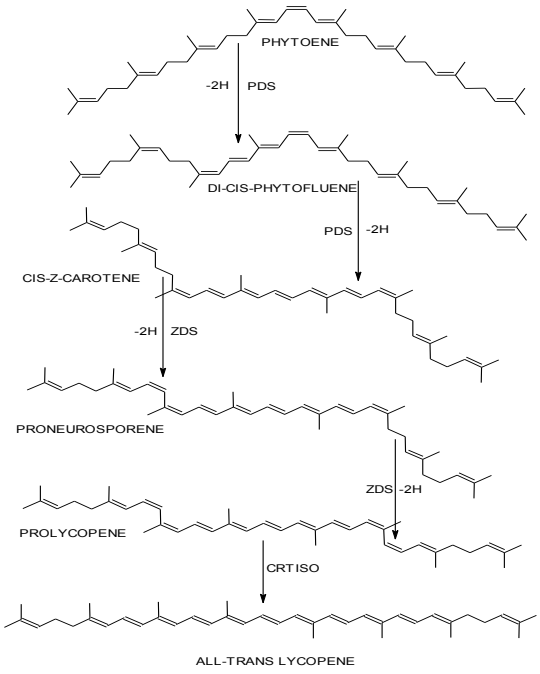


Figure 1.3: Desaturation and isomerization of phytoene. Abbreviations: PDS, phytoene desaturase; ZDS, ζ-carotene desaturase and CRTISO, carotene isomerase.

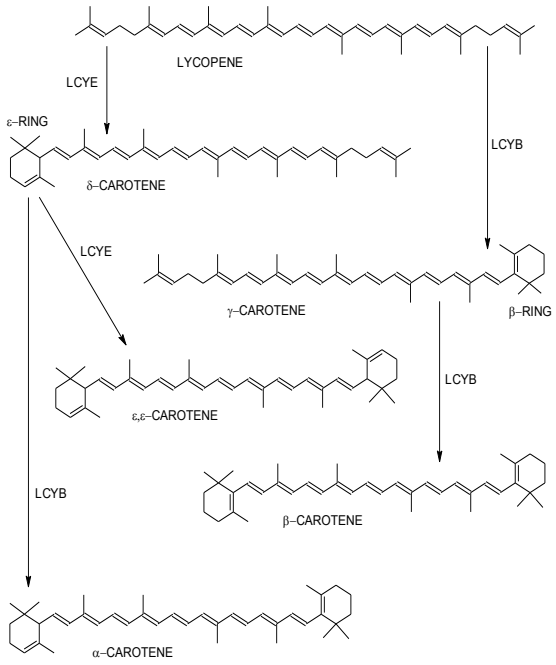


Figure 1.4: Cyclisation of lycopene. Abbreviations: LCYE, ε-cyclase; LCYB, β-cyclase.

Hydroxylation of α and β-carotene (Figure 1.5) will produce the well known xanthophyll pigments zeaxanthin and lutein respectively. Violaxanthin is formed from zeaxanthin through epoxidation (Figure 1.6). This reaction sequence is reversible and de-epoxydation can convert violaxanthin back to zeaxanthin. Neoxanthin is synthesised and derived from violaxanthin (Cunningham and Gantt, 1998; Howitt and Pogson 2006).

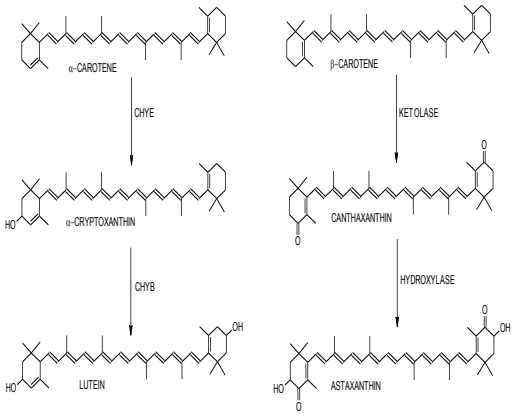


Figure 1.5: Formation of xanthophylls through hydroxylation and addition of a keto group. Abbreviations: CHYE, ε-ring hydroxylase; CHYB, β-ring hydroxylase.

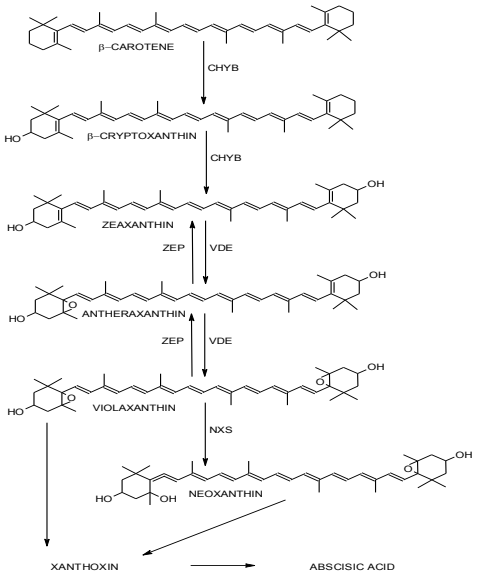


Figure 1.6: Formation of xanthophylls through hydroxylation, epoxidation and deepoxidation. Abbreviations: CHYB, β-ring hydroxylase; VDE, violaxanthin deepoxidase; ZEP, zeaxanthin epoxidase and NXS, neoxanthin synthase.

The study of carotenogenic enzymes remains a very difficult task and, although several crude cell-free preparations from other natural sources have been performed, pure enzymes have not been obtained and the characteristics of the enzyme-catalysed reactions have not been established (Britton, 1989). It is generally believed that carotenoid biosynthesis takes place on a multienzyme complex which is bound to, and may be an integral part of a membrane (Britton, 1989). It is however relatively easy to isolate cell-free preparations which are capable of converting mevalonic acid (MVA), isopentenyl diphosphate (IDP) or GGDP into phytoene (Britton, 1989). Phytoene synthase is considered to be peripheral to the membrane and is generally easily dissociated and

solubilized. The later enzymes for desaturation, cyclization, hydroxylation and other modifications are much more difficult to deal with and are assumed to be membrane-bound. The biosynthesis of phytoene from precursors such as MVA, IDP, GGDP and PPDP has been demonstrated with crude or partially purified enzyme systems from many plants, fungal and bacterial sources (Britton, 1989). Chloroplast systems have been notoriously poor at metabolizing phytoene. In general the most active parts for carotenoid biosynthesis in higher plants are derived from chromoplasts such as in *Narcissus* flowers and in tomato and *Capsicum* fruits (Bramley, 1989). Carotenoid biosynthesis is regulated by several factors, including light (Bramley and Mackenzie, 1987). Many fungi exhibit photoregulation, typically by blue light, and either produce carotenoids only in the light such as *Neurospora* and *Aspergillus*, or show a large increase in carotenogenesis upon illumination such as *Phycomyces* (Rau, 1985). Photoregulation usually occurs at the level of gene expression (Bramley, 1989).

1.7 Genetic manipulation

Until recently, horticultural and agricultural programs, in combination with social marketing, have been used primarily for combating vitamin A deficiency (de Pee et al., 1998). Modern genetic alterations are being used to modify cereals such as rice (*Oryza sativa*) to contain β-carotene (Ye et al., 2000) and increase their content of iron through the use of a ferritin transgene from *Phaseolus vulgare*. All these approaches have the potential to have a major impact on the micronutrient intakes of population groups who derive at least 50 % of their dietary energy from a single cereal staple, such as maize, rice, or cassava (Gibson and Hotz, 2001). There is now increasing interest in developing high yielding genotypes of indigenous wild plants resistant to drought and heat, which are rich

sources of iron and zinc as well as provitamin A carotenoids (Becker, 1986; Kim and Oh, 1996; Malaisse and Parent, 1985). New technologies such as artificial manipulation of chromosome number, the development of addition and substitution lines for specific chromosomes, chemical radiation treatments to induce mutations and chromosome rearrangements, as well as tissue culture approaches such as embryo rescue, in vitro fertilization and protoplast fusion to allow the recovery of interspecific and intergeneric hybrids have been utilized to develop new gene combinations for improving crop cultivars (Simmonds, 1999). The recent technology of genetic engineering can be used to transfer specific cloned genes to single cells from which complete plants can be regenerated via cell and tissue culture (Conner and Jacobs, 2000). These genes may originate from any source of DNA, including inter or intra plant species, microbes, animals or even synthetic DNA sequences designed to encode a specific function. The development of transgenic crops via genetic engineering offers immense opportunities for the incorporation of new genes into crop plants such as resistance to pests, diseases, herbicides and environmental stress, as well as quality traits such as improved post harvest storage, nutritional content and color (Conner and Jacobs, 2000). Even though the benefits and advantages from genetic engineering are imminent, these techniques still need to become more precise to avoid the inadvertent introduction or expression of undesirable genes causing allergenicity, weediness or endanger natural ecosystems.

The issue of food risks, political, economic, social, regulatory and legal issues from transgenic crops must also be considered as public concerns and debates the deployment of these new food production technologies (Kachatourians et al., 2002; Nap et al., 2003; Conner et al., 2003). There are three mechanisms by which food hazards may arise from genetic modification crops which can result in the biosynthesis of new chemical

constituents or elimination of important metabolites such as antioxidants. These include: inserted genes and their expression products; secondary effects of gene expression; and insertional mutagenesis resulting from transgene integration (Conner, 1993). The challenge of this area lies in resolving these societal issues while at the same time meeting the demands of an increasing world population (Kachatourians et al., 2002). Considering these issues before beginning an engineering or breeding program is critical to the potential success of a program. Sourcing all genetic material for transfer from within the germplasm resources for the target crop will help to address this problem (Conner et al., 2007).

1.8 Potatoes

Potato (*Solanum tuberosum*) is the fourth most important food crop in the world following wheat, rice and maize (Visser, 2000). Production worldwide is approximately 293 million tons per annum and covers more than 18 million hectares. Consumption per capita in developing countries is rapidly increasing and has reached 14 kg per annum but is still far less than the European 86 kg or North American 63 kg (Kawchuk, 2002). Potatoes are grown in many countries of the world, representing vastly different physical, biological and political environments. Because of its great adaptability to different climates, not only to the highlands of the tropics but also to the lowlands, it has become a major crop throughout the globe (Van Der Zaag and Horton, 1983). By 2020 about 6 billion peoples will use roots and tubers in their diet and 90% of potato production in developing countries will be in Asia (Swaminathan, 2000). More than 40% of the world's potatoes are already grown in developing countries and that number is expected to increase (Collins, 2000).

Potato has one of the richest genetic resources of any cultivated plant, much of which has been already incorporated into cultivars (Ross, 1986). It is now widely recognized that potatoes provide a rich source of high quality protein, vitamins, minerals, trace elements and fiber (Clayton and Percival, 2000). Starch is a major constituent of potato tubers. Potatoes are only second to soybean in production of protein on a per area basis (Johnson and Lay, 1974), it is superior to all other crops in protein production per unit area and time, only second to sweet potatoes in terms of energy production (Van Der Zaag 1976). Potatoes are well known to contain protein of excellent nutritional quality, with the major storage protein being patatin which accumulates to 40% of total soluble protein in potato tubers (Kaldy, 1972). The nutrient content of an average sized of a serving potato (122 g) contains iron of moderate availability, provides 50% of the recommended daily allowances (RDA) of vitamin C, 10% of the recommended calories plus the vitamins thiamine, niacin and folate which are essential for a child's diet. However their vitamin A, E, selenium and zinc content are generally less than optimal for human nutrition (Fairweather-Tait, 1983). In general, diets based on starchy roots and tubers have lower micronutrient content than those based on unrefined cereals and legumes. However, the latter often contain high levels of phytic acid (myo-inositol hexaphosphate) and polyphenols which inhibit zinc and/or nonhaem iron absorption by forming insoluble complexes in the intestine. Consequently, the bioavailability of micronutrients in diets based on cereals and legumes is often poor (Gibson, 1994). Even carotenoid-rich vegetables have low vitamin A bioavailability and bioefficacy (West et al., 2002).

Sterility is a very serious constraint in potato breeding. As an autotetraploid, the potato can possesses up to four interchangeable alleles at a given locus. This gives the possibility of breeding tetraallelic (heteroallelic) genotypes. The importance of

tetraallelism for yield and other polygenic characters has become evident (Ross, 1986). Because of its tetraploid nature and its vegetative mode of propagation, breeding is a cumbersome and time consuming effort. For this reason potato breeding has been less successful when compared to other important crops. All the above aspects make potato an excellent crop plant to improve by genetic transformation. By using a GM approach the quality of the potato crop can be increased considerably in a relatively short time especially by using the potato plant as factory for the production of high value compounds such as vitamins and proteins (Visser, 2000). The FAO reports that 18.4 million hectares of potatoes were harvested worldwide in 1996. However, both pre- and post-harvest disorders significantly reduce net yield, and therefore both farm profitability and food supplies suffer (Hooker, 1981). Horticultural practices coupled with susceptibility to *Agrobacterium*-mediated transformation, make the potato an ideal candidate for improvement via molecular biology techniques. Using a biotechnological approach, it is possible to introduce desirable traits into existing cultivars, thereby creating new selections capable of producing more or better food for an all-too-hungry world (Rockhold et al, 2001).

As is true for most commercially produced farm commodities, potatoes are the subject of constant research to produce new cultivars with the better agronomic properties such as insect and disease resistance, lower or higher levels of solids, improved yield, improved cold storage, cold tolerance in the field or altered biochemical characteristics (Visser, 2000). Almost all of the related tuberous *Solanum* wild species can be crossed with *S. tuberosum.* It has some interesting characteristics where any selected genotype can be maintained with all its intra and interlocus interactions, and when satisfactory can be multiplied and released as a new cultivar (Ross, 1986). As a vegetatively propagated crop, potato provides many advantages in expressing transgenic traits and heterologous products.

For example, sexual reproduction and meiosis is not necessary for propagation so transgenes do not segregate from one clonal generation to the next. Consequently, germplasm integrity is easily maintained, and the tuber provides an economical eukaryotic expression system. Various strategies have been developed to produce transgenic potatoes with improved agronomic performance, expressing economically important products and exhibiting disease and pest resistance (Kawchuk, 2002). As an autotetraploid, the potato can be regenerated from tissue and protoplasts, and is amenable to transformation (Ross, 1986). According to Horton (1981), the potato will become valuable when the nutritional problems of the future are considered. The cultivated potato is at the forefront of the development of genetic engineering in crop plants, with many of the research targeting potato improvement via the transformation of existing cultivars with specific genes. Many of the initial transgenic crop cultivars to be commercialized in the future are expected to be potatoes with enhanced characteristics (Conner et al., 1997). One of the more challenging issues in potato producing areas of the world is the potential for using genetically engineered potatoes to solve particular production constraint (input traits) or quality/nutrition constraints (output or value-added traits). By more accessible and affordable food, these advanced technologies can be used to aid the disadvantaged peoples of the world; the hungry, the poor, or those whose environments are threatened by heavy pesticide use (Collins, 2000). Augmentation of foods to yield nutritionally balanced and adequate micronutrient content is being achieved with the advances in biotechnology and genetic engineering where value can be added to foods by physiological, biochemical and genetic techniques (Watson, 1999). Plant genetics and genetic engineering can enhance nutritional quality, composition and medically important compounds within plants. The production of these metabolites can be enhanced by learning about the finer aspects of biosynthetic pathways, modification of gene expression levels or transmission of other

genetic controls into such host plants. This should have a direct impact for both overfed and underfed populations (DellaPenna, 1999).

1.9 Genetic manipulation of carotenoid content in potato

Several approaches of genetic manipulation have been performed to increase carotenoid levels in different plant species and tissues. Over the past decade many of the genes encoding the enzymes of the carotenoid biosynthetic pathway such as isopentenyl diphosphate/dimethylallyl diphosphate synthase isomerase, geranylgeranyl diphosphate synthase, phytoene synthase, phytoene desaturase, ζ-carotene desaturase, lycopene α- and β-cyclase, β-carotene hydroxylase, neoxanthin synthase and even the subsequent epoxidase have been cloned from both plant and microbial sources (Herbers, 2003; Cunningham and Gantt, 1998; Hirschberg, 2001; Fraser and Bramley, 2004; Al-Babili et al., 2000). Previous reports describe modifications of the amounts and types of carotenoid that accumulate in experimental and food plants by transgenic manipulation of the carotenoid biosynthetic pathway (Fraser and Bramley, 2004). The over-expression of phytoene synthase has a particularly potent effect on storage organ carotenoid levels and results in increases in total carotenoid content in carrot roots (Hauptmann et al., 1997), tomato fruit (Fraser et al., 2002), canola seed (Shewmaker et al., 1999), *Arabidopsis* seed (Lindgren et al., 2003) and potato tubers (Ducreux et al., 2005). The extent of the increase varied between 1.6 fold in tomato fruit, 50 fold in canola seed up to 6 fold in potato tubers. Transgenic manipulation of potato tubers has been used successfully to elevate carotenoid levels and increase the spectrum of carotenoids that accumulate to significant levels (Romer et al., 2002; Ducreux et al., 2005). In recent work of Diretto et al. (2007b), expression of three *Erwinia* genes encoding phytoene synthase (CrtB), phytoene desaturase/carotene isomerase (CrtI) and

lycopene beta-cyclase (CrtY), under the control of a tuber specific promoter, demonstrated the conversion of geranylgeranyl diphosphate (GGPP) into β-carotene. This resulted in tubers with a deep yellow (golden) phenotype without any adverse leaf phenotypes. Total carotenoids increase up to 20-fold (114 µg/g dry weight) and β-carotene up to 3600-fold (47 µg/g dry weight). This golden potato is the highest carotenoid and β-carotene content reported for biofortified potato as well as for any of the four major staple foods. Consuming 250 gm of this tuber flesh is sufficient to provide 50% of the Recommended Daily Allowance of Vitamin A. This consumption (47 µg/g dry weight) as stated by Diretto *et al*. (2007b), is even better than Golden Rice 2 with 31 µg/g dry weight of β-carotene.

Meanwhile silencing the first step in the epsilon-beta branch, LCYe, increases total carotenoids up to 2.5 fold and β-carotene up to 14 fold (Diretto et al, 2006), whereas silencing the non-heme β-carotene hydroxylases CHY1 and CHY2 in the tuber showed more dramatic changes with total carotenoids increasing up to 4.5 fold and β-carotene up to 38 fold (Diretto et al, 2007a). However, zeaxanthin levels decreased, whereas neoxanthin and violaxanthin stayed the same. CRTISO, LCYb and ZEP were induced in both cases, indicating that they may respond to the balance between individual carotenoid species (Diretto et al, 2007a). Romer et al. (2002) reported that down-regulation of zeaxanthin epoxidase in tubers of *S. tuberosum* led, in some transgenic lines, to a dramatic increase in the zeaxanthin content and the total tuber carotenoid content up to 5.7 fold. In this case the conversion of zeaxanthin to violaxanthin was inhibited leading to accumulation and elevation of zeaxanthin up to 4 to 130 fold. The values reaching up to 40 µg/g dry weight depending on the transgenic lines and tuber development. Ducreux et al. (2005) revealed that expression of a bacterial *crt*B gene encoding phytoene synthase led to

6-fold higher carotenoid levels with violaxanthin, antheroxanthin, lutein and β-carotene were the major carotenoids that accumulated in the transgenics but only lutein levels increase with the increase of β-carotene and total carotenoids. Morris et al. (2006) found that over expression of a bacterial 1-deoxy-D-xylulose 5-phosphate synthase (DXS) gene in potato tubers resulting 2 fold increases in total carotenoid and 6-7 fold increase in phytoene. In all cases, metabolic engineering of potato has led to the production of more β-carotene, phytoene, lutein and zeaxanthin (Romer et al. 2000; Fraser et al. 2002; Ducreux et al. 2005; Diretto et al, 2007a; 2007b), and the accumulation of astaxanthin, a new and high-economic value carotenoid, in potato tubers (Gerjets and Sandmann 2006; Morris et al., 2006b).

Rather than directly regulating carotenoid biosynthesis, recent work by Lu et al. (2006) through transformation of the *Or* gene into wild type cauliflower converts the white color of curd tissue into distinct orange colour with increased levels of β-carotene. *Or* gene, which encodes a DnaJ cysteine-rich domain-containing protein, leads to the formation of large membranous chromoplasts in the cauliflower curd cells which strongly associated with carotenoid accumulation (Li et al. 2001; Lu et al. 2006). Similarly when the *Or* gene under the control of a potato granule-bound starch synthase (GBSS) promoter was introduced into a potato (Li and Van Eck, 2007), the orange-yellow flesh tubers were produced. The total carotenoid levels were 6 fold higher than the non-transformed and control. These orange-yellow flesh tubers is due to the extra orange bodies structures in chromoplasts, which provide a metabolic sink to facilitate accumulation of carotenoids, whereas the tubers in the non-transformed and vector-only controls contain exclusively various sizes of starch grains in amyloplasts. Although previous studies have shown that overexpression of genes in the carotenoid biosynthetic pathway resulted increased levels of

carotenoids, modification of sink capacity also proven as a new strategy to enhance carotenoids in storage tissues of food crops. Manipulation of both tools perhaps can be more effective to enhance carotenoids level quantitatively and qualitatively in order to meet the requirement for human nutrition and health. Hence, genetic engineering improvement of carotenoid content in potato tubers urgently requires more detailed knowledge of the diversity of carotenoid pigments in potatoes and environmental factors influencing their accumulation to better understand tubers carotenogenesis regulation. In order for this to be achieved, it is important to identify the best cultivars for targeting specific genetic manipulations and to understand the key control factors for carotenoid accumulation in potato tubers.

1.10 Goal of this thesis

The aim of this research is to explore the composition and concentration of carotenoids in potato tubers to enable their future enhancement or enrichment through genetic manipulation. To achieve this, six research objectives have been defined:

1. to establish analytical method for carotenoid analysis (Chapter 2)
2. to survey carotenoid content in a wide variety of potato germplasm (Chapter 3)
3. to investigate the stability of carotenoid composition over seasons (Chapter 4)
4. to assess genotype x environment interaction in carotenoid accumulation (Chapter 5)
5. to study the effect of storage and disease upon carotenoid composition (Chapter 6)
6. to assess potato minitubers as a model system for investigating carotenoid biogenesis (Chapter 7)

CHAPTER 2

Materials and methods

2.1 Potato cultivars selection

Potato (*Solanum tuberosum* L.) cultivars representing a diverse collection of genetic material for analysis of carotenoid content were selected for this study (Appendix 1.1). The selected cultivars were planted in field trials and tubers harvested, stored and prepared as described in the relevant chapters.

2.2 Analysis of potato tuber carotenoids

Total carotenoid concentration was determined by spectrophotometry as described by Britton et al. (1995). Individual carotenoid concentration was determined by reverse phase HPLC after saponification as detailed in Morris et al. (2004) with minor alterations as described in section 2.4. Biological samples were prepared in triplicate and each biological sample was further analysed in triplicate. All manipulations were performed on ice and under subdued artificial light conditions with headspaces of containers flushed with oxygen free nitrogen to help prevent carotenoid degradation.

2.2.1 Sampling

The skin of the tubers was removed with a peeler and the remaining tuber tissue was cut into 5 mm slices. For each sample, tuber tissue was pooled from three tubers, mixed, and a random 100 g FW sample that was immediately stored at -20°C. The tuber samples

were freeze-dried for 7 days, after which the samples were ground into fine powder and stored at -80 °C until analysis.

2.2.2 Extraction of carotenoids

The extraction procedure essentially followed the methods described by Morris et al. (2004), Lewis et al. (1998) and Britton et al. (1995) with the following modifications. The number of samples in this study was too large to handle all material in a single day. Therefore, freeze dried material was used to allow samples to be conveniently stored under appropriate conditions in the absence of enzymic reactions. For each sample, 1.0 g of powdered freeze-dried material was mixed with an equal weight of calcium carbonate (1g/g sample) to prevent isomerization and degradation of carotenoids by neutralizing the acidity from any reactions catalyzed by plant enzymes or acids. The tissue was rehydrated by adding 1 ml of distilled water, followed by 5 ml of an acetone and methanol mixture (7:3) to allow efficient solvent penetration. The solution was then allowed to stand overnight in darkness at room temperature. The following day the samples were vortexed and centrifuged for 2 minutes at 13 500 *g* (Heraeus Sepatech Biofuge 13) and the supernatant transferred to a foil covered 50 ml graduated polypropylene centrigfuge tube (Greiner, Raylab Scientific NZ). This procedure was repeated after adding 5 ml of acetone and methanol (7:3) without additional calcium carbonate, until the supernatant or the tissue was colorless (normally two or three times). Combined supernatants were centrifuged at 13 500 *g* for 5 minutes to remove fine particulates and were stored at 4 ºC in the dark prior to analysis. To extract carotenoids an equal volume of diethyl ether and distilled water was added to the combined supernatants. The solution was then allowed to separate and the upper ether layer containing the carotenoids was collected. This procedure was repeated

with diethyl ether alone and the combined upper phase was then dried to completion under a gentle stream of oxygen-free nitrogen. Vials/tubes were then capped and sealed with parafilm to exclude oxygen and immediately stored at -80 °C until subsequent analysis.

2.2.3 Determination of total carotenoid concentration

Total carotenoid concentration was determined by spectrophotometry as described by Lewis et al. (1998). The dried carotenoid was resuspended in 250 µl of ethyl acetate and for determination of total carotenoid, 50 µl of the redissolved sample was then diluted with 950 µl chloroform for spectrophotometric analysis. Carotenoid containing solutions were measured at three different wavelengths: λ 480 nm, 648 nm and 666 nm using Shimadzu UV-160 spectrophotometer. The Wellburn Equation (Wellburn, 1994), in chloroform was applied to obtain the total carotenoid content as described below:

$$C_a = 10.91A_{666} - 1.2A_{648}$$

$$C_b = 16.36A_{648} - 4.57A_{666}$$

$$C_{x+c} = (1000A_{480} - 1.42C_a - 46.09C_b)/202 \ (\mu g/ml)$$

Where C_a = concentration at 666 nm; C_b = concentration at 648 nm; and C_{x+c} = total carotenoid concentration at 480 nm.

2.2.4 Preparation of carotenoid extracts for HPLC analysis (Saponification)

Saponification is an effective means of removing hydrolysed carotenoid esters, which can lead to the formation of artifacts and for destroying chlorophyll which may

interfere the chromatographic separation and shorten the life of the HPLC column (Rodriguez-Amaya and Kimura, 2004). However saponification routinely results in losses of carotenoids especially xanthophylls and it should only be performed when necessary. Of the 250 µl ethyl acetate carotenoid-containing extract, a 200 µl aliquot was dried down to 10-20 µl under a gentle stream of oxygen-free nitrogen. Then 390 µl of acetonitrile and water (9:1) was added to make up the volume to 400 µl. The solution was then further diluted with 400 µl of methanolic potassium hydroxide solution (10%, w/v) to give a final volume of 800 µl, vortexed and allowed to stand overnight in darkness at room temperature. Base carotenoids were then extracted by addition of 2 ml diethyl ether with 0.1% Butylated hydroxytoluene (BHT), followed by addition of 10% NaCl (c. 2 ml) until phase separation was achieved. The upper aqueous phase was removed and re-extracted again with 2 ml diethyl ether with 0.1% BHT and the combined ethereal extracts were washed three times with distilled water to remove excess alkali and cleaved esters. The samples were then dried under a gentle stream of oxygen-free nitrogen and resuspended immediately in 250 µl ethyl acetate. Aliquots of 50 µl were used for spectrophotometric measurement of total carotenoid content to estimate any potential losses of carotenoids following saponification. The remaining sample (200 µl) was retained for analysis of individual carotenoid by HPLC.

2.3 HPLC analysis

The HPLC analysis of saponified carotenoids was performed on an Agilent model 1100 series comprised of a binary pump with autosampler injector, micro vacuum degassers, thermostatted column compartment and a diode array detector according to Morris et al. (2004) with minor alterations listed below. The column used was a Luna C_{18}

end capped 5 µm, 250 x 4.6 mm reverse phase column (Phenomenex Auckland, New Zealand). The solvents used were (A) acetonitrile: water (9:1 v/v) and (B) ethyl acetate. The solvent gradient used developed as follows: 0-40% solvent B (0-20 min), 40-60% solvent B (20-25 min), 60-100% solvent B (25-25.1 min), 100% solvent B (25.1-35 min) and 100-0% solvent B (35-35.1 min) at a flow rate of 1.0 ml min^{-1}. The column was allowed to re-equilibrate in 100% solvent A for 10 min prior to the next injection. The temperature of the column was maintained at 20ºC. The injection volume was 10 µL. Carotenoid standards β-carotene, violaxanthin, lutein, and neoxanthin were isolated from *Eruca sativa* (roquette or rocket salad) by open column chromatography (see section 2.3.1) as described by Kimura and Rodriguez-Amaya (2002), whereas zeaxanthin was obtained commercially from Sigma-Aldrich. Calibration curves were used to calculate the concentration of the respective carotenoids in experimental samples as described by Morris et al. (2004) and Kimura and Rodriguez-Amaya, (2002). The identity of individual carotenoids was confirmed by their spectral characteristics, absorption maximum and retention time as described by Britton et al. (1995). Detection of individual carotenoids was made at the wavelengths of maximum absorption of the carotenoids in the mobile phase: neoxanthin (438 nm), violaxanthin (441 nm), lutein (447 nm), zeaxanthin (452 nm) and β-carotene (454 nm). Compounds were identified by co-chromatography with standards and by elucidation of their spectral characteristics using a photo-diode array detector. Detection for carotenoid peaks was in the range of 350 to 550 nm. Individual carotenoid concentrations were calculated by comparing their relative proportions, as reflected by integrated HPLC peak areas, to total carotenoid content determined by spectrophotometry. The total and individual carotenoid concentration were expressed in terms of microgram per 1.0 g dry weight or freeze-dried matter (µg/g DW)

2.3.1 Isolation of Carotenoid Standards by Open Column Chromatography

Eruca sativa (roquette or rocket salad) was used for isolation of carotenoid standards by open column chromatography (OCC) as described by Kimura and Rodriguez-Amaya (2002). Approximately 200-250 g of leaves were freeze-dried as described in section 2.2.1 and used as the source for standards of neoxanthin, violaxanthin, lutein and β-carotene. The extraction procedure followed the methods described in section 2.2.2.

2.3.2 Partition to Petroleum Ether

To transfer carotenoids from acetone to petroleum ether, 100 ml of petroleum ether was placed in a 500 ml separator funnel. A small portion (50 ml) of the acetone extract was added by slowly flowing along the walls of the funnel and then 300 ml of distilled water was added slowly to the combined supernatants. The solution was then allowed to separate and the lower aqueous-acetone layer was discarded. This operation was repeated until all of the extract has been transferred to petroleum ether. The petroleum ethereal extracts were then washed five times with 200 ml distilled water to remove residual acetone. Then the carotenoid solutions in petroleum ether were concentrated until approximately 10 ml remained.

2.3.3 Preparation of the column

First a chromatographic glass tube with 25 mm external diameter and 300 mm height was mounted on a suction flask and a small amount of glass wool was placed at the bottom of the chromatographic tube. Adsorbent consisting of 100g magnesium oxide and 100g

Hyflosupercel, (1:1 - mixed together and activated for 2 hours at 110°C), were added loosely up to a height of 20 cm. A moderate vacuum from a water aspirator was then applied continuously and the adsorbent surface was pressed down and flattened using a glass rod until the bed height was reduced to approximately 15 cm. Finally the column was topped with 1 cm layer of anhydrous sodium sulfate to prevent residual water from entering the adsorbent. The column then was tested with 100 ml petroleum ether and the water aspirator vacuum adjusted to ensure the adsorbent surface was smooth and the solvent flow even at about 2-3 drops per second. The top of the column was maintained with a cover of petroleum ether until the chromatography process was completed.

2.3.4 Developing the column

The carotenoid sample was maintained in as small a volume as possible to prevent separation and to diminish band broadening before the entire carotenoid sample has reached the adsorbent top. To achieve this, petroleum ethereal extracts were pipetted into the column until the sample layer almost reached the surface of the adsorbent. The MgO:Hyflosupercel (1:1) column was developed starting with 50 ml each of 1% and 8% diethyl ether, followed by (50 ml each) 2%, 5%, 10%, 15%, 20% up to 70% of acetone in petroleum ether. Only the main portion of each band of the desired carotenoid was collected and care was taken to avoid contamination from the other bands to ensure the purity of the standards. Tightly adsorbed carotenoids were eluted by 5% and followed by 10% water in acetone. β-carotene was eluted with 8% diethyl ether in PE, violaxanthin with 15-18% acetone in PE, lutein with 25-40% acetone in PE and neoxanthin with 60-70% acetone in PE. All the desired carotenoids collected from OCC were then washed three times with water in a separatory funnel to remove acetone. To verify the purity of the

standards an aliquot from each isolate was taken and verified through HPLC by checking the chromatogram profile obtained with a photodiode array detector.

2.3.5 Preparation of the standard solution and construction of the standard curves

All aliquots then were dried down under oxygen-free nitrogen and dissolved in 1 ml ethyl acetate (HPLC grade). Immediately before injection 100 µl was filtered through a 0.22 µm PTFE syringe filter (Millipore) directly to sample vials and 10 µl was injected into the chromatograph. Once the desired purity was verified, the concentrations of the pure standards were determined spectrophotometrically, as described in section 2.2.3. Identification of the *E. sativa* carotenoids was carried out as described by Rodriguez-Amaya (1999). This involved the combined use of the retention times, co-chromatography with authentic samples, the visible absorption spectra obtained spectrophotometrically and by the photodiode array detector. Detection was at the wavelengths of maximum absorption of the carotenoids in the mobile phase (max plot): neoxanthin 438 nm; violaxanthin 441 nm; lutein 447 nm; zeaxanthin 452 nm and β-carotene 454 nm. Complete solubilisation of the carotenoid is essential, especially for crystalline samples like zeaxanthin. For the standard curves, triplicate aliquots of 1, 2, 3, 4 and 5 ml were transferred to culture tubes, dried under oxygen-free nitrogen, and just before injection, dissolved in 1 ml HPLC grade ethyl acetate and filtered through a 0.22 µm PTFE syringe filter (Millipore) and 10 µl was analysed by HPLC. Curves constructed with five different concentrations for each carotenoid, in triplicate, were confirmed as linear with a correlation coefficient ≥ 0.95 (Appendix 1.2). The purity of the standard solution was calculated as follows:

$$\% \text{ purity} = \frac{\text{area under the standard peak}}{\text{total area of all peaks}} \times 100$$

The area under the standard peak is obtained at the maximum wavelength of the standard and the total area is obtained from sum of the areas of all peaks at this wavelength. Once the desired purity was obtained (≥ 90%), the concentrations of the pure standards were determined spectrophotometrically, using the following $A_{1cm}^{1\%}$ values: β-carotene, 2592 in petroleum ether; zeaxanthin, 2348 in petroleum ether; lutein, 2550 in ethanol; violaxanthin, 2550 in ethanol and neoxanthin, 2243 in ethanol. A sample of at least 1.0 mg of the pure crystalline substance was weighed accurately (3 decimal places) and dissolved in an accurately measured volume of a suitable solvent. For lutein, violaxanthin and neoxanthin, a petroleum ether solution was prepared in a 5 ml volumetric flask. These were dried under oxygen-free nitrogen and the residue dissolved in a specific volume of ethanol and the absorbance read using ethanol as blank. The concentrations of each pure standard were calculated according to the following formulas:

$$C\ (\mu g/mL) = \frac{\text{absorbance} \times 10^4}{A_{1cm}^{1\%}}$$

The concentrations with the respective standard's % purity then were corrected:

$$\text{Corrected C}\ (\mu g/mL) = \frac{C\ (\mu g/mL) \times \%\ \text{purity}}{100}$$

The concentration of each standard in the mixed standard solution was calculated using the following formula:

$$\text{Concentration}\ (\mu g/mL) = \frac{\text{Corrected C} \times V_{std}\ (mL)}{V}$$

Where Corrected C = concentration in the isolated standard solution, V_{std} = volume taken to prepare the mixture and V = volume of the mixture. For commercial standards such as zeaxanthin, the purity of the standards were also been verified using the same calculation.

The standard curves with five different concentrations for each carotenoid in triplicate were constructed by plotting the area against the concentration. For these carotenoid

experiment curves, the purity of the standards was 90% for neoxanthin and violaxanthin, 98% for zeaxanthin and β-carotene and 92% for lutein. The coefficients of correlation were 0.9858, 0.9961, 0.9726, 0.9950, and 0.9959 respectively (Appendix 1.2). One-point calibration was applied for this study on each day of analysis provided that the point falls on or very close to the curve. Full calibration also was applied every 3 months or when variation of the ratio between concentration and the area of a standard peak exceeded 5% as described by Mantoura and Repeta (1997).

2.4 Measurement of tuber flesh colour intensity

Before evaluation tubers were cut into half from the apical to distal end and blotted dry on a paper towel. Each half of the tuber was measured for its yellow colour intensity by Minolta CR210 chroma meter and CIELAB system. L* a* b* values were determined by taking three measurements at random locations on freshly cut surfaces. The mean value of the two halves of each tuber was used to indicate the yellow-flesh intensity of that tuber. All measurements were first calibrated using Minolta calibration plate with L* (98.07), a* (-0.23) and b* (+1.88). CIE refers to the Commission Internationale de l'É-clairage (International Commission on Illumination) or in the CIE 1976 (L*, a*, b*) colour system, abbreviated CIELAB, the lightness coefficient, L*, ranges from black (0) to white (100), positive a* indicates a hue of red purple and negative a*, of bluish green. Whereas positive b* indicates yellow and negative b* blue. The colour of achromatic or gray is when a* and b* = 0. According to Francis (1980) although the measure of colour's lightness, L*, is correctly reported without further manipulation, a* and b* are merely coordinates that indirectly reflect hue and chroma but are difficult to interpret separately. More importantly, these coordinates are not independent variables.

CHAPTER 3

Characterisation of carotenoid content in tubers of a diverse range of potato cultivars

3.1 Introduction

Colour in potato tubers arises from two main classes of pigments, carotenoids and anthocyanins. Anthocyanins are responsible for the water soluble, vacuolar, pink, red, purple and blue pigments present in coloured potatoes (Lewis et al., 1998b; Mancinelli, 1985), whereas carotenoids are responsible for the orange and yellow lipid soluble pigments in plastids (Van den Berg et al., 2000; Tevini et al., 1984). All carotenoids originate from a single, common precursor, phytoene (Gross, 1991). The color of carotenoids in plants is determined by desaturation, isomerization, cyclization, hydroxylation and epoxidation of the 40-carbon phytoene (Taylor and Ramsay, 2005). The conjugated double-bond structure and nature of end ring groups confer on the carotenoids properties such as colour and antioxidant activity (El-Agamey et al., 2004). A key driver for research aimed at enhancing carotenoid content in storage organs of plants is the emerging evidence of the health benefits associated with carotenoids intake (Taylor and Ramsay, 2005). In order to effectively enhance carotenoid content in crops such as potato, it is critical to first investigate the diversity of carotenoid compounds in a wide range of germplasm to allow the selection of the most appropriate germplasm for manipulation via breeding or molecular genetic methods. Therefore, this chapter describes the quantitative and qualitative determination of carotenoid compounds in tubers of a diverse range of potato cultivars ranging from white to dark yellow. The relationship between tuber flesh colour and carotenoid content was investigated and verified by spectrophotometry and HPLC.

3.2 Experimental Design

3.2.1 New Zealand-grown potato cultivars

A total of 32 potato cultivars were selected to represent a diverse collection of genetic material for analysis of carotenoid content. The selected cultivars were planted (2004) in field trials at Crop and Food Research, Lincoln, Canterbury, New Zealand. Each cultivar was planted in three replicates per plot. The plot size, planting, harvest times and agronomic management of the crop followed the standard protocols set out for New Zealand potato trials as described in detail by Conner et al. (1994). All tubers were harvested (2005) from plots at full maturity following natural plant senescence and maintained at ambient temperatures for three weeks until grading, then placed in cool storage (air at 8°C). Ten undamaged tubers from this season (2004/2005) were selected after harvested from each of three replicate plots for all cultivars.

3.2.2 Netherlands-grown potato cultivars

Twelve selected cultivars were planted (2005) in the plot at field trials at Wageningen, the Netherlands. The plot size, planting, harvest times and agronomic management of the crop followed the standard protocols set out for potato trials at the Laboratory of Plant Breeding, Wageningen University and Research Centre. All tubers were harvested (2005) from plots at full maturity following natural plant senescence and maintained at ambient temperatures for three weeks until grading, then placed in cool storage (air at 8°C). Ten undamaged tubers from this season (2005) were selected after harvest and randomly assigned to three replicates for all cultivars.

3.2.3 Sampling

The skin of the tubers was removed with a peeler and the remaining tuber tissue was cut into 5.0 mm slices. For each sample, tuber tissue was pooled from three tubers, mixed, and a random 100 g sample was immediately frozen at -20^{O}C. The tuber samples were freeze-dried for 7 days, after which the samples were ground into fine powder and stored at -80 °C until analysis.

3.2.4 Extraction of carotenoids

The extraction procedure followed the methods described by Morris et al. (2004), Lewis et al., (1998) and Britton et al., (1995) as detailed in Chapter 2 (section 2.2.2).

3.2.5 Determination of total carotenoid concentration

Total carotenoid concentration was determined by spectrophotometry as described previously by Britton et al., (1995) and Lewis et al., (1998) in Chapter 2 (section 2.2.3).

3.2.6 HPLC analysis

The HPLC analysis of saponified carotenoids was analysed according to Morris et al. (2004) as detailed in Chapter 2 (section 2.4). Carotenoid standards β-carotene, violaxanthin, lutein, and neoxanthin were isolated from *Eruca sativa* (roquette or rocket salad) by open column chromatography (see section 2.3.1) as described by Kimura and Rodriguez-Amaya (2002), whereas zeaxanthin was obtained commercially from Sigma-Aldrich (Auckland, New Zealand).

3.3 Results

3.3.1 Analysis of total carotenoid content in tubers of 32 potato cultivars grown in New Zealand

Potato cultivars representing diverse genetic backgrounds and tuber flesh colours ranging from white, pale yellow, dark yellow through to purple were selected for this study (Appendix 3.1). These 32 cultivars exhibited highly significant differences in total carotenoid content ($P < 0.0001$). As a result, in the 32 potato cultivars grown in New Zealand, the carotenoid content range can be divided into four groups based on colour: dark yellow, pale yellow, cream and white tuber flesh colour as detailed in Table 3.1. The purple-fleshed and pink-fleshed cultivars also have a low carotenoid content similar to the white-fleshed cultivars. There was positive relationship between total carotenoid content and yellow colour intensity of tuber flesh. Agria, a dark yellow-fleshed cultivar, was found to have the highest total carotenoid content (169.57 μg/g DW), substantially higher than all other cultivars tested (Figure 3.1). In contrast, the lowest total carotenoid concentration was found in white-fleshed cultivars such as Moonlight (1.18 μg/g DW).

Table 3.1: Range of total carotenoid content (μg/g DW) for potato cultivars in different tuber flesh colour groups

Flesh Colour	Total carotenoid range (μg/g DW)
Dark Yellow	45.47 - 169.57
Pale Yellow	25.08 - 41.12
Cream to Pale Yellow	23.24
Cream	6.99 - 32.21
Pink	5.50
Purple	2.09
White	1.18 - 26.39

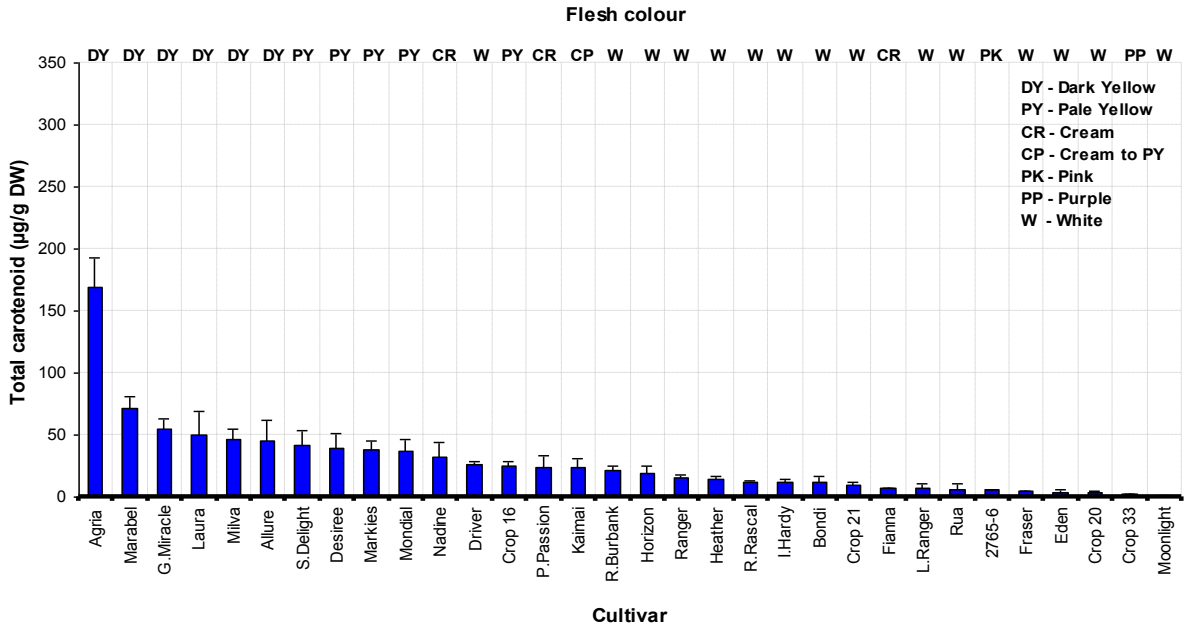


Figure 3.1: Total carotenoid content (µg/g DW) of 32 potato cultivars grown in New Zealand (2004/2005 growing season). Error bars represent ± SE

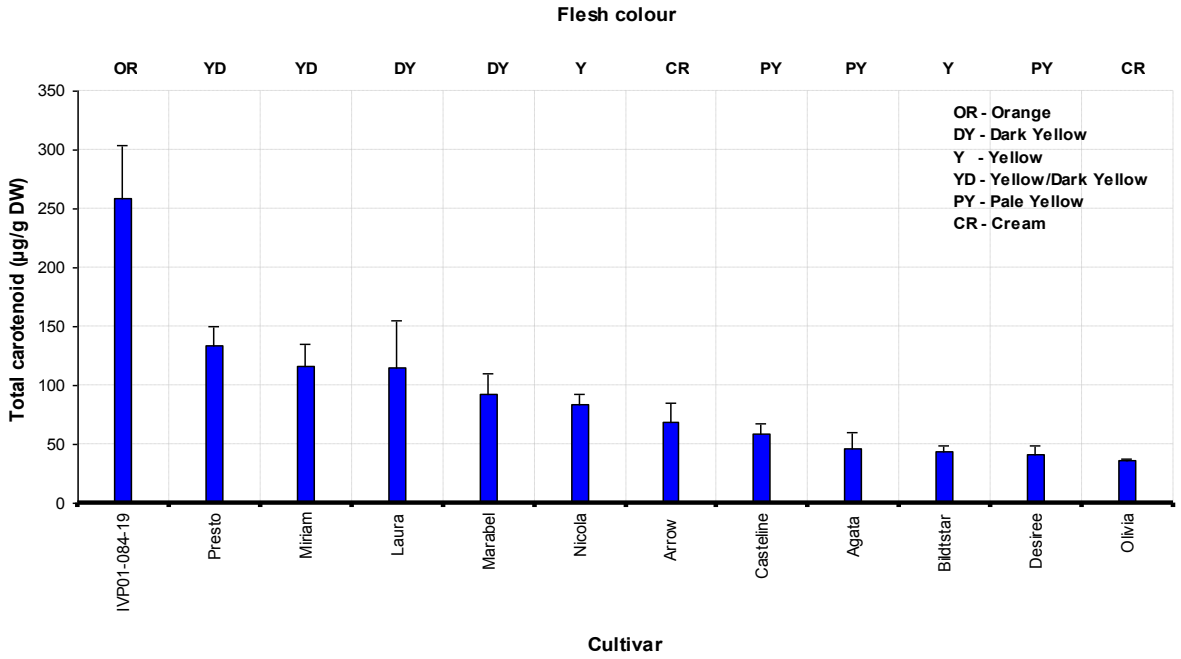


Figure 3.2: Total carotenoid content (µg/g DW) of 12 potato cultivars grown in Netherlands (2005 growing season). Error bars represent ± SE

3.3.2 Analysis of total carotenoid in tubers of 12 potato cultivars grown in the Netherlands

To further investigate the relationship between colour and total carotenoid content, analysis was also performed on 12 cultivars (with cream to orange tuber flesh), grown in the Netherlands in the 2005/2006 growing season. This analysis included three cultivars in common with the cultivars grown in New Zealand and analysed in this study. Analysis of variance established that these 12 cultivars also exhibited highly significant differences in total carotenoid content ($P < 0.0001$). The total carotenoid content in these 12 cultivars ranged from 35.91 µg/g DW in the cream fleshed cultivar Olivia to 258.95 µg/g DW in the orange-fleshed potato genotype IVP01-084-19 (Figure 3.2, Appendix 3.2). This study on cultivars grown in the Netherlands further confirmed the higher concentration of total carotenoids is accompanied with a greater intensity of yellow pigmentation in potato tuber flesh.

3.3.3 HPLC analysis of individual carotenoid pigments of 32 potato cultivars grown in New Zealand

The next step in this analysis was to identify and quantify the specific pigments in each cultivar and to determine whether more intense yellow pigmentation is associated with larger amounts of specific carotenoid compounds or a general increase in all carotenoids. Carotenoid analysis performed by HPLC system detected at least four major carotenoid peaks: neoxanthin, violaxanthin, lutein and β-carotene. Statistical analysis established highly significant differences ($P < 0.0001$) between the 32 cultivars, the individual carotenoid pigments, and their interaction (Table 3.2). The ranges in content for

all individual carotenoid pigments among the six dark yellow-fleshed cultivars studied were higher than for the white flesh cultivars (Table 3.3). As shown in Figure 3.3 and Appendix 3.1, neoxanthin was found highest in Marabel (dark yellow flesh); violaxanthin was highest in Allure (also a dark yellow cultivar), whereas lutein and β-carotene were detected in their highest levels in Agria (dark yellow) and Summer Delight (pale yellow) respectively. Zeaxanthin was not found in any of the 32 cultivars analysed.

The cultivars could be grouped into one of several classes depending on the accumulation of specific carotenoid pigments (Table 3.4 and Figure 3.3). Eleven cultivars ranging from white, cream, pale to dark yellow tuber flesh, such as Driver, Nadine and Marabel, were found to have all four individual carotenoid pigments with a relatively high concentration of neoxanthin and lower concentrations of violaxanthin, lutein and β-carotene. However flesh colour was still strongly correlated to the total amount of carotenoid accumulating. Fifteen cultivars, also varying across the range of white to dark yellow tuber colour, such as Agria, Fianna and Eden, were detected to have three of the four carotenoid pigments; violaxanthin, lutein and β-carotene. The pale yellow cultivar Desiree was a notable exception in this group where it was found to accumulate neoxanthin unlike the other cultivars. A group of three cultivars (Crop 33, 2765-6 and Moonlight) with white, pink and purple flesh only accumulated lutein and β-carotene, whereas another two white tuber flesh cultivars (Ranger and Crop 20) only contained one carotenoid pigment (neoxanthin or β-carotene).

Table 3.2: Analysis of variance for 32 potato cultivars grown in New Zealand during 2004/2005

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Cultivar	31	63430.61867	2046.14899	31.35	0.0001
Error	64	4177.07393	65.26678		
Corrected Total	95	67607.69260			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Cultivar	31	32353.02869	1043.64609	56.34	0.0001
Error	64	1185.56113	18.52439		
Corrected Total	95	33538.58982			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Cultivar	31	3951.491000	127.467452	21.93	0.0001
Error	64	371.978600	5.812166		
Corrected Total	95	4323.469600			

D - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Cultivar	31	49943.47604	1611.07987	52.75	0.0001
Error	64	1954.73453	30.54273		
Corrected Total	95	51898.21057			

E - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Cultivar	31	634.2048292	20.4582203	39.19	0.0001
Error	64	33.4132667	0.5220823		
Corrected Total	95	667.6180958			

Table 3.3: Ranges of carotenoid content among potato cultivar tuber flesh colour groups

Flesh colour	Neoxanthin	Violaxanthin	Lutein	β-Carotene	Total Carotenoid
Dark Yellow	46.32 - 69.21	0.04 – 32.70	0.20 – 160.63	0.01 – 8.54	45.47 – 169.57
Pale yellow	34.78 – 36.78	0.07 – 2.67	0.97 – 24.83	0.01 – 13.62	25.08 – 41.12
Cream	21.92 – 31.10	0.02 – 0.06	1.05 – 6.89	0.01 – 0.04	6.99 – 32.21
White	10.59 – 24.08	0.01 – 7.64	0.40 – 21.03	ND – 3.76	1.18 – 26.39

ND – non-detectable

In general the highest carotenoid concentrations, either in total or individual carotenoid pigments were detected in yellow fleshed cultivars. It can be concluded that the intensity of yellow fleshed cultivars is strongly associated with the concentration of total and individual carotenoids. However the relative distributions of individual carotenoids within each colour grouping (dark yellow, pale yellow, cream and white) did not necessary correlate to the levels of total carotenoids. In other words, high levels of carotenoids in dark yellow lines may result from the accumulation of different levels of individual carotenoid pigments.

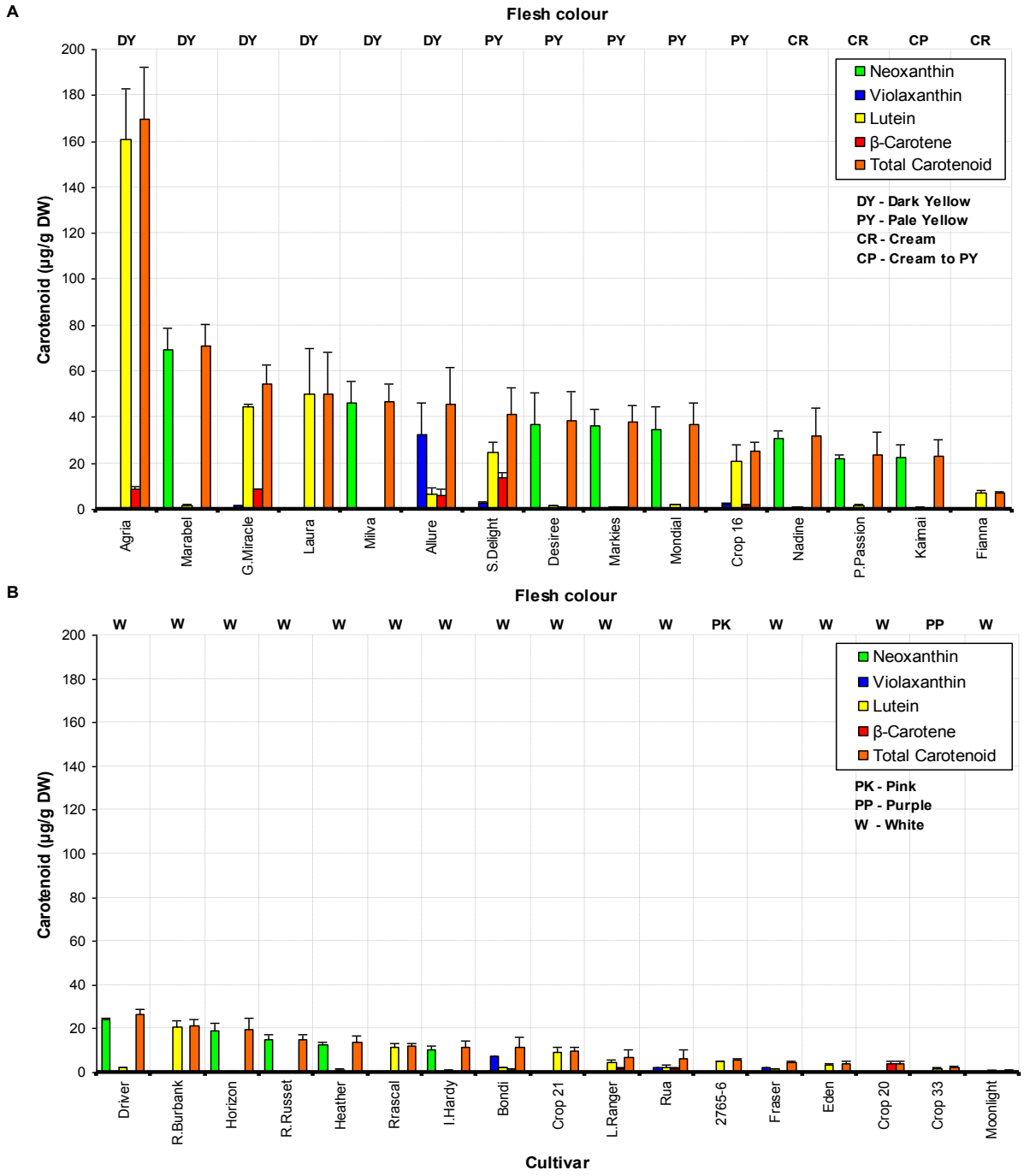


Figure 3.3: Comparison of total carotenoid levels and contributing individual carotenoid types between commercially grown potato tubers harvested in the 2004/2005 growing season in New Zealand.
A – Individual and total carotenoid content (µg/g DW) of 32 potato cultivars grown in New Zealand (2004/2005 growing season) with dark yellow to cream flesh colour
B - Individual and total carotenoid content (µg/g DW) of 32 potato cultivars grown in New Zealand (2004/2005 growing season) with white, pink and purple flesh colour
Zeaxanthin not included due to undetected levels in all samples. Error bars represent ± SE.

Table 3.4: Relative distributions of individual carotenoid from one to four types of carotenoid with 32 cultivars

Cultivar	Total Carotenoid (µg/g DW)	Flesh colour	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)
Cultivars with 4 carotenoid pigments						
Marabel	71.34	Dark Yellow	69.21	0.26	1.80	0.07
Milva	46.57	Dark Yellow	46.32	0.04	0.20	0.01
Markies	37.96	Pale Yellow	36.21	0.07	0.97	0.71
Mondial	36.89	Pale Yellow	34.78	0.17	1.93	0.01
Nadine	32.21	Cream	31.10	0.02	1.05	0.04
Driver	26.39	White	24.08	0.01	2.03	0.27
P. Passion	23.87	Cream	21.92	0.05	1.89	0.01
Kaimai	23.24	P Yellow/Cream	22.37	0.06	0.77	0.04
Horizon	19.50	White	19.07	0.02	0.40	0.01
Heather	13.91	White	12.47	0.01	1.41	0.01
Ilam Hardy	11.70	White	10.59	0.01	1.09	0.00
Cultivars with 3 carotenoid pigments						
Agria	169.57	Dark Yellow	ND	0.40	160.63	8.54
G. Miracle	54.52	Dark Yellow	ND	1.59	44.4	8.54
Laura	50.31	Dark Yellow	ND	0.04	50.25	0.01
Allure	45.47	Dark Yellow	ND	32.7	6.67	6.04
S. Delight	41.12	Pale Yellow	ND	2.67	24.83	13.62
Desiree	38.76	Pale Yellow	36.78	ND	1.38	0.60
Crop 16	25.08	Pale Yellow	ND	2.25	21.21	1.63
R. Burbank	21.16	White	ND	0.07	21.03	0.05
Red Rascal	12.22	White	ND	0.33	11.79	0.09
Crop 19	11.46	White	ND	7.64	2.43	1.39
Crop 21	9.90	White	ND	0.28	9.46	0.16
Fianna	6.99	Cream	ND	0.06	6.89	0.03
L. Ranger	6.77	White	ND	0.06	4.70	2.01
Rua	6.08	White	ND	2.07	2.39	1.62
Fraser	4.68	White	ND	2.40	1.75	0.52
Eden	4.12	White	ND	0.24	3.64	0.24
Cultivars with 2 carotenoid pigments						
2765-6	5.50	Pink	ND	ND	5.07	0.44
2765-5	2.09	Purple	ND	ND	1.71	0.38
Moonlight	1.18	White	ND	ND	0.83	0.35
Cultivars with 1 carotenoid pigments						
Ranger	15.31	White	15.31	ND	ND	ND
Crop 20	3.76	White	ND	ND	ND	3.76

ND – non-detectable

3.3.4 HPLC analysis of individual carotenoid pigments of 12 potato cultivars grown in the Netherlands

In comparison to the New Zealand-grown cultivars analysed, only three major carotenoids were detected in the extracts of the 12 yellow flesh cultivars grown in the Netherlands. These were neoxanthin, violaxanthin and lutein. No traces of zeaxanthin and β-carotene were found in any of the 12 cultivars tested. The analysis of variance established revealed highly significant differences ($P < 0.0001$) between the 12 cultivars, the individual carotenoid pigments, and their interaction (Table 3.5). Ranges in carotenoid content among all 12 cultivars yellow to orange fleshed varieties studied were as follows: neoxanthin (28.20 – 228.01 µg/g DW), violaxanthin (undetectable levels – 30.29 µg/g DW) and lutein (0.08 – 0.66 µg/g DW).

As shown in Figure 3.4 and Appendix 3.2, all cultivars were found to accumulate individual carotenoid pigments in a similar manner with different concentrations and reflecting changes in tuber colour. There was found to be a strong association between individual carotenoid pigments and total carotenoid content within 12 yellow to orange flesh cultivars grown in Netherlands.

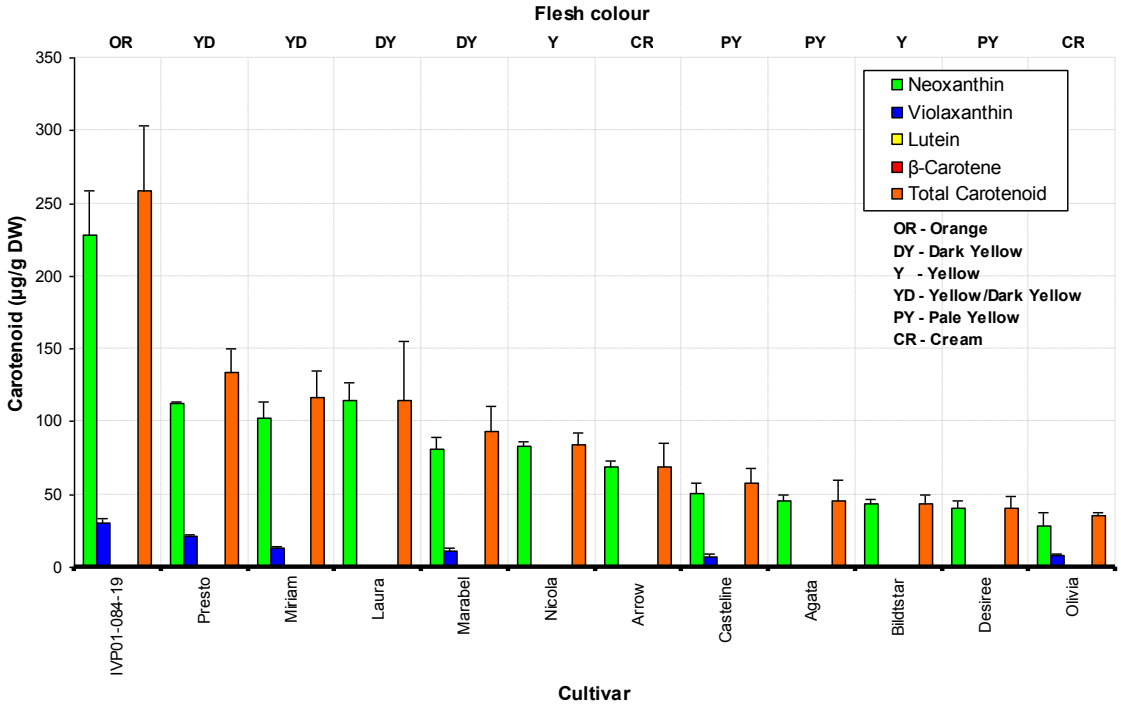


Figure 3.4: Individual and total carotenoids content (µg/g DW) of 12 potato cultivars grown in Netherlands (2005 growing season). Zeaxanthin not included due to undetected levels in all samples. Error bars represent ± SE

Table 3.5: Analysis of variance for 12 potato cultivars grown in Netherlands during 2005

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	11	128207.2029	11655.2003	575.47	0.0001
Error	24	486.0768	20.2532		
Corrected Total	35	128693.2797			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	11	97323.06652	8847.55150	466.94	0.0001
Error	24	454.75527	18.94814		
Corrected Total	35	97777.82179			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	11	3104.968256	282.269841	167.64	0.0001
Error	24	40.409667	1.683736		
Corrected Total	35	3145.377922			

Lutein, β-carotene and zeaxanthin not included due to trace amounts or undetected levels in all samples.

3.4 Discussion

Tuber flesh colour in potato tubers ranges from white, cream, yellow through to orange and is strongly associated with the presence of carotenoids. The yellow fleshed trait in potato tubers is controlled by a single gene which has been mapped to the third chromosome, with yellow flesh dominant over white flesh (Bonierbale et al., 1988; Jacobs et al., 1995). Caldwell et al. (1945) reported that the range of total carotenoids content of 22 white fleshed varieties and breeding clones lay between 0.14 and 1.87 µg/g FW. Two years later, Brunstetter and Wiseman (1947) observed the total carotenoids content of white flesh 'Katahdin' to be 3.0 µg/g FW. Subsequently, Iwanzik et al. (1983) found that the sum of the carotenoids in yellow flesh European varieties ranged from 0.27 to 3.42 µg/g FW. Later, Gross (1991) concluded that the total carotenoid content in potato tubers was between 0.27 and 2.43 µg/g FW. Yellow-fleshed cultivars had a higher carotenoid content carotenoid content than the white-fleshed cultivars. Brown (2005) found that the carotenoid content ranged from 0.50 to 1.0 µg/g FW in white fleshed varieties to 20 µg/g FW in deep yellow to orange fleshed cultivars. Brown et al. (2004) also divided cultivars into white, yellow and dark yellow categories on the basis of colour which corresponded to 0.50 to 1.0, 1.5 to 2.5 and 5.0 to 7.0 µg/g FW total carotenoid range groupings. These previous reports are in agreement with the present study where similar results were observed. The total carotenoid content of dark yellow-fleshed cultivars always much higher than white-fleshed cultivars (Figure 3.1) whereas carotenoid content range for dark yellow group always much more higher than pale yellow, cream and white (Table 3.1). Strong positive relationships were found between tuber flesh colour intensity and total carotenoid level in both the New Zealand (Figure 3.1) and Netherland (Figure 3.2) cultivars. A positive linear relationship between tuber flesh colour and total carotenoid

content has been also reported in 13 German cultivars (Iwanzik et al. 1983). Similarly, a survey of 11 diploid potato clones found that the yellow-fleshed diploid potatoes contained 4 to 22 times more carotenoids than the white fleshed cultivars (Lu et al. 2001). According to Brown (2005) and Brown et al. (2003), yellow flesh cultivars are believed to have higher antioxidant activity than white flesh cultivars due to the presence of lutein and zeaxanthin pigments. Both of these pigments are strongly related to the yellow flesh colour and are known to have antioxidant activity. Previous studies have established that the carotenoid profiles in potato tubers are dominated mostly by lutein, zeaxanthin and violaxanthin. There is just a trace of either alpha- or beta- carotene (Fossen and Andersen, 2000; Fossen et al., 2003; Iwanzik et al., 1983; Mazza and Muniati, 1993; Rodriguez-Saona et al., 1998). Based on uv/vis absorbance spectrophotometry and HPLC analysis of 32 potato cultivars grown in New Zealand and 12 cultivars grown in the Netherlands, the carotenoids found present in potato tubers were neoxanthin, violaxanthin, lutein and β-carotene. These four compounds were the predominant carotenoids found in 32 potato cultivars grown in New Zealand whereas neoxanthin, violaxanthin and a trace of lutein were the major carotenoid found in yellow to orange fleshed cultivars grown in the Netherlands. No traces of zeaxanthin was found in dark yellow to pale yellow potato cultivars in both cases whereas previous studies reported that zeaxanthin was detected as one of the major carotenoid (Tevini et al., 1984; Lu, et al., 2001). Brown et al. (1993) and Gross (1991) stated that white and yellow fleshed potatoes have similar composition of carotenoids, but the yellow colour is due to higher concentrations of certain carotenoids. Yellow fleshed potatoes are generally characterised by higher contents of lutein and violaxanthin (Nesterenko and Sink, 2003). Gross (1991) also reported that lutein was the major pigment found in white potatoes and the second major pigment found in those with yellow flesh. β-carotene and neoxanthin were identified at low levels in the potato tuber.

Similarly Brown (2005) reported that lutein was always present in white fleshed potato and violaxanthin was the second most common carotenoid in yellow fleshed potatoes. Iwanzik et al. (1983) also found that lutein and violaxanthin have been identified as major carotenoids in yellow flesh potatoes whereas Brown et al. (1993) reported that lutein and zeaxanthin have been identified as major carotenoids in orange flesh potatoes.

Similar to previous reports, this study found that within each of the dark yellow, pale yellow and white fleshed colour groups of potato cultivars, different compositions of individual carotenoid pigments can be found. As shown in Table 3.4, some of dark yellow fleshed cultivars (Marabel and Milva) were found with four different carotenoid pigments, whereas the other four cultivars (Agria, Laura, Golden Miracle and Allure) with dark yellow flesh had only three carotenoid compounds. Similar results also were found in pale yellow and white fleshed cultivars. Some of white cultivars were detected with high concentration of lutein (Russet Burbank) and some were detected with high concentration in neoxanthin (Driver) or violaxanthin (Allure). In addition, neoxanthin and violaxanthin were identified as major carotenoids in yellow to orange fleshed cultivars grown in the Netherlands (Figure 3.4). Lutein was detected at low level, whereas zeaxanthin and β-carotene were not found at all. Overall, lutein and neoxanthin were found to be major carotenoids present in 32 potato cultivars grown in New Zealand whereas neoxanthin was the major carotenoid found in 12 potato cultivars grown in Netherlands.

Carotenoid biosynthesis is essential for plant growth and development as accessory pigments in photosynthesis, as photoreceptors preventing photooxidative damage, as precursors of some scents and the growth regulator ABA, and as antioxidants (Hirschberg, 2001; Cunningham, 2002; Fraser and Bramley, 2004; Howitt and Pogson, 2006). All

individual carotenoids originate from phytoene and type of carotenoids in potato tubers is determined by desaturation, isomerization, cyclization, hydroxylation and epoxidation. The significant branch point in carotenoid biosynthesis is the cyclization of lycopene (Figure 1.4). The introduction of two β rings to lycopene can lead to the formation of β-carotene and on another branch point the formation of α-carotene take place with both ε-ring and β ring present. Neoxanthin, violaxanthin and zeaxanthin are derived from the β-carotene pathway, whereas lutein is derived from the α-carotene pathway. In 32 New Zealand grown cultivars, eleven cultivars were detected to have all four types of individual carotenoids with a relatively high concentration of neoxanthin. Sixteen cultivars were detected to have three carotenoid pigments, of which 12 cultivars were detected with high lutein, 3 cultivars with high violaxanthin and only Desiree accumulated high neoxanthin. A group of three cultivars accumulated only lutein and β-carotene, whereas another two cultivars contained only a single carotenoid pigment (neoxanthin or β-carotene). This result demonstrates that carotenoid composition and accumulation level vary with potato cultivars. The identification of such a genetic basis to significant levels of carotenoid within tubers from potato germplasm has provided the impetus to optimize carotenoid levels using both breeding (Brown et al., 1993; Lu et al., 2001) and transgenic strategies (Romer et al., 2002). Various regulatory factors in the carotenoid biosynthesis pathway may be responsible for the genetic differences in carotenoid content in potato tubers. It seems that genetic factors play an important role in grouping these 32 cultivars into four categories. Selection of the right cultivar with the right capability to accumulate carotenoids will determine whether or not certain genetic manipulation strategies for carotenoid biosynthesis will succeed. Understanding the mechanism that controls carotenoid biosynthesis and exploring the diversity of carotenoid compounds in a wide

range of germplasm will contribute greatly to breeding potatoes with enhanced β-carotene or other carotenoids.

Biosynthesis of β-carotene requires enzyme activity of PSY, PDS, ZDS and LCY-B whereas HYD and LCY-E will diminish β-carotene. The manipulation of the genes encoding all these enzymes is crucial and critical to genetically engineer the pathway for carotenoid accumulation especially provitamin A as all the genes are needed for accumulation of the carotenoid building blocks, IPP and GGPP (Wurtzel, 2001). When the first effort was made to engineer the rice endosperm, a gene encoding PSY was introduced. As a result phytoene accumulated in the rice endosperm (Burkhardt et al., 1997), but no compounds downstream of phytoene such as lutein and β-carotene accumulated. Subsequently, when the genes encoding PSY, LCY-B and a bacterial CRTI (which mediates the four desaturation reactions mediated by phytoene desaturase, PDS, and ζ-carotene desaturase, ZDS) were introduced, β-carotene accumulated, resulting in golden rice (Ye et al., 2000). However some of the transgenic lines also accumulated significant levels of lutein and zeaxanthin which could only been produced if HYD enzyme activity was present. This result suggested that different plant species will react differently towards the stability of individual carotenoids accumulated in transgenic plants. Furthermore, the response in monocotyledonous and dicotyledonous species is also likely to differ. For example, over-expression of a phytoene desaturase gene resulted in an enhanced cyclization reaction in tomato fruit (Romer et al., 2000), whereas in rice grains phytoene desaturase gene led to an induction of cyclase and hydroxylase activity (Ye et al., 2000).

Attempts to modify the carotenoid content of potato tubers have focused on manipulation of various steps in the carotenoid pathway. In recent work involving

transgenic manipulation of potato tubers, carotenoid levels were successfully elevated by the expression of three *Erwinia* genes encoding phytoene synthase (CrtB), phytoene desaturase/carotene isomerase (CrtI) and lycopene beta-cyclase (CrtY), under the control of a tuber specific promoter. This resulted in the conversion of geranylgeranyl diphosphate (GGPP) into β-carotene and produced the golden potato with the highest total carotenoid (114 µg/g DW) and β-carotene (47 µg/g DW) ever been reported (Romer et al., 2002; Ducreux et al., 2005; Diretto et al., 2007b). The β-carotene content of golden potato is even higher than Golden Rice 2 (31 µg/g DW) (Diretto et al., 2007b). The potato cultivar Desiree has been used widely in genetic engineering programmes to enhance certain individual carotenoid such as β-carotene (Ducreux et al., 2005; Morris et al., 2006b; Diretto et al., 2007b). The results in this chapter suggest that other cultivars may be more appropriate. For example, neoxanthin was found highest in Marabel (69.21 µg/g DW); violaxanthin was highest in Allure (32.7 µg/g DW), whereas lutein and β-carotene were detected in their highest levels in Agria (160.63 µg/g DW) and Summer Delight (13.62 µg/g DW) respectively. The highest total carotenoid reported in this chapter was in Agria (169.57 µg/g DW).

These findings highlight the potential to manipulate both total carotenoid content and the type of carotenoids that are produced in potato tubers for genetic manipulation purposes. In this case the best cultivar to enhance level of β-carotene is either Agria which accumulates the highest total carotenoid, or Summer Delight with the highest level of β-carotene, but not Desiree which accumulate only trace element of β-carotene. The total carotenoid content of Desiree reported by Morris et al. (2004) and Ducreux et al. (2005) was 4.9 µg/g DW which accumulated predominantly violaxanthin (1.49 µg/g DW) followed by lutein (0.9 µg/g DW), neoxanthin (0.75 µg/g DW), antheraxanthin (0.45 µg/g

DW) and zeaxanthin (0.15 µg/g DW). The carotenoid content and profiles of Desiree increased and changed following overexpression of the gene encoding phytoene synthase (CrtB) (Ducreux et al., 2005). The total carotenoid content increased up to 38.32 µg/g DW and accumulated predominantly lutein (11.88 µg/g DW), β-carotene (11.11 µg/g DW) and violaxanthin (9.2 µg/g DW). Agria has a dark yellow tuber flesh, while Summer Delight and Desiree are pale yellow. Agria accumulated only lutein whereas Desiree only accumulated neoxanthin in this chapter. In contrast, previous research reported that Desiree also accumulated violaxanthin (Morris et al. 2004; Ducreux et al. 2005). Lutein is derived from the α-carotene pathway (Figure 1.5), whereas neoxanthin is derived from the β-carotene pathway (Figure 1.6). In order to enhance β-carotene in Desiree, five mechanisms need to be thoroughly understood because this involves neoxanthin, violaxanthin, antheraxanthin, zeaxanthin and ABA biosynthesis whereas in Agria it involves only α-carotene. The question remains; which mechanism will stimulate carotenogenesis or β-carotene or other carotenoids more effectively and to much higher levels. One possibility is that by restricting the supply of lutein in Agria will increase carotenogenic metabolic flux to the other branch point involving β-carotene. By using the Desiree cultivar Diretto et al. (2006) already demonstrated that by silencing the first dedicated step in the beta-epsilon-branch of carotenoid biosynthesis, lycopene epsilon cyclase (*LCY-e*), significantly increased β-carotenoid levels. β-carotene increased (up to 14-fold) and total carotenoids increased up to 2.5-fold. Furthermore, no major changes in tuber productivity or leaf carotenoid composition were observed in any of the lines, suggesting that silencing of *LCY-e* is a viable strategy for changing tuber carotenoid composition without affecting agronomic performance. It is therefore important to carry out these genetic manipulations using parental material with higher total carotenoid content, such as Agria in order to produce tubers with enhanced or elevated carotenoid content such as β-carotene.

From the study, the white to dark yellow tuber flesh colour is closely associated with total carotenoid content, with no particular individual carotenoid pigments being responsible for any particular tuber flesh colour. Breithaupt and Bamedi (2002) also found similar relationship and noted the differences between location and between fresh and frozen samples. Another interesting aspect of this study was the comparison of three cultivars Laura, Marabel and Desiree were grown in both New Zealand and the Netherlands (Appendix 3.3). The total carotenoid of Marabel and Desiree were similar but Laura grown in Netherlands was found to have 2 times higher carotenoid content than grown in New Zealand. Laura in New Zealand was found to contain high lutein levels, low level of violaxanthin and β-carotene, whereas Laura in Netherlands was high in neoxanthin with only a trace amount of lutein. Desiree and Marabel had similar total carotenoids when grown in the two countries, with high neoxanthin and low lutein concentrations. However, violaxanthin was absent from Desiree in New Zealand and β-carotene was absent in Netherlands, whereas for Marabel neoxanthin, violaxanthin, lutein and β-carotene were detected in New Zealand but with an absence of β-carotene in Netherlands. Lu et al. (2001) have showed that environmental factors can exert some influences on the expression of yellow flesh intensity and Haynes et al. (1996) reported that there were significant differences in yellow flesh intensity across environments. In general, it can be concluded from the above comparison that individual carotenoid pigments and total carotenoid content can differ with type of location or environment. Given these results, it was hypothesised that there was a strong possibility of genotype x environment interactions influencing carotenoid composition of potato tubers. Therefore a study was implemented to investigate this in greater depth and the results presented in the next two chapters.

CHAPTER 4

Stability of carotenoid composition in a wide range of potato genotypes over two seasons

4.1 Introduction

The previous chapter reports marked differences in carotenoid composition for one of three cultivars grown in New Zealand and the Netherlands (section 3.4, Appendix 3.3). This suggests that environmental conditions can influence the presence of specific carotenoid compounds and their concentration in potato tubers. This chapter investigates this phenomenon and its implications further by investigating the stability of carotenoid composition in a wide range of potato genotypes over two seasons in both New Zealand and the Netherlands. Reflectance colorimeter measurement was also made in an attempt to provide a high-throughput system for rapid estimation of the yellow colour intensity of potato tubers.

4.2 Experimental Design

4.2.1 New Zealand-grown potato cultivars

The same 32 potato cultivars grown during the 2004/2005 season (Chapter 3) were planted again in the 2006/2007 growing season at a different site within the potato field trials at Crop and Food Research, Lincoln, Canterbury, New Zealand. Each cultivar was planted in three replicate plots. The plot size, planting, harvest times and agronomic management of the crop followed the standard protocols set out for New Zealand potato trials as described in detail by Conner et al. (1994). All tubers were harvested from plots at

full maturity following natural plant senescence and maintained at ambient temperatures for three weeks until grading, then placed in cool storage (air at 8°C). Ten undamaged tubers were selected after harvested from each of three replicate plots for all cultivars.

4.2.2 Netherlands-grown potato cultivars

Ten of the twelve potato cultivars grown during the 2005 season (Chapter 3) were replanted within field trials at Wageningen, the Netherlands during 2006. The plot size, planting, harvest times and agronomic management of the crop followed the standard protocols set out for potato trials at the Laboratory of Plant Breeding, Wageningen University and Research Centre. All tubers were harvested from plots at full maturity following natural plant senescence and maintained at ambient temperatures for three weeks until grading, then placed in cool storage (air at 8°C). Ten undamaged tubers were selected after harvest and randomly assigned to three replicates for all cultivars.

4.2.3 Sampling

The skin of the tubers was removed with a peeler and the remaining tuber tissue was cut into 5.0 mm slices. For each sample tuber tissue was pooled from three tubers, mixed, and a random 100 g FW sample was immediately frozen at -20^{O}C. The tuber samples were freeze-dried for 7 days, after which the samples were ground into fine powder and stored at -80 °C until analysis.

4.2.4 Extraction of carotenoids

The extraction procedure followed the methods described by Morris et al. (2004), Lewis et al., (1998) and Britton et al., (1995) as detailed in Chapter 2 (section 2.2.2).

4.2.5 Determination of total carotenoid concentration

Total carotenoid concentration was determined by spectrophotometry as described previously by Britton et al., (1995) and Lewis et al., (1998) in Chapter 2 (section 2.2.3).

4.2.6 HPLC analysis

The HPLC analysis of saponified carotenoids was analysed according to Morris et al. (2004) as described detailed in Chapter 2 (section 2.4). Carotenoid standards β-carotene, violaxanthin, lutein, and neoxanthin were isolated from *Eruca sativa* (roquette or rocket salad) by open column chromatography (see section 2.3.1) as described by Kimura and Rodriguez-Amaya (2002), whereas zeaxanthin was obtained commercially from Sigma-Aldrich (Auckland, New Zealand).

4.2.7 Measurement of tuber flesh colour intensity

Potato tuber flesh colour was measured for its yellow colour intensity by Minolta CR210 chroma meter and CIELAB system according to Francis (1980) as described detailed in Chapter 2 (section 2.4). L* a* b* values were determined by taking three measurements at random locations on freshly cut surfaces. The mean value of the two halves of each tuber was used to indicate the yellow-flesh intensity of that tuber. All measurements were first calibrated using Minolta calibration plate with L* (98.07), a* (-0.23) and b* (+1.88).

4.3 Results

4.3.1 Genotype x growing season interaction for 32 New Zealand-grown potato cultivars

Thirty two cultivars were analysed for total and individual carotenoid content from two growing seasons of 2004/2005 (Chapter 3) and 2006/2007 (this chapter). Analysis of variance on the 2006/2007 data confirmed the previous findings by exhibiting highly significant differences ($P < 0.0001$) between the 32 cultivars and the individual carotenoid pigments (Table 4.1). When the data is pooled from both growing seasons, further analysis of variance established highly significant differences ($P < 0.0001$) between the 32 cultivars, the carotenoid pigments, the seasons, and all combinations of interactions (Table 4.2). This clearly demonstrates that growing season can have an important influence on the accumulation of carotenoids. The importance of the interaction components emphasises that the changes in carotenoid composition are complex and the responses are not consistent across cultivars. Examination of the summarised data reveals that, of the 32 cultivars analysed, 20 cultivars exhibit an increase and 12 cultivars a decrease in total carotenoid content in the second season (Figures 4.1 and 4.2). The cultivars that increased in carotenoid content had an average increase of 83.57%, while those that decreased in carotenoid content had an average decrease of 43.73%. A comparison over the two seasons of the ranges of total carotenoid content in each tuber flesh colour is summarised in Table 4.3. Overall, the total carotenoid content of 32 different cultivars in 2006/2007 growing season ranged from 0.91 to 129.08 µg/g DW as compared to 1.18 to 169.57 µg/g DW in 2004/2005. The cultivar with the largest difference between years was Crop 16 (276.16% increase), whereas in the opposite extreme Milva exhibited a marked decline (88% decrease). Other cultivars with marked changes in total carotenoid content were Markies

with a relatively higher amount in 2006/2007 (129.08µg/g DW) and Agria with a relatively higher amount in 2004/2005 (169.57 µg/g DW) (Appendix 4.1).

Table 4.1: Analysis of variance for 32 potato cultivars grown in New Zealand during 2006/2007

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	31	121208.1829	3909.9414	190.42	0.0001
Error	64	1314.1424	20.5335		
Corrected Total	95	122522.3253			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	31	1793.170596	57.844213	8.41	0.0001
Error	64	440.162200	6.877534		
Corrected Total	95	2233.332796			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	31	846.9302958	27.3203321	85.94	0.0001
Error	64	20.3452667	0.3178948		
Corrected Total	95	867.2755625			

D - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	31	911.1429823	29.3917091	58.99	0.0001
Error	64	31.8886667	0.4982604		
Corrected Total	95	943.0316490			

E - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	31	205.2018500	6.6194145	112.95	0.0001
Error	64	3.7506000	0.0586031		
Corrected Total	95	208.9524500			

F - Analysis of variance for zeaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	31	101150.8809	3262.9316	351.75	0.0001
Error	64	593.6750	9.2762		
Corrected Total	95	101744.5559			

Table 4.2: Analysis of variance for 32 potato cultivars over two growing seasons (2004/2005 and 2006/2007) in New Zealand

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
YEAR	1	2208.8568	2208.8568	51.49	0.0001
CULTIVAR	31	148776.6366	4799.2463	111.87	0.0001
YEAR*CULTIVAR	31	35862.1649	1156.8440	26.97	0.0001
Error	128	5491.2163	42.9001		
Corrected Total	191	192338.8747			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
YEAR	1	3851.99375	3851.99375	303.28	0.0001
CULTIVAR	31	19739.61602	636.76181	50.13	0.0001
YEAR*CULTIVAR	31	14406.58327	464.7284	36.59	0.0001
Error	128	1625.72333	12.70096		
Corrected Total	191	39623.91637			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
YEAR	1	1.884169	1.884169	0.61	0.4345
CULTIVAR	31	3001.279665	96.815473	31.59	0.0001
YEAR*CULTIVAR	31	1797.141631	57.972311	18.91	0.0001
Error	128	392.323867	3.065030		
Corrected Total	191	5192.629331			

D - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
YEAR	1	4267.07510	4267.07510	274.93	0.0001
CULTIVAR	31	24687.47579	796.37019	51.31	0.0001
YEAR*CULTIVAR	31	26167.14323	844.10139	54.39	0.0001
Error	128	1986.62320	15.52049		
Corrected Total	191	57108.31732			

E - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
YEAR	1	24.9841021	24.9841021	86.05	0.0001
CULTIVAR	31	358.1071479	11.5518435	39.79	0.0001
YEAR*CULTIVAR	31	481.2995312	15.5257913	53.47	0.0001
Error	128	37.1638667	0.2903427		
Corrected Total	191	901.5546479			

F - Analysis of variance for zeaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
YEAR	1	31706.09005	31706.09005	6836.03	0.0001
CULTIVAR	31	50575.44045	1631.46582	351.75	0.0001
YEAR*CULTIVAR	31	50575.44045	1631.46582	351.75	0.0001
Error	128	593.6750	4.6381		
Corrected Total	191	133450.6459			

Table 4.3: Range of total carotenoid content for potato cultivars in different tuber flesh colour groups

Flesh Colour	Total carotenoid (µg/g DW) range for 2005/2006	Total carotenoid (µg/g DW) range for 2006/2007
Dark Yellow	45.47 - 169.57	5.59 - 126.28
Pale Yellow	25.08 - 41.12	22.44 - 129.08
Pale Yellow to Cream	23.24	41.96
Cream	6.99 - 32.21	17.37 - 34.84
Pink	5.50	3.13
Purple	2.09	3.04
White	1.18 - 26.39	0.91 - 46.88

Examination of individual carotenoid profiles in the 32 cultivars grown in 2004/2005 revealed that only four major carotenoids were detected (neoxanthin, violaxanthin, lutein and β-carotene). In 2006/2007, these four compounds, plus zeaxanthin, were detected (Figures 4.1 and 4.2). Comparison of carotenoid profiles of 32 potato cultivars from the 2004/2005 and 2006/2007 season also showed variations in individual carotenoid composition and content of cultivars between the seasons. For example, in Markies (Figure 4.1), zeaxanthin was not detected in 2004/2005 season, whereas in 2006/2007 season zeaxanthin was detected, but violaxanthin was absent. The major carotenoids were neoxanthin in the 2004/2005 season and zeaxanthin in the 2006/2007 season. Likewise, Agria had a high concentration at lutein, with the absence of neoxanthin and zeaxanthin, in the 2004/2005 season, all five major carotenoids were found in the 2006/2007 season with zeaxanthin accounting for the about 80% of the total carotenoids (Figure 4.1). These data highlight the major effect the growth season can have on the presence and accumulation of carotenoid compounds (Table 4.2). In 2004/2005 lutein was detected in most of the cultivars whereas zeaxanthin was detected in most of the cultivars in 2006/2007. Generally, the ranking of 32 cultivars remained quite similar from year to year with 3 dark yellow-fleshed cultivars (Laura, Allure and Agria), 1 pale yellow-fleshed cultivar (Summer Delight), 2 cream-fleshed cultivars (Nadine and Purple Passion) and 8 white to pink fleshed cultivars (Ilam Hardy, Ranger, Eden, Moonlight, Crop 21, Bondi, Crop 33 and 2765-6) remain in the same rank or with slightly changes. These data indicated that

genotype ability to accumulate carotenoids was relatively stable among these 14 cultivars. Other cultivars behaved were quite different between seasons. For example Milva in 2005/2006 was in the rank of 5 but in 2006/2007 was in the rank of 27.

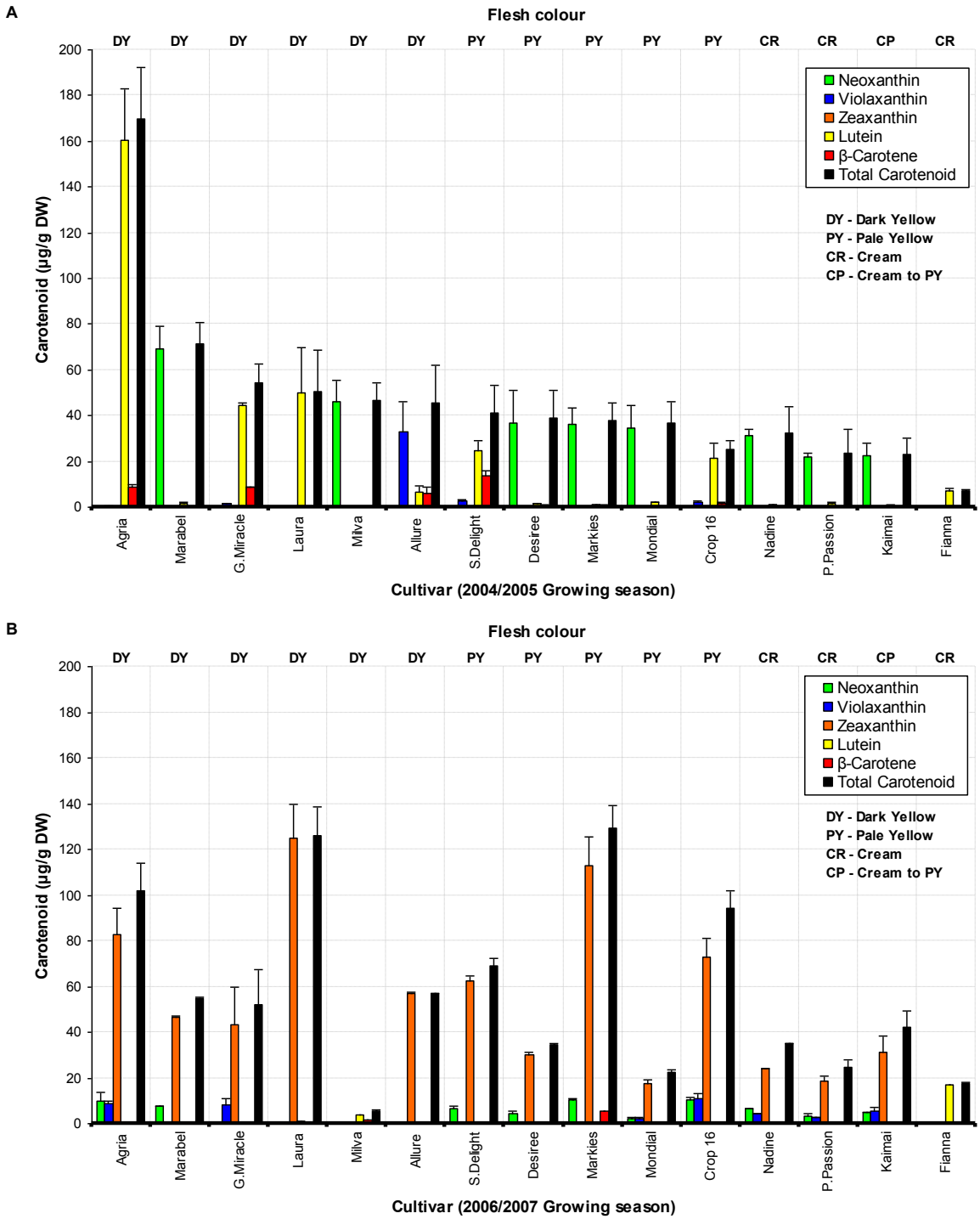


Figure 4.1: Analysis of the effect of growing season on carotenoid accumulation in 32 potato cultivars
A - Individual and total carotenoids content (µg/g DW) of dark yellow to cream fleshed cultivars for 2004/2005 growing season (from chapter 3)
B - Individual and total carotenoids content (µg/g DW) of dark yellow to cream fleshed cultivars for 2006/2007 growing season
Error bars represent ± SE.

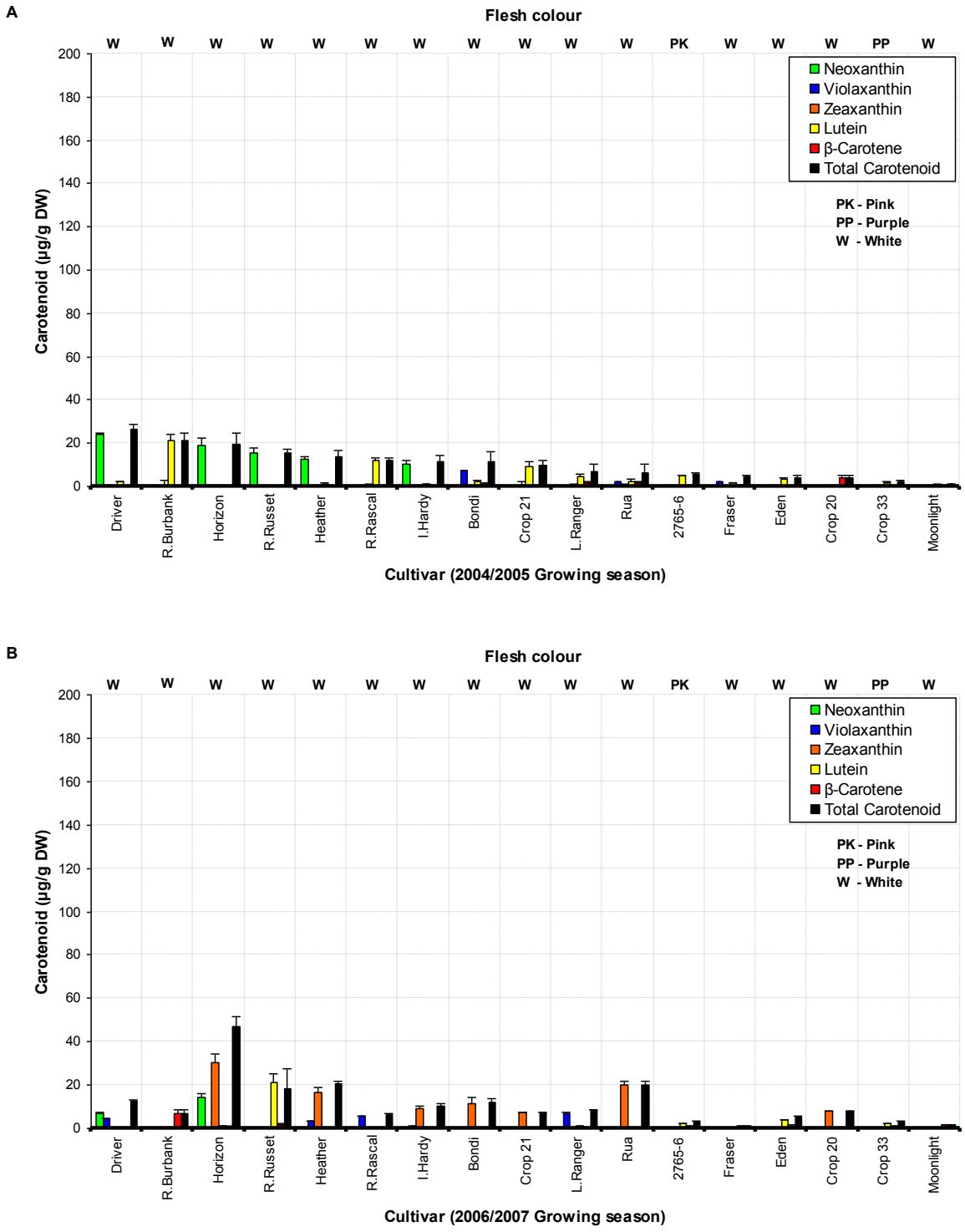


Figure 4.2: Analysis of the effect of growing season on carotenoid accumulation in 32 potato cultivars.
A - Individual and total carotenoids content (µg/g DW) of white, pink and purple fleshed cultivars for 2004/2005 growing season (from Chapter 3)
B - Individual and total carotenoids content (µg/g DW) of white, pink and purple fleshed cultivars for 2006/2007 growing season
Error bars represent ± SE.

4.3.2 Tuber flesh intensity colour for 32 potato cultivars

The average of yellow-flesh intensity (b*) of the 32 New Zealand-grown potato cultivars in 2006/2007 season were estimated by the Minolta chroma meter CR-210 measuring in CIELAB. Values ranged from 14.42 to 30.57 (Appendix 4.2), with the ranges for the tuber flesh colour groups, pink to purple, white, cream and pale yellow to dark yellow, summarised in Table 4.4. In general, yellow flesh intensity range values were consistently higher from purple/pink group up to the dark yellow group. Laura, with dark yellow tuber flesh, was observed to have substantially highest value of yellow intensity (30.57), whereas 2765-5 with purple tuber flesh was found to have the lowest value of 14.42. Even though yellow intensity values were not independent values and correlation coefficient values were not significant, thus from the observation and measurement taken it can be concluded that the greater the value of total carotenoid contents the higher the yellow flesh intensity.

Table 4.4: Range of yellow flesh intensity (b*) for different tuber flesh colour groups

Tuber flesh	b*
Dark Yellow	22.94 - 30.57
Pale Yellow	25.45 - 29.10
Cream	24.87 - 28.51
White	22.30 - 25.03
Pink	20.56
Purple	14.42

4.3.3 Genotype x growing season interactions for 10 potato cultivars grown in the Netherlands

Ten cultivars grown in the Netherlands were analysed for total and individual carotenoid content from two growing seasons, 2005 (Chapter 3) and 2006 (this chapter) to investigate the stability of their carotenoid profile. Analysis of variance on the 2006 data confirmed the previous findings by exhibiting highly significant differences ($P < 0.0001$) between the ten cultivars and the individual carotenoid pigments (Table 4.5). Similar to the results from the New Zealand-grown cultivars, analysis of variance on the pooled data from both growing seasons established highly significant differences ($P < 0.0001$) between the ten cultivars, the carotenoid pigments, the seasons, and all combinations of interactions (Table 4.6). This further reinforces that growing season can have a marked influence on the accumulation of carotenoids. The changes in carotenoid composition are complex and also change with cultivar as indicated by the interaction components. The total carotenoid content for all these cultivars ranged from 40.77 to 258.95 µg/g DW in 2005 as compared to 5.65 to 319.88 µg/g DW in 2006 (Figure 4.3). The genotype IVP01-084-19 (258.95 µg/g DW) was detected to have the highest amount of carotenoid content and Desiree (40.77 µg/g DW) the lowest in 2005, whereas in 2006 Laura (319.88 µg/g DW) was the highest and Casteline (5.65 µg/g DW) was the lowest. Of the ten genotypes analysed, two cultivars (Laura and Marabel) increased in total carotenoid content between 2005 and 2006, whereas an eight cultivars decreased, especially genotype IVP01-084-19 (Appendix 4.3).

Comparison of carotenoid profiles of the ten Netherlands-grown cultivars from the 2005 and 2006 seasons showed variations in the profiles of individual carotenoid compounds (Figures 4.3). For example Agatha in 2005 accumulated mostly neoxanthin with traces of violaxanthin and β-carotene, whereas in 2006 lutein and β-carotene were the major carotenoids with only traces of neoxanthin and violaxanthin. Similar marked changes were also apparent for Laura, which contained 54.3% neoxanthin, 38.3% violaxanthin, 7.4% lutein and just a trace of β-carotene in 2005, whereas in 2006 it contained 99.5% of neoxanthin and just a trace of violaxanthin and lutein. Surprisingly, no zeaxanthin was detected in either season in potato tubers grown in the Netherlands.

Table 4.5: Analysis of variance for 10 potato cultivars grown in the Netherlands during 2006

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	9	294939.1508	32771.0168	30.61	0.0001
Error	20	21411.7925	1070.5896		
Corrected Total	29	316350.9433			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	9	66188.27933	7354.25326	4.83	0.001
Error	20	30423.58793	1521.17940		
Corrected Total	29	96611.86727			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CULTIVAR	9	65747.82103	7305.31345	63.12	0.0001
Error	20	2314.76180	115.73809		
Corrected Total	29	68062.58283			

Lutein, β-carotene and zeaxanthin not included due to trace amounts or undetected levels in most of the samples.

Table 4.6: Analysis of variance for 10 potato cultivars over two growing seasons (2005 and 2006) in the Netherlands

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
YEAR	1	6501.0450	6501.0450	11.93	0.001
CULTIVAR	9	213392.0020	23710.2224	43.51	0.0001
YEAR*CULTIVAR	9	199261.0272	22140.1141	40.63	0.0001
Error	40	21796.1547	544.9039		
Corrected Total	59	440950.2289			

B - Analysis of variance for neoxanthin content (µg/g DW)

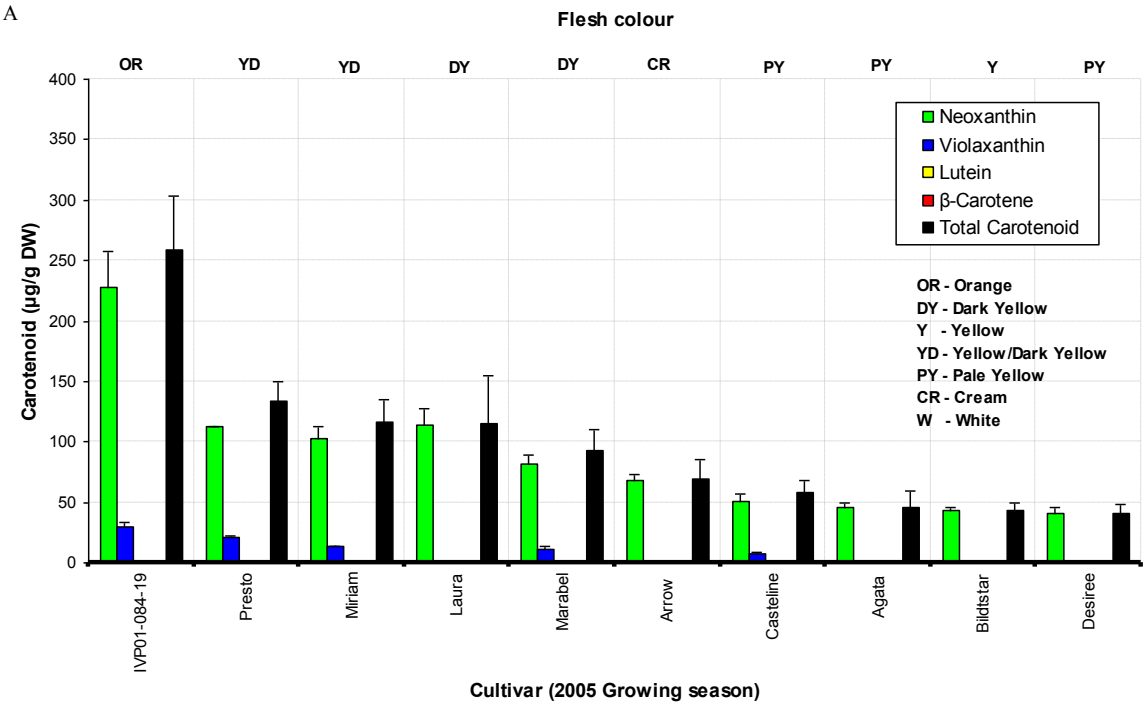
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
YEAR	1	35545.64920	5545.64920	46.24	0.0001
CULTIVAR	9	78578.11558	8730.90173	11.36	0.0001
YEAR*CULTIVAR	9	74954.03065	8328.22563	10.83	0.0001
Error	40	30750.7619	768.7690		
Corrected Total	59	219828.5573			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
YEAR	1	4168.00011	4168.00011	70.91	0.0001
CULTIVAR	9	31196.33043	3466.25894	58.97	0.0001
YEAR*CULTIVAR	9	37479.56309	4164.39590	70.85	0.0001
Error	40	2351.02420	58.77560		
Corrected Total	59	75194.91783			

No samples available for Nicola and Olivia in 2006 growing season
Lutein, β-carotene and zeaxanthin not included due to trace amounts or undetected levels in most of the samples.

A



B

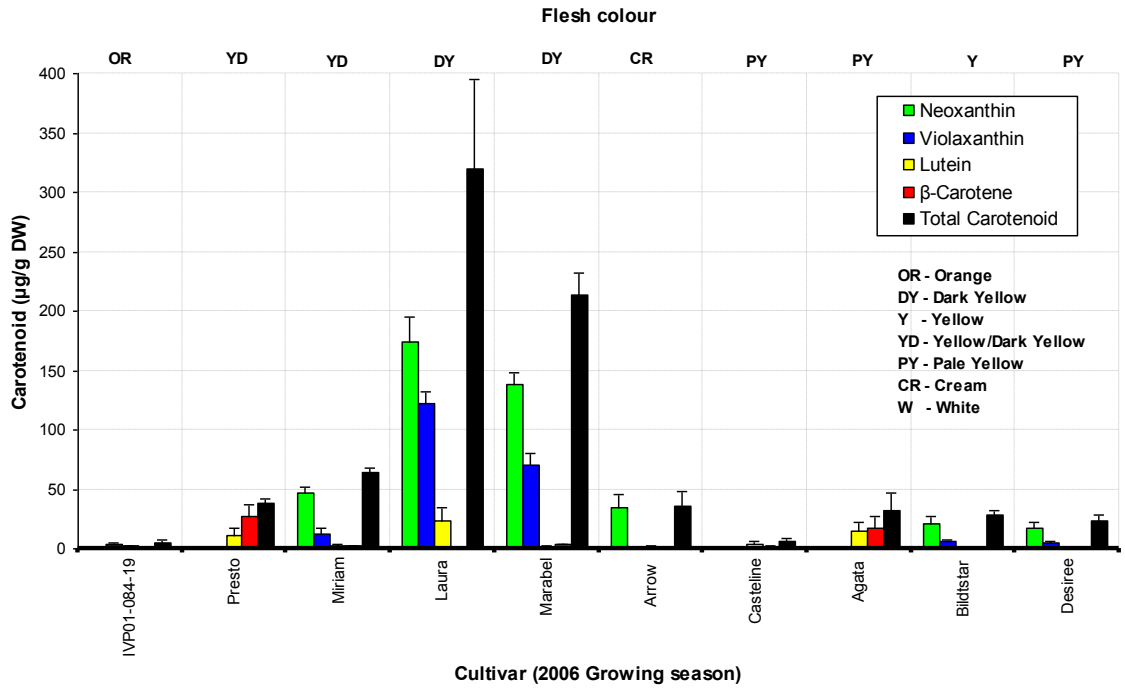


Figure 4.3: Analysis of the effect of growing season on carotenoid accumulation in ten potato cultivars grown in the Netherlands.

A - Individual and total carotenoids content (µg/g DW) of orange to pale yellow fleshed cultivars for the 2005 growing season

B - Individual and total carotenoids content (µg/g DW) of orange to pale yellow fleshed cultivars for the 2006 growing season

Error bars represent ± SE. Zeaxanthin not presented due to undetected levels

No samples available for Nicola and Olivia in 2006 growing season

4.4 Discussion

Considerable research interest has recently focused on the development of both transgenic and traditional breeding methods to increase total and individual carotenoid composition in potatoes (Lu et al., 2001; Brown et al., 1993; Romer et al., 2002; Ducreux et al., 2004). Unfortunately little information is available on the influence of the environment on carotenoid content in potatoes, especially growing seasons and locations. Genotype and environment interactions have been reported to account for variation in free amino acids, protein, and sugar composition (Hsi et al., 1981; Oupadissakoon et al., 1980; Dawson and McIntosh, 1973; Amaya et al., 1977, 1978; Basha et al., 1976; Young et al., 1974a, b; Young, 1979, 1980). In addition, the total glycoalkaloid content of potato tubers was found strongly influenced by environmental effects during the growing season (Friedman and McDonald, 1997) even though there are also strong genetic effects (Sanford and Sinden, 1972; Sanford et al., 1995). Seasonal differences, growing conditions, locations, genotypes and postharvest storage conditions are among the factors that can be significantly affecting the quality and nutritional value of potatoes (Haynes et al., 1996; Griffiths et al., 2007; Anderson and Smith, 2006; Jing et al., 2007). The bioavailability of carotenoids is a complex issue and depends on many factors (Fraser and Bramley, 2004). In this study of inter-seasonal and genotype interactions, the data revealed that variations in total carotenoid content and the concentration of individual carotenoid pigments is due to strong relationship between genotype and growing seasons. This assumption is supported by Chloupek and Hrstkova (2005), in their observations of 26 crops over a 43 year period growing seasons; where yield adaptability over time was controlled largely by weather and small variations from year to year in agronomical practices. In other words, major factors influencing yield are location, year and their interactions. They also observed that yield

variation of the 26 crops, including potato, in the Europe was greater than in the USA by nearly two times. In another case, the level of polyphenols in potatoes has been reported to have significant difference with environmental conditions and genetics (Chloupek and Hrstkova, 2005). In potato a strong relationship and interaction between the intensity of the yellow colour in tuber flesh, total carotenoid content and growing locations has also been reported. Lu et al., (2001) demonstrated that environmental factors can exert some influences on the expression of yellow tuber flesh intensity. The correlation between genotypes and environment can be indicative of the particular potato cultivar for best adapted to certain location. For example in 2004/2005 growing season in New Zealand, Agria was found to have a substantially higher carotenoid content relative to other cultivars with mostly lutein and no zeaxanthin, whereas in 2006/2007 Agria contained all five carotenoids with relatively high concentration of zeaxanthin (Figure 4.1).

A notable difference between the two seasons was the accumulation of zeaxanthin in 2006/2007 and the absence of zeaxanthin in 2004/2005. The agronomical practices and location for 2006/2007 season were not the same as previous season. In the 2004/2005 season chemical fertilizers were applied, whereas during the 2006/2007 season organic matter (chicken manure) was used massively. The distance between the two site locations was only about 3 kilometres. It is therefore important to make further studies in soil type of the location and soil acidity test. Organic matter can increase the acidity of the soil and pH can affect epoxidation and de-epoxydation reactions in the xanthophyll cycle (Rockholm and Yamamoto, 1996). Hydroxylation of α and β-carotene will produce lutein and zeaxanthin respectively. Violaxanthin is formed from zeaxanthin through epoxidation and de-epoxydation can convert violaxanthin back to zeaxanthin. This reaction sequence is reversible and mediated by pH (Cunningham and Gantt, 1998; Howitt and Pogson 2006).

Epoxidation will occur in the dark or under low light and activity is optimal near pH 7.5 (Hager, 1975; Siefermann and Yamamoto, 1975) whereas de-epoxydation activity is active at pH below 6.5 and optimal at approximately pH 5.2 (Rockholm and Yamamoto, 1996).

Zeaxanthin occurs only in trace amounts under physiological conditions *in vivo* or without stress condition (Ruban et al. 1994, Lee and Thornber 1995, Verhoeven et al. 1999). However, during irradiance stress or high-light exposure, zeaxanthin is formed upon de-epoxidation through operation of the reversible xanthophyll cycle (Yamamoto 1979, 1985). Although zeaxanthin accumulates during irradiance stress, such association is usually only transient. Upon recovery under low-light or in darkness, zeaxanthin will disappear (Yamamoto 1985). Nevertheless, it was concluded from recent *in vitro* studies (Croce et al. 1999, Hobe et al. 2000), upon analysis of zeaxanthin accumulating *Arabidopsis thaliana* mutants (Pogson et al. 1996, Pogson et al. 1998, Tardy and Havaux 1996), and from the green alga *Scenedesmus obliquus* (Heinze et al. 1997), that zeaxanthin could replace lutein and violaxanthin under irradiance stress.

There are two possibilities to explain the accumulation of zeaxanthin in 2006/2007 season and not previous season:

i. The conversion of individual carotenoids such as violaxanthin, neoxanthin to zeaxanthin is due to irradiance stress condition from high-light exposure. This will promote the conversion of other carotenoids to zeaxanthin from the β-carotene and α–carotene branch point. As a result zeaxanthin concentration will increase. This reaction will restrict the supply of precursors for ABA biosynthesis and the plant responds by increasing carotenogenic metabolic flux to compensate for this restriction (Ruban et al., 1994; Farber et al., 1997). Polle

et al. (2001) also concluded that zeaxanthin can successfully replace lutein and violaxanthin under irradiance stress condition.

ii. The presence and absence of zeaxanthin is in response to changes in pH. Acidity will trigger the de-epoxidation reaction by the conversion of violaxanthin and other precursors of ABA to zeaxanthin, whereas alkaline conditions will induce lutein or the supply of precursors for ABA biosynthesis which will lead to the conversion of zeaxanthin to violaxanthin, neoxanthin or other precursors for ABA biosynthesis through epoxidation reaction (Morosinotto et al., 2003).

Overall this study clearly demonstrated that the growing season can strongly influence the total and individual pigment content of carotenoids in potato tubers, which can be significantly affecting the quality and nutritional value of potatoes. Therefore, in addition to genotypic factors, environmental factors also play an important role in determining the accumulation of individual carotenoids in potato tubers, especially in Agria and Desiree. Between seasons, lutein has been transformed into zeaxanthin in Agria, whereas neoxanthin has been transformed into zeaxanthin in Desiree. These results clearly indicate that selection for high or low carotenoid tuber levels cannot be made on the basis of a single year's results. However, valid comparisons can be made between data from different years if the material being stored and grown under similar environmental conditions. This study suggests that environmental factors such as seasonal climatic variation may influence the accumulation of potato tuber carotenoids content and composition. Clearly, further studies utilizing potato plant material grown under different environmental conditions is required to confirm this hypothesis.

CHAPTER 5

Environmental stability of potato tuber carotenoid composition

5.1 Introduction

The previous chapter reports differences in carotenoid composition in a wide range of potato genotypes over two seasons in both New Zealand and the Netherlands (section 4.3). This data suggests that growing seasons can influence the accumulation of specific carotenoid compounds and their concentration in potato tubers. In addition, measurement of yellow hue component confirmed that the higher the total carotenoid content the greater the yellow intensity colour.

This chapter investigates further the environmental stability of carotenoid content in potato tubers and its implications by measuring carotenoid composition in eight cultivars grown at three different locations in New Zealand and two cultivars grown at eight different locations in the Netherlands. Reflectance colorimeter measurement was also made in order to further confirm the yellow intensity and total carotenoid content relationship of the potato tubers.

5.2 Experimental Design

5.2.1 New Zealand-grown potato cultivars

A total of eight potato cultivars (Agria, Laura, Marabel, Desiree, Fianna, Ranger Russet, Van Rosa, Vt^n 62-33-3) were selected for analysis of carotenoid content. The selected cultivars were planted in three replicate plots at three different locations in New Zealand: Palmerston North and Pukekohe (both in the North Island) and Lincoln (South Island) during the 2006/2007 growing season. The plot size, planting, harvest times and agronomic management of the crop followed the standard protocols set out for New Zealand potato trials as described in detail by Conner et al. (1994). All tubers were harvested from plots at full maturity following natural plant senescence and maintained at ambient temperatures for three weeks until grading, then placed in cool storage (air at 8°C). Ten undamaged tubers were selected after harvested from each of three replicate plots for all cultivars.

5.2.2 Netherlands-grown potato cultivars

Two cultivars (Agria and Desiree) were selected and tubers harvested from potato field trials planted in eight different locations in the Netherlands during 2006 (Metslawier, Friesland; Bant, Flevoland; Rilland, Zeeland; Nagele, Flevoland; Emmeloord, Flevoland; Wierum, Friesland; Wieringerwerf, Noord-Holland; Wageningen, Gelderland). Each cultivar was planted in three replicates per plot. The plot size, planting, harvest times and agronomic management of the crop followed the standard protocols for potato trials at each site. All tubers were harvested from plots at full maturity following natural plant

senescence and maintained at ambient temperatures for three weeks until grading, then placed in cool storage (air at 8°C). Ten undamaged tubers were selected after harvest and randomly assigned to three replicates for both cultivars.

5.2.3 Sampling

The skin of the tubers was removed with a peeler and the remaining tuber tissue was cut into 5.0 mm slices. For each sample tuber tissue was pooled from three tubers, mixed, and a random 100 g FW sample was immediately frozen at -20^{O}C. The tuber samples were freeze-dried for 7 days, after which the samples were ground into fine powder and stored at -80 °C until analysis.

5.2.4 Extraction of carotenoids

The extraction procedure followed the methods described by Morris et al. (2004), Lewis et al., (1998) and Britton et al., (1995) as detailed in Chapter 2 (section 2.2.2).

5.2.5 Determination of total carotenoid concentration

Total carotenoid concentration was determined by spectrophotometry as described previously by Britton et al., (1995) and Lewis et al., (1998) in Chapter 2 (section 2.2.3).

5.2.6 HPLC analysis

The HPLC analysis of saponified carotenoids was analysed according to Morris et al. (2004) as described detailed in Chapter 2 (section 2.4). Carotenoid standards β-carotene, violaxanthin, lutein, and neoxanthin were isolated from *Eruca sativa* (roquette or rocket salad) by open column chromatography (see section 2.3.1) as described by Kimura and Rodriguez-Amaya (2002), whereas zeaxanthin was obtained commercially from Sigma-Aldrich (Auckland, New Zealand).

5.2.7 Measurement of tuber flesh colour intensity

Potato tuber flesh colour was measured for its yellow colour intensity by Minolta CR210 chroma meter and CIELAB system according to Francis (1980) as described detailed in Chapter 2 (section 2.4). L* a* b* values were determined by taking three measurements at random locations on freshly cut surfaces. The mean value of the two halves of each tuber was used to indicate the yellow-flesh intensity of that tuber. All measurements were first calibrated using Minolta calibration plate with L* (98.07), a* (-0.23) and b* (+1.88).

5.3 Results

5.3.1 Genotype x location interactions for 8 potato cultivars in New Zealand

Potato tuber samples from the 2006/2007 growing season of eight different cultivars were grown at Lincoln, Palmerston North and Pukekohe were analysed for carotenoid content and composition to determine the effect of different locations on carotenoid accumulation. In virtually all instances the analysis of variance established significant to highly significant differences between the eight cultivars, the three locations and their interaction for all the carotenoid pigments (Table 5.1). This clearly demonstrates that growing locations can have an important influence on the accumulation of carotenoids. The importance of the interaction components emphasises that the changes in carotenoid composition are complex and the responses are not consistent across cultivars.

The major carotenoids identified in tubers derived from all eight cultivars from all three locations were neoxanthin, violaxanthin, zeaxanthin, lutein and β-carotene. However, the content of individual carotenoid compounds in most cultivars varied with the location in which they were grown. This is indicative of the highly significant interactions between cultivars and environment in the analysis of variance (Table 5.1). The data (Figure 5.1 and Appendix 5.1) showed that Laura grown in Palmerston North contained the highest amount of total carotenoid (350.15 μg/g DW) followed by Laura from Pukekohe and Lincoln. Among the cultivars tested, Vt^n 62-33-3 from Palmerston North contained the lowest amount of total carotenoid (2.76 μg/g DW) followed by Vt^n 62-33-3 from Lincoln and Ranger Russet from Pukekohe. Dark yellow potato tuber flesh cultivars from Palmerston North contained a relatively higher amount of total carotenoid compared to other locations.

Except for Agria and Laura which had consistently higher carotenoid content at al three sites, the Lincoln-grown cultivars generally contained more total carotenoid than other locations. Examination of carotenoid profiles of the potatoes grown at different locations showed that the location had a major effect on the total and individual carotenoid contents (Figure 5.1, Appendix 5.1). In addition, comparison of carotenoid profiles of these potato tubers ranging from white to dark yellow in three different locations also demonstrated tremendous fluctuation and variation in the individual carotenoids. For example, Agria from Lincoln contained relatively high concentration of zeaxanthin, but Agria from the two other locations contained relatively high concentrations of zeaxanthin as well as neoxanthin and violaxanthin. For variation in individual carotenoid, violaxanthin was not present in Laura from Lincoln, but was detected from the other two locations. In another example, neoxanthin and β-carotene were present only in Lincoln-grown Desiree; they were absent in Palmerston North and Pukekohe. Three individual carotenoid compounds (neoxanthin, violaxanthin and zeaxanthin) were absent from Ranger Russet in Pukekohe, whereas only neoxanthin and violaxanthin were absent in Palmerston North and Lincoln. These data suggest that although location had an effect on the total carotenoid and yellow intensity within same cultivars, they also had effect on the individual carotenoid content and carotenoid composition of the potatoes in same cultivar.

Table 5.1: Analysis of variance for 8 potato cultivars (2006/2007 growing season) grown at three different locations in New Zealand

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	2	17408.9284	8704.4642	10.96	0.0001
CULTIVAR	7	594867.9324	84981.1332	107.03	0.0001
LOCATION x CULTIVAR	14	92205.3299	6586.0950	8.29	0.0001
Error	48	38112.0759	794.0016		
Corrected Total	71	742594.2667			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	2	484.808033	242.404017	6.12	0.0043
CULTIVAR	7	7125.848487	1017.978355	25.69	0.0001
LOCATION x CULTIVAR	14	2823.862500	201.704464	5.09	0.0001
Error	48	1901.72607	39.61929		
Corrected Total	71	12336.24509			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	2	2268.497058	1134.248529	36.66	0.0001
CULTIVAR	7	8407.550599	1201.078657	38.82	0.0001
LOCATION x CULTIVAR	14	5620.224497	401.444607	12.98	0.0001
Error	48	1485.07173	30.93899		
Corrected Total	71	17781.34389			

D - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	2	31.346419	15.673210	2.38	0.1031
CULTIVAR	7	2096.134743	299.447820	45.53	0.0001
LOCATION x CULTIVAR	14	276.517269	19.751234	3.00	0.0023
Error	48	315.724467	6.577593		
Corrected Total	71	2719.722899			

E - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	2	35.6547028	17.8273514	208.28	0.0001
CULTIVAR	7	79.55039944	11.3577135	132.70	0.0001
LOCATION x CULTIVAR	14	169.4269639	12.1019260	141.39	0.0001
Error	48	4.1084000	0.0855917		
Corrected Total	71	288.6940611			

F - Analysis of variance for zeaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	2	6170.6158	3085.3079	6.88	0.0024
CULTIVAR	7	380153.2147	54307.6021	121.08	0.0001
LOCATION x CULTIVAR	14	36271.4734	2590.8195	5.78	0.0001
Error	48	21530.0554	448.5428		
Corrected Total	71	444125.3592			

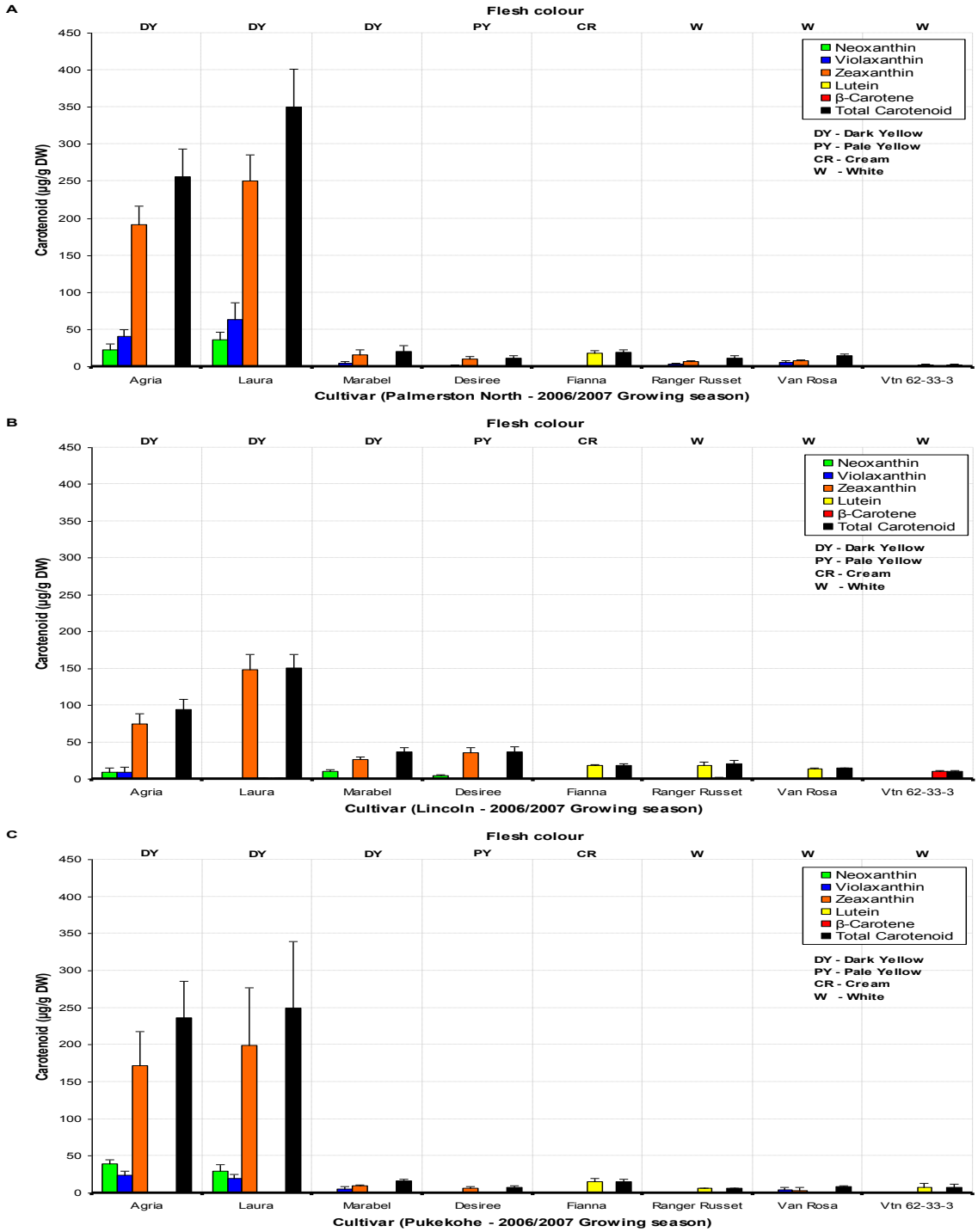


Figure 5.1: Analysis of total and individual carotenoid compounds (µg/g DW) of potato cultivars (2006/2007 growing season) grown at three different locations in New Zealand

A - Individual and total carotenoids content (µg/g DW) of eight potato cultivars grown at Palmerston North

B - Individual and total carotenoids content (µg/g DW) of eight potato cultivars grown at Lincoln

C - Individual and total carotenoids content (µg/g DW) of eight potato cultivars grown at Pukekohe

Error bars represent ± SE.

5.3.2 Tuber flesh intensity colour for potato cultivars grown at three different locations in New Zealand

There is also strong relationship between the intensity of the yellow colour in tuber flesh and total carotenoid content. Large differences were detected for yellow-flesh intensity (b*) from all three locations. The average of b* (Appendix 5.2) for the eight potato cultivars from Lincoln, Palmerston North and Pukekohe as measured by the Minolta chroma meter CR-210 measuring in CIELAB, varied between the colour groups (Table 5.2). Colour measurements of tuber flesh colours from white, cream and pale yellow to dark yellow for all cultivars grown at 3 different locations is presented in Figure 5.2.

Table 5.2: Range of yellow flesh intensity (b*) for different tuber flesh colour groups from three different locations in New Zealand

	Dark Yellow	Pale Yellow	Cream	White
Lincoln	27.42 - 32.14	25.20	25.09	23.05 - 26.27
Palmerston North	31.25 - 34.83	28.67	26.09	24.23 - 25.47
Pukekohe	26.96 - 33.37	26.89	25.26	23.30 - 26.15

Dark yellow, pale yellow and cream tuber flesh cultivars from Palmerston North were observed to have substantially highest value of yellow intensity (Table 5.2 and Figure 5.2) whereas white tuber flesh cultivars from Lincoln were found to have relatively lowest value of yellow intensity. It can be concluded that environmental conditions can influence the presence of specific carotenoid compounds and their concentration in potato tubers and also influencing tuber flesh colour intensity even within the same cultivar.

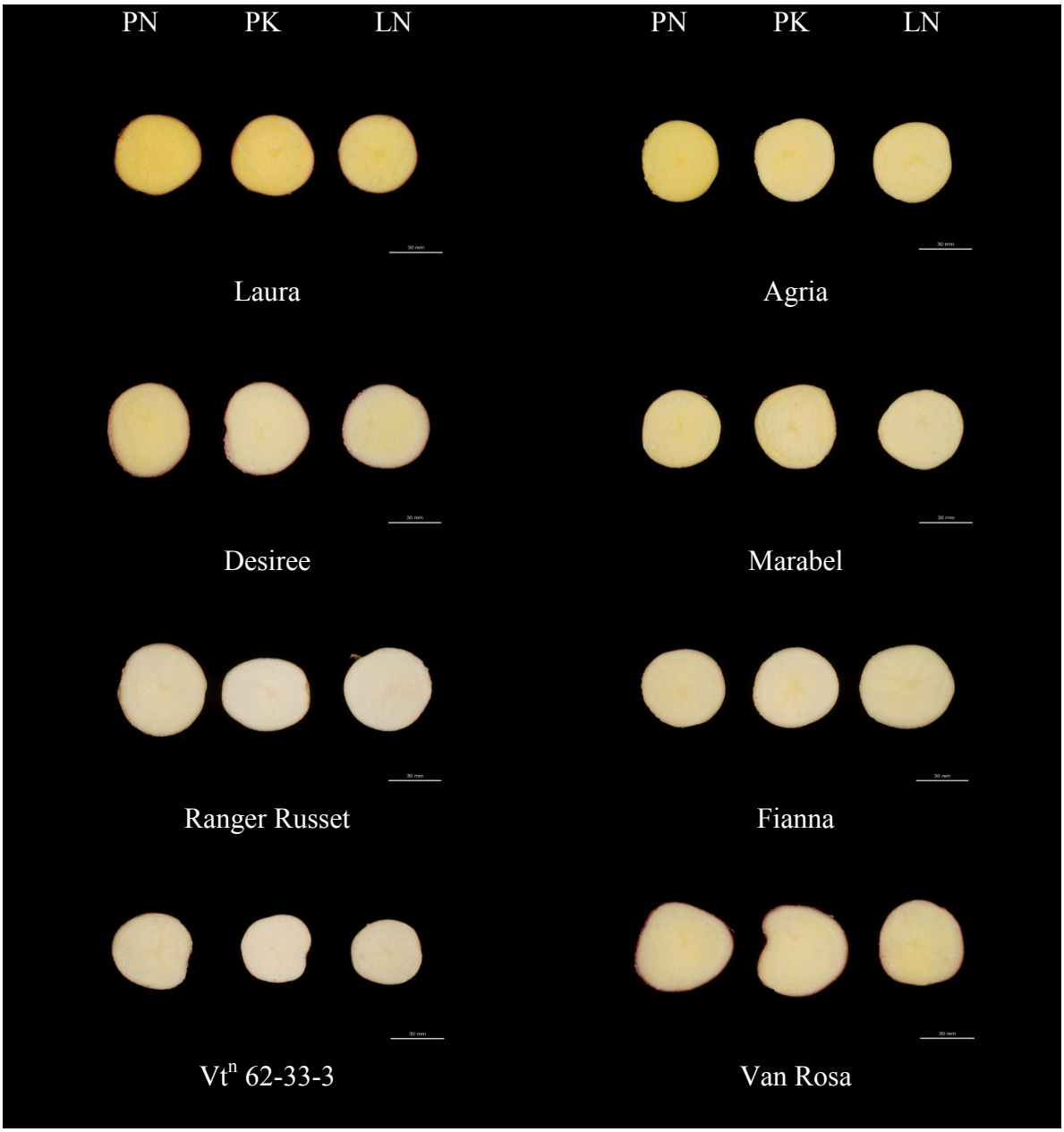


Figure 5.2: Comparison of tuber flesh colour and yellow hue component (b*) for eight cultivars grown at three different locations

	PN	PK	LN
Agria	32.60	31.85	31.41
Laura	34.83	33.37	32.14
Marabel	31.25	26.96	27.42
Desiree	28.67	26.89	25.20
Fianna	26.09	25.26	25.09
Ranger Russet	24.23	23.30	23.05
Van Rosa	25.47	26.15	26.27
Vt^n 62-33-3	25.30	24.31	24.87

PN – Palmerston North, PK – Pukekohe, LN – Lincoln

5.3.3 Genotype x location interactions for Agria and Desiree grown in the Netherlands

Effect of location and genotype on potato tubers carotenoid content was further studied by examining the Agria and Desiree carotenoid composition grown at eight different locations in the Netherlands. The analysis of variance established significant to highly significant differences between the two cultivars, the eight locations, and their interaction for all the carotenoid pigments (Table 5.3).

The data showed that carotenoid composition of both cultivars varied significantly for all locations. Examination of carotenoid composition and profiles (Figure 5.3 and Appendix 5.3) of Agria and Desiree cultivars grown at different locations showed marked variations in the individual carotenoid compounds. These observations reflect the complex interactions determined in the analysis of variance (Table 5.3). Four carotenoids pigments were detected (neoxanthin, violaxanthin, lutein and β-Carotene). No zeaxanthin was detected at any of the locations.

In Desiree the total carotenoid content was very low at Metslawier, Bant, Rilland and Nagele, and much higher at Wierum, Wieringerwerf and Wageningen. When the total carotenoid content was high in Desiree, neoxanthin and violaxanthin were the predominant pigments, whereas when total carotenoid content was low, lutein and β-carotene were predominant (Figure 5.3, Appendix 5.3). For Desiree grown in Metslawier, Bant, Rilland and Nagele only β-carotene were detected. When Desiree was grown at Wierum, Wieringerwerf and Wageningen major differences in the relative proportion of various carotenoid compounds were apparent. Neoxanthin, lutein and β-carotene were detected in

Desiree from Wierum, whereas only lutein and β-carotene from Wieringerwerf. All four individual carotenoids were detected from Wageningen, where the highest total carotenoid content was observed in Desiree. Higher concentration of lutein was detected in Desiree from Wieringerwerf, whereas neoxanthin was found higher in Wierum and Wageningen. Violaxanthin was detected only in Desiree from Wageningen.

The carotenoid composition in Agria exhibited similar patterns to Desiree. Total carotenoid content were also much at Metslawier, Bant, Rilland and Emmeloord and substantially higher at Wierum, Wieringerwerf and Wageningen. Neoxanthin and violaxanthin were predominant when the total carotenoid content was high and lutein and β-carotene were predominant when the total carotenoid content was low (Figure 5.3, Appendix 5.3). Agria from Metslawier, Bant, Rilland and Emmeloord showed slight variations only in total carotenoid content, with lutein and β-carotene being predominant except at Emmeloord where lutein was absent. Neoxanthin, violaxanthin, lutein and β-carotene were detected in Agria grown in Wierum, Wieringerwerf and Wageningen.

Table 5.3: Analysis of variance for carotenoid content of Agria and Desirees (2006 growing season) grown at eight different locations in Netherlands

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	7	113282.5847	16183.2264	98.45	0.0001
CULTIVAR	1	46501.9950	46501.9950	282.88	0.0001
LOCATION x CULTIVAR	7	75755.2893	10822.1842	65.83	0.0001
Error	32	5260.4014	164.3875		
Corrected Total	47	240800.2704			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	7	50440.48793	7205.78399	151.60	0.0001
CULTIVAR	1	20010.37505	20010.37505	420.98	0.0001
LOCATION x CULTIVAR	7	34210.89610	4887.27087	102.82	0.0001
Error	32	1521.0466	47.5327		
Corrected Total	47	106182.8057			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	7	9248.046158	1321.149451	23.17	0.0001
CULTIVAR	1	3990.912133	3990.912133	69.98	0.0001
LOCATION x CULTIVAR	7	7634.543700	1090.649100	19.12	0.0001
Error	32	1824.99860	57.03121		
Corrected Total	47	22698.50059			

D - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	7	273.6225479	39.0889354	11.40	0.0001
CULTIVAR	1	14.7741021	14.7741021	4.31	0.0460
LOCATION x CULTIVAR	7	164.5717146	23.5102449	6.86	0.0001
Error	32	109.6828667	3.4275896		
Corrected Total	47	562.6512313			

E - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOCATION	7	144.7902250	20.6843179	12.00	0.0001
CULTIVAR	1	51.3774083	51.3774083	29.79	0.0001
LOCATION x CULTIVAR	7	117.6886917	16.8126702	9.75	0.0001
Error	32	55.1804667	1.7243896		
Corrected Total	47	369.0367917			

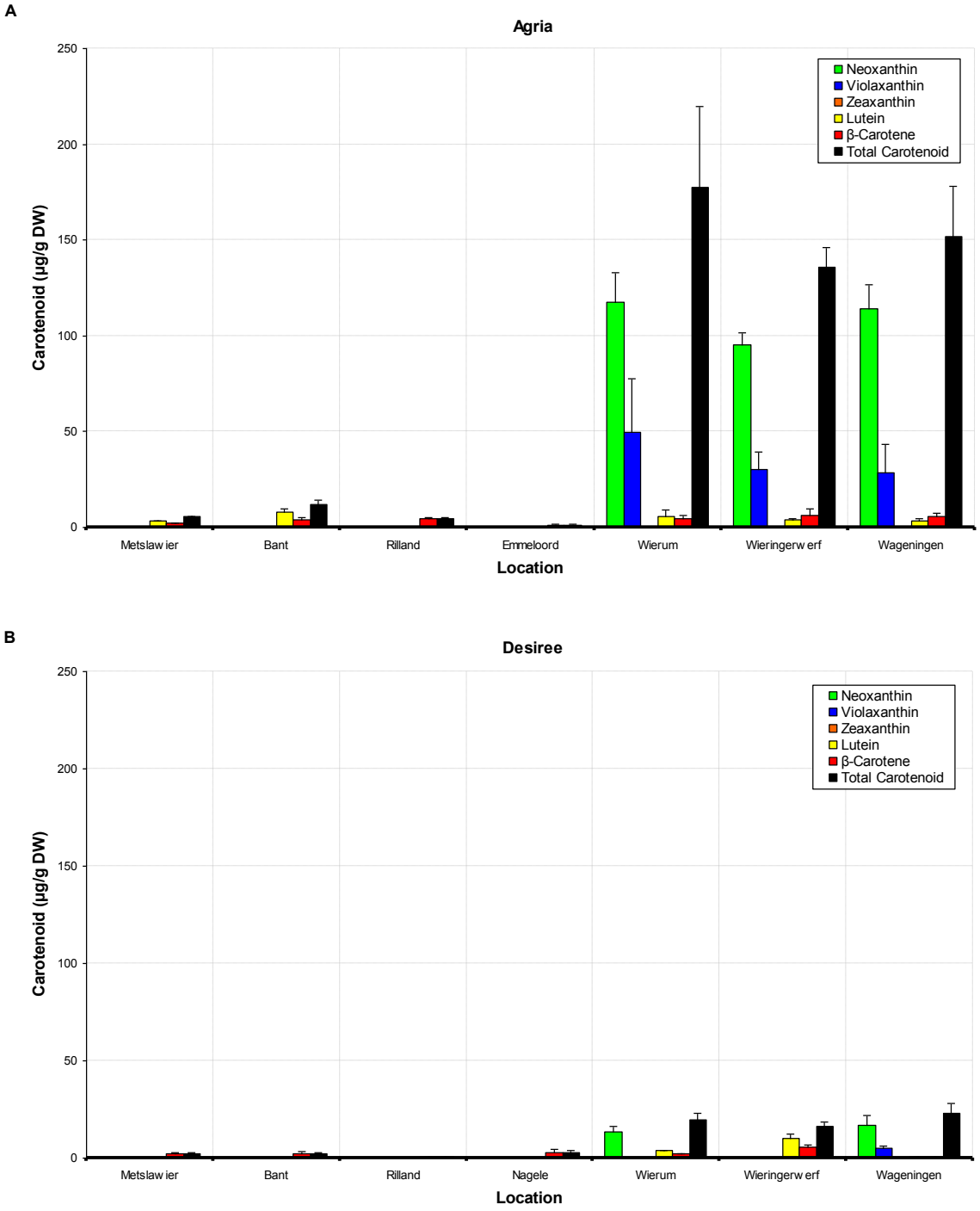


Figure 5.3: Analysis of total and individual carotenoid content (µg/g DW) of Agria and Desiree (2006 growing season) grown at eight different locations in Netherlands
A - Individual and total carotenoids content (µg/g DW) of Agria
B - Individual and total carotenoids content (µg/g DW) of Desiree
No samples available for Agria from Nagele and Desiree from Emmeloord. Zeaxanthin not included due to undetected levels in all samples. Error bars represent ± SE.

5.4 Discussion

It has been reported that crops with similar biology, environment and agronomical practices tended to have similar ability to change (Chloupek and Hrstkova, 2005). Diamond (2002) stated that as crops spread across the world they became exposed to different condition of environment and gradually became adapted to their new environments. Ability to change to different environments has been reported at both the species and the cultivar levels (Dencic et al. 2000; Banziger and Cooper, 2001). The bioavailability of carotenoids is a complex issue and depends on many factors such as location, year, cultivar and their interactions (Fraser and Bramley, 2004). In this study on the influences of location and genotype interactions on carotenoid accumulation, the data revealed that variations in both the total carotenoid content and the individual carotenoid compounds exhibit strong relationships between genotype and environment (Figures 5.1-5.3; Tables 5.1-5.3). Genotype x environment interactions on biochemical composition has been previously reported for phenolics accumulation in potatoes. Hamouz et al. (1999) reported that potatoes cultivated on loam soils in warm dry regions with low altitudes over a three year period contained a lower amount of total phenolics than those cultivated in cooler and more humid regions on sandy loam soil. In a similar study they found that organically grown potatoes contained higher levels of phenolics than did the same varieties grown in a conventional manner. It is hypothesized that the chemically untreated plants defend themselves against unfavorable extrinsic factors with higher levels of polyphenols. Furthermore, it is known that tubers that are exposed to abiotic and biotic stresses increase their production of phenolics as a defence mechanism (Lewis et al. 1998b; Hamouz et al. 1999).

Al-Saikhan (2000) also reported the same results with others over levels of lutein, neoxanthin, violaxanthin, zeaxanthin, and total carotenoids for five yellow-fleshed and one white-fleshed variety grown in two locations (Colorado and Texas). Total carotenoid levels as well as levels of lutein, violaxanthin and zeaxanthin were significantly higher in Texas-grown tubers. In addition, significant differences were also found between varieties for all carotenoids analysed (Al-Saikhan, 2000). Our results demonstrated that genotypes grown at different locations in both New Zealand and the Netherlands vary significantly in terms of total carotenoid content and profiles of individual carotenoid compounds. There is also strong relationship and interaction between the intensity of the yellow colour in tuber flesh, total carotenoid content and locations. For example Laura in Palmerston North was found to have more intense yellow colour compared to Laura grown in Pukekohe and Lincoln (Figure 5.2), which is reflected by the total carotenoid content (Figure 5.2). This supports previous claims of significant differences in yellow flesh intensity across environments (Haynes et al., 1996). In a subsequent study by the same group, it was determined that yellow-flesh intensity was significantly affected by environment, with the general trend of decreasing yellow flesh intensity from south to north (Haynes et al. 1996).

Beside location, other environmental effects such as temperature, light, mineral uptake, salinity and irrigation were also found to have an impact in carotenoid development in other plant species and organs (Collins et al., 2006). For examples high temperatures (>32°C) and excessive light intensity were found affected lycopene synthesis and degradation in tomato and grapefruit (Goodwin and Jamikorn, 1952; Tomes, 1963; Adergoroye and Joliffe, 1987; Hamauzu et al., 1998). Alba et al. (2000) added that red light will stimulate carotenoid accumulation in tomato, whereas far-red light blocks carotenoid accumulation. In contrast Vogele (1937) observed that lycopene synthesis is not

affected by temperatures in the range of 20 to 37°C in watermelon, lycopene content is reduced between 4 to 21°C in red grapefruit (Meredith and Young, 1971; Purcell et al., 1968). Increased rates of potassium (K) and phosphorus (P) also were found to increase lycopene content up to 20 to 30% in hydroponically grown tomatoes (Dumas et al., 2003; Trudel and Ozbun, 1971). Meanwhile, water availability and salinity were observed to increase lycopene content in tomatoes (Dumas et al., 2003; De Pascale et al., 2001; Krauss et al., 2006). These reports suggest that both above ground and underground environmental factors may influence the accumulation of carotenoids in potato tubers when grown at different locations. As stated in the previous chapter, correlation between genotypes and environment can be indicative of the particular potato cultivar best adapted to a certain location. In other words, there are certain locations where a specific cultivar accumulates a high level of carotenoid content and yellow intensity. For example Laura was found to have intensely yellow pigment and highest carotenoid content with high zeaxanthin in Palmerston North compared to Pukekohe and Lincoln. Conversely, a high level of neoxanthin and violaxanthin and no zeaxanthin were detected in Laura grown in the Netherlands. Therefore suitable cultivars, accompanied by suitable agronomy, have the potential to improve the nutritional quality and flavour of potato. However, some of potato cultivars were found to be more sensitive to changes in environment. For example, in the Netherlands Desiree and Agria grown in Metslawier, Bant, and Rilland were observed to have lower carotenoid content, but when grown in Wierum, Wieringerwerf and Wageningen the carotenoid content increased dramatically.

In Chapter 4 it was hypothesized that light and pH influenced the absence and presence of zeaxanthin in potato tubers throughout 2 growing seasons. To further investigate this hypothesis, we have tested the environmental stability of carotenoid

content in potato tubers and its implications by measuring carotenoid composition in eight cultivars grown at three different locations in New Zealand and two cultivars grown at eight different locations in the Netherlands. A key difference among the locations tested is temperature, with the North Island (Palmerston North and Pukekohe) of New Zealand being warmer than the South Island (Lincoln) and the Netherlands. Earlier in this chapter general factors influencing carotenoid composition and content were discussed. These included:

i. The ability of crops to change or adapt to new environments
ii. Influence of locations
iii. Influence of growing seasons
iv. Influence of cultivars

More specifically, the influence of genotype x environment interactions that can lead to changes of biochemical compositions can include:

i. Soil type – loam and sandy loam
ii. Temperature – cooler and warmer
iii. Light – humid and dry
iv. Location – low and high altitude
v. Defence mechanism against abiotic and biotic stress – polyphenols and phenolics
vi. Agricultural practices such as fertilizer and irrigation

All of these factors may affect biochemical composition such as carotenoid compound and yellow flesh intensity, but none have explained the mechanism of these changes physiologically or in term of biochemistry. Therefore, based on the results observed it is

hypothesised that the mechanism of the changes, especially the absence and presence of zeaxanthin, is as an indicator of environmental stress.

According to Havaux and Niyogi (1999) when light energy absorbed by plants becomes excessive relative to the capacity of photosynthesis, the xanthophyll violaxanthin is reversibly deepoxidized to zeaxanthin (violaxanthin cycle). Such environmental influences on the violaxanthin cycle can be inferred from the results of comparing yellow flesh and white flesh cultivars at three different locations (Lincoln, Pukekohe and Palmerston North). Only lutein accumulated in white flesh cultivars with no neoxanthin, violaxanthin and zeaxanthin being detected, suggesting no functional activity of violaxanthin deepoxidase (VDE). Two major consequences of this phenomenon are the absence of zeaxanthin formation in white flesh cultivars and the presence of zeaxanthin in yellow flesh cultivars such as Agria and Laura. Similar results were obtained for 32 cultivars grown in New Zealand for the second season (2006/2007). There are two possibility mechanisms that regulate the differences in carotenoid biosynthesis between cultivars with white or yellow flesh tuber:

i. The availability or the abundance of carotenogenic gene transcripts.
ii. The abundance or the presence of structures of sequestering or producing carotenoids.

These two possibility mechanisms have been described by Howitt and Pogson (2006). For the first mechanism, transcriptional regulation of carotenoid biosynthesis genes in carrot has been recently studied by Clotault et al. (2008). They identified eight genes encoding carotenoid biosynthesis enzymes during the development of white, yellow, orange, and red carrot roots. The genes were phytoene synthase (PSY1 and PSY2), phytoene desaturase (PDS), ζ-carotene desaturase (ZDS1 and ZDS2), lycopene Ɛ-cyclase

(LCYE), lycopene β-cyclase (LCYB), and zeaxanthin epoxidase (ZEP). All eight genes were present in the white cultivar even though it did not contain carotenoids, although the expression of some genes might possibly be suppressed or switched off. However, the high expression of genes encoding LCYE was noted in white cultivars with the accumulation of lutein. The enzyme from this gene is involved in lycopene channeling into the pathway branch ending at lutein, which is the major carotenoid in white cultivars. The results revealed that no zeaxanthin and violaxanthin deepoxidase gene expression were detected and the accumulation of total carotenoids and major carotenoids in yellow and white cultivars were therefore due to transcriptional level of genes directing the carotenoid biosynthesis pathway. The absence of precursors for ABA such as violaxanthin and neoxanthin could be explained by the absence of violaxanthin deepoxidase in white cultivars.

The transcript levels of the genes encoding the carotenogenic enzymes were also determined during potato tuber development by Morris et al. (2004) in a dark yellow cultivar (DB375\1) and pale yellow cultivars (Desiree and Pentland Javelin). Similar gene sequences to those found in carrot also detected in potato. Among the extra genes characterised are 1-deoxy-D-xylose-5-phosphate reductoisomerase (DXR); isopentenyl pyrophosphate isomerase (IPI) and β-carotene hydroxylase (CHYB). Higher transcript levels of PDS, PSY and LCYE were detected in tissues of swelling stolons and developing tubers of high carotenoid-accumulating *S. phureja* accession (DB375\1) and also in Desiree and Pentland Javelin. This accounts for DB375\1 and other yellow flesh potato cultivars accumulating more total carotenoids and zeaxanthin than the other cultivars classified as light yellow tuber flesh by Morris et al. (2004). Another interesting point is zeaxanthin epoxidase transcript levels were lower in DB375\1 tissues, which lead to the

accumulation of neoxanthin, zeaxanthin, violaxanthin and antheraxanthin. In contrast, in white carrots zeaxanthin epoxidase transcripts levels were higher, leading to the accumulation of lutein. Surprisingly no VDE transcripts were detected at all for both examples. Morris et al. (2004) also reported that an inverse relationship between the zeaxanthin epoxidase transcript level and the total tuber carotenoid content. The role of ZEP and VDE appears important and strongly correlates with the presence and absence of zeaxanthin and tuber flesh color. This is in agreement with the results reported by Brown et al. (1993), who also correlated the tuber orange flesh trait in potato with the content of zeaxanthin. Genetic studies have attributed the orange flesh phenotype with high tuber zeaxanthin to the presence of an allele at the Y locus, designated Or, which is dominant over Y and y controlling yellow and white flesh, respectively. The Y gene has been mapped to chromosome three by Bonierbale et al. (1988). Therefore, the availability and functional carotenogenic gene expression is important for the control of the carotenoid biosynthesis pathway in potato tubers. In a recent paper, Lu et al. (2006) demonstrated that the *Orange (Or)* gene mutation in cauliflower induces differentiation of β-carotene-containing chromoplasts in the non-pigmented curd tissue. Chromoplasts are carotenoid-accumulating plastids found in many fruits and flowers whereas the *Or* gene is a dominant mutation that confers an orange pigmentation to the curd and the apical meristem of cauliflower, which are normally colorless. This gene is highly expressed in tissues containing non-green plastids, such as apical shoots and young leaves in cauliflower and Arabidopsis (Lu et al., 2006). The *Or* gene mutation confers the accumulation of high levels of β-carotene in various tissues that normally devoid of carotenoids. Therefore, by manipulating or selecting appropriate potato cultivars that possess structure that can accumulate carotenoids (e.g. chromoplasts versus amyloplasts) may provide an important mechanism to enhance carotenoid content. The *Or* gene has been introduced into the potato

cultivar Desiree where expression of the *Or* transgene in tubers of transgenic lines led to enhanced levels of violaxanthin, lutein, and β-carotene with a total carotenoid level of approximately 6-fold higher than the non-transformed or vector-only controls (Lu et al., 2006).

In conclusion, the differences in carotenoid profile and tuber flesh color from different growing seasons, locations and cultivars can be explained by the regulation of genes especially ZEP and VDE, presence of structure sequestering carotenoids and environmental stress. As stated by Havaux and Niyogi (1999), white flesh cultivars, which have a limited capacity to tolerate excessive light, exhibited an increased susceptibility to photooxidative damage. In contrast, yellow flesh cultivars which carotenoid content are much higher can specifically tolerate excessive light and also many environmental stress conditions by regulating ZEP and VDE. Selecting the appropriate potato cultivars for the appropriate environmental conditions and appropriate agronomic practices is not only important for yield production, but also for nutritional value and quality of potatoes (Troyer, 2003; Denison et al., 2003). Identifying which environmental factors can influence the accumulation of specific individual carotenoid pigments should be a key research initiative. This could be more important than selecting potato genotypes with higher carotenoid content as parents in a breeding program for the development of new potato cultivars with enriched nutrients. Carotenoid activity and specific carotenoid compounds in a wide range of genotypes has already been identified and quantified (Chapter 3). Growing season (Chapter 4) and different locations (this chapter) have also been established to influence the accumulation of total carotenoid content and individual carotenoid compounds. The next chapter will study the composition of carotenoids by evaluating environmental factors associated with storage and disease of potato tubers.

CHAPTER 6

Influence of post harvest storage time and disease on carotenoid biosynthesis

6.1 Introduction

Potato has the richest source of genetic variability among the cultivated crop plants (Ross, 1986) and this large reservoir of genetic variation can provide useful new traits for potato variety development (Hanneman and Bamberg, 1986). There are more than 2000 species in the *Solanum* genus and 180 of which form tubers (Hawkes, 1978). Therefore the feasibility of incorporating exotic germplasm into cultivated potatoes is very high (Peloquin, 1982). Improving the nutritional values of potato is an important breeding goal and understanding the regulation, genetics and inheritance of carotenoid biosynthesis is vital to achieve this. Environmental conditions can have a marked influence on the accumulation of carotenoids in potato tubers. This thesis has already established the variation in specific carotenoid compounds in response to growing seasons (Chapter 4) and locations (Chapter 5). The main objective of this study was to determine the effect of disease and storage time on carotenoids content in selected genotypes potato tubers.

6.2 Experimental Design

6.2.1 Potato genotypes

Analysis of carotenoid content was determined on a total of 17 potato genotypes for storage time, whereas 24 potato genotypes were available to investigate the impact of disease. The genotypes included Agria and Vt^n62-33-3, a series of clones derived from hybridisation of these two genotypes, as well as two unrelated genotypes, Iwa and Gladiator. The selected genotypes were planted in field trials at Crop and Food Research, Lincoln. The plot size, planting, harvest times and agronomic management of the crop followed the standard protocols set out for New Zealand potato trials. All tubers were harvested from plot at full maturity following natural plant senescence and maintained at ambient temperatures for three weeks until grading, then placed in cool storage (air at 8°C). Three to five undamaged tubers were selected after harvested from each of three replicate plots for all genotypes.

6.2.2 Storage time treatments

All tubers (2004/2005 growing season) were stored in darkness at cool temperatures (air at 8°C) for 4 weeks, 12 months and 24 months

6.2.3 Scab score

All selected tubers for scored for symptoms of powdery scab, caused by the protozoan pathogen *Spongospora subterranea* f. sp. *subterranean*, using an ordinal scale where '0' indicates no disease and '7' means the tuber skin surface is completely covered in scabs (Baldwin et al., 2006). The tubers were then grouped into categories of those without scab, lower scab scores and higher scab scores.

6.2.4 Sampling

The skin of the tubers was removed with a peeler and the remaining tuber tissue was cut into five mm slices. For each sample tuber tissue was pooled from three tubers, mixed, and a random 100 g sample was immediately frozen at -20^{O}C. The tuber samples were freeze-dried for 7 days, after which the samples were ground into fine powder and stored at -80 °C until analysis.

6.2.5 Extraction of carotenoids

The extraction procedure followed the methods described by Morris et al. (2004), Lewis et al. (1998) and Britton et al. (1995) as mentioned detailed in Chapter 2 (section 2.2.2).

6.2.6 Determination of total carotenoid concentration

Total carotenoid concentration was determined by spectrophotometry as described previously by Britton et al., (1995) and Lewis et al., (1998). All the calculations are described in detail in section 2.2.3.

6.2.7 HPLC analysis

The HPLC analysis of saponified carotenoids was analysed according to Morris et al. (2004) as described detailed in Chapter 2 (section 2.4). Carotenoid standards β-carotene, violaxanthin, lutein, and neoxanthin were isolated from *Eruca sativa* (roquette or rocket salad) by open column chromatography (see section 2.3.1) as described by Kimura and Rodriguez-Amaya (2002), whereas zeaxanthin was obtained commercially from Sigma-Aldrich (Auckland, New Zealand).

6.3 Results

6.3.1 Analysis of carotenoid content of potato tubers in response to 12 months storage time

Statistical analysis revealed that there were highly significant differences ($P< 0.0001$) between genotypes, storage time, carotenoid content, and all combinations of interactions (Table 6.1). The importance of the interaction components emphasises that the changes in carotenoid composition are complex and the responses to storage time are not consistent across potato genotypes. As demonstrated in Figure 6.1 and Appendix 6.1, after harvest and four weeks storage time, lutein or β-carotene were detected in all genotypes at varying and generally low levels. Two exceptions were lines AV198 and AV367 in which lutein practically accounts for all carotenoid accumulating. Neoxanthin and violaxanthin were detected in about half of the genotypes, and when present made up a relatively high proportion of carotenoid levels. Zeaxanthin was not detected in any of the genotypes. After 12 months of storage time, neoxanthin, violaxanthin, lutein and β-carotene were detected in all genotypes. About half of the genotypes analysed contained varying amounts of zeaxanthin. After extended storage time four genotypes (AV177, AV198, AV318 and AV438) exhibited increased total carotenoid content, whereas the range of individual carotenoid compounds were lower, except for zeaxanthin.

Table 6.1: Analysis of variance of storage time (year 0, 1 & 2) on carotenoid content of 17 potato genotypes

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
STORAGE	2	222096.3370	111048.1685	749.71	0.0001
GENOTYPES	16	351340.5540	21958.7846	148.25	0.0001
STORAGE*GENOTYPES	32	710506.1576	22203.3174	149.90	0.0001
Error	102	15108.318	148.121		
Corrected Total	152	1299051.367			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
STORAGE	2	11147.1562	5573.5781	48.08	0.0001
GENOTYPES	16	228075.5075	14254.7192	122.96	0.0001
STORAGE*GENOTYPES	32	286763.0453	8961.3452	77.30	0.0001
Error	102	11825.2653	115.9340		
Corrected Total	152	537810.9742			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
STORAGE	2	48063.2564	24031.6282	2374.06	0.0001
GENOTYPES	16	82717.5907	5169.8494	510.72	0.0001
STORAGE*GENOTYPES	32	139910.7679	4372.2115	431.93	0.0001
Error	102	1032.5054	10.1226		
Corrected Total	152	271724.1204			

D - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
STORAGE	2	75319.2650	37659.6325	17175.9	0.0001
GENOTYPES	16	58647.0507	3665.4407	1671.74	0.0001
STORAGE*GENOTYPES	32	117656.1082	3676.7534	1676.90	0.0001
Error	102	223.6437	2.1926		
Corrected Total	152	251846.0676			

E - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
STORAGE	2	3011.230970	1505.615485	11095.4	0.0001
GENOTYPES	16	1096.414043	68.525878	504.99	0.0001
STORAGE*GENOTYPES	32	2331.115875	72.847371	536.84	0.0001
Error	102	13.841067	0.135697		
Corrected Total	152	6452.601954			

F - Analysis of variance for zeaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
STORAGE	2	33267.18946	16633.59473	262.33	0.0001
GENOTYPES	16	34418.99188	2151.18699	33.93	0.0001
STORAGE*GENOTYPES	32	46796.94981	1462.40468	23.06	0.0001
Error	102	6467.5409	63.4073		
Corrected Total	152	120950.6720			

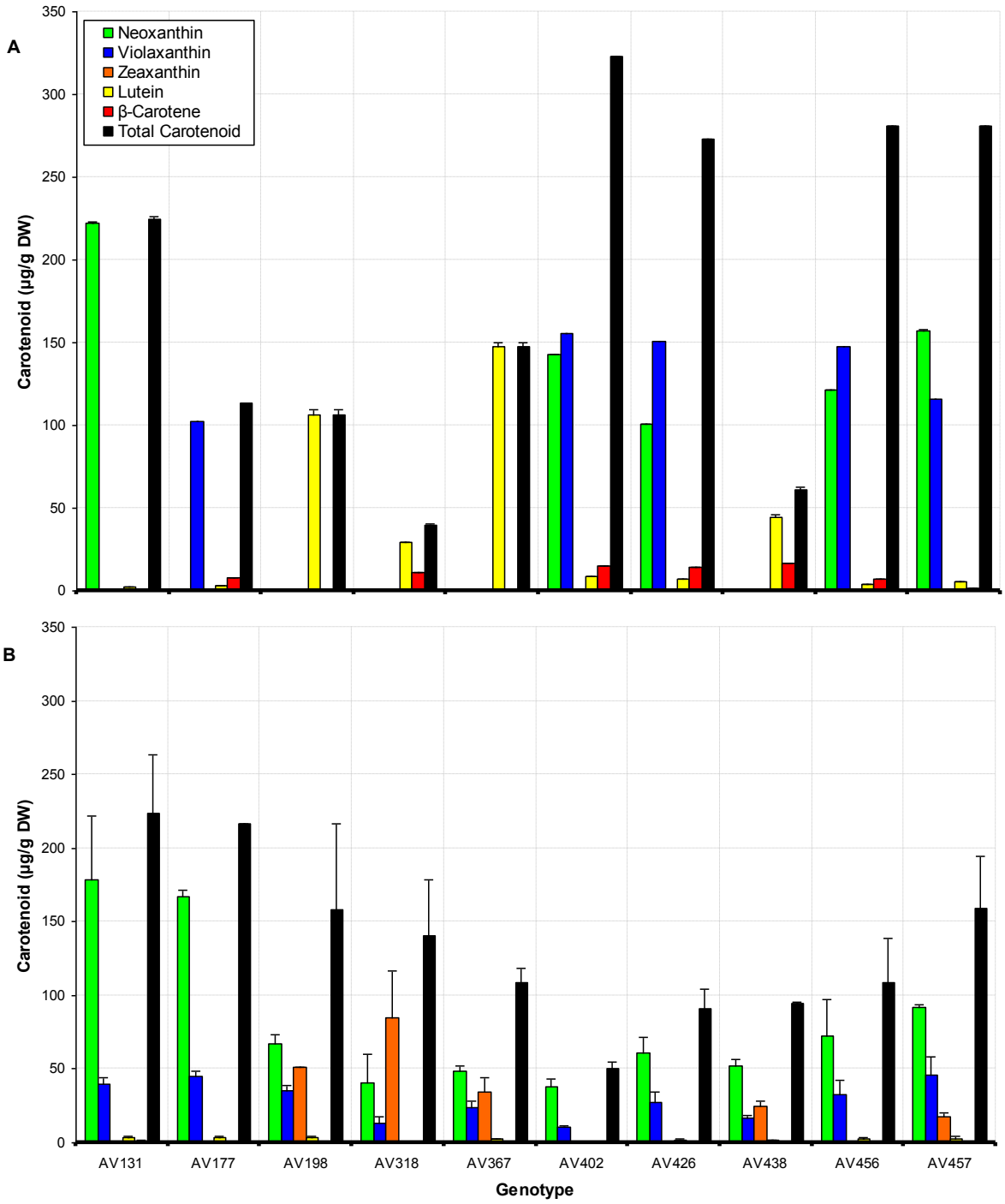


Figure 6.1: Analysis of the effect of storage time for four weeks and 12 months at 8°C in the dark on carotenoid accumulation in specific genotypes of potato.
A - Individual and total carotenoids content (µg/g DW) of ten potato genotypes stored for four weeks
B - Individual and total carotenoids content (µg/g DW) of ten potato genotypes stored for twelve months
Error bars represent ± SE.

6.3.2 Analysis of carotenoid content of potato tubers in response to 24 months storage time

The changes in carotenoid content over 24 months were investigated in a different set of genotypes due to deterioration of some samples resulting in blemished and rotten tubers. As stated in section 6.3.1 statistical analysis revealed that there were highly significant differences ($P<0.0001$) between genotypes, storage time, carotenoids content, and all interactions (Table 6.1). After harvested and storage for four weeks, lutein and β-carotene were detected as major carotenoids followed by violaxanthin and neoxanthin (Figure 6.1 and Appendix 6.1). The relative proportion of carotenoid levels was very high in neoxanthin, violaxanthin and lutein compared to other carotenoids. Zeaxanthin was not detected in any of the genotypes. After 24 months of storage time, violaxanthin, zeaxanthin and lutein were detected in all genotypes, with neoxanthin and β-carotene detected in 91% of genotypes. Six genotypes (Iwa, Vt^n 62-33-3, AV115, AV198, AV242, AV318) were found to have increased in total carotenoids, whereas ranges in carotenoid content were lower after extended storage time, except for zeaxanthin. β-carotene was not detected in any of the genotypes.

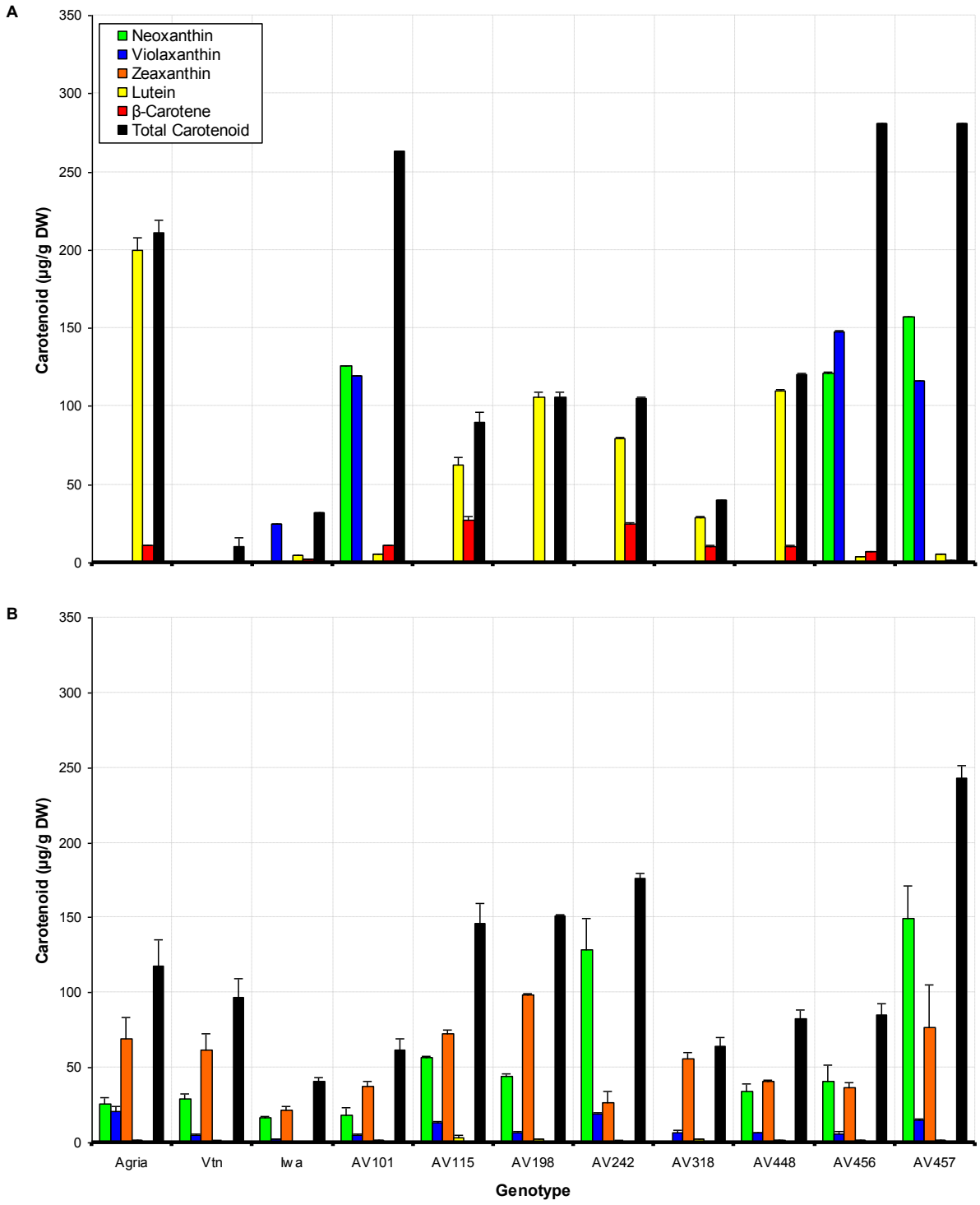


Figure 6.2: Analysis of the effect of storage time for four weeks and 24 months at 8°C in the dark on carotenoid accumulation in specific genotypes of potato.
A - Individual and total carotenoids content (µg/g DW) of 11 potato genotypes over four weeks storage time
B - Individual and total carotenoids content (µg/g DW) of 11 potato genotypes over twenty four months storage
time. Error bars represent ± SE.

6.3.3 Analysis of carotenoid content in potato tubers in response to the presence of powdery scab symptoms

Analysis of variance established confirmed that there were highly significant differences ($P<0.0001$) between potato genotypes, powdery scab score, carotenoid content, and all combinations of interactions (Table 6.2). The importance of the interaction components emphasises that the changes in carotenoid composition are complex and the variation in response to disease symptoms is not consistent across genotypes. As illustrated in Figure 6.3 and Appendix 6.2, lutein and β-carotene were detected 96% and 87% respectively of all genotypes without scab, whereas 46% had both violaxanthin and neoxanthin. In most genotypes neoxanthin, violaxanthin and lutein made up the major proportion of the total carotenoids. Zeaxanthin was not detected in any of the genotypes. With the presence of powdery scab symptoms on the tubers (lower scab scores), lutein and β-carotene were detected in all genotypes but the amount decreased whereas the amount of violaxanthin and neoxanthin were increased and were found 50% and 25% of the genotypes. Zeaxanthin remained undetected. In about half total carotenoid content increased.

For potato tubers infected with higher scab scores, violaxanthin, lutein and β-carotene were detected present in all genotypes, whereas neoxanthin was only present in about a quarter of the genotypes. Zeaxanthin remained undetected. The predominant carotenoids in highly infected tubers were neoxanthin and violaxanthin. About half of the genotypes exhibited increased total carotenoid content compared to tubers with lower scab scores or the absence of powdery scab symptoms. With the presence of higher scab score powdery scab symptoms on the tubers, lutein and neoxanthin were found decreased whereas violaxanthin, β-carotene and total carotenoid amount were increased significantly.

Table 6.2: Analysis of variance of disease (all combinations) on carotenoid content of 24 potato genotypes

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SCAB	2	42416.5146	21208.2573	15771.4	0.0001
GENOTYPES	23	887360.0299	38580.8709	28690.4	0.0001
SCAB*GENOTYPES	43	900039.4794	20931.1507	15565.3	0.0001
Error	137	184.228	1.345		
Corrected Total	205	1830000.252			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SCAB	2	119471.7820	59735.8910	1463679	0.0001
GENOTYPES	23	232465.7533	10107.2067	247652	0.0001
SCAB*GENOTYPES	43	354355.1524	8240.8175	201921	0.0001
Error	137	5.5913	0.0408		
Corrected Total	205	706298.2791			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SCAB	2	78845.7017	39422.8509	239250	0.0001
GENOTYPES	23	469494.7303	20412.8144	123881	0.0001
SCAB*GENOTYPES	43	480039.9593	11163.7200	67750.4	0.0001
Error	137	22.574	0.165		
Corrected Total	205	1028402.966			

D - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SCAB	2	20023.96781	10011.98390	16400.0	0.0001
GENOTYPES	23	44173.51142	1920.58745	3146.00	0.0001
SCAB*GENOTYPES	43	85549.09277	1989.51379	3258.91	0.0001
Error	137	83.6365	0.6105		
Corrected Total	205	149830.2085			

E - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SCAB	2	73.105154	36.552577	418.18	0.0001
GENOTYPES	23	5461.177002	237.442478	2716.46	0.0001
SCAB*GENOTYPES	43	6115.329348	142.216962	1627.03	0.0001
Error	137	11.97500	0.08741		
Corrected Total	205	11661.58650			

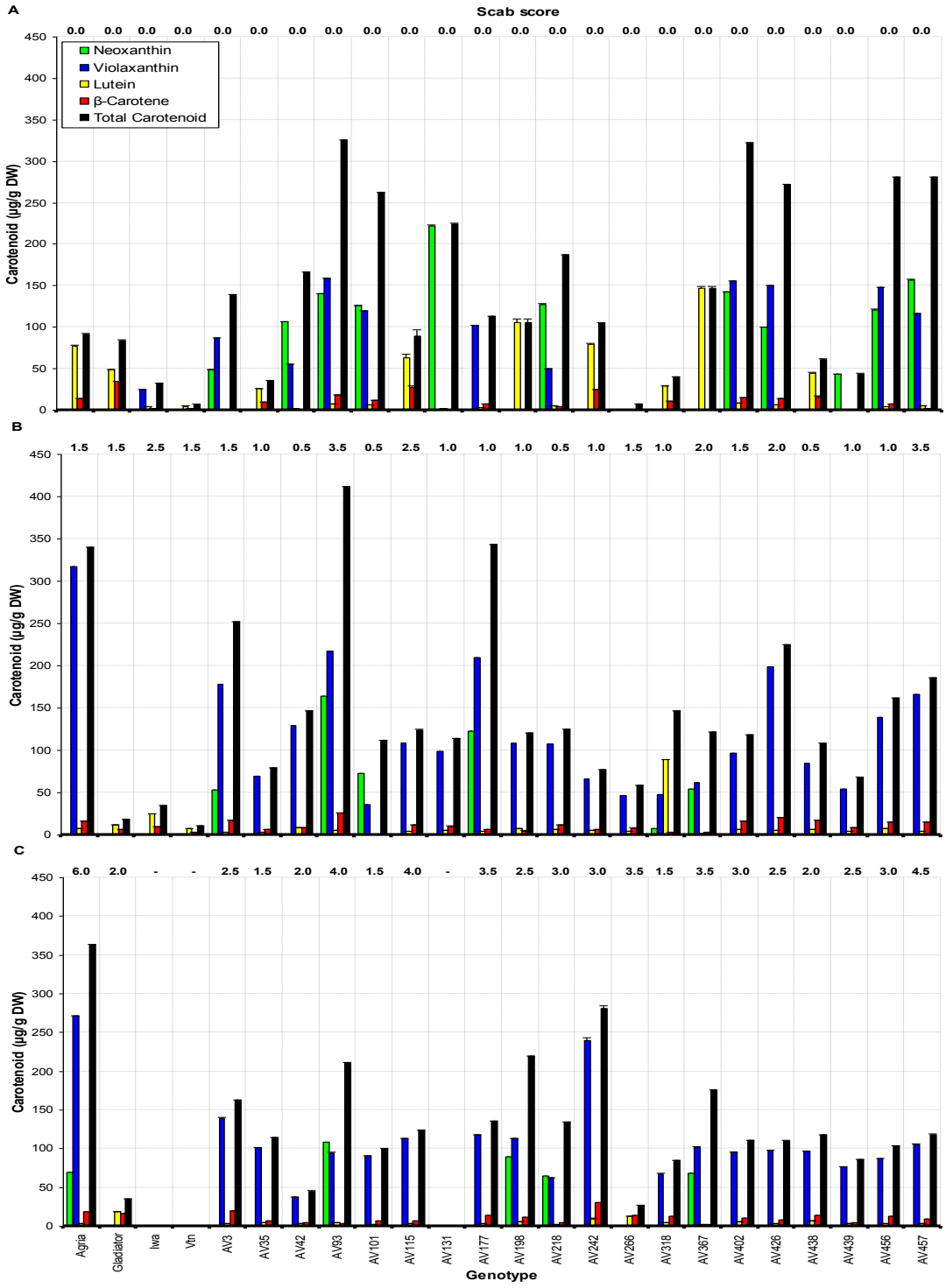


Figure 6.3: Analysis of carotenoid content in potato tubers in response to the presence of powdery scab symptoms

A - Individual and total carotenoids content (µg/g DW) of 24 potato genotypes without scab

B - Individual and total carotenoids content (µg/g DW) of 24 potato genotypes with lower scab score

C - Individual and total carotenoids content (µg/g DW) of 24 potato genotypes with higher scab score

Error bars represent ± SE. Zeaxanthin not included due to undetected levels in all samples.

No samples available for Iwa, Vt^{n} 62-33-3 and AV131 with higher scab score (-).

6.4 Discussion

Little is known about the effects of storage time and disease particularly on carotenoid biosynthesis. So far only Lopez et al. (2008), Blessington et al. (2007), Griffiths et al. (2007) and Morris et al. (2004) reported on effect of storage time on carotenoid biosynthesis and there is no report at all on the effect of disease on carotenoids. Lopez et al. (2008) observed that tubers stored at 5˚C for 6 months greatly enhanced tuber colour and carotenoid content in the *Or* transgenic tubers to a level of 10-fold over controls (5.50 µg/g DW). Violaxanthin and lutein were the major carotenoids detected in controls whereas in *Or* transgenic tubers besides violaxanthin and lutein, β-carotene also increased. In addition to that phytofluene and ζ-carotene levels were also increased in the *Or* transgenic tubers during long term cold storage time. The total carotenoid contents were between 28.22 – 31.19 µg/g DW before the storage time and 56.42 – 71.58 µg/g DW after the storage time. Blessington et al. (2007) reported that total carotenoid content decreased, but that of lutein increased with storage potato tubers stored at 20°C and analysed after 0, 10, 20, 75 and 110 days in storage time. Meanwhile Griffiths et al. (2007) revealed that 12 weeks of storage time significantly reduced the total carotenoid content of tubers from 38 lines of *S. phureja* and reducing the storage time temperature from 10 to 4˚C also lowered the carotenoid content. Lutein was observed the most stable and least likely to be reduced while the levels of carotenoids derived from β-carotene (violaxanthin, antheraxanthin and zeaxanthin) were significantly reduced during storage time at either temperature. A previous study by Morris et al. (2004) also showed a decrease in total carotenoids upon storage time in a *S. phureja* line and Desiree. Following 9 months storage time at 4 °C the levels of zeaxanthin and antheraxanthin decreased, whereas the level of lutein increased. There was only a small decrease in total carotenoid content, however in Desiree the main changes were decrease in the level of violaxanthin and an increase in lutein.

These phenomena have been explained as followed as stated by Lopez et al. (2008), Blessington et al. (2007), Griffiths et al. (2007) and Morris et al. (2004):

(i) any lutein chemically destroyed by, for example, reaction with potentially damaging oxidants was replaced at a faster rate than the other major carotenoids;

(ii) the activity of enzymes and candidate enzymes (carotenoid cleavage dioxygenases) that catabolize the β-carotene-derived carotenoids such as zeaxanthin, antheraxanthin and violaxanthin was greater than those utilizing lutein as their substrate;

(iii) transformation of the free carotenoids to fatty acid esters, provided no change in total carotenoids, although in this study total carotenoid content increased;

(iv) the carotenoid sequestering structures formed in the tubers continually provide a sink force pulling the biosynthetic pathway toward more carotenoid formation;

(v) the carotenoid sequestering structures formed in the tubers may also help to slow down carotenoid degradation especially in the intense yellow flesh tubers;

(vi) ZEP activity have been reduced and therefore restricts the supply of precursors for ABA biosynthesis and the plant responds by increasing carotenogenic metabolic flux to compensate for this restriction;

(vii) LCYB, LCYE and CHYB activities have been reduced and restricts the supply of lutein; and

(viii) due to induction mechanism such as physical changes like sprouting and subsequent dehydration, concentration and induced stress.

From all previous research and the results of this study, potato tuber storage time between 1 to 9 months resulted in total carotenoid decreases concomitantly with increase of lutein. This is in agreement with the first point listed above stating that any chemically degradation of lutein will be replaced at a faster rate than the other major carotenoids. As a result total carotenoids decreased. However, an exception is the intense yellow flesh tubers like *Or* transgenic tubers and Agria, total carotenoid increased concomitantly with the increased of lutein and β-carotene. This is in agreement with the fourth and fifth points above stating that the carotenoid sequestering structures formed in the tubers continually provide a sink force pulling the biosynthetic pathway toward more carotenoid formation and also help to slow down carotenoid degradation. In addition, ZEP activity is reduced and therefore restricts the supply of precursors for ABA biosynthesis and the plant responds by increasing lutein and β-carotene to compensate for this restriction. Total carotenoid content always associated with tuber flesh color and in recent work by Edwards et al. (2002), yellow-fleshed tubers when stored at 8.3 °C for 84 days had higher hue angles chroma values compared to the white-fleshed potatoes. These values were consistently higher and the chroma values were maximal for most yellow-fleshed tubers.

There is no previous report on the storage time for more than 12 months and our works on storage time for 24 months revealed the breakdown of lutein and β-carotene into zeaxanthin, violaxanthin and neoxanthin. This can be explained if the activity of enzymes that catabolize neoxanthin, zeaxanthin and violaxanthin was greater than those utilizing lutein as their substrate after 24 months of storage time. Furthermore LCYB, LCYE and

CHYB activities may have been reduced to restrict the supply of lutein. To compensate for this restriction, ZEP activity may have been induced to supply for ABA precursors such as zeaxanthin, violaxanthin and neoxanthin. Interestingly, the Vt^n 62-33-3 (white flesh) and Agria (yellow flesh) carotenoid profiles were similar after 24 months storage time. For the first 4 weeks Agria accumulated high concentration of lutein and β-carotene whereas no individual carotenoids were detected in Vt^n 62-33-3. After 24 months storage time total carotenoid for Agria decreased, whereas for Vt^n 62-33-3 it increased, and both genotypes contained zeaxanthin, violaxanthin and neoxanthin.

The presence of disease symptoms also had a major influence on the accumulation of carotenoids in potato tubers. Genotypes with powdery scab symptoms on the tubers exhibited marked changes in their profile of carotenoid compounds (Figure 6.3, Appendix 6.2). Genotypes resistant and susceptible to powdery scab were included among those tested. Vt^n 62-33-3 and Gladiator are resistant, whereas Agria and Iwa are susceptible (Baldwin et al. 2008). The series of AV lines represent individuals from a population segregating for powdery scab resistance/susceptibility. An indication of the resistance/susceptible response to powdery scab in each genotype is apparent from the powdery scab scores illustrated in Figure 6.3. There appears to be no association between the changes in carotenoid composition in response to powdery scab symptoms and the resistance/susceptible status of the potato genotypes to the disease.

In this case phenomenon of plant self defence mechanism towards disease such as powdery scab might also being applied using all the possibilities mentioned above. The difference between disease and storage time is the tubers were infected while in the field whereas for storage time the event took place after harvested. In comparisons (Figure 6.3),

lutein and β-carotene were also detected in all genotypes without scab while on the tubers with lower scab scores, lutein and β-carotene were detected in all genotypes but the amount decreased. For potato tubers infected with higher scab scores, pronounced violaxanthin concentration was detected present in all genotypes, whereas zeaxanthin remained undetected. According to Britton (2008), it is now recognized that abscisic acid which is one of the main hormone-like growth regulating substances in plants, play an apparent role in pathogenic defence by inducing gene transcription for protease inhibitors in response to wounding. The active form of ABA is a derivative of violaxanthin and neoxanthin. Therefore in our results on the effect of disease on carotenoid demonstrated that the pronounced violaxanthin concentration was accumulated after the tubers infected with powdery scab, which can explain why no zeaxanthin was detected at all at this stage due to the apparent role of ABA.

According to Blessington et al. (2007) the increase and decrease in particular carotenoid compounds is most probably due to low stability of specific carotenoid compounds. Furthermore, stimulation of antioxidant synthesis such as carotenoid is known to occur with stress, which may have increased at the end of the storage period due to dehydration (Friedman, 1997, Ghanekar et al., 1984; Kang and Saltveit, 2002). Activation of systemic host defence systems upon pathogen infection is also known to involve stress response genes, which can also involve responses to oxidative stress (Desender et al., 2007). Physical changes such as sprouting and dehydration are believed to be responsible for this phenomenon as well (Blessington et al., 2007). The response of potato genotypes to such environments appeared to be very important and the magnitude of these changes was found to be highly genotype dependent.

CHAPTER 7

Assessment of potato minitubers as a model system for carotenoid biogenesis

7.1 Introduction

Carotenoids are synthesized and localised in the plastids of higher plants. Chloroplasts store carotenoids in thylakoid membranes while chromoplasts store high levels of carotenoids in membranes, oil bodies, or other crystalline structures within the stroma (Cunningham and Gantt, 1998; Howitt and Pogson, 2006). In potato tubers, carotenoids are reported to be compartmentalised to amyloplasts (Lopez-Juez and Pyke, 2005). Amyloplasts are colourless plastids specialized for storage of starch granules (DellaPenna and Pogson, 2006). Potatoes may become major sources of carotenoids in the diet, but the extent of environmental and genetic influences on plant carotenoid biosynthesis are poorly understood. Carotenoid biosynthesis is regulated by several factors and is susceptible to geometric isomerisation in the presence of oxygen, light and heat which causes colour loss and oxidation. The main problems associated with carotenoid accumulation arise from the inherent instability of pigments (Bramley and Mackenzie, 1987; Cinar, 2004). In this chapter carotenoid biogenesis is investigated in potato minitubers as a potential model system for rapid initiation, extraction and analysis of carotenoids by providing stringent control of genetic, developmental and environmental factors. The value of this experimental system for investigating variables controlling carotenoid accumulation is then tested by assessing the effects of environmental variables, such as drought stress, light intensity and nutrient strength on carotenoid accumulation.

7.2 Experimental Design

7.2.1 Tissue culture and minituber initiation

Virus-free *in vitro* plants of cultivars Agria and Desiree were obtained from the New Zealand Institute for Crop & Food Research Ltd. These were incubated in a growth room at 24ºC day and night temperature, with a 16-h photoperiod at 80-85 μmol m^{-2} s^{-1} under cool white fluorescent light. Every 4 weeks the *in vitro* plants were subcultured as nodal cuttings on potato multiplication medium (PMM) composed of Murashige and Skoog (1962) salts and vitamins supplemented with 30g/L sucrose, 40 mg/L ascorbic acid, 500 mg/L casein hydrolysate and 10 g/L agar following the method of Conner et al. (1991). Media were adjusted to pH 5.7 and sterilized by autoclaving (15 min, 121ºC) and 50 ml aliquots poured into pre-sterilised 290 ml plastic pottles (80 mm diameter x 60 mm high; Vertex Plastics, Hamilton, New Zealand).

For minituber initiation, individual shoots of 3-4 nodes from vigorously growing four-week-old cultures were transferred into 40 ml of liquid tuber initiation medium (TIM) in 250 ml polycarbonate culture vessels (7 cm diameter x 8 cm high). The TIM contained the same constituents as PMM, except with the addition of 80 g/L sucrose, 5 mg/L benzyladenine, 2.5 mg/L ancymidol and no agar. Nine shoots were placed upright into each culture vessel and were incubated in darkness at 25ºC. Minitubers were classified as such when their diameter exceeded 2 mm, and normally grew up to more than 5 mm diameter within 4 weeks.

7.2.2 Effect of environmental factors on carotenoid biosynthesis

In three independent experiments the influence of light, water-stress and nutrient availability on carotenoid biosynthesis were tested in both Agria and Desiree. Minitubers harvested after 4 weeks from two culture vessels were pooled for each of three replicates established under the following conditions:

1. Light versus darkness by incubation under cool-white, fluorescent lamps (80-85 μmol m^{-2} s^{-1}; 16 h photoperiod) with dark condition imposed by carefully wrapping the culture vessels in aluminium foil.
2. Incubation in darkness with and without 50mM PEG 4000 to impose water-stress.
3. Incubation in darkness at three concentrations of MS salts (one tenth, half and full-strength).

7.2.3 Minituber extraction and analysis of carotenoids

Minitubers were harvested and pooled for each replicated each treatment, cut in half and freeze dried as combined skin and flesh samples. All the methods for extraction and analysis of total and individual carotenoid were followed the methods described in Chapter 2.

7.3 Results

7.3.1 Effect of light on carotenoid accumulation in potato minitubers

Statistical analysis showed that there was highly significance difference ($P < 0.0001$) in carotenoid content in Agria minitubers developing in the dark and light (Table 7.1). Agria minitubers accumulated four individual carotenoids compounds (violaxanthin, zeaxanthin, lutein and β-carotene) when developing in both dark and light. The two predominant carotenoids were violaxanthin and zeaxanthin. Neoxanthin was not detectable in either dark or light treatments. However, development of Agria minitubers in light resulted in an approximate doubling of the total carotenoid content compared minitubers developing in darkness (Appendix 7.1, Figure 7.1). The amount of each individual carotenoid also approximately doubled upon development in light, especially for violaxanthin and zeaxanthin.

Analysis of variance comparing Desiree minitubers grown in the dark and light also established highly significant differences ($P < 0.0001$) in carotenoid content (Table 7.2). As shown in Appendix 7.1 and Figure 7.1, five individual carotenoids (neoxanthin, violaxanthin, zeaxanthin, lutein and β-carotene) were found in Desiree minitubers grown in darkness, but upon development in light only four (neoxanthin, violaxanthin, lutein and β-carotene) were detected, with an absence of zeaxanthin. After development in light, total carotenoid content approximately doubled, and reflected an increase in neoxanthin and violaxanthin.

Table 7.1: Analysis of variance of carotenoid biosynthesis analysis on carotenoid content of Agria minitubers in response to light

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	42713.15627	42713.15627	18662.5	0.0001
Error	4	9.15487	2.28872		
Corrected Total	5	42722.31113			

B - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	8332.826667	8332.826667	64528.9	0.0001
Error	4	0.516533	0.129133		
Corrected Total	5	8333.343200			

C - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	17.64735000	17.64735000	70589.4	0.0001
Error	4	0.00100000	0.00025000		
Corrected Total	5	17.64835000			

D - Analysis of variance for zeaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	12356.06640	12356.06640	9114.83	0.0001
Error	4	5.42240	1.35560		
Corrected Total	5	12361.48880			

Neoxanthin and β-carotene not included due to trace amounts or undetected levels in most of the samples.

Table 7.2: Analysis of variance of carotenoid biosynthesis analysis on carotenoid content of Desiree minitubers in response to light

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	15994.97402	15994.97402	31665.9	0.0001
Error	4	2.02047	0.50512		
Corrected Total	5	15996.99448			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	1500.052817	1500.052817	33101.6	0.0001
Error	4	0.181267	0.045317		
Corrected Total	5	1500.234083			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	19895.04167	19895.04167	551619	0.0001
Error	4	0.14427	0.03607		
Corrected Total	5	19895.18593			

D - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	52.98481667	52.98481667	317909	0.0001
Error	4	0.00066667	0.00016667		
Corrected Total	5	52.98548333			

E - Analysis of variance for zeaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	3672.405600	3672.405600	39723.2	0.0001
Error	4	0.369800	0.092450		
Corrected Total	5	3672.775400			

β-carotene not included due to trace amounts or undetected levels in most of the samples.

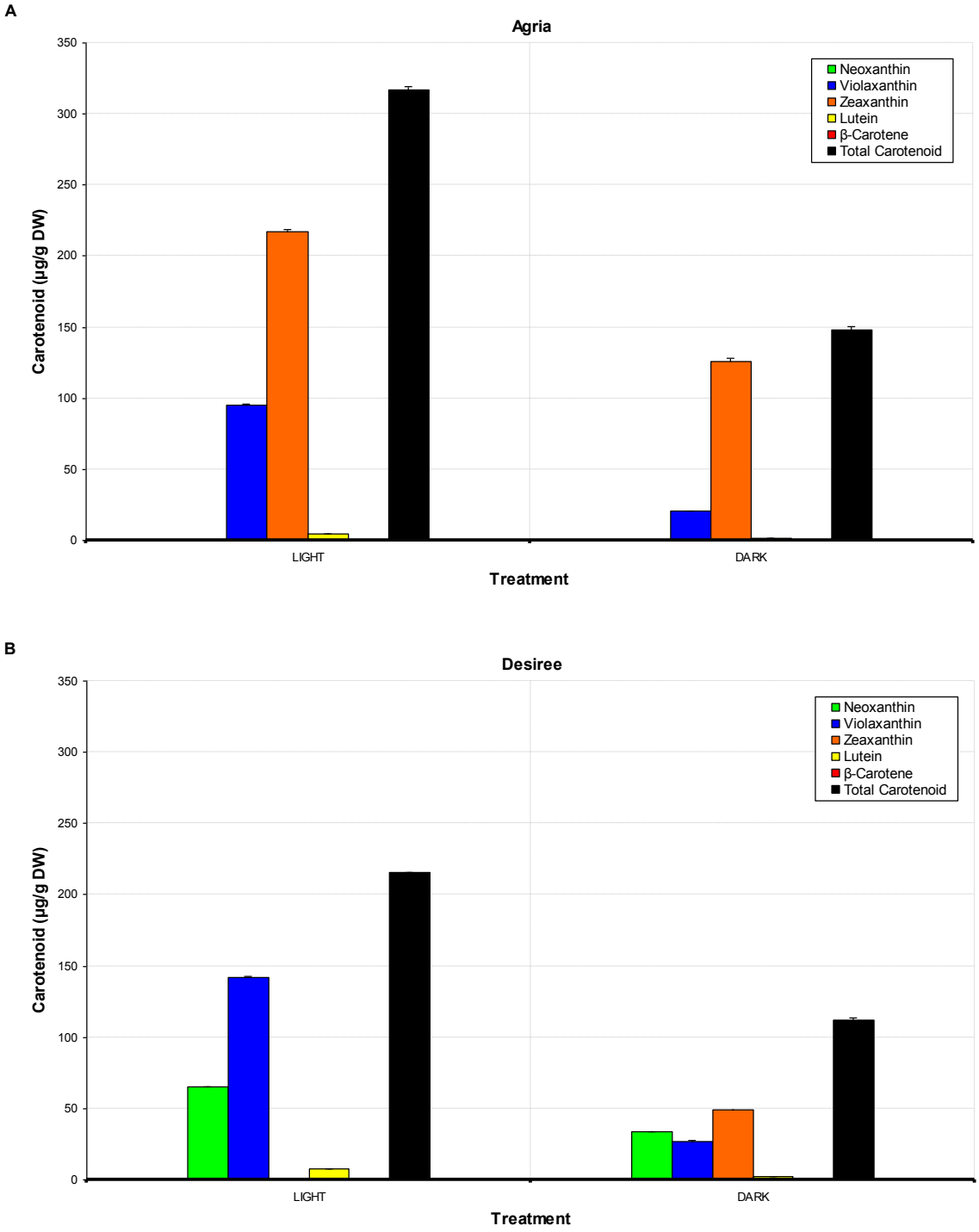


Figure 7.1: Analysis of carotenoid content (µg/g DW) of Agria and Desiree minitubers in response to light
A - Individual and total carotenoid content (µg/g DW) of Agria minitubers developing in light and dark
B - Individual and total carotenoid content (µg/g DW) of Desiree minitubers developing in light and dark
Error bars represent ± SE.

7.3.2 Effect of PEG on carotenoid accumulation in potato minitubers

Analysis of variance showed that there was a highly significance difference ($P < 0.0001$) in carotenoid content in response to the water stress treatment during development of Agria minitubers (Table 7.3). Agria minitubers developing in the presence of PEG (Appendix 7.1, Figure 7.2) exhibited an increased total carotenoid content. This increase reflected a substantially higher amount of violaxanthin and occurred despite the total absence of zeaxanthin in the presence of PEG.

Analysis of variance also established highly significant differences ($P < 0.0001$) in carotenoid content for Desiree minitubers developing in the presence of water stress (Table 7.4). As shown in Appendix 7.1 and Figure 7.2, total carotenoid content increased in minitubers developing in the PEG treatment. This reflected an increase in both neoxanthin and violaxanthin, with traces of lutein being observed in both treatments.

Table 7.3: Analysis of variance of carotenoid biosynthesis analysis on carotenoid content of Agria minitubers in response to water stress

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	829.7856000	829.7856000	28.57	0.0059
Error	4	116.1801333	29.0450333		
Corrected Total	5	945.9657333			

B - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	13126.46827	13126.46827	556.67	0.0001
Error	4	94.32127	23.58032		
Corrected Total	5	13220.78953			

C - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	45.21015000	45.21015000	564.77	0.0001
Error	4	0.32020000	0.08005000		
Corrected Total	5	45.53035000			

D - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	5.41500000	5.41500000	640.83	0.0001
Error	4	0.03380000	0.00845000		
Corrected Total	5	5.44880000			

E - Analysis of variance for zeaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	8993.107350	8993.107350	6606.51	0.0001
Error	4	5.445000	1.361250		
Corrected Total	5	8998.552350			

Neoxanthin not included due to trace amounts or undetected levels in most of the samples.

Table 7.4: Analysis of variance of carotenoid biosynthesis analysis on carotenoid content of Desiree minitubers in response to water stress

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	1359.918150	1359.918150	340.08	0.0001
Error	4	15.995200	3.998800		
Corrected Total	5	1375.913350			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	448.7620167	448.7620167	372.50	0.0001
Error	4	4.8188667	1.2047167		
Corrected Total	5	453.5808833			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	325.0176000	325.0176000	454.67	0.0001
Error	4	2.8594000	0.7148500		
Corrected Total	5	327.8770000			

D - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	11.50935000	11.50935000	5115.27	0.0001
Error	4	0.00900000	0.00225000		
Corrected Total	5	11.51835000			

E - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	1	1.11801667	1.11801667	9583.00	0.0001
Error	4	0.00046667	0.00011667		
Corrected Total	5	1.11848333			

Zeaxanthin not included due to trace amounts or undetected levels in most of the samples.

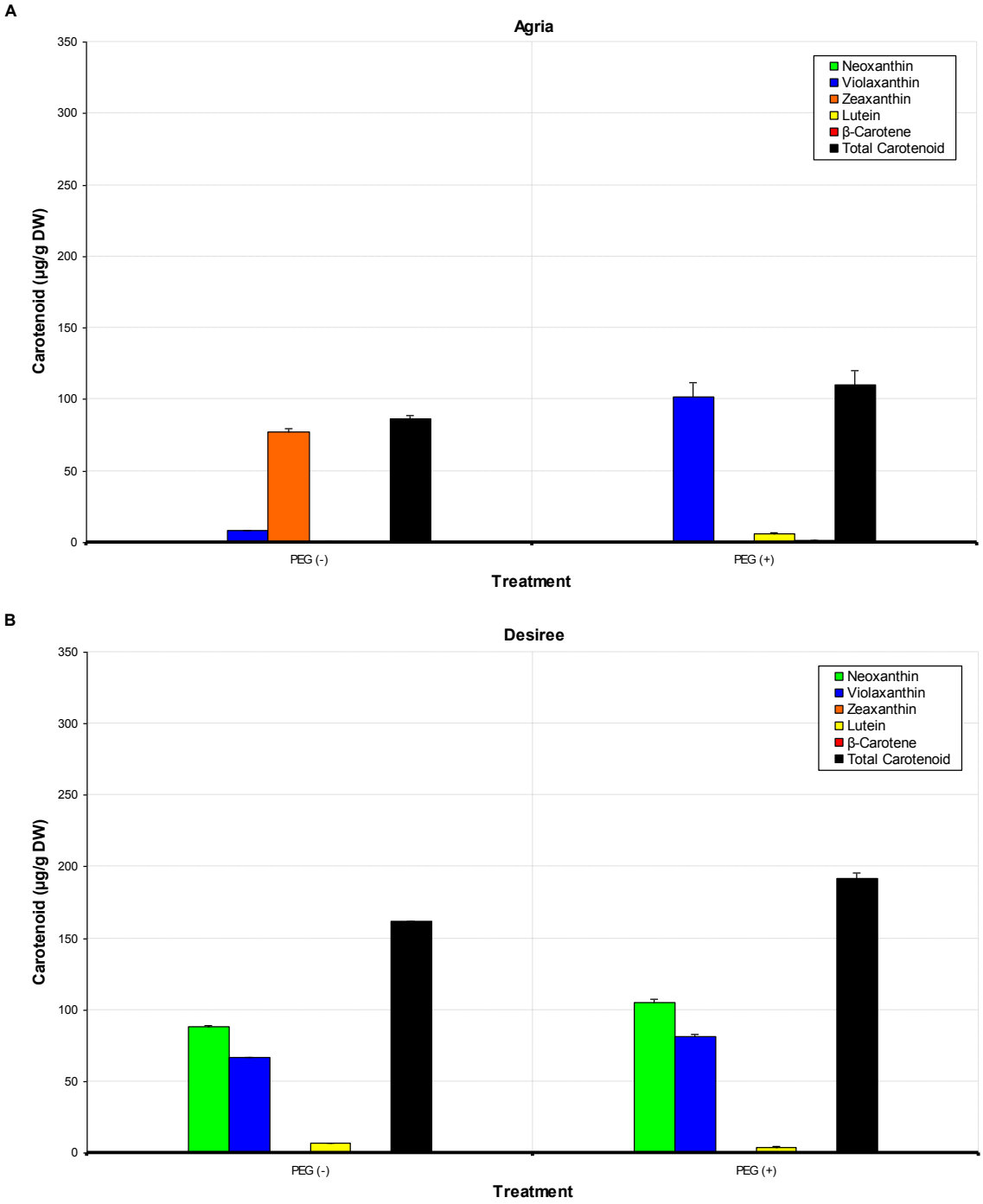


Figure 7.2: Analysis of carotenoid content (µg/g DW) of Agria and Desiree minitubers in response to water stress

A - Individual and total carotenoid content (µg/g DW) of Agria upon development wit h and without PEG treatment

B - Individual and total carotenoid content (µg/g DW) of Desiree upon development with and without PEG treatment

Error bars represent ± SE.

7.3.3 Effect of nutrient stress on carotenoid accumulation in potato minitubers

Nutrient stress during Agria minituber development resulted in a highly significance difference ($P < 0.0001$) in carotenoid content (Table 7.5). When MS salt strength increased from 0.1x to 0.5x, total carotenoid, violaxanthin and β-carotene content decreased, accompanied by a slight increase in lutein concentration. However, when MS salt strength increased from 0.5x to 1.0x, total carotenoid, violaxanthin and β-carotene increased, whereas lutein concentration decreased (Appendix 7.1, Figure 7.3).

Analysis of variance also established highly significant differences ($P < 0.0001$) in carotenoid content in Desiree minitubers developing in varying MS salt strengths (Table 7.6). As shown in Appendix 7.1 and Figure 7.3, when MS salt strength increased from 0.1x to 0.5x, total carotenoid content slightly increased due to minor changes in neoxanthin and lutein. In contrast, upon further increases in MS salt strength, 0.5x to 1.0x, total carotenoid content and individual carotenoids, especially neoxanthin, violaxanthin and lutein, decreased. No changes were observed in β-carotene when MS salt strength increased from 0.1x to 0.5x for the development of Desiree minitubers.

Table 7.5: Analysis of variance of carotenoid biosynthesis analysis on carotenoid content of Agria minitubers in response to nutrient stress

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	15395.19529	7697.59764	1739.21	0.0001
Error	6	26.55553	4.42592		
Corrected Total	8	15421.75082			

B - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	15676.71269	7838.35634	1999.43	0.0001
Error	6	23.52180	3.92030		
Corrected Total	8	15700.23449			

C - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	15.07962222	7.53981111	1156.02	0.0001
Error	6	0.03913333	0.00652222		
Corrected Total	8	15.11875556			

D - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	6.83886667	3.41943333	2024.66	0.0001
Error	6	0.01013333	0.00168889		
Corrected Total	8	6.84900000			

Neoxanthin and zeaxanthin not included due to trace amounts or undetected levels in most of the samples.

Table 7.6: Analysis of variance of carotenoid biosynthesis analysis on carotenoid content of Desiree minitubers in response to nutrient stress

A - Analysis of variance for total carotenoid content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	1934.360600	967.180300	1015.91	0.0001
Error	6	5.712200	0.952033		
Corrected Total	8	1940.072800			

B - Analysis of variance for neoxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	304.7912000	152.3956000	540.15	0.0001
Error	6	1.6928000	0.2821333		
Corrected Total	8	306.4840000			

C - Analysis of variance for violaxanthin content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	554.1194000	277.0597000	1797.53	0.0001
Error	6	0.9248000	0.1541333		
Corrected Total	8	555.0442000			

D - Analysis of variance for lutein content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	14.44802222	7.22401111	2399.12	0.0001
Error	6	0.01806667	0.00301111		
Corrected Total	8	14.46608889			

E - Analysis of variance for β-carotene content (µg/g DW)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	0.00020000	0.00010000	Infty	0.0001
Error	6	0.00000000	0.00000000		
Corrected Total	8	0.00020000			

Zeaxanthin not included due to trace amounts or undetected levels in most of the samples.

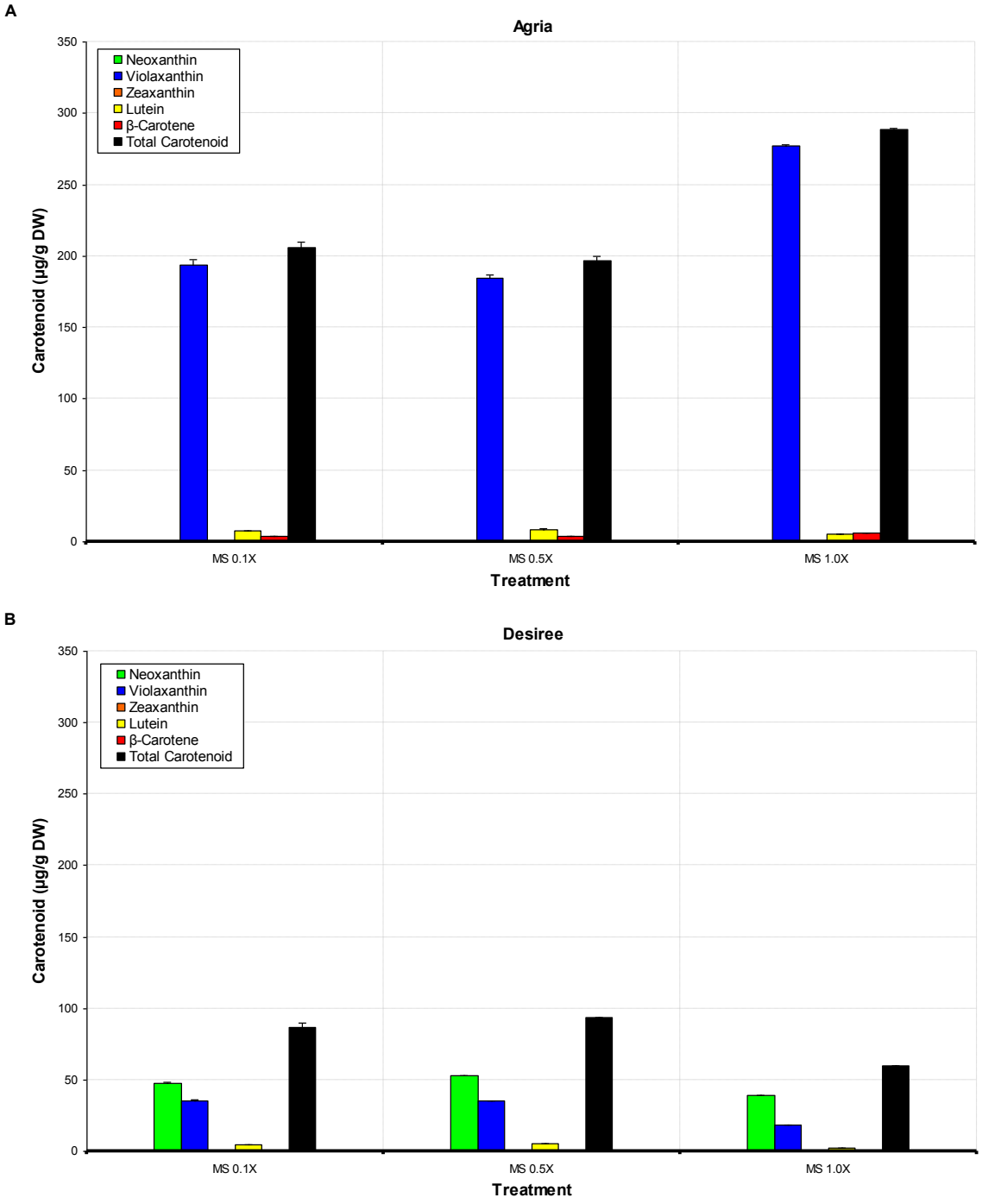


Figure 7.3: Analysis of carotenoid content (µg/g DW) of Agria and Desiree minitubers in response to nutrient levels

A - Individual and total carotenoids content (µg/g DW) of Agria upon 0.1x, 0.5 xs and 1.0x MS salt stress

B - Individual and total carotenoids content (µg/g DW) of Desiree upon 0.1x, 0.5 xs and 1.0x MS salt stress Error bars represent ± SE.

7.4 Discussion

The development of potato minitubers through in vitro system has proved to be an effective experimental system for investigating the environmental factors involved in regulating carotenoid biosynthesis. This potential model system has been used because of several advantages over the use of field grown tubers:

i. rapid initiation of minitubers within four weeks from establishing the experiment rather than whole growing season in the field;
ii. the environmental conditions are easy to control because of the small size of the plantlets;
iii. potato minitubers were easily exposed to different types of environmental treatments effect
iv. variation between tubers was minimised; and
v. extraction and analysis of carotenoids can be done by using potato minitubers.

Buchanan et al. (2000) defined environmental stress as external conditions that adversely affect growth, development, or productivity. Plant responses to stress by many ways such as altered gene expression, trigger cellular metabolism and changes in growth rates and crop yields. There are two types of stress:

i. biotic - imposed by other organisms; and

ii. abiotic - arising from an excess or deficit in the physical or chemical environment.

Abiotic or physical and chemical environmental conditions can cause stress and influence carotenoid biosynthesis and of this light, water stress and nutrient are among the important factors. Resistance or sensitivity of plants to stress depends on the species, genotype and development age. There are three stress resistance mechanisms:

i. avoidance mechanisms - prevents exposure to stress;

ii. tolerance mechanisms - permit the plant to withstand stress; and

iii. acclimation - alter their physiology in response stress.

Plants growing in full sunlight often receive and absorb more light than they are able to use for photosynthesis. Carotenoids have as an important role in the protection of photosynthetic organisms against excessive light (Siefermann-Harms, 1987 and Frank and Cogdell, 1996), and these functions have been demonstrated *in vitro* in photosystem II complexes (Telfer et al*.,* 1994)). Light is a major stress factor in plants resulting in photoinhibition and photooxidation in photosynthetic tissues and caused the loss in productivity. Light also one of the main factors regulating carotenoid biosynthesis (Bramley and Mackenzie, 1987). Recent studies have demonstrated a clear correlation between the dissipation of excess excitation energy and the formation of zeaxanthin from violaxanthin in the light-harvesting complexes of plants (Young et al., 1997; Baker and Bowyer, 1994 and Long et al., 1994). Under these situation violaxanthin is reversibly deepoxidized by violaxanthin deepoxidase to zeaxanthin (Pfundel and Bilger, 1994; Demmig-Adams and Adams, 1996 and Eskling et al., 1997). In other words, violaxanthin become an efficient accessory pigment in weak light and zeaxanthin become an efficient photoprotector in strong light (Havaux and Niyogi, 1999). This correlation also has been found true for a wide range of environmental conditions such as water stress and extreme temperature and not just under excess light (Demmig-Adams and Adams, 1993).

In this chapter light exposure to Agria and Desiree minitubers leads to the similarity that both total and individual carotenoids were elevated up to 2-fold higher on a μg/g DW basis than the total and individual carotenoids produced by dark treatment except for

violaxanthin. The results were consistent with Demmig-Adams and Adams (1992b) and Sapozhnikov et al. (1957), who both observed high violaxanthin in sun-grown crop plants. However, they are not in agreement with Havaux and Niyogi (1999) who found high violaxanthin in the dark and high zeaxanthin in strong light. Lutein and total carotenoid content also were increased in accordance with their observations and others (Thayer and Bjorkman, 1990; Demmig-Adams and Adams, 1992a; Johnson et al., 1993a). In this study lutein and total carotenoid in Agria and Desiree minitubers also increased with light. Yamamoto et al. (1962) demonstrated that the changes were due to the stoichiometric and cyclical conversions among violaxanthin, antheraxanthin and zeaxanthin. Light induces the de-epoxidase reaction and required acidity for de-epoxidase activity which can be generated by ATP hydrolysis or supplied by buffer (Hager, 1969; Yamamoto et al., 1972; Rockholm and Yamamoto, 1996). The de-epoxidase is stereospecific for xanthophylls and because of that the polyene chain of the carotenoid must be all-trans. Otherwise, neoxanthin, which is 9-cis, is an inactive substrate and becomes active when isomerized to the all-trans form (Yamamoto and Higashi, 1978).

Swamy and Smith (1999) reported that the phytohormone abscisic acid (ABA) plays a regulatory role in many physiological processes in plants. Different stress conditions such as water, drought, cold, light, and temperature result in increased amounts of ABA. The action of ABA involves modification of gene expression and analysis of responsive promoters revealed several potential *cis*- and *trans*-acting regulatory elements. In some of the controls in Agria and Desiree minituber experiments zeaxanthin was detected. The occurrence of zeaxanthin might be in response to the brief exposure of samples to light. Every week all minitubers samples were checked and observed for contamination and size

of minitubers. This brief exposure to light might trigger the accumulation of zeaxanthin in some of the minituber control samples.

The presence of zeaxanthin in Agria and Desiree minitubers developing on dark-grown plants could be explained by changes in gene expression in response to stress. Abiotic stress can alter gene expression and trigger cellular metabolism in plants (Buchanan et al., 2000). Stress recognition may activate signal transduction pathways that transmit information within the individual cell and throughout the plant. This may induce changes in gene expression that modify growth and development and even influence the carotenoid biosynthesis. A stress will trigger and alter cellular metabolism, and as a result zeaxanthin accumulated as a precursor to ABA biosynthesis. Furthermore, resistance or sensitivity of plants to stress depends on the species, genotype and development age. In our experiment of potato minitubers, we used 4 week old potato minitubers compared to chapter 3 and 4 which used potato tubers that harvested after one season. There is evidence that different developmental age will accumulate different carotenoid profiles as revealed by Morris et al. (2004). In their study, 28 day stolons similar to our 4 week minitubers, were detected to have highest total carotenoid compared to 80-day developing tubers and 9-month mature tubers. Zeaxanthin was detected in both cases. Morris et al (2004) also reported similar results whereby the orange flesh tubers of DB375/1 were detected with high zeaxanthin, whereas pale yellow Desiree was detected with high violaxanthin. In addition yellow flesh cultivars were found to have capability and ability to produce more carotenoids compared to white flesh cultivars. Yellow flesh cultivars with high carotenoid content are able to withstand stress particularly light with tolerance mechanism (Buchanan et al., 2000). As a result, in Desiree minitubers accumulated violaxanthin and neoxanthin when exposed to light, whereas in Agria zeaxanthin and violaxanthin accumulated. In the

experiment involving nutrient stress (Figure 7.3), total and individual carotenoids of Agria increased with increased nutrient concentration. In contrast, in Desiree total and individual carotenoids initially increased with increasing nutrient level, but then decreased at higher nutrient levels. Again we observed the accumulation of neoxanthin and violaxanthin in Desiree and only violaxanthin in Agria.

Another important environmental stress is water deficit. Water related stresses could affect plants if the environment contains insufficient water to meet basic needs. Among environmental conditions that can lead to water deficit are drought, hypersaline conditions, low temperatures and transient loss of turgor at midday (Buchanan et al., 2000). Under conditions of water stress roots synthesize ABA and transport it into the shoots, with ABA being an essential mediator in triggering plant responses especially carotenoid biosynthesis to adverse environmental stimuli (Zeevaart and Creelman, 1988). In crops such as winter wheat, potatoes and alfalfa, a large increase in the ABA content was observed during hardening and cold acclimation (Chen et al., 1983; Luo et al., 1992; Wrightman, 1979 and Lalk and Dorffling, 1985). However according to Lalk and Dorffling (1985) the extent of the ABA response depends on varietal differences for example in winter wheat, a freeze-resistant variety of wheat had a higher ABA level than a less resistant variety. Similarly for potato species, an increase in ABA was observed in *Solanum commersonii*, but not in *S. tuberosum*, which failed to acclimate at –3°C. The PEG treatments simulated drought stress and caused the total and individual carotenoid concentrations increased slightly in both cultivars (Figure 7.2).

In ABA biosynthesis, oxidative cleavage is the first committed reaction and is believed to be the key regulatory step. Since many kinds of stresses induce ABA synthesis,

ABA may be considered a plant stress hormone (Swamy and Smith, 1999). It regulates several important aspects of plant growth and development. Recent studies have demonstrated a pivotal role for ABA in modulation at the gene level of adaptive responses for plants in adverse environmental conditions (Orr et al., 1986; Ramagopal, 1987; Singh et al., 1987; Pena-Cortes et al., 1989). ABA is also involved in several other physiological processes such as stomatal closure, embryo morphogenesis, development of seeds, and synthesis of storage proteins and lipids (Thomas, 1993), germination (Koornneef et al., 1989), leaf senescence (Zeevaart and Creelman, 1988), and defense against pathogens (Dunn et al., 1990). Nevertheless, ABA acts as a mediator in controlling adaptive plant responses to environmental stresses (Ingram and Bartels, 1996). In several instances, it has been implicated in signal transduction at the single-cell level (Jeffrey and Giraudt, 1998). Therefore, based on the results in this study environmental growing conditions such as light, dark, water stress and nutrient concentration affect and activate carotenoid biosynthesis significantly. Furthermore, the presence of disease symptoms and storage period also had a major influence on the accumulation of carotenoids in potato tubers (Chapter 6). As other environmental stress response, disease or pathogen infection can lead to oxidative stress responses which involve stress response genes (Desender et al., 2007), storage period which can lead to physical changes such as sprouting and dehydration are also believed to be responsible for this phenomenon as well (Blessington et al., 2007).

The results from this chapter suggest that a regulatory step for the carotenoid biosynthetic pathway versus environmental stress is mediated by ABA and involves the epoxidation of zeaxanthin to violaxanthin by ZEP during the first committed step in ABA biosynthesis. Zeaxanthin appears to be a key factor and indicator for the presence of environmental stress. Due to the presence and time of occurrence of environmental stress,

some genotypes accumulated merely violaxanthin and neoxanthin in order to generate xanthoxin or precursors of ABA biosynthesis pathway. Not surprisingly, the response of potato genotypes to such environments appeared to be highly genotype dependent and time duration exposed to stress. Another factor is the activity of functional enzymes and candidate enzymes that regulate carotenoid biosynthesis which will determine type and quantity of individual carotenoids. By understanding the environmental factors that affected carotenoid biosynthesis, it should be possible to enhance the amount and type of carotenoid that accumulates in potato tubers.

CHAPTER 8

General discussion and conclusions

8.1 Research aim and objectives

The aim of this study is to explore the composition and concentration of carotenoids in potato tubers to enable their future enhancement or enrichment through genetic manipulation. In order to achieve this goal, in chapter 3, the carotenoid content and composition of a wide variety of potato germplasm was surveyed. To extend this study, in chapter 4, the consistency of this composition was investigated and found to vary over two seasons in both New Zealand and the Netherlands. The next logical step, in chapter 5, was to further study this phenomenon and its implications by investigating the environmental stability of carotenoid composition, or genotype x environment interaction, in relation of carotenoid accumulation in eight cultivars at three different locations in New Zealand and two cultivars at seven different locations in Netherlands. Later in chapter 6, the effects of storage and disease upon carotenoid accumulation were determined in selected genotypes of potato tubers. Lastly, in order to complete this task and study, an effective model system using potato minitubers were established to investigate tuber carotenogenesis and determine parameters influencing carotenoid composition.

8.2 Genotype sources of carotenoids

Potato cultivars exhibit a wide variation in skin and flesh colour. Colours in these tissues range from pink, red, purple and blue derived from anthocyanin pigments and pale yellow derived from carotenoid pigments. For this study thirty two potato cultivars grown in New Zealand and twelve cultivars grown in Netherlands with wide range of tuber flesh colour were evaluated to identify and quantify the carotenoids present, as well as to determine the relationship between tuber flesh colour intensity and carotenoid content. This investigation revealed that individual cultivars not only vary in total carotenoid content, but also vary in carotenoid composition (Figures 3.3 and 3.4). There were highly significant differences ($P < 0.0001$) between total carotenoid content and individual carotenoid compounds with all cultivars tested (Tables 3.2 and 3.5). Increasing yellow colouration was found to correlate with increasing carotenoid content. In the thirty-two potato cultivars ranging from white to dark yellow grown in New Zealand, the main carotenoids identified were lutein, neoxanthin, violaxanthin and β-carotene. Agria, a dark yellow-fleshed cultivar, was found to have the highest total carotenoid content (169.57 µg/g DW), substantially higher than all other cultivars tested (Figure 3.1). As shown in Figure 3.3 and Appendix 3.1, neoxanthin was found highest in Marabel (dark yellow flesh) and detected in only 13 cultivars (10.59 to 69.21µg/g DW); violaxanthin was found highest in Allure (also a dark yellow cultivar) and was detected only in this cultivar (32.76 µg/g DW). Whereas lutein and β-carotene were found in most of the cultivars but the concentration varied from 0.00 to 160.63 µg/g DW and 0.00 to 13.62 µg/g DW, respectively. Their highest levels were detected in Agria (dark yellow) and Summer Delight (pale yellow) accordingly. Zeaxanthin was not found in any of the 32 cultivars analysed.

In 12 cultivars grown in the Netherlands (sections 3.3.2 and 3.3.4), the analysis of variance also established highly significant differences ($P < 0.0001$) in total carotenoid content and individual carotenoid compounds (Table 3.5). In these cultivars ranging from cream to dark orange the main carotenoids identified were neoxanthin, violaxanthin and lutein. IVP01-084-19, an orange-fleshed cultivar, was found to have the highest total carotenoid content (258.95 µg/g DW). As shown in Figure 3.4 and Appendix 3.2, neoxanthin (28.20 to 228.01 µg/g DW) was detected in all cultivars but was highest in IVP01-084-19 (orange flesh); lutein (0.08 to 0.66 µg/g DW) was also detected in small quantities in all cultivars; whereas violaxanthin (0.00 to 30.29 µg/g DW) was found in only 6 cultivars and predominant in IVP01-084-19. In cultivars grown in New Zealand and the Netherlands there appears to be little correlation between the presence of individual carotenoid compounds accumulating and colour intensity. However, tuber flesh colour was found to correlate with total carotenoid content both in New Zealand-grown and the Netherlands-grown cultivars. Surprisingly when the three cultivars of Laura, Marabel and Desiree grown in both New Zealand and Netherlands were compared (Appendix 3.3), Laura in New Zealand was found to contain high lutein, whereas Laura in Netherlands was high in neoxanthin. Violaxanthin was not detected in Desiree grown in New Zealand, whereas β-carotene was not detected in Desiree grown in Netherlands. β-carotene was detected in Marabel grown in New Zealand, but absent in Marabel grown in Netherlands. Due to these observations the cultivars were analysed over a second growing season in both New Zealand and the Netherlands.

8.3 Cultivars x growing seasons

Thirty-two potato cultivars grown in New Zealand and ten cultivars grown in Netherlands were analysed over two growing seasons to assess stability in carotenoids composition. The data showed that for thirty-two New Zealand-grown potato cultivars, they contained neoxanthin, violaxanthin, lutein and β-carotene in the first season, whereas in the second season they contained an additional carotenoid of zeaxanthin (section 4.3.1). In contrast, for the ten potato cultivars grown in the Netherlands neoxanthin, violaxanthin and lutein were detected in both seasons. Examination of carotenoid profiles of the potato tubers grown in two different seasons showed that the cultivars and the season had a major effect on the total carotenoid content and the individual carotenoid compounds. For the 32 cultivars grown in New Zealand, high concentrations of neoxanthin and lutein were found in the first season, whereas high concentrations of zeaxanthin were found in the second season (Figure 4.1 and 4.2). Neoxanthin and violaxanthin were found to be predominant in the first season for cultivars grown in the Netherlands, whereas lutein and β-carotene were the most common carotenoids detected in second season (Figure 4.3). In addition, comparison of both cultivars and growing seasons revealed highly significant difference. These data suggested that although for some cultivars, growing season had an effect on the carotenoid profile; some cultivars exhibited no effect on the total and individual carotenoid content and composition of the potato tubers analysed. Reflectance colorimeter measurement of yellow hue component confirmed that the higher the total carotenoid content, the greater the yellow intensity colour.

It appears that environmental factors such as seasonal climatic variation may have been influenced the accumulation of potato tuber carotenoids content and composition. Clearly, further study utilizing potato plant material grown under different environmental conditions is required to confirm this hypothesis.

8.4 Cultivars x locations

Eight cultivars were grown at three different locations in New Zealand and two cultivars grown at eight different locations in the Netherlands to further investigate the stability of carotenoid composition. The results revealed that genotypes from different locations in both New Zealand and the Netherlands varied significantly in term of total carotenoid content and individual carotenoid compounds. Six cultivars grown at three different locations in New Zealand were found to have markedly different carotenoid composition. Only Agria and Fianna were detected with similar carotenoid profiles. Reflectance colorimeter measurement of yellow hue component again confirmed the relationship between the intensity of the yellow colour in tuber flesh and total carotenoid content, as well as confirming the interaction between colour and locations. Laura grown in Palmerston North was found to have more intense yellow colour compared to other locations (Figure 5.2).

Meanwhile Agria and Desiree grown in Netherlands at eight different locations (section 5.3.3) were also found to have different carotenoid compositions. Agria and Desiree grown in Metslawier, Bant, and Rilland were observed to have similar carotenoid compounds and lower carotenoid content, but when grown in Wierum, Wieringerwerf and Wageningen, different individual carotenoid composition and higher total carotenoid

content were observed (Figure 5.3). The reflectance colorimeter measurement of yellow hue component could be performed for Agria and Desiree grown in the Netherlands since only freeze dried samples could be provided due to biosecurity issues for importation of samples into New Zealand. These results suggest that beside seasonal climatic variation between two growing seasons, environmental factors such as location, soil type and agronomic practices also could influence the accumulation of carotenoids in potato tubers. Correlation between genotypes and environment can be indicative of the particular potato cultivar best adapted to certain locations (Fraser and Bramley, 2004; Al-Saikhan, 2000; Haynes et al. 1996; Collins et al. 2006). In other words, there are particular locations where specific cultivars accumulate certain carotenoid compounds with high levels of carotenoid content and intense yellow tuber flesh colour. This raises the importance and need for an effective potential model system to investigate in depth the environmental factors that influence tuber carotenogenesis or controlling carotenoid accumulation. A rapid system to produce whole plants with tubers is needed to replace production of tubers in the field or glasshouse in order to make valid assumptions about what is occurring in the field.

8.5 Storage and disease

Besides cultivars, growing season and location, the levels of total carotenoids, neoxanthin, violaxanthin, zeaxanthin, lutein and β-carotene in potatoes are strongly influenced and affected by disease and post harvest storage conditions. The storage of tubers over a two year period and disease (powdery scab), as described in chapter 6, appears to have distinct effects on carotenoid biosynthesis, the magnitude of the effects being dependent on the cultivar, time of storage, and the scab score. Results show that storage for 12 and 24 months resulted in the accumulation of neoxanthin, violaxanthin and

zeaxanthin with a concomitant decreased of lutein, β-carotene and total carotenoid content (Figures 6.1 and 6.2). Whereas genotypes infected with disease (lower to higher scab score) resulted in accumulation of highly violaxanthin followed by β-carotene and total carotenoids (Figure 6.3), with a concomitant decreased in neoxanthin and lutein. This might be explained by a number of possibilities as described further in chapter 6 and among those as reported by Griffiths et al. (2007): (i) any lutein chemically destroyed by reaction was replaced at a faster rate than the other major carotenoids, (ii) the activity of enzymes that catabolise the β-carotene-derived carotenoids such as zeaxanthin and violaxanthin was greater in the potato tubers than those utilizing lutein as their substrate and (iii) transformation of the free carotenoids to fatty acid esters.

8.6 Potato tuber carotenogenesis model system assessment

The previous chapters report marked differences in carotenoid composition over a wide range of potato genotypes under different environmental conditions. The data suggests that cultivars, growing seasons, location, storage and disease can all influence the accumulation of specific carotenoid compounds and their concentration in potato tubers. Therefore in chapter 7, potato minitubers of Agria and Desiree were used as a potential model system to investigate variables that controlling carotenoid accumulation. The production of potato minitubers of Agria and Desiree through tissue culture system was established as an effective tool or model system for assessing environmental factors that affected carotenoid accumulation in the field. The value of this experimental system was tested and proven by assessing the effects of environmental variables, such as drought stress, light intensity and nutrient strength on carotenoid accumulation.

When these observations are related back to previous chapters, it becomes apparent that the accumulations of certain carotenoid pigments are susceptible to environmental conditions. For example, light intensity could influence the accumulation of zeaxanthin in Agria (Figure 7.1). Interestingly, all samples of Agria grown in New Zealand except harvested from the first season of 2004/2005 were found with high amount of zeaxanthin. Based on section 7.3.2 and 7.3.3, it can be concluded that water stress and nutrient strength can influence the accumulation of violaxanthin. Violaxanthin was found higher at North Island (Palmerston North and Pukekohe), which are warmer whereas at South Island (Lincoln), which is colder, violaxanthin was found lower even at different growing seasons, further suggesting that violaxanthin accumulation is susceptible to changes in the environment. Light and water stress were strongly related with absent of zeaxanthin (Figure 7.1 and 7.2), whereas nutrient strength influenced the accumulation of other carotenoids (Figure 7.3). Agria grown in Metslawier, Bant, Rilland and Emmeloord were found with lutein and β-carotene predominantly, whereas neoxanthin and violaxanthin were detected predominant in Agria grown in Wierum, Wieringerwerf and Wageningen. No zeaxanthin was detected in both cases. Meanwhile in Desiree, comparison of individual carotenoids over two seasons established that zeaxanthin was found in second season but not in the first season. Based on Desiree minitubers experiment, it can be concluded that light can influenced the presence of zeaxanthin. All samples from 3 different locations (Lincoln, Palmerston North and Pukekohe) also were detected with zeaxanthin but the amount of neoxanthin and violaxanthin varied. In section 7.3.1 to 7.3.3 the amount of neoxanthin and violaxanthin increased with the presence of light, water stress and nutrient strength. This sensitivity of zeaxanthin to environmental changes may account for the absence of zeaxanthin in Desiree grown in Netherlands.

8.7 Conclusion

The effects of the cultivars, growing seasons, locations, storage, disease and genotype x environment interactions have been studied on carotenoid content in potato. Despite significant results in our understanding of carotenogenesis in potato tubers and the role of abscisic acid in plant stress tolerance, the control mechanisms regulating overall carotenoid biosynthesis and accumulation still remain an enigma. Each factor had an effect on the carotenoids content and profile; however, the most influential factor appeared to be cultivar selection. Of all the cultivars and genotypes tested in this research we found that Fianna is the most stable cultivar of all parameters tested which accumulated merely lutein even though planted in three different locations and two different seasons. There may be a stimulation, induction, or degradation of some compounds due to environmental or storage factors on carotenoid biosynthesis; however, the magnitude of these effects is not as great as genetic control. However, the effects of environment and storage cannot be denied and should be further investigated. By identifying the key genes controlling carotenoid biosynthesis a greater understanding of how gene actions influence carotenoid accumulation and composition in response to interactions with environmental factors will emerge.

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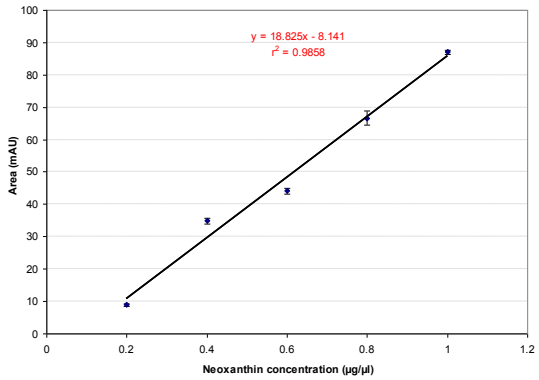
Appendix 1.1

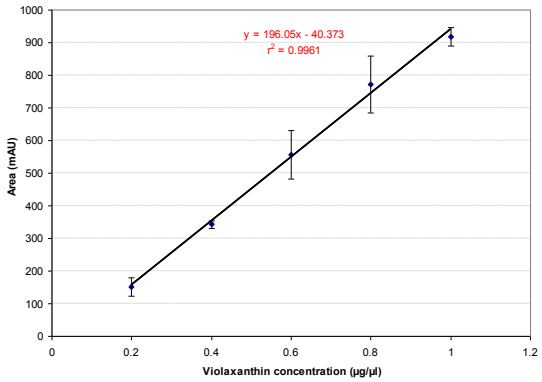
List of potato cultivars with flesh colour and parentage origin for surveying wide germplasm of carotenoid grown in New Zealand and Netherlands

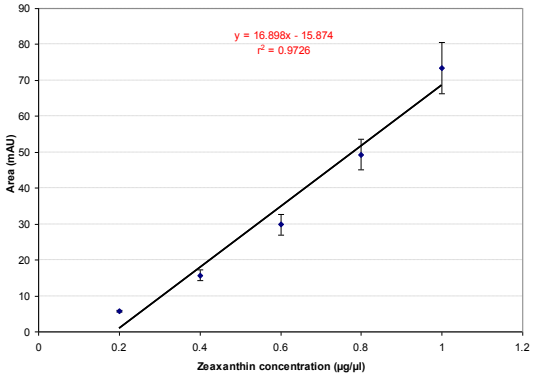
Cultivar	Flesh Colour	Parentage
2765-6	Pink	1463-1 x 2390-1
Agata	Pale Yellow	BM 52.72 x Sirco
Agria	Dark Yellow	Quarta x Selmo
Allure (Crop 15/2852-5)	Dark Yellow	Astarte x SVP AM 66-42
Arrow	Cream	Solaro x Fresco
Bildtstar	Yellow	Winda x Saturna
Bondi (Crop 19)	White	Ranger Russet x Karaka
Casteline	Pale Yellow	Safrane x 85 F 276.5
Crop 16 (2885-1)	Pale Yellow	Agria x Fraser
Crop 20 (1021/1)	White	Fianna x L115-1
Crop 21 (2279-3)	White	1053-57 x Maris Bard
Crop 25 (937/3)	Pale Yellow	Kaimai x L115-1
Crop 33 (2765-5)	Purple	1463-1 x 2390-1
Desiree	Pale Yellow	Urgenta x Depesche
Driver	White	993.60 x V394
Eden	White	10899AD(14) x Maris Piper
Fianna	Cream	K062/660 x AM 66/42
Fraser	White	676-34 x Whitu
Gladiator	White	B5281-1 x Vt^n 62-33-3
Golden Miracle (Crop 22/2886-3)	Dark Yellow	Agria x 2221-12
Heather	White	143/27 x DXMP70
Horizon	White	1053-53 x Baillee
Ilam Hardy	White	Arran Pilot x Katahdin
IVP01-084-19	Orange	96-4622-20 x IVP92-027-9
Iwa	White	119-224 x [Sebago x Harford]
Kaimai	Cream to Pale Yellow	Rua x V394
Laura	Dark Yellow	Rosella x B6140-12
Lone Ranger (Crop 18)	White	Ranger Russet x V394
Marabel	Dark Yellow	Nena x MA 75-364
Markies	Pale Yellow	Agria x Fianna
Milva	Dark Yellow	Nena x Dunja
Miriam	Yellow to Dark Yellow	Culpa x ST6948-2006
Mondial	Pale Yellow	Spunta x SVP Ve 66-295
Moonlight	White	1463.1 x V394
Nadine	Cream	((Maris Piper x Desiree) x S. vernei) x (Pentland Dell x S. vernei)
Nicola	Yellow	Clivia x 6430/1011
Olivia	Cream	W72-22-489 x VK69-491
Presto	Yellow to Dark Yellow	MV982.034.87 x Marabel
Purple Passion (Crop 32)	Cream	Red Rascal x Picador
Ranger Russet	White	Butte x A6595-3
Red Rascal (1830-11)	White	Tekau x Desiree
Rua	White	Katahdin x Harford
Russet Burbank	White	Mutant of Burbank
Summer Delight (Crop 17)	Pale Yellow	1858.21 x V394
Van Rosa	White	Sport of Desiree
Vt^n 62-33-3	White	((V24/20 x Ulster Knight)1 x Profijt)15 x (VRN I-3 x Profijt)5

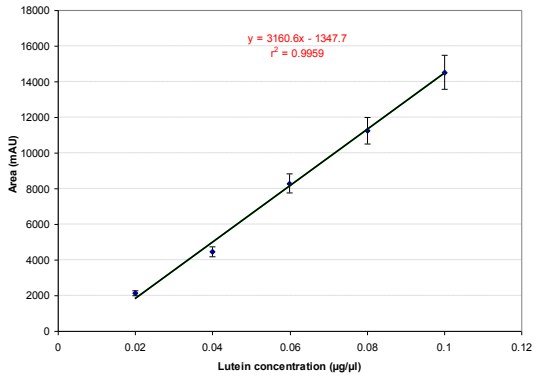
Appendix 1.2

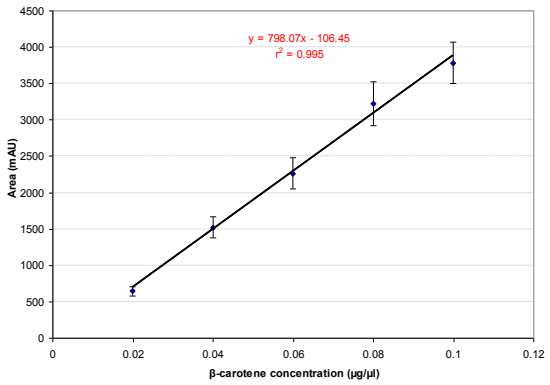
Standard curves of neoxanthin, violaxanthin, zeaxanthin, lutein and β-carotene











Appendix 3.1

Neoxanthin, violaxanthin, lutein, zeaxanthin, β-carotene, total carotenoid content (µg/g DW) and flesh colour of 32 New Zealand-grown cultivars harvested in 2005

Cultivar	Flesh colour	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
Agria	DY	ND	0.40 ± 0.06	ND	160.63 ± 22.2	8.54 ± 1.18	169.57 ± 22.91
Marabel	DY	69.21 ± 9.75	0.26 ± 0.04	ND	1.80 ± 0.25	0.07 ± 0.01	71.34 ± 9.26
G.Miracle	DY	ND	1.59 ± 0.04	ND	44.40 ± 1.09	8.54 ± 0.21	54.52 ± 8.19
Laura	DY	ND	0.04 ± 0.02	ND	50.25 ± 19.8	0.01 ± 0.01	50.31 ± 18.21
Milva	DY	46.32 ± 9.25	0.04 ± 0.01	ND	0.20 ± 0.04	0.01 ± 0.00	46.57 ± 7.99
Allure	DY	ND	32.7 ± 13.58	ND	6.67 ± 2.76	6.04 ± 2.50	45.47 ± 16.46
S.Delight	PY	ND	2.67 ± 0.48	ND	24.83 ± 4.48	13.62 ± 2.46	41.12 ± 12.04
Desiree	PY	36.78 ± 14.1	ND	ND	1.38 ± 0.53	0.60 ± 0.23	38.76 ± 12.36
Markies	PY	36.21 ± 7.37	0.07 ± 0.01	ND	0.97 ± 0.20	0.71 ± 0.14	37.96 ± 7.48
Mondial	PY	34.78 ± 9.65	0.17 ± 0.05	ND	1.93 ± 0.54	0.01 ± 0.00	36.89 ± 9.21
Nadine	CR	31.10 ± 2.89	0.02 ± 0.00	ND	1.05 ± 0.10	0.04 ± 0.00	32.21 ± 11.75
Driver	W	24.08 ± 0.81	0.01 ± 0.00	ND	2.03 ± 0.07	0.27 ± 0.01	26.39 ± 2.56
Crop 16	PY	ND	2.25 ± 0.71	ND	21.21 ± 6.74	1.63 ± 0.52	25.08 ± 4.05
P.Passion	CR	21.92 ± 1.78	0.05 ± 0.00	ND	1.89 ± 0.15	0.01 ± 0.00	23.87 ± 10.0
Kaimai	CP	22.37 ± 5.89	0.06 ± 0.02	ND	0.77 ± 0.20	0.04 ± 0.01	23.24 ± 7.18
R.Burbank	W	ND	0.07 ± 0.01	ND	21.03 ± 2.92	0.05 ± 0.01	21.16 ± 3.35
Horizon	W	19.07 ± 3.30	0.02 ± 0.00	ND	0.40 ± 0.07	0.01 ± 0.00	19.50 ± 5.12
R. Russet	W	15.31 ± 2.22	ND	ND	ND	ND	15.31 ± 2.00

ND – Non-detectable, DY – Dark Yellow, PY – Pale Yellow, CR – Cream, W – White, CP – Cream to Pale Yellow, PK – Pink, PP - Purple

Cultivar	Flesh colour	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
Heather	W	12.47 ± 1.45	0.01 ± 0.00	ND	1.41 ± 0.16	0.01 ± 0.00	13.91 ± 2.99
R.Rascal	W	ND	0.33 ± 0.03	ND	11.79 ± 1.22	0.09 ± 0.01	12.22 ± 1.15
I.Hardy	W	10.59 ± 1.29	0.01 ± 0.00	ND	1.09 ± 0.13	0.00 ± 0.00	11.70 ± 2.71
Bondi	W	ND	7.64 ± 0.50	ND	2.43 ± 0.16	1.39 ± 0.09	11.46 ± 4.75
Crop 21	W	ND	0.28 ± 0.06	ND	9.46 ± 2.07	0.16 ± 0.03	9.90 ± 1.91
Fianna	CR	ND	0.06 ± 0.01	ND	6.89 ± 1.13	0.03 ± 0.01	6.99 ± 0.66
L.Ranger	W	ND	0.06 ± 0.01	ND	4.70 ± 0.94	2.01 ± 0.40	6.77 ± 3.39
Rua	W	ND	2.07 ± 0.76	ND	2.39 ± 0.88	1.62 ± 0.60	6.08 ± 4.33
2765-6	PK	ND	ND	ND	5.07 ± 0.35	0.44 ± 0.03	5.50 ± 0.88
Fraser	W	ND	2.40 ± 0.17	ND	1.75 ± 0.12	0.52 ± 0.04	4.68 ± 0.42
Eden	W	ND	0.24 ± 0.03	ND	3.64 ± 0.38	0.24 ± 0.03	4.12 ± 1.26
Crop 20	W	ND	ND	ND	ND	3.76 ± 1.43	3.76 ± 1.45
Crop 33	PP	ND	ND	ND	1.71 ± 0.66	0.38 ± 0.15	2.09 ± 0.63
Moonlight	W	ND	ND	ND	0.83 ± 0.20	0.35 ± 0.08	1.18 ± 0.06

ND – Non-detectable, DY – Dark Yellow, PY – Pale Yellow, CR – Cream, W – White, CP – Cream to Pale Yellow, PK – Pink, PP - Purple

Appendix 3.2

Neoxanthin, violaxanthin, zeaxanthin, lutein, β-carotene and total carotenoid content of 12 Netherlands grown cultivars harvested in 2005

Cultivar	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
IVP01-084-19	228.01 ± 30.18	30.29 ± 2.70	ND	0.66 ± 0.28	ND	258.95 ± 44.35
Presto	113.10 ± 0.03	20.90 ± 1.13	ND	0.28 ± 0.03	ND	134.28 ± 15.55
Miriam	102.96 ± 10.21	13.01 ± 1.23	ND	0.32 ± 0.02	ND	116.29 ± 18.35
Laura	114.26 ± 12.87	0.00 ± 0.00	ND	0.55 ± 0.12	ND	114.81 ± 40.22
Marabel	81.44 ± 7.59	11.16 ± 2.15	ND	0.28 ± 0.04	ND	92.89 ± 17.38
Nicola	83.66 ± 2.39	0.00 ± 0.00	ND	0.59 ± 0.19	ND	84.25 ± 7.82
Arrow	68.48 ± 4.82	0.00 ± 0.00	ND	0.45 ± 0.00	ND	68.93 ± 16.37
Casteline	51.20 ± 6.37	6.94 ± 1.79	ND	0.15 ± 0.03	ND	58.29 ± 9.30
Agata	45.32 ± 4.21	0.00 ± 0.00	ND	0.33 ± 0.00	ND	45.65 ± 13.79
Bildtstar	43.68 ± 2.64	0.00 ± 0.00	ND	0.11 ± 0.01	ND	43.79 ± 5.48
Desiree	40.49 ± 5.35	0.00 ± 0.00	ND	0.28 ± 0.10	ND	40.77 ± 7.86
Olivia	28.20 ± 9.29	7.63 ± 1.57	ND	0.08 ± 0.01	ND	35.91 ± 1.71

ND-Non-detectable

Appendix 3.3

Comparison of Desiree, Laura and Marabel grown in New Zealand and Netherlands.

Cultivar	Year	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
Desiree (NZ)	2005	36.78 ± 14.1	ND	ND	1.38 ± 0.53	0.60 ± 0.23	38.76 ± 12.36
Desiree (ND)	2006	40.49 ± 5.35	0.00 ± 0.00	ND	0.28 ± 0.10	ND	40.77 ± 7.86
Laura (NZ)	2005	ND	0.04 ± 0.02	ND	50.25 ± 19.8	0.01 ± 0.01	50.31 ± 18.21
Laura (ND)	2006	114.3 ± 12.87	0.00 ± 0.00	ND	0.55 ± 0.12	ND	114.81 ± 40.22
Marabel (NZ)	2005	69.21 ± 9.75	0.26 ± 0.04	ND	1.80 ± 0.25	0.07 ± 0.01	71.34 ± 9.26
Marabel (ND)	2006	81.44 ± 7.59	11.16 ± 2.15	ND	0.28 ± 0.04	ND	92.89 ± 17.38

ND – Non-detectable, NZ – New Zealand, ND - Netherlands

Appendix 4.1

Neoxanthin, violaxanthin, lutein, zeaxanthin, β-carotene and total carotenoid content (μg/g DW) of 32 New Zealand cultivars harvested in 2007

Cultivar	Flesh color	Neoxanthin (μg/g DW)	Violaxanthin (μg/g DW)	Zeaxanthin (μg/g DW)	Lutein (μg/g DW)	β-Carotene (μg/g DW)	Total Carotenoid (μg/g DW)
206	-	27.89 ± 1.58	15.9 ± 0.75	137.92 ± 1.59	0.24 ± 0.01	3.15 ± 0.07	185.13 ± 0.66
Markies	PY	10.67 ± 0.19	ND	112.89 ± 12.6	0.31 ± 0.03	5.22 ± 0.18	129.08 ± 10.30
Laura	DY	0.00 ± 0.00	ND	124.66 ± 15.1	0.92 ± 0.05	0.71 ± 0.05	126.28 ± 12.28
Crop 25	PY	ND	ND	119.75 ± 0.04	2.04 ± 0.02	1.18 ± 0.00	122.93 ± 0.11
Agria	DY	10.08 ± 3.57	8.86 ± 0.86	82.87 ± 11.5	0.13 ± 0.03	0.13 ± 0.07	102.07 ± 11.71
Crop 16	PY	10.21 ± 1.31	10.7 ± 2.30	72.92 ± 8.14	0.13 ± 0.03	0.37 ± 0.02	94.34 ± 7.48
S. Delight	PY	6.57 ± 1.28	ND	62.29 ± 2.54	0.11 ± 0.01	0.14 ± 0.01	69.12 ± 3.13
Allure	DY	ND	ND	56.78 ± 0.80	0.14 ± 0.00	0.46 ± 0.01	56.82 ± 0.31
Marabel	DY	7.49 ± 0.17	ND	46.66 ± 0.24	0.53 ± 0.08	0.01 ± 0.00	54.69 ± 0.44
G.Miracle	DY	0.00 ± 0.00	8.26 ± 2.66	43.43 ± 16.2	0.31 ± 0.19	0.24 ± 0.12	52.24 ± 15.33
Horizon	W	14.59 ± 1.54	ND	30.59 ± 3.77	0.90 ± 0.12	0.80 ± 0.15	46.88 ± 4.55
Kaimai	CP	4.97 ± 0.09	5.51 ± 1.72	31.18 ± 7.13	0.09 ± 0.01	0.22 ± 0.04	41.96 ± 7.26
Nadine	CR	6.54 ± 0.02	4.14 ± 0.02	23.97 ± 0.09	0.18 ± 0.00	0.10 ± 0.00	34.84 ± 0.26
Desiree	PY	4.39 ± 0.90	0.00 ± 0.00	30.08 ± 1.41	0.09 ± 0.06	0.02 ± 0.01	34.58 ± 0.51
CR4	-	3.82 ± 0.24	3.39 ± 0.26	22.13 ± 2.80	0.10 ± 0.02	0.04 ± 0.00	29.48 ± 2.68
P.Passion	CR	3.55 ± 0.82	2.50 ± 0.47	18.46 ± 2.48	0.07 ± 0.00	0.03 ± 0.01	24.61 ± 3.07
Mondial	PY	2.41 ± 0.27	2.42 ± 0.14	17.54 ± 1.45	0.04 ± 0.00	0.03 ± 0.00	22.44 ± 0.86
Heather	W	ND	3.24 ± 0.28	16.73 ± 2.08	0.40 ± 0.06	0.11 ± 0.01	20.47 ± 1.42
Rua	W	ND	0.00 ± 0.00	19.79 ± 2.04	0.44 ± 0.05	0.11 ± 0.01	20.34 ± 1.70
R.Russet	W	0.00 ± 0.00	ND	ND	21.3 ± 3.71	1.99 ± 0.38	18.34 ± 9.11
Fianna	CR	ND	ND	ND	16.8 ± 0.80	0.60 ± 0.10	17.37 ± 0.61

ND – Non-detectable, DY – Dark Yellow, PY – Pale Yellow, CR – Cream, W – White, CP – Cream to Pale Yellow, PK – Pink, PP - Purple

Cultivar	Flesh color	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
Guard	-	ND	11.4 ± 0.19	ND	1.47 ± 0.13	0.52 ± 0.20	13.43 ± 0.12
Driver	W	7.08 ± 0.62	4.65 ± 0.03	0.30 ± 0.01	0.27 ± 0.01	0.14 ± 0.01	12.44 ± 0.46
Iwa	W	ND	1.64 ± 0.16	10.45 ± 0.27	0.08 ± 0.01	0.07 ± 0.02	12.25 ± 0.38
Bondi	W	ND	0.00 ± 0.00	11.50 ± 2.68	0.19 ± 0.05	0.08 ± 0.02	11.77 ± 2.26
Ilam	W	ND	1.21 ± 0.15	9.16 ± 1.15	0.15 ± 0.02	0.05 ± 0.01	10.57 ± 1.10
Hardy							
Lone	W	ND	7.10 ± 0.15	ND	0.98 ± 0.02	0.37 ± 0.01	8.45 ± 0.14
Ranger							
Crop 20	W	ND	ND	7.75 ± 0.23	ND	0.04 ± 0.00	7.79 ± 0.20
Crop 21	W	ND	ND	7.44 ± 0.11	ND	0.07 ± 0.00	7.51 ± 0.09
R.Burbank	W	ND	ND	ND	ND	6.98 ± 1.77	6.98 ± 1.44
R.Rascal	W	ND	5.65 ± 0.15	ND	0.63 ± 0.02	0.28 ± 0.01	6.56 ± 0.14
Milva	DY	ND	ND	ND	4.00 ± 0.18	1.59 ± 0.02	5.59 ± 0.18
Eden	W	ND	ND	ND	3.95 ± 0.22	1.43 ± 0.07	5.39 ± 0.31
2765-6	PK	ND	ND	ND	2.09 ± 0.02	1.03 ± 0.18	3.13 ± 0.26
Crop 33	PP	ND	ND	ND	2.04 ± 0.22	1.00 ± 0.18	3.04 ± 0.36
Gladiator	W	ND	ND	ND	1.63 ± 0.01	0.77 ± 0.00	2.40 ± 0.12
Moonlight	W	ND	ND	ND	ND	1.47 ± 0.26	1.46 ± 0.21
Fraser	W	ND	ND	ND	ND	0.91 ± 0.26	0.91 ± 0.30

ND – Non-detectable, DY – Dark Yellow, PY – Pale Yellow, CR – Cream, W – White, CP – Cream to Pale Yellow, PK – Pink, PP - Purple

Appendix 4.2

Means of tuber flesh intensity colour for 32 potato cultivars New Zealand grown using Minolta chroma meter CR-210 measuring in CIELAB L* = lightness, a* = bluish-green (-)/red-purple (+) hue component, b* = yellow (+)/blue (-) hue component, C* [$(a^{*2} + b^{*2})^{½}$] = chroma, h° (from arctangent b*/a*) = hue angle (0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue).

Cultivar	Flesh colour	L*	a*	b*	C*	h°	Total Carotenoid (µg/g DW)
Markies	PY	89.49	-9.78	29.10	30.70	108.50	129.08
Laura	DY	88.83	-9.81	30.57	32.10	107.73	126.28
Agria	DY	86.17	-9.33	26.82	28.39	109.10	102.07
Crop 16	PY	87.19	-9.25	26.78	28.33	108.97	94.34
Summer Delight	PY	88.07	-9.73	26.30	28.03	110.23	69.12
Golden Miracle	DY	87.19	-9.30	28.65	30.12	107.93	56.82
Marabel	DY	89.49	-9.69	27.26	28.93	109.50	54.69
Allure	DY	87.80	-9.55	27.36	28.97	109.17	52.24
Horizon	W	87.83	-8.93	22.30	24.02	111.73	46.88
Kaimai	CP	90.83	-10.36	28.51	30.33	109.90	41.96
Nadine	CR	85.58	-8.67	21.93	23.58	111.47	34.84
Desiree	PY	86.85	-9.25	25.99	27.58	109.50	34.58
Purple Passion	CR	88.36	-9.69	26.37	28.09	110.13	24.61
Mondial	PY	86.71	-9.38	25.45	27.12	110.17	22.44
Heather	W	89.02	-9.02	23.44	25.11	111.00	20.47
Rua	W	88.51	-9.22	23.19	24.95	111.60	20.34
Ranger Russet	W	87.86	-8.57	22.96	24.50	110.40	18.34
Fianna	CR	88.36	-9.17	24.87	26.50	110.17	17.37
Driver	W	89.11	-9.25	24.39	26.07	110.70	12.44
Bondi	W	89.15	-8.92	24.47	26.04	109.97	11.77
Ilam Hardy	W	89.37	-9.00	23.48	25.14	110.90	10.57
Lone Ranger	W	89.48	-2.98	24.13	25.83	110.90	8.45
Crop 20	W	89.88	-9.31	25.15	26.81	110.23	7.79
Crop 21	W	88.03	-8.73	22.52	24.14	111.10	7.51
Russet Burbank	W	89.39	-9.07	24.26	25.89	110.43	6.98
Red Rascal	W	89.00	-8.76	23.88	25.43	110.07	6.56
Milva	DY	86.85	-8.52	22.94	24.46	110.33	5.59
Eden	W	93.30	-10.38	28.35	30.18	110.03	5.39
2765-6	PK	88.03	-4.08	20.56	20.96	101.13	3.13
Crop 33	PP	75.77	-3.47	14.42	14.83	103.40	3.04
Moonlight	W	87.89	-8.90	23.97	25.57	110.30	1.46
Fraser	W	88.71	-9.24	25.03	26.68	110.20	0.91

DY – Dark Yellow, PY – Pale Yellow, CR – Cream, W – White, CP – Cream to Pale Yellow, PK – Pink, PP - Purple

Appendix 4.3

Neoxanthin, violaxanthin, zeaxanthin, lutein, β-carotene and total carotenoid content of 12 Netherlands grown cultivars harvested in 2006

Cultivar	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
CE675	192.25 ± 39.56	121.40 ± 21.01	ND	19.82 ± 5.40	2.81 ± 1.89	336.27 ± 59.91
Laura	173.86 ± 21.66	122.38 ± 9.68	ND	23.64 ± 11.40	0.00 ± 0.00	319.88 ± 74.99
Marabel	137.92 ± 10.79	70.05 ± 10.75	ND	1.76 ± 0.14	3.32 ± 0.47	213.04 ± 19.35
Markies	72.92 ± 5.67	20.03 ± 4.87	ND	3.33 ± 1.44	1.71 ± 0.78	97.99 ± 2.34
Miriam	47.22 ± 4.65	12.34 ± 5.13	ND	2.05 ± 1.05	2.67 ± 0.13	64.29 ± 4.00
Milva	23.98 ± 9.21	7.16 ± 3.01	ND	0.72 ± 0.33	1.31 ± 0.85	33.17 ± 10.56
Agata	0.00 ± 0.00	0.00 ± 0.00	ND	14.86 ± 7.95	16.85 ± 9.84	31.71 ± 15.68
Bildtstar	20.99 ± 5.69	6.38 ± 1.31	ND	0.45 ± 0.12	0.61 ± 0.30	28.43 ± 3.78
Arrow	34.08 ± 11.65	ND	ND	1.43 ± 0.83	0.80 ± 0.51	36.30 ± 11.59
Presto	0.00 ± 0.00	ND	ND	11.66 ± 5.09	26.89 ± 10.56	38.55 ± 3.17
IVP01-084-19	0.00 ± 0.00	3.54 ± 0.87	ND	1.96 ± 0.86	ND	5.49 ± 1.53
Casteline	ND	ND	ND	3.89 ± 2.22	1.76 ± 0.91	5.65 ± 2.81
Fianna	ND	ND	ND	ND	0.36 ± 0.19	0.36 ± 0.17
Ranger Russet	ND	ND	ND	7.62 ± 0.55	4.03 ± 0.41	11.65 ± 0.87

ND – Non-detectable, DY – Dark Yellow, PY – Pale Yellow, CR – Cream, W – White, CP – Cream to Pale Yellow, PK – Pink, PP – Purple

Appendix 5.1

Total and individual carotenoid compounds (µg/g DW) of 8 potato cultivars (2006/2007 growing season) grown at three different locations in New Zealand

Cultivar	Site	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
Agria	LN	9.57 ± 5.40	9.42 ± 6.57	74.36 ± 14.60	0.20 ± 0.13	0.24 ± 0.17	93.80 ± 14.43
(DY)	PN	22.57 ± 8.47	41.28 ± 9.05	191.91 ± 24.68	0.31 ± 0.12	0.16 ± 0.02	256.21 ± 37.27
	PK	39.64 ± 4.89	23.95 ± 6.12	171.75 ± 46.58	0.35 ± 0.07	0.27 ± 0.15	235.96 ± 49.36
Laura	LN	0.00 ± 0.00	ND	148.61 ± 20.43	1.05 ± 0.15	0.61 ± 0.15	150.27 ± 19.39
(DY)	PN	36.48 ± 9.59	63.05 ± 23.38	250.26 ± 34.83	0.28 ± 0.06	0.09 ± 0.01	350.15 ± 50.82
	PK	29.21 ± 9.44	20.11 ± 5.04	199.67 ± 77.21	0.30 ± 0.29	0.24 ± 0.20	249.53 ± 89.88
Marabel	LN	10.11 ± 2.30	ND	26.31 ± 4.00	0.23 ± 0.08	0.01 ± 0.00	36.67 ± 5.68
(DY)	PN	ND	4.53 ± 2.39	15.44 ± 7.49	0.04 ± 0.01	ND	20.01 ± 8.86
	PK	ND	5.67 ± 2.89	10.18 ± 0.49	0.07 ± 0.04	0.00 ± 0.00	15.92 ± 2.59
Desiree	LN	4.21 ± 1.64	0.00 ± 0.00	35.55 ± 7.04	0.15 ± 0.06	0.04 ± 0.02	36.94 ± 6.33
(PY)	PN	ND	1.52 ± 0.19	10.22 ± 2.98	0.04 ± 0.02	ND	11.78 ± 2.84
	PK	ND	0.90 ± 0.21	6.82 ± 2.04	0.03 ± 0.02	ND	7.75 ± 2.03
Fianna	LN	ND	ND	ND	18.04 ± 1.81	0.65 ± 0.07	18.69 ± 1.74
(CR)	PN	ND	ND	ND	18.31 ± 3.49	0.52 ± 0.27	18.83 ± 3.34
	PK	ND	ND	ND	15.23 ± 4.32	0.10 ± 0.18	15.33 ± 3.82
Ranger	LN	0.00 ± 0.00	ND	0.00 ± 0.00	18.62 ± 4.73	1.56 ± 0.54	20.18 ± 4.58
(W)	PN	ND	3.84 ± 0.25	7.29 ± 0.56	0.27 ± 0.08	0.01 ± 0.00	11.41 ± 3.29
	PK	ND	ND	ND	6.21 ± 0.54	0.30 ± 0.03	6.50 ± 0.47
Van Rosa	LN	ND	ND	0.00 ± 0.00	13.75 ± 1.01	0.68 ± 0.33	14.42 ± 1.02
(W)	PN	ND	5.80 ± 2.18	8.28 ± 1.25	0.10 ± 0.05	ND	14.89 ± 1.86
	PK	ND	4.53 ± 3.56	3.74 ± 3.38	0.07 ± 0.03	0.01 ± 0.00	8.35 ± 1.76
Vtn	LN	ND	ND	ND	ND	10.07 ± 1.10	10.07 ± 1.00
(W)	PN	ND	ND	ND	2.76 ± 0.83	ND	2.76 ± 0.74
	PK	ND	ND	ND	7.76 ± 5.02	ND	7.75 ± 4.49

ND – Non-detectable, DY – Dark Yellow, PY – Pale Yellow, CR – Cream, W – White, CP – Cream to Pale Yellow, PK – Pink, PP – Purple
LN – Lincoln, PN – Palmerston North, PK - Pukekohe

Appendix 5.2

Means of tuber flesh intensity colour for 8 potato cultivars grown at three different locations in New Zealand using a Minolta chroma meter CR-210 measuring in CIELAB L* = lightness, a* = bluish-green (-)/red-purple (+) hue component, b* = yellow (+)/blue (-) hue component, C* $[(a^{*2} + b^{*2})^{½}]$ = chroma, h° (from arctangent b*/a*) = hue angle (0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue).

Cultivar	Flesh colour	L*	a*	b*	C*	h°	Total Carotenoid (µg/g DW)
Laura LN	Dark Yellow	89.00	-10.38	32.14	33.77	107.83	150.27
Laura PK		89.32	-9.87	33.37	34.79	106.40	249.53
Laura PN		89.70	-10.14	34.83	36.27	106.17	350.15
Agria LN	Dark Yellow	89.66	-10.58	31.41	33.14	108.53	93.80
Agria PK		89.87	-10.00	31.85	33.38	107.40	235.96
Agria PN		88.91	-10.05	32.60	34.11	107.10	256.21
Marabel LN	Dark Yellow	89.21	-10.39	27.42	29.32	110.70	36.67
Marabel PK		87.54	-9.87	26.96	28.71	110.03	15.92
Marabel PN		90.30	-11.10	31.25	33.16	109.50	20.01
Desiree LN	Pale Yellow	86.14	-9.90	25.20	27.07	111.33	36.94
Desiree PK		89.52	-10.21	26.89	28.75	110.73	7.75
Desiree PN		87.99	-10.52	28.67	30.53	110.10	11.78
Fianna LN	Cream	87.63	-9.27	25.09	26.74	110.23	18.69
Fianna PK		89.36	-9.47	25.26	26.97	110.50	15.33
Fianna PN		88.48	-9.14	26.09	27.64	109.23	18.83
Ranger LN	White	89.42	-9.13	23.05	24.79	111.57	20.18
Ranger PK		91.08	-9.23	23.30	25.05	111.57	6.50
Ranger PN		89.11	-9.13	24.23	25.89	110.57	11.41
Van Rosa LN	White	86.07	-9.59	26.27	27.96	110.00	14.42
Van Rosa PK		88.04	-9.43	26.15	27.80	109.77	8.35
Van Rosa PN		86.64	-9.40	25.47	27.14	110.17	14.89
Vtn LN	White	88.80	-9.27	24.87	26.54	110.40	10.07
Vtn PK		91.43	-9.16	24.31	25.98	110.57	7.75
Vtn PN		89.79	-9.34	25.30	26.96	110.20	2.76

LN – Lincoln, PN – Palmerston North, PK - Pukekohe

Appendix 5.3

Total and individual carotenoid compounds (µg/g DW) of Agria and Desiree grown at 8 different locations in Netherlands harvested in 2006

Cultivar	Site	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
Desiree	HZPC	ND	ND	ND	ND	2.15 ± 0.75	2.15 ± 0.68
	Agrico	ND	ND	ND	ND	2.45 ± 0.69	2.45 ± 0.63
	MR	ND	ND	ND	ND	0.62 ± 0.11	0.62 ± 0.19
	MN	ND	ND	ND	ND	2.85 ± 1.50	2.85 ± 1.34
	VRW	13.65 ± 2.43	ND	ND	3.99 ± 0.21	1.97 ± 0.11	19.60 ± 3.47
	VRWT	ND	ND	ND	10.33 ± 1.96	5.70 ± 0.98	16.02 ± 2.63
	RH	17.09 ± 4.65	5.12 ± 0.96	ND	0.58 ± 0.14	0.30 ± 0.06	23.09 ± 5.11
Agria	HZPZ	ND	ND	ND	3.42 ± 0.07	2.31 ± 0.07	5.73 ± 0.00
	Agrico	ND	ND	ND	7.79 ± 1.91	3.97 ± 0.90	11.77 ± 2.45
	MR	ND	ND	ND	0.00 ± 0.00	4.80 ± 0.44	4.80 ± 0.39
	VRE	ND	ND	ND	ND	1.04 ± 0.50	1.04 ± 0.48
	VRW	117.31 ± 15.57	49.44 ± 27.93	ND	5.66 ± 3.23	4.82 ± 1.72	177.23 ± 42.59
	VRWT	95.19 ± 6.14	30.14 ± 9.24	ND	4.12 ± 0.62	6.21 ± 3.53	135.66 ± 10.60
	RH	114.19 ± 12.57	28.62 ± 14.48	ND	3.67 ± 0.92	5.56 ± 2.02	152.05 ± 25.96
	RH1	144.92 ± 17.79	71.43 ± 6.82	ND	2.58 ± 0.38	9.63 ± 0.47	228.56 ± 11.15

ND – Non-detectable, HZPZ-Metslawier, Friesland; Agrico-Bant, Flevoland; MR- Rilland, Zeeland; MN-Meyer-NOP, VRE- Nagele, Flevoland; VRW-Wierum, Friesland; VRWT- Wieringerwerf, Noord-Holland; RH- Wageningen, Gelderland

Appendix 6.1

Individual and total carotenoid content (µg/g DW) of potato tubers in response to 12 months storage and 24 months storage

LINE (AV)	Year	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
F10/60	0	ND	ND	ND	109.94 ± 0.63	10.73 ± 0.16	120.66 ± 0.70
(448)	2	33.87 ± 5.26	6.28 ± 0.75	40.56 ± 1.00	1.57 ± 0.25	0.09 ± 0.02	82.35 ± 5.96
F10/66	0	ND	102.25 ± 0.17	ND	3.36 ± 0.01	7.75 ± 0.01	113.36 ± 0.16
(177)	1	167.37 ± 4.44	45.01 ± 3.67	ND	3.64 ± 0.71	0.41 ± 0.07	216.43 ± 0.00
F12/50	0	ND	ND	ND	199.90 ± 7.89	10.95 ± 0.43	210.85 ± 8.33
(Agria)	2	25.76 ± 3.95	20.64 ± 3.41	69.55 ± 14.31	1.45 ± 0.31	0.15 ± 0.01	117.55 ± 17.95
F12/64	0	ND	ND	ND	29.29 ± 0.31	10.74 ± 0.11	40.035 ± 0.32
(318)	1	40.59 ± 19.9	13.20 ± 4.13	85.23 ± 31.56	1.02 ± 0.10	0.09 ± 0.10	140.12 ± 38.84
	2	ND	6.65 ± 2.02	55.58 ± 4.39	2.12 ± 0.10	0.16 ± 0.02	64.51 ± 5.30
F13/61	0	100.45 ± 0.03	150.9 ± 0.04	ND	7.03 ± 0.00	14.49 ± 0.00	272.83 ± 0.06
(426)	1	61.05 ± 10.4	27.51 ± 6.66	ND	2.09 ± 0.15	0.03 ± 0.00	90.68 ± 14.03
F14/52	0	222.05 ± 1.08	ND	ND	2.54 ± 0.05	0.37 ± 0.00	224.97 ± 1.08
(131)	1	178.22 ± 43.7	39.95 ± 4.04	ND	3.96 ± 0.74	1.24 ± 0.39	223.37 ± 39.90
F14/63	0	ND	25.11 ± 0.00	ND	4.60 ± 0.00	2.49 ± 0.00	32.2 ± 0.00
(Iwa)	2	16.66 ± 0.59	2.09 ± 0.38	21.80 ± 2.65	0.55 ± 0.01	0.02 ± 0.00	41.12 ± 2.33

ND – Non-detectable

LINE (AV)	Year	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
F15/65	0	ND	ND	ND	147.5 ± 2.24	ND	147.51 ± 2.24
(367)	1	48.32 ± 3.74	23.83 ± 4.12	34.18 ± 9.63	2.30 ± 0.15	0.01 ± 0.00	108.63 ± 9.49
F15/67	0	ND	ND	ND	106.0 ± 3.50	ND	106.05 ± 3.49
(198)	1	67.06 ± 6.02	35.65 ± 3.22	51.13 ± 0.31	3.84 ± 0.45	0.65 ± 0.00	158.32 ± 58.63
	2	43.90 ± 1.80	6.75 ± 0.75	98.55 ± 0.70	2.14 ± 0.35	ND	151.35 ± 0.33
F15/71	0	125.98 ± 0.03	119.4 ± 0.03	ND	6.01 ± 0.00	11.59 ± 0.00	262.99 ± 0.06
(101)	2	18.38 ± 4.90	4.85 ± 0.61	37.32 ± 3.24	1.33 ± 0.09	0.03 ± 0.01	61.92 ± 7.22
F16/49	0	ND	ND	ND	44.37 ± 1.31	16.37 ± 0.62	60.74 ± 1.91
(438)	1	52.37 ± 4.62	16.82 ± 1.53	24.37 ± 3.60	1.37 ± 0.10	0.02 ± 0.00	94.95 ± 0.50
F16/62	0	ND	ND	ND	ND	ND	10.45 ± 5.91
(Vtn)	2	29.61 ± 2.68	4.98 ± 0.66	61.53 ± 10.97	1.16 ± 0.10	0.02 ± 0.00	97.30 ± 11.76
F16/69	0	ND	ND	ND	62.67 ± 4.69	27.12 ± 2.24	89.79 ± 6.91
(115)	2	56.78 ± 1.06	13.40 ± 0.97	72.51 ± 2.93	3.66 ± 0.94	0.01 ± 0.00	146.36 ± 12.90
F17/64	0	142.9 ± 0.04	155.8 ± 0.04	ND	8.87 ± 0.00	15.29 ± 0.00	322.86 ± 0.07
(402)	1	37.88 ± 5.64	10.87 ± 0.47	ND	1.10 ± 0.10	0.14 ± 0.00	49.99 ± 5.09
F17/71	0	157.26 ± 0.40	116.2 ± 0.29	ND	5.38 ± 0.01	1.93 ± 0.00	280.8 ± 0.00
(457)	1	91.68 ± 2.10	46.30 ± 12.47	17.29 ± 3.05	2.92 ± 1.25	0.54 ± 0.04	158.73 ± 35.44
	2	149.57 ± 22.03	14.92 ± 1.28	77.26 ± 28.24	1.48 ± 0.48	0.03 ± 0.00	243.26 ± 7.98
F18/62	0	121.3 ± 0.37	147.9 ± 0.45	ND	4.25 ± 0.01	7.25 ± 0.02	280.74 ± 0.00
(456)	1	72.69 ± 24.61	32.89 ± 9.15	ND	2.43 ± 1.12	0.94 ± 0.12	108.95 ± 29.40
	2	41.29 ± 10.46	6.04 ± 1.07	36.87 ± 3.39	1.28 ± 0.30	0.01 ± 0.00	85.49 ± 6.89
F18/69	0	ND	ND	ND	79.73 ± 0.72	25.18 ± 0.23	104.91 ± 0.95
(242)	2	128.56 ± 21.03	19.15 ± 1.19	26.92 ± 7.40	1.23 ± 0.06	0.02 ± 0.00	175.88 ± 3.89

ND – Non-detectable

Appendix 6.2

Individual and total carotenoid content (µg/g DW) of potato tubers in response to the presence of powdery scab symptoms

LINE	AV (*)	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
F10/66	177(0.0)	ND	102.25 ± 0.17	ND	3.36 ± 0.01	7.75 ± 0.01	113.365 ± 0.16
F10/17	177(1.0)	122.36 ± 0.13	210.21 ± 0.22	ND	4.39 ± 0.00	6.60 ± 0.01	343.55 ± 0.29
F10/17	177(3.5)	ND	117.97 ± 0.20	ND	4.03 ± 0.01	13.94 ± 0.02	135.94 ± 0.25
F10/58	Gla (0.0)	ND	ND	ND	49.26 ± 0.20	35.06 ± 0.14	84.315 ± 0.29
F5/19	Gla (1.5)	ND	ND	ND	11.61 ± 0.17	6.73 ± 0.10	18.335 ± 0.22
F5/19	Gla (2.0)	ND	ND	ND	18.83 ± 0.19	16.03 ± 0.16	34.86 ± 0.39
F11/54	3(0.0)	48.89 ± 0.07	87.14 ± 0.12	ND	1.53 ± 0.00	1.62 ± 0.00	139.18 ± 0.16
F5/38	3(1.5)	53.21 ± 0.07	178.47 ± 0.24	ND	3.47 ± 0.00	17.27 ± 0.02	252.415 ± 0.29
F5/38	3(2.5)	ND	139.94 ± 0.23	ND	3.40 ± 0.01	19.99 ± 0.03	163.33 ± 0.22
F11/56	Aga(0.0)	ND	ND	ND	77.28 ± 1.34	14.05 ± 0.24	91.33 ± 1.59
F7/27	Aga(1.5)	ND	317.33 ± 0.07	ND	7.15 ± 0.00	16.16 ± 0.00	340.64 ± 0.06
F7/27	Aga(6.0)	69.18 ± 0.13	271.20 ± 0.53	ND	3.95 ± 0.01	19.39 ± 0.04	363.72 ± 0.00
F11/67	42(0.0)	106.93 ± 0.00	55.74 ± 0.00	ND	2.20 ± 0.00	1.49 ± 0.00	166.37 ± 0.00
F7/32	42(0.5)	ND	129.05 ± 0.37	ND	8.26 ± 0.02	9.10 ± 0.03	146.41 ± 0.00
F7/32	42(2.0)	ND	37.22 ± 0.28	ND	3.69 ± 0.03	4.34 ± 0.03	45.255 ± 0.29
F12/50	Aga(0.0)	ND	ND	ND	199.9 ± 7.89	10.95 ± 0.43	210.85 ± 8.33
F3/27	Aga(3.0)	ND	271.15 ± 0.24	ND	5.25 ± 0.00	37.29 ± 0.03	313.7 ± 0.26
F3/27	Aga(4.0)	71.54 ± 0.04	337.31 ± 0.20	ND	5.77 ± 0.00	34.47 ± 0.02	449.095 ± 0.22

ND – Non-detectable, (*) – scab score

LINE	AV (*)	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
F12/64	318(0.0)	ND	ND	ND	29.29 ± 0.31	10.74 ± 0.11	40.035 ± 0.32
F5/25	318(1.0)	7.39 ± 0.00	47.40 ± 0.02	ND	88.71 ± 0.04	3.61 ± 0.00	147.115 ± 0.06
F5/25	318(1.5)	ND	66.84 ± 1.12	ND	4.29 ± 0.07	13.34 ± 0.22	84.46 ± 0.00
F5/25	318(3.0)	ND	30.93 ± 0.28	ND	3.45 ± 0.03	4.42 ± 0.04	38.805 ± 0.29
F12/56	218(0.0)	127.89 ± 0.29	49.84 ± 0.11	ND	5.15 ± 0.01	4.47 ± 0.01	187.34 ± 0.00
F7/19	218(0.5)	ND	107.39 ± 0.06	ND	6.39 ± 0.00	11.46 ± 0.01	125.24 ± 0.06
F7/19	218(3.0)	64.99 ± 0.69	62.23 ± 0.66	ND	2.34 ± 0.02	4.50 ± 0.05	134.06 ± 0.00
F13/61	426(0.0)	100.45 ± 0.03	150.86 ± 0.04	ND	7.03 ± 0.00	14.49 ± 0.00	272.83 ± 0.06
F5/17	426(2.0)	ND	199.11 ± 0.23	ND	5.16 ± 0.01	20.41 ± 0.02	224.685 ± 0.26
F5/17	426(2.5)	ND	98.21 ± 0.31	ND	4.03 ± 0.01	8.05 ± 0.03	110.285 ± 0.26
F13/67	93(0.0)	140.65 ± 0.24	158.87 ± 0.28	ND	7.90 ± 0.01	18.13 ± 0.03	325.54 ± 0.00
F1/17	93(3.5)	164.20 ± 1.13	217.10 ± 1.49	ND	5.14 ± 0.04	26.05 ± 0.18	412.49 ± 0.00
F1/17	93(4.0)	108.55 ± 0.13	95.01 ± 0.12	ND	4.31 ± 0.01	3.42 ± 0.00	211.295 ± 0.22
F14/52	131(0.0)	222.05 ± 1.08	ND	ND	2.54 ± 0.05	0.37 ± 0.00	224.97 ± 1.08
F2/31	131(1.0)	ND	98.56 ± 1.22	ND	5.51 ± 0.07	10.27 ± 0.13	114.35 ± 0.00
F14/63	Iwa(0.0)	ND	25.11 ± 0.00	ND	4.60 ± 0.00	2.49 ± 0.00	32.2 ± 0.00
F2/27	Iwa(2.5)	ND	ND	ND	24.96 ± 0.15	9.36 ± 0.06	34.32 ± 0.06

ND – Non-detectable, (*) – scab score

LINE	AV (*)	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
F14/66	439(0.0)	43.09 ± 0.77	ND	ND	0.66 ± 0.01	0.04 ± 0.00	43.78 ± 0.78
F2/25	439(1.0)	ND	54.70 ± 0.33	ND	4.58 ± 0.03	8.87 ± 0.05	68.155 ± 0.34
F2/25	439(2.5)	ND	76.91 ± 0.06	ND	3.83 ± 0.00	5.26 ± 0.00	85.99 ± 0.32
F15/67	198(0.0)	ND	ND	ND	106.05 ± 3.50	ND	106.05 ± 3.49
F4/23	198(1.0)	ND	108.71 ± 0.38	ND	7.12 ± 0.02	4.89 ± 0.02	120.725 ± 0.32
F4/23	198(2.5)	89.79 ± 0.17	113.35 ± 0.22	ND	5.37 ± 0.01	11.38 ± 0.02	219.89 ± 0.32
F15/71	101(0.0)	125.98 ± 0.03	119.41 ± 0.03	ND	6.01 ± 0.00	11.59 ± 0.00	262.99 ± 0.06
F2/23	101(0.5)	72.59 ± 0.46	36.34 ± 0.23	ND	1.25 ± 0.01	1.24 ± 0.01	111.42 ± 0.00
F2/23	101(1.5)	ND	91.22 ± 0.08	ND	2.39 ± 0.00	7.26 ± 0.01	100.87 ± 0.07
F15/65	367(0.0)	ND	ND	ND	147.51 ± 2.24	ND	147.51 ± 2.24
F1/19	367(2.0)	54.20 ± 0.31	62.37 ± 0.36	ND	2.25 ± 0.01	3.05 ± 0.02	121.87 ± 0.00
F1/19	367(3.5)	68.13 ± 0.23	101.9 ± 0.35	ND	2.70 ± 0.01	2.67 ± 0.01	175.42 ± 0.00
F16/62	Vtn(0.0)	ND	ND	ND	ND	ND	10.45 ± 5.91
F4/20	Vtn(0.0)	ND	ND	ND	4.90 ± 0.19	2.16 ± 0.08	7.06 ± 0.22
F4/20	Vtn(1.5)	ND	ND	ND	7.36 ± 0.18	3.53 ± 0.08	10.895 ± 0.22
F16/69	115(0.0)	ND	ND	ND	62.67 ± 4.69	27.12 ± 2.24	89.79 ± 6.91
F3/17	115(2.5)	ND	108.46 ± 0.23	ND	3.87 ± 0.01	11.99 ± 0.03	124.315 ± 0.22
F3/17	115(4.0)	ND	113.53 ± 0.32	ND	3.53 ± 0.01	6.60 ± 0.02	123.65 ± 0.25

ND – Non-detectable, (*) – scab score

LINE	AV (*)	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
F16/49	438(0.0)	ND	ND	ND	44.37 ± 1.31	16.37 ± 0.62	60.74 ± 1.91
F5/33	438(0.5)	ND	85.06 ± 0.21	ND	6.15 ± 0.01	16.94 ± 0.04	108.145 ± 0.22
F5/33	438(2.0)	ND	97.16 ± 0.22	ND	6.61 ± 0.01	14.09 ± 0.03	117.865 ± 0.22
F17/70	266(0.0)	ND	ND	ND	ND	ND	7.5 ± 0.00
F3/22	266(1.5)	ND	46.25 ± 0.44	ND	4.14 ± 0.04	8.10 ± 0.08	58.48 ± 0.39
F3/22	266(3.5)	ND	ND	ND	13.41 ± 0.08	14.06 ± 0.08	27.47 ± 0.13
F17/71	457(0.0)	157.26 ± 0.40	116.23 ± 0.29	ND	5.38 ± 0.01	1.93 ± 0.00	280.8 ± 0.00
F7/17	457(3.5)	ND	165.94 ± 0.18	ND	4.61 ± 0.00	15.26 ± 0.02	185.82 ± 0.16
F7/17	457(4.5)	ND	105.34 ± 0.37	ND	3.82 ± 0.01	9.50 ± 0.03	118.655 ± 0.32
F17/64	402(0.0)	142.92 ± 0.04	155.78 ± 0.04	ND	8.87 ± 0.00	15.29 ± 0.00	322.86 ± 0.07
F3/25	402(1.5)	ND	96.25 ± 0.28	ND	5.98 ± 0.02	16.37 ± 0.05	118.605 ± 0.29
F3/25	402(3.0)	ND	95.23 ± 0.06	ND	5.59 ± 0.00	10.64 ± 0.01	111.46 ± 0.06
F18/69	242(0.0)	ND	ND	ND	79.73 ± 0.72	25.18 ± 0.23	104.91 ± 0.95
F4/18	242(1.0)	ND	65.83 ± 1.20	ND	5.14 ± 0.09	6.30 ± 0.12	77.27 ± 0.00
F4/18	242(3.0)	ND	240.33 ± 3.43	ND	9.97 ± 0.14	30.85 ± 0.44	281.145 ± 3.27
F18/62	456(0.0)	121.3 ± 0.37	147.94 ± 0.45	ND	4.25 ± 0.01	7.25 ± 0.02	280.74 ± 0.00
F7/30	456(1.0)	ND	139.54 ± 0.61	ND	7.40 ± 0.03	14.72 ± 0.06	161.66 ± 0.00
F7/30	456(3.0)	ND	87.42 ± 0.02	ND	3.27 ± 0.00	13.43 ± 0.00	104.12 ± 0.00
F18/49	35(0.0)	ND	ND	ND	25.79 ± 0.17	9.91 ± 0.15	35.7 ± 0.28
F5/30	35(1.0)	ND	69.22 ± 0.06	ND	3.73 ± 0.00	6.34 ± 0.01	79.295 ± 0.06
F5/30	35(1.5)	ND	101.89 ± 0.24	ND	5.19 ± 0.01	6.91 ± 0.02	113.99 ± 0.22

ND – Non-detectable, (*) – scab score

Appendix 7.1

Individual and total carotenoid content (µg/g DW) of Agria and Desiree minitubers for carotenoid biosynthesis analysis

Cultivar	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Zeaxanthin (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)	Total Carotenoid (µg/g DW)
AGRIA						
LIGHT	ND	95.05 ± 0.66	216.95 ± 1.50	4.85 ± 0.03	0.04 ± 0.00	316.88 ± 2.18
DARK	ND	20.51 ± 0.29	126.19 ± 1.78	1.42 ± 0.02	0.02 ± 0.00	148.14 ± 2.09
PEG (-)	ND	8.51 ± 0.26	77.43 ± 2.34	0.47 ± 0.01	0.01 ± 0.00	86.41 ± 2.61
PEG (+)	ND	102.06 ± 9.71	ND	5.96 ± 0.57	1.91 ± 0.18	109.94 ± 10.46
MS 0.1X	ND	193.97 ± 3.93	ND	7.56 ± 0.15	4.11 ± 0.08	205.65 ± 4.17
MS 0.5X	ND	184.48 ± 2.68	ND	8.69 ± 0.13	3.80 ± 0.06	196.96 ± 2.86
MS 1.0X	ND	277.38 ± 0.96	ND	5.56 ± 0.02	5.79 ± 0.02	288.72 ± 1.00
DESIREE						
LIGHT	65.31 ± 0.09	142.35 ± 0.19	ND	7.93 ± 0.01	0.05 ± 0.00	215.63 ± 0.28
DARK	33.69 ± 0.42	27.18 ± 0.34	49.48 ± 0.61	1.99 ± 0.02	0.03 ± 0.00	112.37 ± 1.39
PEG (-)	88.21 ± 0.54	66.71 ± 0.41	ND	6.98 ± 0.04	0.05 ± 0.00	162.25 ± 0.00
PEG (+)	105.51 ± 2.13	81.43 ± 1.64	ND	4.21 ± 0.08	0.91 ± 0.02	192.06 ± 3.87
MS 0.1X	47.27 ± 1.30	35.04 ± 0.96	ND	4.73 ± 0.13	0.03 ± 0.00	87.07 ± 2.39
MS 0.5X	52.93 ± 0.00	35.11 ± 0.00	ND	5.66 ± 0.00	0.03 ± 0.00	93.73 ± 0.00
MS 1.0X	38.77 ± 0.00	18.43 ± 0.00	ND	2.63 ± 0.00	0.02 ± 0.00	59.84 ± 0.00

ND – Non-detectable