Genetic characterisation of agronomic and morphological traits and the development of DNA markers associated with total glycoalkaloid content in the tubers of tetraploid potato (*Solanum tuberosum* L.)

Jacob van Dam

Genetic characterisation of agronomic and morphological traits and the development of DNA markers associated with total glycoalkaloid content in the tubers of tetraploid potato (*Solanum tuberosum* L.)

Promotoren: Prof. dr. ir. P.C. Struik
Hoogleraar in de Gewasfysiologie
Prof. dr. D. Levy
Plant physiologist, ARO The Volcani Center, Bet Dagan, Israel

Co-promotor: Dr. I. Levin
Geneticist, ARO The Volcani Center, Bet Dagan, Israel

Samenstelling promotiecommissie:
Prof. dr. L.H.W. van der Plas (Wageningen Universiteit)
Dr. ir. A.J. Haverkort (PRI, Wageningen Universiteit en Research centrum)
Dr. ir. L.T. Colon (PRI, Wageningen Universiteit en Research centrum)
Dr. G.J. de Vrije (ATO, Wageningen Universiteit en Research centrum)

**Genetic characterisation of agronomic and morphological traits
and the development of DNA markers associated with
total glycoalkaloid content in the tubers of tetraploid potato
(*Solanum tuberosum* L.)**

Jacob van Dam

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. ir. L. Speelman
in het openbaar te verdedigen
op donderdag 12 september 2002
des namiddags te half twee in de Aula

Abstract

Van Dam, J., 2002. Genetic characterisation of agronomic and morphological traits and the development of DNA markers associated with total glycoalkaloid content in the tubers of tetraploid potato (*Solanum tuberosum* L.). PhD Thesis, Wageningen University, The Netherlands, 101 pp., with English and Dutch summaries.

Glycoalkaloids are secondary metabolites that are characterised by an undesirable taste, and which are known to be toxic when consumed in large quantities. Some wild potato germplasms that are used for the introgression of genes encoding heat tolerance contain high concentrations of glycoalkaloids in the tubers. However, heat tolerance is not necessarily associated with high glycoalkaloid content in the tubers and it is feasible to detect heat tolerant clones that will not accumulate excessive glycoalkaloid levels in the tubers when exposed to heat stress. Breeding and selection should therefore take place to lower the glycoalkaloid content in potato tubers and DNA markers can significantly facilitate the selection process.

The main objective of this study was to develop DNA markers associated with total glycoalkaloid (TGA) content in the potato tubers. For this purpose two tetraploid resource populations segregating for TGA-content were constructed and analysed in two consecutive experiments. The populations were characterised for the genetic control of total glycoalkaloid content in the tubers. Although tuber glycoalkaloid content was the most important trait under study, additional agronomic and morphological traits were also recorded and analysed. These additional traits were used to characterise the phenotypic and genetic architectures of the two segregating populations used in this study.

In both populations, the parental clones differed markedly in TGA-content and the progeny population was normally distributed for this trait after logarithmic transformation. Broad sense heritability estimate of TGA-content was 0.54 in Cara × LT7 and 0.50 in NT8 × LT7 and the trait proved to be inherited in a non-dominant manner. The minimum number of genes contributing to TGA-content was estimated to be between 3 and 7. None of the other traits recorded in this study showed a statistically significant genetic association with TGA-content. This suggests that tuber TGA-content may be genetically modulated without any significant adverse effects on other agronomic traits. Broad sense heritability was estimated for all traits assessed. Heritability estimates that were similar in both populations ranged from 0.1 for the number of main stems to 0.5 for total tuber glycoalkaloid content. In both populations, most heritabilities estimated were about 0.3. Main agronomic traits, such as tuber dormancy, tuber weight, and maturity, appeared to be controlled by several additive genes.

Three hundred and forty-two Inter-Simple Sequence Repeat (ISSR) single primers or two such primer combinations were used to amplify random PCR products. Two single ISSR markers were found significantly associated with TGA-content. A multiple regression analysis was also carried out using a 'stepwise' procedure. In this analysis TGA-content was the dependent variable whereas the polymorphic PCR products and all possible two-way interactions among them were the independent variables. The resulting best model consisted of

an interaction between two loci in addition to a single locus effect. This interaction suggests that the expression of TGA-content is partially modulated by two interacting loci.

A single copy DNA marker for a morphological leaf characteristic, vein depth, was generated in a study of the association of molecular markers with morphological characteristics. ISSR primers were used to generate random PCR products followed by the design of a CAPS marker. A multiple regression analysis showed that the best model consisted of the ISSR-PCR product and a CAPS-PCR product. The results suggest that these products represent separate alleles at the same DNA locus. Vein depth is apparently controlled by one or several genes that modulate the lamina expansion, either during cell division or during cell elongation. Vein depth is shown to be regulated in an additive way and hence allelic differences are supposedly accountable for small differences in lamina growth. In this study, the feasible generation of a single copy DNA marker in tetraploids and its possible use in the study of tetrasomic inheritance are demonstrated.

Progress has been made in the development of a DNA marker associated with TGA-content in potato tubers. When the development of such DNA marker will be completed, it may be valuable in programmes which include the introgression of genes from wild species while discarding genotypes high in TGA-content, contributing considerably to breeding potatoes adapted to warm climates.

Contents

Preface

The thesis work, from start to finish, took approximately seven and a half years. Besides working on my thesis, many events happened in this period of time. Among them were happy occasions, such as getting married and becoming father of three wonderful daughters. There were also sad and painful experiences, such as coping with many terror attacks, including those on family, friends and acquaintances.

The successful completion of my thesis work is not merely a merit of myself. "G'd was with Joseph and he was a successful man…" (Genesis 39:2). First and foremost I would like to express my gratefulness to the Almighty, who enabled me to carry out this demanding and challenging task.

Furthermore, I am grateful to tens of people who participated in many different ways to complete the project; from colleagues who created a good atmosphere at the department to the day-labourers who filled pots with potting soil. During these years, several people contributed substantially to my work. My appreciation is much deeper than a few words can say.

My supervisor at the Volcani Center, Israel, was David Levy. He was involved in almost all aspects of planning and operational aspects of the research carried out, resulting in many fruitful discussions. Most of the period of the thesis work he was Head of the Institute for Field and Garden Crops at the Volcani Center. Nevertheless, he was always available whenever I needed his advice.

When the project started, Ilan Levin was not immediately involved in my research. First, he gave some advice. Before he was aware of it, he stood in the middle of my work. With his background in the genetics of tomato and chicken, those potatoes were initially hard to grasp. He taught me everything related to the bio-molecular lab work.

Paul Struik and I know each other for some ten years. He was the supervisor of my main MSc research at the Department of Agronomy, Wageningen University, The Netherlands. He continued to be my scientific mentor when he agreed to be "promotor" for my PhD research. With Paul I experienced the relativity of distance. As I immigrated to Israel immediately after my MSc graduation, we communicated mainly through e-mail. There was no difference whatsoever between the way he assisted me in Beth Dagan and the way he guided me before in Wageningen, with his quick responses, sharp analyses, and no less important, pleasant mentorship. Whenever necessary, he kept me going with a few stimulating words.

I owe a lot of credit to the technicians of our team. Yehoshua Yitzhak was the backbone of all fieldwork. He taught me the practical side of the fieldwork, with all its ins and outs. Besides his extended experience, it was always nice to be in his company. Edna Fogelman helped me to feel at home in the lab. She is a very dedicated worker and she had always the patience to explain to me whatever needed. We learned together the practice of several marker systems and learned from each other.

Baruch Bar-Tel needs a special word of thank. He is the one who actually enabled me to work on my PhD, while concurrently working for him at the Israel Plant Breeders' Rights Testing Unit. He trained me to see the morphological details of a wide variety of plants, including potato. Some of these morphological characteristics of the potato plant have been used in this work. Baruch and I have a lot in common, such as growing up in The Netherlands in the periphery ('mediene'), studying in Wageningen, and immigrating to Israel at a similar age. The good relations both at work and privately, created special ties.

For choosing the most appropriate method for the biochemical analysis of glycoalkaloids, I thank Martin Weissenberg for his advice.

Gon van Laar did a very nice job at the very end of my thesis work by editing my manuscript quickly and professionally.

Where would I be without family? Most important of all is my wife Thalia, who stimulated me continuously to finish the project. She has been a constant inspiration. My parents supported me from the very beginning until the very end. Simon, my one and only brother, did the same. Moreover, he lent me his computer when mine was on the fringe of collapse in a critical stage of writing the manuscript. My parents-in-law helped me creating time whenever I requested. Last but not least, my little daughters contributed to my thesis work by either refusing to fall asleep or waking up in the middle of the night, enabling me to utilize many nightly hours.

Tishri 5763 / September 2002

Jacob van Dam

CHAPTER 1

General introduction

The earliest years of potato production in Israel

In Israel, history for most matters begins some 3000 years ago. Potatoes are an exception. Their appearance in the area is a new phenomenon. The cultivation of potatoes in the country started some years preceding the foundation of the State of Israel in 1948, at the end of the 19th century (Levy *et al.*, 1992). Potato was grown in Yavne'el in the Galilee and in Ein Ganim in the coastal area. Seed potatoes were imported from Russia. Potato was grown as a winter crop in the rainy season, although not quite successfully (Rozenblatt, 1946). Later, experiments were carried out to grow potato in the Jordan Valley. The purpose was to supply potatoes to the UK market in March and early April, preceding the earliest supply from Western Europe, usually in May and June. The quality was considered excellent, but the tuber size was too small (Salaman, 1928). In the beginning of the 1930s, two technical improvements were introduced in Ramat Gan, in the coastal area: instalment of irrigation, and cultivation as a spring crop. With these changes, both the winter rain and the spring sun were utilised, and subsequently the area of potato cultivation increased (Rozenblatt, 1946).

Due to the outbreak of the Second World War, no seed potatoes could be imported and solutions had to be sought for the storage of potatoes. One of the first times that potatoes were stored, storage was done in the cool wine cellars of Mikve Yisra'el in the early 1940s. Also due to the war, to ensure food supply, farmers started to grow potato in all parts of the country and in all seasons. In the spring of 1941, the only seed potatoes available were tubers harvested from the previous winter crop. Hence, various methods of dormancy breaking were employed. The use of local tubers for seed diminished the dependency on foreign seed potatoes and caused a considerable increase in the area of potato growth (Rozenblatt, 1946).

Current potato production

Potato is one of the largest and most important vegetables grown in Israel in an estimated total area of 11,000 hectare (Peleg, 2001). Potato is grown in two seasons: spring (planting in January-February, harvesting in May-July) and autumn (planting in September, harvesting in January-March) (double cropping). Yields in spring are usually higher than in autumn. Comparable results were reported from Tunisia, which

has a climate similar to the one in Israel (Fahem & Haverkort, 1988). In spring, the crop is mainly grown in the semi-arid Negev (70%) and in the Sharon area in the coastal area (30%). In autumn, by far most potatoes are grown in the Negev.

From 1960 until 1997, potato production (fresh weight) comprised a relatively constant proportion of the total vegetable production, ca. 18%. In that period, vegetable production almost quadruplicated and so did the potato production (Ben David, 1999). The cropped area is still increasing. Table 1 shows the potato production in Israel and in some other countries.

Table 1. Potato production in Israel, compared with some other countries (FAO, 2001).

	Area harvested (1000 ha)			Yield (kg ha^{-1})			Production (1000 MT)		
	1997	1998	1999	1997	1998	1999	1997	1998	1999
Israel	7	8	8	39543	41675	41675	277	333	333
Netherlands	180	179	183	44319	29294	44809	7973	5249	8200
USA	544	562	539	38816	38427	40238	21116	21581	21700
Russian Fed.	3352	3265	3250	11049	9623	9600	37040	31419	31200
World	18301	18213	17993	16478	16108	16358	301556	293377	294332

Seed potatoes for the spring season are imported from European seed companies usually during December and January. In January 2002, an amount of 19,000 tonnes of seed potatoes was imported (Y. Peleg, pers. comm.). The origin of these seed potatoes is shown in Figure 1, which is also representative for the situation in the previous years. Seed potatoes for the autumn season are locally grown.

From the crop planted in spring, most of the harvest is either sold on the local market as ware potatoes, a part is exported as early potato and some as baker potatoes. Another part of the yield is stored as seed for the autumn crop. Most of the harvest of the autumn crop is sold on the local market as ware or exported as ware or 'baby' potatoes.
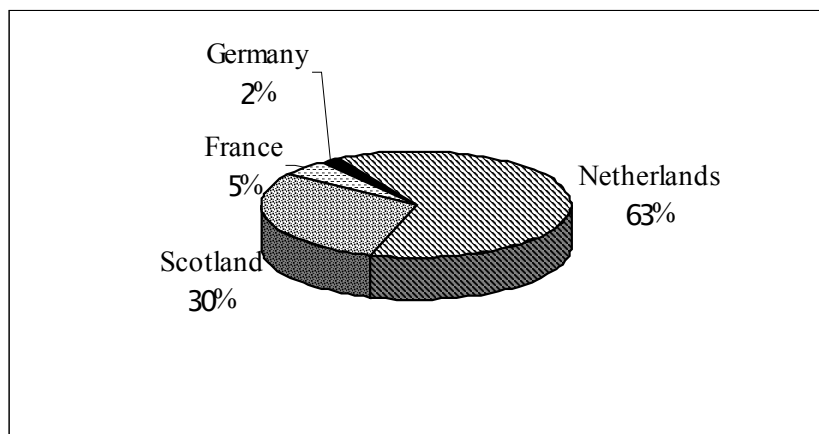

Figure 1. The origin of seed potatoes imported to Israel in 2002.

Cultivars

The main cultivar grown in the early years was Up-to-date, which was fairly adapted to the local climate, and to a lesser extent the cultivar Arran Banner, which was more susceptible to heat stress than Up-to-date. During the Second World War, the British introduced several cultivars, such as Kerr's Pink and Great Scott, but these were strongly negatively affected by the high temperatures (Rozenblatt, 1946).

Today, according to Mr. Y. Peleg of the Vegetable Board, the main cultivars grown in Israel are Mondial and Désirée, which account for almost half of all potatoes grown. These two cultivars are mainly sold on the local market as ware potatoes. Other cultivars are either used for the local processing industry or for export.

Currently, locally-bred cultivars (Idit, Ori and Zohar) protected by Israeli plant breeders' rights, show a potential commercial value. Another locally bred cultivar, Zahov, has been submitted for plant breeders' rights (B. Bar-Tel, pers. comm.) and is especially suited for crisping. Zahov is being compared with the cultivar Hermes, and preliminary results show that this Israeli cultivar performs equally well or slightly better, both in Israel and in the UK, with high yields and good quality characteristics (E. Margalit, pers. comm.). These four cultivars were bred for adaptation to the Mediterranean climate (Levy *et al*., 2001).

Growth, yield, and tuber quality in warm climates

The climate has major effects on the physiology of the potato crop. Most modern cultivars perform best in a temperate climate with moderate temperatures and a long photoperiod.

Effects of temperature on production processes
High temperatures decrease the net assimilation rate, causing a reduction of the carbohydrate synthesis (Midmore & Prange, 1992). At high temperatures, plants are taller, have smaller leaves, have a lower stem to leaf ratio and have a delayed foliage maturity (Marinus & Bodlaender, 1975; Ben Khedher & Ewing, 1985; Van Dam *et al*., 1996). Also at high temperatures, tuber yield and the ratio of tuber to haulm dry matter are reduced, and tubers are lower in percentage dry matter (Marinus & Bodlaender, 1975; Ben Khedher & Ewing, 1985). At night, high temperatures have an adverse effect both on tuber fresh weight and tuber dry weight, particularly when occurring before tuber initiation (Murti *et al*., 1976). The onset of expolinear tuber growth and the onset of linear tuber bulking are delayed at high temperatures. Moreover, high temperatures also result in lower absolute tuber growth rates and lower relative rates of

partitioning of dry matter to the tubers (Gawronska *et al.*, 1992; Van Dam *et al.*, 1996).

Effects of high temperature on tuber formation

The number of tuber incipients is reduced at high temperatures, which reduces the demand for photosynthate translocation to the tubers (Prange *et al.*, 1990). High soil temperatures have an adverse effect on tuber initiation, tuber yield, and tuber dry weight and enhance secondary growth (Midmore, 1984; Reynolds & Ewing, 1989) and other physiological disorders, such as heat sprouts, translucent ends and hollow heart (Storey & Davis, 1992).

The starch content of potato tubers is decreased at high temperatures by inhibition of the conversion of sugars into starch (Krauss & Marschner, 1984; Mohabir & John, 1988). Permanent or temporary high soil temperature during tuber development affects starch content more directly via disturbed carbohydrate metabolism within the tuber and less via the supply of assimilates (Krauss & Marschner, 1984). In the hormonal control of tuber formation, gibberellic acid plays a key role in many of the sequential events that together result in tuber formation. Under non-inducing conditions, such as high temperature, gibberellin levels and activities are high and decrease under inducing conditions (Struik *et al.*, 1999).

Effects of light on tuber formation and production processes

Long photoperiods delay stolon and tuber initiation, delay and reduce partitioning of dry matter to the tubers and also reduce absolute maximum tuber bulking rates. These effects result in a low harvest index (Struik & Ewing, 1995; Van Dam *et al.*, 1996). Early genotypes are less affected by high temperatures than later genotypes (Snyder & Ewing, 1989). Shorter daylengths hasten plant development from emergence till the end of leaf growth. The magnitude of the effect depends on the cultivar (Kooman *et al.*, 1996).

High irradiance is favourable for potato growth, as it increases the net assimilation rate (Sale, 1973; Menzel, 1985).

Comparison of the growing conditions during the cropping seasons in Israel and The Netherlands

Significant temperature differences exist between Israel and Western Europe. In The Netherlands, for example, both the average maximum temperature and the average minimum temperature during the growing season are significantly lower than during both growing seasons in Israel (Figure 2). Whereas in Israel main difficulties in crop growth arise due to hot weather conditions, in The Netherlands major difficulties arise

at the beginning of the growth season due to low temperatures, sometimes even below or just above zero degrees Celsius.

In Israel, potatoes are grown in warm semi-arid regions. Although they are irrigated during most of both growing seasons, they are nevertheless exposed to various degrees of water stress resulting from high ambient temperatures and relatively low air humidity, a combination which is very common during the spring and summer seasons. Partial leaf wilting, commonly seen, may lead to loss of tuber yield and quality (Levy, 1983a). High temperatures greatly reduce both tuber yield and tuber quality. In Israel, the main problems in potato production, related to the warm climate are: delayed tuber initiation in autumn, competition between tuber and haulm growth in late spring and summer, reduced yields due to sporadic hot spells, reduced dry matter concentration, black spot and internal brown spots. Tuber disorders are especially severe when harvest in the spring season is delayed and the plants are exposed to the heat of July-August. Transient drought occurring during the spring and summer at the period of tuber growth, causes a reduction in quality and number of harvestable tubers rather than in number of tubers present. Moreover, the effect of drought is enhanced by the restricted uptake of water by the notably shallow roots. To induce tuberization it is not necessary to have a moist medium around the stolon tips or to have uptake of water by stolon roots (Struik & Van Voorst, 1986).
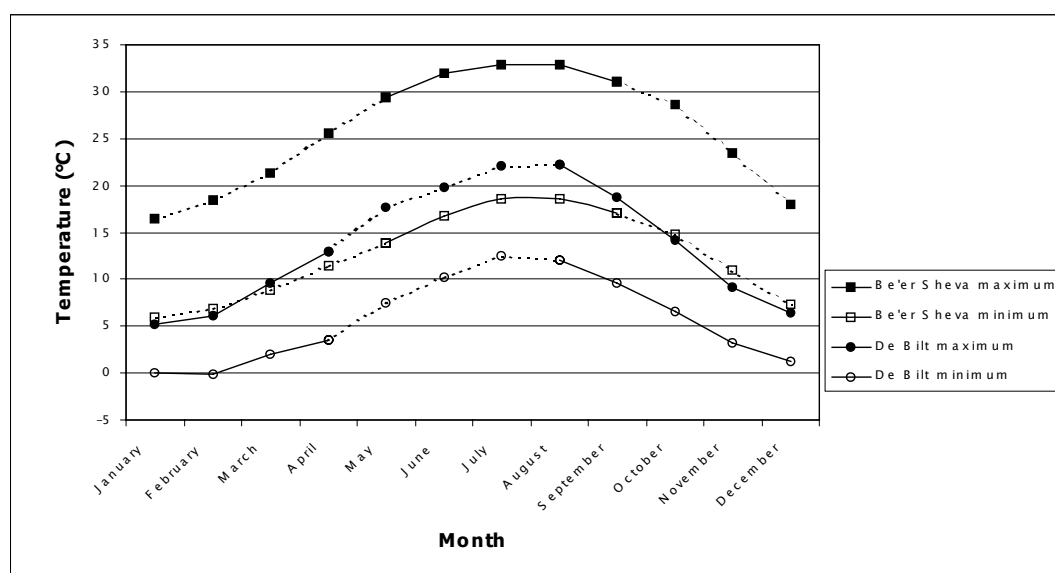
Figure 2. Monthly average maximum temperatures (closed squares) and minimum temperatures (open squares) in Be'er Sheva, in the Negev, where most of the Israeli potatoes are grown, and in De Bilt, The Netherlands (closed circles for maximum and open circles for minimum temperatures). Growing seasons are presented as dashed lines, off-seasons as continuous lines. Israel has two growing seasons: spring and autumn (see text). In The Netherlands the growing season is in summer (April-August).

The spring and the autumn growing seasons in Israel significantly differ in light intensity. In spring, when the sky is clear during most of the season, light intensities are considerably higher than in autumn, which is advantageous for tuber growth (Arazi, 1990).

Breeding

Adaptation of the potato to the tropics, subtropics, semi-arid and arid conditions has been an important task of breeders, since in vast areas of Africa and Asia, the yielding capacity and the high nutritional value of the potato is much needed (Levy, 2000). Specific cultivars can be bred that are specifically appropriate for growth in hot climates. An example of such a cultivar is Norchip, which was reported to have outstanding heat tolerance (Ben Kheder & Ewing, 1985).

With some exceptions, almost all potato cultivars from Europe and North America show significant adverse effects of high temperatures on tuber yield and quality. Therefore, locally bred heat-tolerant cultivars can contribute considerably to the potato production in hot climates (Levy, 1984). A high rate of sucrose synthesis, mediated by sucrose-phosphate synthase activity during periods of high photosynthesis is an important factor positively affecting the performance of heat-tolerant genotypes (Basu & Minhas, 1991). Dry matter production and its accumulation in the tubers are the most important measures of the adaptation of potato cultivars to hot climates (Levy, 1983b). The sensitivity of tuber development to high air temperatures can be seen as physiologically distinct from its sensitivity to high soil temperatures. This may imply that sensitivity to air or soil temperatures, respectively, could be under separate genetic control, and therefore both should be considered when breeding for heat-tolerant potatoes (Reynolds & Ewing, 1989). The degree of osmotic regulation and turgor maintenance, as well as the capacity to develop extensive root systems, was suggested as selective criteria for adaptation to hot, dry climates (Levy, 1983a). Heat tolerance is genetically controlled (Levy *et al*., 1991; Midmore & Prange, 1991; Veilleux *et al*., 1997). Most breeders are of the opinion that selection is more effective if carried out in the region where a resultant variety is to be grown (Kehoe, 1992). A screening procedure developed for efficient selection of heat-tolerant clones in segregating populations was described by Levy *et al*. (1991). The screening is based on a comparison of the performance of progenies grown under controlled cool and hot conditions. Screening of progenies provides a valuable and direct method of identifying parental material (Caligari, 1992b). At the International Potato Center (CIP) in Peru, breeding efforts have been made since its foundation thirty years ago to develop cultivars adapted to warm environments. Sufficient genetic variability in a

wide genetic base led to improvement of earliness, a highly desirable characteristic for growing potatoes under stress conditions, especially heat (International Potato Center, 1984). Advanced clones adapted for hot environments from CIP are used in Israeli breeding programmes.

Heat tolerance and glycoalkaloids

Sources for heat tolerance can be found in wild potato germplasms. In breeding progammes, accessions of potato species are used for the introgression of genes encoding heat tolerance, such as *S. acaule*, *S. stoloniferum*, *S. bulbocastanum*, *S. demissum*, *S. brachycarpum*, *S. andigena*, *S. phureja*, *S. berthaultii*, *S. chacoense* and *S. microdontum* (Midmore & Prange, 1991; Veilleux *et al.*, 1997). When genes encoding heat tolerance can be incorporated in advanced breeding lines or successful existing cultivars, new cultivars could be bred that contain both the good characteristics of such clones for ware production and the suitability for growth under heat stress. However, some wild potato species, or in some cases only several accessions of a species, used for introgression of genes encoding heat tolerance, contain high concentrations of glycoalkaloids in the tubers. Glycoalkaloids belong to a broad category of secondary metabolites. These naturally occurring substances have pronounced and various physiological activities in animals and humans (Kutchan, 1995). In potato tubers, glycoalkaloids consist for 90-95% of α-solanine and α-chaconine. These two compounds can be detected as total glycoalkaloids (TGA), by hydrolysis of glycosides of solanidine, which is a common precursor in the metabolic pathway of both α-solanine and α-chaconine (Van Gelder, 1989). Under normal conditions of human consumption, the amounts of potato alkaloids ingested are not harmful. Sometimes, however, alkaloid quantities can increase to toxic, and in rare instances, fatal levels (Woolfe, 1987). The level of glycoalkaloids in the tubers is genetically controlled and varies widely among cultivars (Sanford & Sinden, 1972; Dimenstein *et al.*, 1997). Besides α-solanine and α-chaconine, other glycoalkaloids occur in other plant organs as well. For example, accession 80-1 of *S. chacoense* has a high content of leptine in the foliage. This trait is attempted to be introgressed for resistance against the Colorado potato beetle, which should reduce the use of pesticides (Medina *et al.*, 2002).

Introgression of heat tolerance can concur with an obviously unintentional introduction of high levels of total glycoalkaloids (TGA) in the tubers. However, heat tolerance is not necessarily associated with high glycoalkaloid content in the tubers and it is feasible to detect heat tolerant clones that will not accumulate excessive glycoalkaloid levels in the tubers when exposed to heat stress (Veilleux *et al.*, 1997).

Therefore, all promising clones and new cultivars should be examined for their TGA-content in the tubers. The Israeli Extension Service performs trials for many European seed companies for TGA-content in the tubers under the Israeli climatic conditions.

In Israel, the consciousness among potato growers about glycoalkaloids in potato tubers increased at the end of the 1980s. The cultivar Atica, which was widely grown for export, was reported to have a bitter taste in a particular season. Examinations revealed relatively high levels of TGA in the tubers. This cultivar is not grown in Israel anymore, and cultivars exhibiting a tendency to accumulate glycoalkaloids in the tubers are routinely discarded (Z. Dar, pers. comm.).

DNA markers for tuber TGA-content

High levels of TGA-content in the tubers are undesirable. The procedure for obtaining a reliable estimate for TGA-content, as carried out in Israel, is time consuming and laborious. Promising clones are planted for several years in both spring and winter season, and samples are examined for TGA-content. The procedure takes about one working week per sample, although several samples can be processed simultaneously.

In specifically selected populations, differences in fingerprint patterns can be correlated to specific phenotypes. Defined probes can be subsequently used for a marker-assisted selection (MAS). By the use of such markers, a repeated test under field conditions can be replaced, thus speeding up the selection process (Wenzel *et al.*, 2000). DNA markers could alleviate the direct TGA analysis. In theory, when the DNA markers cover all major genes and gene interactions, an assessment could be made of the potential TGA-content in the tubers of each clone. This implicates that analysing a DNA sample extracted from young leaves of a seedling grown from true potato seed (TPS), could be used for the assessment of the potential TGA-content in the tubers before the first tuber has been formed. This would speed up breeding considerably and reduce the need for repeated field trials, consequently reducing the cost of development of new cultivars. Presently, marker-assisted selection works efficiently for traits encoded by a single gene. The application of DNA markers for more complex characteristics with a quantitative inheritance (QTLs) is yet to be fully evaluated (Wenzel *et al.*, 2000).

Challenges

The tetraploid nature of all modern potato cultivars complicates the process of breeding, in comparison to diploid species. The complication is caused by four pairs of chromosomes that take part in the meiosis in tetrasomic inheritance. In the most

extreme case, eight different alleles can undergo mitotic segregation. This means that the outcome of a cross is less predictable due to the high number of possible allelic permutations during meiosis.

Potato is characterised by a strong self-incompatibility. Selfing occurs occasionally, but the plants are weak. This makes the breeding of near isogenic lines impossible. Both tetrasomic inheritance and self-incompatibility limit the ability to regulate and control breeding and selection. This is even more complicated for multigenic traits, where several genes affect the characteristics. DNA markers could potentially enhance breeding by the identification and selection of genetic material in an early stage of crop growth, and thus speed up the development of new cultivars.

Outline of the thesis

This thesis can be divided into two major complementary parts. The first part (Chapters 2 and 3) addresses the quantitative genetic characterisation of two resource populations, designed for the identification of DNA markers associated with TGA-content in the tubers. Before any attempt to identify DNA markers, knowledge should be gained about the genetics of the characteristic studied. The second part (Chapters 4 and 5) addresses the biomolecular analyses of these two populations leading to the identification of DNA markers associated with tuber TGA-content and vein depth of leaflets.

Chapter 2 describes a genetic analysis of TGA-content and briefly of some agronomic and morphological traits, which were carried out on two segregating populations representing the first clonal generation. Genetic parameters related to TGA-content were estimated, and the suitability of these resource populations to develop DNA markers associated with TGA-content was assessed. Other traits described in this chapter were assessed for their possible genetic association with tuber TGA, which could thus serve as morphological markers. Moreover, these traits give some indication of the populations in which genetic control was described for TGA-content. Whereas in Chapter 2 other traits were described briefly as in the context of TGA-content, in Chapter 3 over 30 agronomic and morphological traits were analysed extensively from the two experiments that were carried out. Genetic parameters related to these traits, such as tuber weight, tuber number, tuber dormancy, plant height, terminal leaflet shape, and the number of inflorescences were assessed.

Chapter 4 describes an association between TGA-content in the tubers and several PCR-based DNA markers, generated by Inter-Simple Sequence Repeats (ISSR) primers. The association consisted of a single ISSR product and an interaction between two loci represented by PCR products. The involvement is shown of an interaction

between two loci in the control of glycoalkaloid metabolism in potato tubers. In Chapter 5, a DNA marker is presented for the morphological characteristic of leaflet vein depth, which is a spin-off from the search for molecular markers for TGA-content in the tubers. To understand the biological relevance of the alleles detected, the findings are discussed within the framework of the morphogenesis of the leaf and a tetrasomic inheritance. We found this characteristic appropriate to show the feasibility of generating molecular markers at this ploidy level.

Chapter 6 describes some background of the parental clones that were used in the experiments conducted for this study.

In the general discussion of the thesis (Chapter 7) I will review overall results and interpretations of DNA markers associated with TGA-content in the tubers.

CHAPTER 2

Genetic characterisation of tetraploid potato (*Solanum tuberosum* L.) emphasising genetic control of total glycoalkaloid content in the tubers[*]

Jacob van Dam[1], Ilan Levin[1], Paul C. Struik[2] & David Levy[1]

[1] Institute of Field and Garden Crops, The Volcani Center, POB 6, Bet Dagan 50250, Israel

[2] Department of Plant Sciences, Crop and Weed Ecology Group, Wageningen University, Haarweg 333, 6709 RZ Wageningen, The Netherlands

Abstract

Two segregating resource populations were constructed, one of 216 clones using the tetraploid clones Cara and LT7 as parents and one of 176 clones using the tetraploid clones NT8 and LT7 as parents. The populations were used, in replicates, to estimate genetic parameters for total glycoalkaloid (TGA) content in the tubers, and for other agronomic and morphological traits such as tuber weight, maturity and plant height.

In both populations, the parental clones differed markedly in TGA-content and the progeny population was normally distributed for this trait after logarithmic transformation. Broad sense heritability estimate of TGA-content was 0.54 in Cara × LT7 and 0.50 in NT8 × LT7 and the trait proved to be inherited in a non-dominant manner. The minimum number of genes contributing to TGA-content was estimated to be between 3 and 7. None of the other traits recorded in this study showed a statistically significant genetic association with TGA-content. This suggests that tuber TGA-content may be genetically modulated without any significant adverse effects on other agronomic traits. The genetic parameters estimated in this study indicate that these populations are suitable for DNA-marker analysis for TGA-content in tubers.

Key words: *Solanum tuberosum* L., glycoalkaloids, broad sense heritability, genetic correlation

Dagan, 50250, Israel. No. 114/99.

Introduction

Breeding potatoes adapted to hot and dry climates is considered to be one of the main objectives of modern potato breeding programmes (Caligari, 1992a). To obtain cultivars adjusted to such conditions, wild potato germplasms are often being used for the introgression of genes encoding heat-tolerance. Such introgressions often cause increased levels of total glycoalkaloids (TGA) in the potato tubers, as several of these wild germplasms contain naturally high TGA-levels. High levels of TGA may be toxic to humans and cause an undesirable taste (Storey & Davies, 1992). Levels of TGA should therefore be included as a major selection criterion in introgression programmes utilising species containing high TGA-levels as donors of genes, such as *Solanum vernei* which contains genes encoding resistance to potato cyst nematodes (Van Gelder & Scheffer, 1991) and *Solanum chacoense* which contains genes encoding for heat-tolerance (Veilleux *et al*., 1997). It is generally agreed that average TGA-levels in the tubers should remain below 20 mg per 100 g fresh weight (FW). It has been suggested, however, that breeding should aim at maximum TGA-levels of 6 - 7 mg per 100 g FW due to consumer-safety considerations (Van Gelder, 1989).

Content of TGA, like many other economically important traits, is determined by several genes (Dale & Mackay, 1994). Extensive field trials and several years of testing are, therefore, required to quantify TGA-content. Also, TGA-content is assessed in mature tubers and requires a costly and laborious biochemical analysis. This analysis is particularly inefficient and expensive when large numbers of seedlings need to be analysed as is usually the case in typical breeding programmes. Genetic markers could potentially alleviate these problems, enabling the identification and selection of genetic material at an early stage of crop growth.

Several selection criteria can be used in practical breeding:

- *Morphological traits*, characterised by discrete distribution that can be measured with minimal experimental error.
- *Quantitative traits* that are characterised by an exceptionally high heritability and encoded by genes that co-segregate with a trait of interest.
- *Molecular markers*, such as isozymes, RNA and DNA markers, characterised by a discrete distribution similar to morphological traits.

In an effort to identify genetic markers linked to genes encoding TGA-content, two segregating resource populations were designed and constructed. The tetraploid parental clones used, were LT7 that is characterised by high average TGA-content, and two clones, Cara and NT8 that share relative low levels of TGA. (Dimenstein *et al*., 1997). The search for genetic markers at the tetraploid level is important, since the relevance of markers identified at the mono- and diploid level to the performance of

cultivated tetraploid species is not clear (Bradshaw, 1994).

Analysis of TGA-content and of agronomic and morphological traits was carried out on two segregating populations representing the first clonal generation. Genetic parameters related to TGA-content were estimated, and the suitability of these resource populations to develop DNA markers linked to TGA-content was assessed.

Materials and methods

Plant material and culture

Two segregating potato populations were constructed, one population using the tetraploid clones Cara and LT7, and a second population using NT8 and LT7 as parents. The parental clones were chosen on the basis of their known divergent tuber TGA-content and genetic background. LT7 is characterised by a high TGA-content in the tubers (Dimenstein *et al.*, 1997), and both Cara and NT8 are characterised by a low TGA-content in the tubers (Dimenstein *et al.*, 1997; Levy *et al.*, 1995). Cara is an Irish cultivar from the Oak Park Research Center (Stegemann & Schnick, 1985), LT7 is a clone from the International Potato Center (CIP), Peru (International Potato Center, 1982), and NT8 is a neotuberosum clone from the Cornell University, USA. Cara, LT7 and NT8 are unrelated, late maturing and have fair yields under Israeli climatic conditions (Levy *et al.*, 1991; K.M. Paddock, pers. comm., 1998).

Plants from true potato seed (TPS) originated from the two crosses were grown in a greenhouse and tubers were obtained in two separate experiments. After harvest, tubers of the progeny clones were kept in storage under ambient conditions and were stored in a cold store (4 °C) as soon as sprouts emerged. The clones were transferred from the cold store to room temperature one week before planting. The experimental design was a complete randomised block design with three blocks containing one replicate of each of the available progeny clones. On 15 September 1995, 216 progeny clones of Cara × LT7 were planted, and on 18 September 1997, 176 progeny clones of NT8 × LT7 were planted in Bet Dagan, Israel. Also, five replicates of each parental clone per block were included in each of the two experiments. A tuber of about 10 g was planted per replicate in potting soil in 10 l pots at 1 cm depth. Pots were placed in a 50 mesh screenhouse under ambient conditions. To prevent stress, water and nutrients were supplied twice a day by means of drip irrigation. The longest main stem was trained on a string. The plants were kept healthy throughout the growing season. Tubers were harvested when 95% of the leaves had senesced naturally. The tubers were stored under ambient conditions loosely covered with a black plastic sheet, and were regularly monitored till sprouts emerged and then moved to the cold store.

Observations

All observations were made on single plants. In total, more than thirty different variables were observed or assessed, representing a broad range of different quantitative and qualitative traits (Table 1). The quantitative traits were total glycoalkaloid (TGA) content in the tubers and agronomic traits, such as tuber weight and tuber number. The qualitative traits were morphological traits that are easy to record, such as plant height and depth of leaflet vein. The assessment of these traits, except TGA-content, will be described in detail in Chapter 3.

TGA-content, expressed as mg solanine per 100 g FW tuber tissue, was assessed within 2 to 6 months after cold storage. TGA-content in tubers was hardly affected by storage (data not shown). The chemical analysis was carried out according to Dimenstein *et al.* (1997), a procedure based on the colorimetric Van Gelder method (Morgan *et al.*, 1985; Van Gelder, 1989). To avoid the confounding effect of tuber size on TGA-content (Wolf & Duggar, 1946; Storey & Davies, 1992), only size class 2 tubers (35 - 60 mm) were used, since most progeny clones included tubers of this size class.

Quarters of five different tubers (or less, subject to availability) were sliced in longitudinal direction and blended. Ten gram samples were taken and placed for 30 min. in methanol:chloroform (2:1, v:v), and filtrated thereafter. To the filtrate, 0.8% aqueous solution of sodium sulphate was added twice to separate the methanol and chloroform layers. The upper methanolic layer containing TGA was evaporated, and the residue was rinsed twice with 5% acetic acid, then 25% aqueous solution of ammonium hydroxide was added. The reaction mixture was heated to 70 °C and TGA were allowed to precipitate overnight at 4 °C. The precipitate was dried, dissolved in 0.5% aqueous solution of phosphoric acid, and Clark reagent was added. Absorbancy was measured using a spectrophotometer and TGA-content was calculated based on α-solanine as standard.

Data analysis

Statistical analyses were carried out using the statistical package JMP Statistical Discovery Software (version 3.1.5, SAS Institute). The effect of the parental clones was analysed using a cross classified analysis of variance. Main effects in the model included the assignment of clone, block and clone × block interaction. Analyses of the progeny clones were carried out using a randomised block design with no interaction, because each clone was represented by one replicate per block.

Broad sense heritability, phenotypic-, genetic- and environmental correlation between TGA-content and all other traits recorded was calculated as described by Falconer (1989). The Shapiro-Wilk W-test was used to calculate whether the

Table 1. Agronomic and morphological traits recorded. Scores assigned are 1 (very small/weak) to 9 (very large/strong).

Trait	Distribution	Abbreviation
Agronomic traits:		
Total tuber glycoalkaloid content (mg /100 g fresh tuber)	Continuous	TGA
Total tuber weight (g)	Continuous	TWT
Total number of tubers	Discontinuous	TNT
Maturity (days after planting)	Continuous	MT
Morphological traits:		
Plant height (cm)	Continuous	HT
Total number of nodes main stem	Discontinuous	NNT
Depth of veins	Score 1-9	DV
Waviness of leaf margin	Score 1-9	WM
Necrotic leaf spots	Score 1-9	NLS

distribution frequency for each trait in the progeny was normal. Traits were also tested for normality of the distribution frequency after transformation to common logarithm ($\log_{10}$). A trait that was characterised by normal distribution after transformation to the common logarithm was analysed using transformed values.

The minimum number of genes contributing to TGA-content was estimated, based on Wright's method (Lande, 1981) with a few modifications, because the method was designed for diploid species. In the calculation, the variance of the F_1 was used instead of the variance of the F_2, because the distribution of values in a tetraploid F_1 is similar to that of a diploid F_2. Also, variances of both parental clones were assumed to be equal. *Solanum tuberosum* L. is generally assumed to behave as autotetraploid, although the use of other species may turn the behaviour of clones to a more allotetraploid one (Hawkes, 1992). Therefore, the outcome using Wright's method was adjusted for the extra number of possible chromosomal combinations in the meiosis compared to diploids, i.e. division by 2 for complete allotetraploid-behaviour and division by 4 for complete autotetraploid-behaviour.

Results and discussion

Characterisation of the populations
Means, standard errors and the statistical significance of the components of variation

included in the analysis of the parental clones of the cross Cara × LT7 are presented in Table 2. Comparison of the parental clones, Cara and LT7. Presented are means, standard errors (SE), and significance probabilities for clone, block, and clone × block effect. Abbreviations are explained in Table 1.

Trait	Cara		LT7		Source of variation		
					Clone	Block	C × B
	Mean	SE	Mean	SE	p(F) [a]	p(F) [a]	p(F) [a]
Agronomic traits:							
TGA	5.98	0.84	43.8	4.1	0.000	0.759	0.868
TWT	1107	38	954	29	0.007	0.804	0.901
TNT	10.7	1.0	12.6	1.1	0.087	0.028	0.000
MT	129	2.2	126	1.7	0.272	0.058	0.639
Morphological traits:							
HT	96.9	2.5	94.2	2.54	0.789	0.752	0.604
NNT	30.7	0.65	28.2	0.76	0.055	0.157	0.715
DV	3.00	0.00	2.93	0.07	0.327	0.383	0.383
WM	3.13	0.22	3.80	0.37	0.149	0.704	0.704
NLS	4.60	0.29	5.80	0.20	0.003	0.732	0.274

[a] $P < 0.05$ indicates a statistically significant effect.

Table 2. Results of similar analyses carried out on the progeny clones of the cross Cara × LT7 are presented in Table 3. Also included in Table 3 are the Shapiro-Wilk W-test for the normality of distribution before as well as after logarithmic transformation. Means and standard errors obtained for the NT8 × LT7 population were similar and are therefore not shown. Comparisons between broad sense heritability estimates obtained separately for each of the two populations in this study and estimates reported elsewhere are presented in Table 4. Highest phenotypic-, genetic- and environmental correlations obtained between the transformed values of TGA-content and traits recorded on Cara × LT7 progeny clones are presented in Table 5. Figure 1 shows the frequency distribution of the mean values of TGA-content within the progeny after logarithmic transformation.

Genetic estimates, such as heritability values, obtained in this study may be biased by genotype × environment interactions due to the variance component method used (Yildirim & Çalişkan, 1985), and estimates obtained may apply specifically to the germplasm used (Dudley & Moll, 1969; Falconer, 1989). Nevertheless, results and genetic estimates obtained in this study usually agree with results obtained elsewhere. The parental clones used in this study were chosen on the basis of the marked

difference between their average TGA-content. This difference was confirmed in this

Table 3. Analysis of Cara × LT7 progeny clones. Presented are means, standard errors (SE), significance probabilities for clone and block effect, proportion of total variation accounted for by the statistical model used (R^2), normality of the frequency distribution of means before and after transformation to common logarithm. Abbreviations are explained in Table 1.

Trait	Mean	SE	Clone p(F) [a]	Block p(F) [a]	R^2	Normal distribution p(W) [b]	Normal distribution log_{10} p(W) [b]
Agronomic traits:							
TGA [c]	19.0	0.75	0.000	0.000	0.73	0.000	0.395
TWT	996	14.9	0.000	0.000	0.66	0.946	0.000
TNT [c]	15.7	0.38	0.000	0.953	0.56	0.000	0.899
MT	130	0.46	0.000	0.003	0.59	0.000	0.000
Morphological traits:							
HT	92.3	1.05	0.000	0.243	0.65	0.877	0.335
NNT	29.6	0.19	0.000	0.252	0.58	0.183	0.000
DV	3.27	0.04	0.000	0.054	0.56	0.000	0.000
WM	3.78	0.08	0.000	0.000	0.66	0.000	0.000
NLS	4.55	0.10	0.000	0.000	0.64	0.297	0.000

[a] $P < 0.05$ indicates a statistically significant effect; [b] $P \geq 0.05$ indicates a normal distribution; [c] analysis of variance carried out on values transformed to a common logarithm.
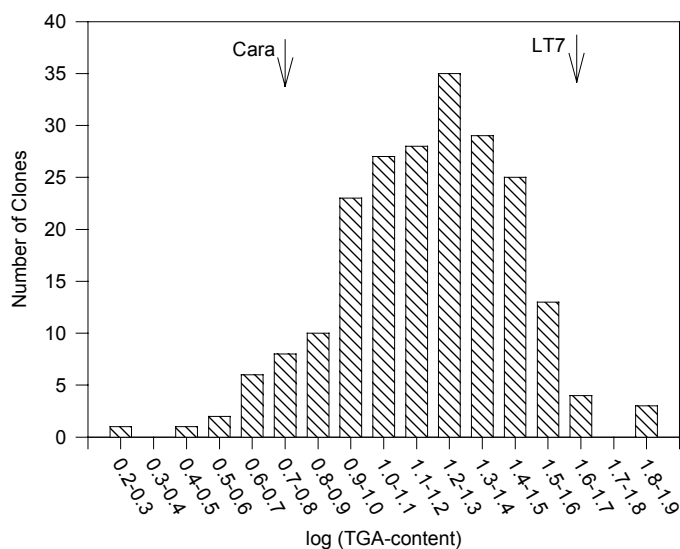

Figure 1. Frequency distribution of mean TGA-content (mg/100 g FW) observed in the progeny population after transformation to a common logarithm. Arrows indicate the mean values of

parental clones.

Table 4. Comparison of broad sense heritabilities estimated in this study with estimates reported elsewhere.

Trait	Cara × LT7 h^2_{BS}	NT8 × LT7 h^2_{BS}	Reported h^2_{BS}	Reference	Experimental design
Agronomic traits:					
TGA	0.54 [a]	0.50 [a]	0.86-0.89	Sanford & Sinden (1972)	10 crosses, 2 years
			0.25-0.26	Ross *et al.* (1978)	3 clones, 4 years, 5 locations
TWT	0.45	0.24	0.59	Chaudhary & Sharma (1984)	339 F_1C_2 clones
			0.33	Gopal *et al.* (1994)	135 F_1C_3 clones
			0.55	Du Plooy *et al.* (1996)	14 clones, 2 locations, 3 planting dates
TNT	0.44 [a]	0.48	0.67	Chaudhary & Sharma (1984)	339 F_1C_2 clones
			0.30	Gopal *et al.* (1994)	135 F_1C_3 clones
			0.64	Du Plooy *et al.* (1996)	14 clones, 2 locations, 3 planting dates
MT	0.33	0.17	0.58	Tai & Young (1984)	20 clones, 3 years
			0.62	Lynch & Kozub (1988)	9 clones, 3 years, 5 locations
Morphological traits:					
HT	0.42	0.23	0.58	Tai & Young (1984)	20 clones, 3 years
			0.63	Yildirim & Çalişkan (1985)	11 clones, 2 year, 3 locations
NNT	0.31	0.20	n.a.		
DV	0.31	0.30	n.a.		
WM	0.43	0.31	n.a.		
NLS	0.40	n.a.	n.a.		

[a] broad sense heritability estimated after transformation to common logarithm.

n.a. not available.

Table 5. Phenotypic-, genetic- and environmental correlation with TGA-content after transformation to common logarithm of Cara × LT7, with at least one correlation $\geq |0.15|$. Abbreviations are explained in Table 1.

Correlated trait	Phenotypic correlation	Genetic correlation	Environmental correlation
NLS	0.08	−0.01	0.15
MT	−0.16	0.00	−0.27
TNT	0.07	−0.01	0.15
TWT	0.23	0.03	0.32

study (P < 0.0001, Table 2). Although few other traits, such as TWT, significantly differed between the parental clones, most of the traits recorded, covering a broad range of agronomic and morphologic characteristics, did not show such a statistical difference (Table 2). These traits, on the other hand, differed statistically between the progeny clones (P < 0.0001, Table 3). These results suggest that these traits are subjected to dominant rather than additive variance and confirm results of Golmirzaie *et al.* (1994), who reported that genes with non-additive effects determine many characters of interest in potatoes.

Estimates of broad sense heritability obtained in this study in both populations (Table 4) are each based on variation within a single full sib family. Heritability estimate of TGA-content was 0.54 in Cara × LT7, and 0.50 in NT8 × LT7. These estimates were within the range of estimates reported elsewhere, as were most other heritability estimates obtained in this study.

Total glycoalkaloid content
Total glycoalkaloid (TGA) content in the tubers differed markedly between the parental clones used (P < 0.0001, Table 2). Other traits that also differed significantly (P < 0.05) between the parental clones may suggest a co-segregation between genes encoding these traits and genes encoding TGA-content. However, the genetic correlation between TGA-levels and the broad range of traits recorded in this study were exceptionally low and insignificantly different from zero (Table 5).

Significant interactions between cultivar and environment have been demonstrated for TGA-content (Dale & Mackay, 1994). However, a cultivar with a relatively high level of TGA under one set of environmental conditions will tend to have high levels wherever it is grown (Sinden *et al.*, 1984; Dale & Mackay, 1994). This was supported by the statistically insignificant genotype × block interaction observed in this study using the parental clones (Table 2).

Higher than normal levels of glycoalkaloids tend to persist among several of the offspring resulting from breeding programmes introducing germplasm from wild species (Valkonen *et al.*, 1996). Three clones resulting from Cara × LT7 had an exceptionally high TGA-content (> 65.3 ± 6.2 mg/100 g FW). However, clones containing high levels of TGA do not necessarily have to be discarded from breeding programmes. For example, the notorious cultivar Lenape, removed from commerce due to its high TGA-content, was the male parent of the cultivar Atlantic, which did not exceed TGA-content of 18.0 mg/100 g FW in repeated tests (Sinden *et al.*, 1984).

Most of the progeny (95%) had an intermediate TGA-content ranging between the average values of the parental clones. A transformation to common logarithm was used for TGA-content, because clones with a higher mean TGA-content were more variable than clones with a lower content (scale effect). After transformation, TGA-content was closer to a normal distribution (Figure 1, Table 3), which confirms findings by Sanford & Sinden (1972). The frequency distribution of raw data indicates a skewed distribution towards low TGA-content (data not shown). Similar behaviour was noted by Ross (1966), who suggested that most cultivars and breeding hybrids have two or more dominant alleles for low solanine synthesis. However, when adjusting for the scale effect (Figure 1), a normal distribution pointing towards a non-dominant mode of inheritance for TGA-content was demonstrated. Other workers could not confirm Ross' findings either (Dale & Mackay, 1994). Sanford & Sinden (1972) reported that TGA-content seems to be genetically controlled in a polygenic manner. Using Wright's method (Lande, 1981), the minimum number of genes contributing to TGA-content in this population was estimated to be 3 to 7.

Our results demonstrate that TGA-content is controlled by a relatively low number of genes and is characterised by a relatively high heritability estimate. Our results also suggest that TGA-content is not genetically associated with a large number of traits representing important agronomic and morphological characteristics. It is therefore concluded that TGA-content may be genetically modulated without any significant adverse effects on other agronomic traits, and that the search for DNA markers for TGA-content in the tubers is justified. The number of genes estimated, combined with the relatively high broad sense heritability estimates obtained in this study for the two populations analysed indicate that this populations are suitable for DNA-marker analysis for TGA-content in the tubers.

CHAPTER 3

Genetic characterisation of two tetraploid potato (*Solanum tuberosum* L.) resource populations for genetic control of agronomic and morphological traits[*]

Jacob van Dam[1], Ilan Levin[1], Paul C. Struik[2] & David Levy[1]

[1] Institute of Field and Garden Crops, The Volcani Center, POB 6, Bet Dagan 50250, Israel
[2] Department of Plant Sciences, Crop and Weed Ecology Group, Wageningen University, Haarweg 333, 6709 RZ Wageningen, The Netherlands

Abstract

Two segregating resource populations were constructed, one of 216 clones using the tetraploid clones Cara and LT7 as parents and one of 176 clones using the tetraploid clones NT8 and LT7 as parents. The populations were used, in replicates, to estimate genetic parameters for over 30 agronomic and morphological traits, including tuber weight, tuber dormancy and plant height. Broad sense heritability was estimated for all traits assessed. Heritability estimates that were similar in both populations ranged from 0.1 for the number of main stems to 0.5 for total tuber glycoalkaloid content. In both populations, most heritabilities estimated were about 0.3. Main agronomic traits, such as tuber dormancy, tuber weight, and maturity, appeared to be controlled by several additive genes. Typical morphological traits, such as leaf size and number of leaflets per leaf, appeared to be controlled by dominance gene effects.

Key words: Population genetics, broad sense heritability, plant morphology

Introduction

New potato cultivars are generally produced by cross-pollination followed by vegetative propagation. They should comprise yield traits, including morphological and physiological factors, resistance to pests and diseases, and quality traits with respect to different utilisation purposes (Ross, 1986). Agronomic traits, such as tuber number and tuber weight, are important components that define the attainable economical value of a potato cultivar within a particular environment and for a specific market. Knowledge of the inheritance of each of these traits can help the breeder to choose the best parents and to make proper selections from their progeny, either by identifying promising offspring or discarding inferior genotypes.

The economically interesting parts of the potato crop are obviously the tubers. Therefore, traits that are not directly related to tubers may seem unimportant. However, morphological characteristics could be linked to economically important traits. Due to the complexity of many agronomic traits, which are mostly controlled by many genes, such links can simplify breeding by using them as morphological markers. An advantage of the use of such morphological markers is that they can be obtained quickly and reliably by simple, reproducible observations. Knowledge of the genetics of such morphological characteristics is therefore useful. Ortiz & Huamán (1994) pointed out that despite the tremendous amount of phenotypic variability that exists in morphological and tuber characteristics of the tuber-bearing *Solanum* species, knowledge of the mode of inheritance of several of these traits is still scarce. The main reasons are the tetrasomic inheritance and the strong inbreeding depression, which makes the production of inbred lines difficult. Moreover, a better understanding of the inheritance of both tuber traits and above-ground morphology will enhance the overall understanding of potato genetics.

In this study, analyses of agronomic and morphological traits were carried out on two segregating populations after clonal propagation. Genetic parameters, such as broad sense heritability, frequency distribution, mid-parent value and progeny mean were estimated for each trait. The inheritance of these traits is discussed, based on these genetic parameters.

Materials and methods

Plant material and culture
Two segregating potato populations were constructed, one population Cara × LT7, and a second population NT8 × LT7. The tetraploid parents were chosen on the basis of their known divergent tuber total glycoalkaloid (TGA) content and genetic

background. Methods, results and discussion related to TGA-content were described at length by Van Dam *et al.* (1999), and will not be discussed further in this paper. LT7 is characterised by a high TGA-content in the tubers (Dimenstein *et al.*, 1997; Van Dam *et al.*, 1999), and both Cara and NT8 are characterised by a low TGA-content in the tubers (Levy *et al.*, 1995; Dimenstein *et al.*, 1997; Van Dam *et al.*, 1999). Cara is an Irish cultivar from the Oak Park Research Centre (Stegemann & Schnick, 1985). LT7 is a clone from the International Potato Center (CIP), Peru (International Potato Center, 1982). NT8 is a neotuberosum clone, selected in Israel from true seed received from Cornell University, Ithaca, NY, USA. Cara, LT7 and NT8 are unrelated, late maturing and have fair yields under Israeli climatic conditions (Levy *et al.*, 1991; K.M. Paddock, pers. comm., 1998).

Specific experimental procedures

Experiment 1 Plants grown from true potato seed (TPS) originated from the cross Cara × LT7, were grown in a greenhouse. After harvest, the F_1C_1 tubers of the progeny clones were kept in storage under ambient conditions and were stored in a cold store (4 °C) as soon as first sprouts emerged. The clones were transferred from the cold store to room temperature one week before planting. The experimental design was a complete randomised block design with three blocks each containing one replicate of each of the available progeny clones. On 15 September 1995, 216 progeny clones of Cara × LT7 were planted in Bet Dagan, Israel.

Experiment 2 Plants grown from an F_1C_1 originated from the cross NT8 × LT7, were grown in a screen house. The tubers were planted in March 1997. When both parental clones and all progeny clones had formed small tubers, they were harvested immature in one day. These F_1C_2 tubers were kept in storage under ambient conditions and were stored in a cold store (4 °C) as soon as first sprouts emerged. The clones were transferred from the cold store to room temperature one week before planting. The experimental design was a complete randomised block design with three blocks each containing one replicate of each of the available progeny clones. On 18 September 1997, 176 progeny clones of NT8 × LT7 were planted in Bet Dagan, Israel.

General experimental procedures
In both experiments, five replicates of each parental clone per block were included. A tuber of about 10 g was planted per replicate in potting soil in 10 l pots at 1 cm depth. Pots were placed in a 50 mesh screenhouse under ambient conditions. To prevent stress, water and nutrients were supplied twice a day by means of drip irrigation. The

longest main stem was trained on a string. The plants were kept healthy throughout the growing season. Tubers were harvested when 95% of the leaves had senesced naturally. For the assessment of tuber dormancy, tubers were kept under ambient conditions loosely covered with a black plastic sheet. When sprouts of about 2 mm were observed, tubers were moved to the cold store.

Observations

All observations were made on single plants. In Experiment 1, 37 different variables and in Experiment 2, 31 variables were assessed, representing a broad range of different quantitative and qualitative traits (Table 1). The quantitative traits were agronomic traits, such as tuber weight and tuber number. The qualitative traits were morphological traits, such as depth of leaf vein, leaf coalescence and terminal leaflet shape.

Most morphological traits were visually assessed during growth of the plants, mostly by assigning a score on a scale from 1 (very small/weak) to 9 (very large/strong). This method is a common practice among breeders (Caligari, 1992a) and it is recommended for morphological characteristics by UPOV (International Union of Protection of New Varieties of Plants) for the description of cultivars for Plant Breeders' Rights (UPOV, 1986). Length of the dormancy period was defined as number of weeks from harvest until 50% of the tubers had at least one sprout of > 2 mm at ambient temperatures. Scores from 1 (very short) to 9 (very long) represent the shortest (9 weeks) to the longest (20 weeks) dormancy period of the progeny. Tuber number and tuber weight were recorded directly after harvest. The tubers were graded into four size (diameter) classes: class 1 = 10-35 mm (minitubers), class 2 = 35-60 mm (seed tubers), class 3 = 60-85 mm (ware tubers), and class 4 = >85 mm (oversized tubers). The method for classifying tuber number distribution and tuber size distribution is explained in Figure 1.

Maturity was calculated as the number of days from planting to yellowing of 95% of the leaves (senescence). Secondary growth of tubers (knobbiness), a physiological disorder, was visually assessed by a score from 1 (very weak) to 9 (very strong). Plant height was measured and total number of nodes was counted as well as the number of nodes till first, second and third inflorescence, if present. For terminal leaflet shape, a scale was assigned from 1 to 3, representing length to width ratio (1=elliptic, 2=ovate, 3=orbicular). For leaf coalescence, a zero value (0) was also included to represent a complete absence, in addition to values ranging from 1 (very weak) to 9 (very strong). Necrotic leaf spots (NLS), a physiological disorder, was visually assessed by a score from 1 (very weak) to 9 (very strong).

Table 1. Agronomic and morphological traits recorded in Experiments 1 and 2. Scores assigned are 0 (absent) and 1 (very small/weak) to 9 (very large/strong), unless stated differently. Tuber size classes are Class 1 = 10-35 mm (minitubers), Class 2 = 35-60 mm (seed tubers), Class 3 = 60-85 mm (ware tubers), Class 4 = >85 mm (oversized tubers).

Trait	Distribution	Abbreviation	Experiment
Agronomic traits:			
Dormancy	Score 1-9	TDM	Exp 1
Stem number	Discontinuous	SN	Exp 1, Exp 2
Total number of tubers	Discontinuous	TNT	Exp 1, Exp 2
Tuber number per size class	Discontinuous	TNC1-4	Exp 1, Exp 2
Tuber number distribution	Score 1-9	TND	Exp 1, Exp 2
Total tuber weight (g)	Continuous	TWT	Exp 1, Exp 2
Tuber weight per size class (g)	Continuous	TWC1-4	Exp 1, Exp 2
Tuber size distribution	Score 1-9	TSD	Exp 1, Exp 2
Maturity (days after planting)	Continuous	MT	Exp 1, Exp 2
Tuber secondary growth	Score 1-9	TSG	Exp 1, Exp 2
Tuber eye depth	Score 1-9	TDE	Exp 1, Exp 2
Total tuber glycoalkaloid content (mg /100 g fresh tuber)	Continuous	TGA	Exp 1, Exp 2
Morphological traits:			
Plant height (cm)	Continuous	HT	Exp 1, Exp 2
Total number of nodes main stem	Discontinuous	NNT	Exp 1, Exp 2
Number of nodes till 1st/ 2nd/ 3rd inflorescence	Discontinuous	NNI1-3	Exp 1
Average node length [a]	Continuous	ANL	Exp 1, Exp 2
Stem thickness of main stem	Score 1-9	ST	Exp 1
Leaf size	Score 1-9	LS	Exp 1, Exp 2
Leaf compactness	Score 1-9	LC	Exp 1
Number of leaflets per leaf	Score 1-9	LLN	Exp 1
Terminal leaflet size	Score 1-9	LLS	Exp 1, Exp 2
Terminal leaflet shape	Score 1-3 [d]	LSH	Exp 1
Depth of veins	Score 1-9	DV	Exp 1, Exp 2
Waviness of leaflet margin	Score 1-9	WM	Exp 1, Exp 2
Frequency of coalescence between terminal and axillary leaflet [b]	Score 0-9	CAF	Exp 1, Exp 2
Frequency of coalescence between axillary leaflets [b]	Score 0-9	CBF	Exp 1, Exp 2
Intensity of CAF [c]	Score 0-9	CAI	Exp 1, Exp 2
Necrotic leaf spots	Score 1-9	NLS	Exp 1, Exp 2
Number of inflorescences	Discontinuous	NI	Exp 1

[a] calculated: HT/NNT;

[b] proportion of leaves per plant;

[c] proportion of coalesced leaflets;

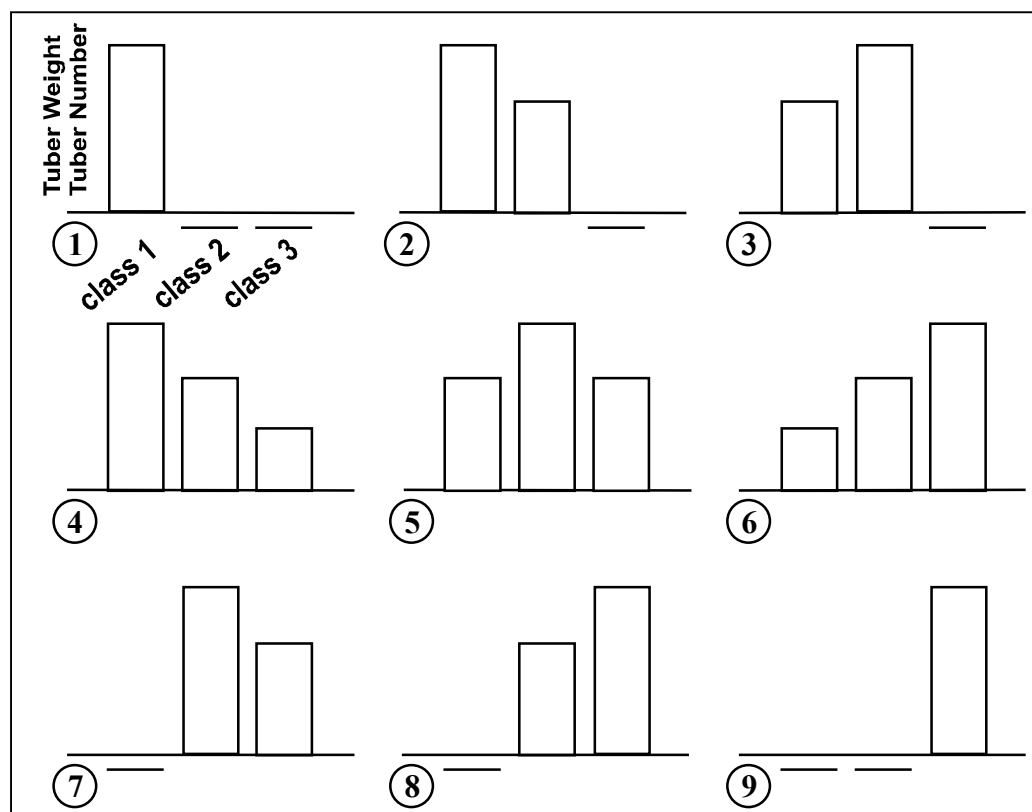[d] 1=elliptic, 2=ovate, 3=orbicular.

Figure 1. Scores from 1 to 9 were assigned for tuber number distribution (TND) and tuber size distribution (TSD), respectively. For that purpose, tubers were graded into three size (diameter) classes: class 1 = 10-35 mm (small tubers), class 2 = 35-60 mm (seed tubers), class 3 = > 60 mm (ware tubers and oversized tubers). Distinction between ware tubers and oversized tubers (Table 1) resulted in too small subgroups. The score reflects the skewness of the distribution. Values for tuber weight or tuber number on vertical axis are arbitrary. Only a relative difference between two adjacent size classes was taken into account, not their absolute values. The relative differences among the size classes are visualised in the graphs. Score 5 represents a hardly skewed distribution shape, which in many cases was normally distributed. Extreme scores 1 and 9 represent presence of only small and only large tubers, respectively. Absence of a size class is represented by horizontal line below the base line. For TND, every single tuber ≥ 10 mm was taken into account. Absence for TSD, which has a continuous distribution, is defined as less than 1% weight compared to the adjacent weight class. It should be noted that extreme values (i.e. score 1 and 9) for size distribution are theoretical. They were not observed in the experiments conducted.

Data analysis Statistical analyses were carried out using the statistical package JMP Statistical Discovery Software (version 3.1.5, SAS Institute). The effect of the parental clones was analysed using a cross classified analysis of variance. Main effects in the model included clone, block and clone × block interaction. Analyses of the progeny

clones were carried out using a randomised block design with no interaction, because each clone was represented by only one replicate per block.

Broad sense heritability was calculated as described by Falconer (1989). The Shapiro-Wilk W-test was used to calculate whether the distribution frequency for each trait in the progeny was normal. Traits were also tested for normality of the distribution frequency after transformation to common logarithm ($\log_{10}$). A trait that was characterised by normal distribution after transformation to the common logarithm was analysed using transformed values.

Results and discussion

The two experiments described in this study were conducted in two consecutive autumn seasons in the same screenhouse and using the same equipment. The 10-day average maximum and minimum temperatures were similar in the two growing seasons (Figure 2).

For each characteristic observed, a short introduction is given, describing either its agronomical importance or a morphological explanation of the trait.
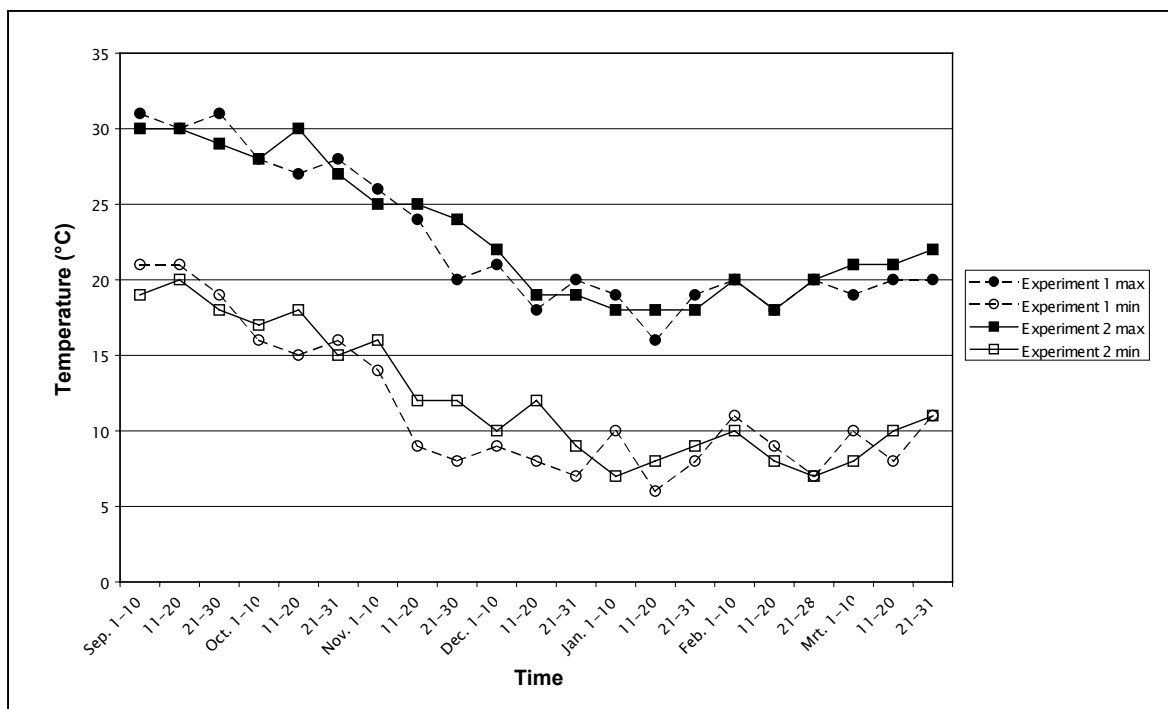

Figure 2. Maximum and minimum temperatures in seasons 1995/1996 (Experiment 1) and 1997/1998 (Experiment 2).

Agronomic traits

Tuber dormancy (TDM) This trait was only observed in Experiment 1. Tuber dormancy is the time from harvest till sprouting. The length of the dormant period is of particular importance where two crops can be grown each year (Allen *et al*., 1992), as after harvest of the first crop, dormancy has to be released before planting the subsequent second crop.

Cara and LT7 were not significantly different, and the mid-parent value was not significantly different from the progeny mean. The large range of dormancy periods among the progeny (9 to 20 weeks) and the gradual frequency distribution of the progeny within these weeks suggest that several genes control tuber dormancy. This agrees with results obtained with molecular markers. Freyre *et al*. (1994) reported in diploid potato the identification of six putative QTLs (quantitative trait loci) associated with dormancy. Van den Berg *et al*. (1996) detected QTLs on nine chromosomes that affected tuber dormancy, either alone or through epistatic interactions in reciprocal backcrosses between *S. tuberosum* and *S. berthaultii*. Frequency distribution of tuber dormancy was skewed towards lower scores. We concluded that short dormancy is dominant over long dormancy. This is in agreement with Buso *et al*. (2000), who also reported dominance for shorter tuber dormancy.

Tuber dormancy was highly heritable (0.58; Table 7), similar to results of Thompson *et al*. (1980) who reported a broad sense heritability of 0.57. This shows that tuber dormancy is mainly genetically controlled (Bogucki & Nelson, 1980; Van Ittersum, 1992; Struik & Wiersema, 1999).

Stem number (SN) The number of main stems per plant is one of the elements determining stem density, which subsequently affects tuber yield (Beukema & Van der Zaag, 1990; Allen & Wurr, 1992). It also affects tuber size and tuber number distribution (Struik & Wiersema, 1999).

In both experiments, parental clones were not significantly different and no significant difference between mid-parent value and progeny mean could be observed, although tubers of Experiment 1 were physiologically older than tubers used in Experiment 2. Besides seed age, the number of stems can be influenced by crop husbandry techniques that enhance the emergence of sprouts and their development into productive stems, such as seed size, pre-sprouting treatment, storage temperature, duration of storage and cultivar, as well as seed density (Struik & Wiersema, 1999). Broad sense heritability in this experiment appeared to be low in both experiments (0.13 and 0.16, respectively; Table 7).

Tuber number (TNT, TNC1-4) The number of tubers per plant is one of the major parameters determining tuber size distribution. The best way of manipulating tuber number is by manipulating seed rate, size of the seed tubers and their physiological age. Storage conditions and pre-sprouting treatments strongly influence the number of tubers per plant (Struik & Wiersema, 1999).

In Experiment 1, the parental clones were not significantly different in total tuber number. In size classes 2 and 4, Cara had a significantly higher tuber number than LT7 (Table 2). Tuber number in size classes 1 and 3 were not significantly different. In Experiment 2, parental clone NT8 had a significantly lower total number of tubers than LT7. This agrees with Lynch *et al.* (2001), who reported considerable differences among cultivars for the number of tubers per plant. Also in Experiment 2, NT8 had a significantly lower number of tubers than LT7 in size classes 1 and 2, but not in size class 3 (Table 3). The total number of tubers appeared mainly to be determined by the number of tubers in the lowest size class.

Tuber number is by definition discontinuous. However, when the range in numbers is large enough and there are enough units in a population, number can be considered as continuous in a frequency distribution (Falconer, 1989). In Experiment 1, frequency distributions of the progeny of both total number of tubers and tuber number of size class 1 were normal after common logarithmic transformation, but not those of size classes 2, 3 and 4 (Table 4). In Experiment 2, total tuber number had − after common logarithmic transformation − a frequency distribution somewhat skewed towards higher tuber numbers. Tuber number of size class 1 was normally distributed after common logarithmic transformation. Tuber number of size class 2 was normally distributed, but tuber numbers of size classes 3 and 4 were not normally distributed (Table 5). These results show that not only for a single clone, but also for a progeny, the frequency distribution of total number of tubers is mainly determined by the distribution of small tubers.

Heritability estimates of total number of tubers and of tuber number of the size class 1 were high (Table 7). Tuber number of the various size classes varied considerably in their heritability estimates. Wurr (1977) suggested that differences in tuber number for different genotypes do not result from a difference in number of potential tuber sites, but from some control over tuber initiation. Struik *et al.* (1990) claimed that tuber set, rather than tuber initiation, determined total tuber number. After the tubers have been set, the growth into various size grades is the result of competition among the tuber settings and growth rate of the individual tubers (Struik *et al.*, 1990; 1991).

Table 2. Comparison of the parental clones in Experiment 1, Cara and LT7. Presented are means, standard errors (SE), and significance probabilities for clone, block, and clone × block effect. Abbreviations are explained in Table 1.

Trait	Cara		LT7		Source of variation		
					Clone	Block	C × B
	Mean	SE	Mean	SE	p(F) [a]	p(F) [a]	p(F) [a]
Agronomic traits:							
TDM	3.87	0.09	3.53	0.17	0.099	0.706	0.451
SN	1.60	0.19	1.53	0.17	0.780	0.071	0.369
TNT	10.7	1.0	12.6	1.1	0.087	0.028	0.000
TNC1	2.87	0.51	3.87	0.87	0.209	0.056	0.002
TNC2	4.53	0.58	5.93	0.61	0.050	0.056	0.006
TNC3	2.87	0.43	2.80	0.34	0.893	0.041	0.157
TNC4	0.40	0.13	0.00	0.00	0.001	0.018	0.018
TND	5.08	0.19	5.00	0.31	0.810	0.063	0.157
TWT	1107	38	954	29	0.007	0.804	0.901
TWC1	27.5	5.0	26.5	5.7	0.871	0.146	0.003
TWC2	319	38	462	54	0.020	0.145	0.026
TWC3	576	82	465	62	0.280	0.184	0.452
TWC4	184	63	0	0	0.002	0.032	0.032
TSD	6.07	0.15	5.73	0.32	0.302	0.053	0.170
MT	129	2.2	126	1.7	0.272	0.058	0.639
TSG	2.11	0.35	1.60	0.31	0.125	0.032	0.614
TDE	3.00	0.00	3.80	0.44	0.099	0.182	0.182
TGA	5.98	0.84	43.8	4.1	0.000	0.759	0.868
Morphological traits:							
HT	96.9	2.50	94.2	2.54	0.789	0.752	0.604
NNT	30.7	0.65	28.2	0.76	0.019	0.149	0.572
NNI1 [b]	22.2	1.10	21.2	0.63	0.264	0.146	0.444
NNI2 [b]	28.1	1.39	28.4	1.36	0.572	0.206	0.521
NNI3 [b]	0.00	0.00	0.00	0.00	-	-	-
ANL	3.19	0.07	3.36	0.11	0.233	0.338	0.947
ST	5.00	0.39	4.42	0.25	0.240	0.533	0.271
LS	5.27	0.18	5.53	0.92	0.402	0.587	0.835
LC	5.93	0.27	4.60	0.29	0.003	0.661	0.661
LLN	5.53	0.24	5.67	0.25	0.718	0.675	0.876
LLS	5.27	0.18	5.00	0.20	0.327	0.383	0.383
LSH	2.00	0.00	2.00	0.00	-	-	-
DV	3.00	0.00	2.93	0.07	0.327	0.383	0.383
WM	3.13	0.22	3.80	0.37	0.149	0.704	0.704
CAF	1.47	0.51	1.47	0.54	1.000	0.450	0.747
CBF	0.00	0.00	1.40	0.40	0.001	0.157	0.157
CAI	1.33	0.45	1.20	0.39	0.827	0.558	0.303
NLS	4.60	0.29	5.80	0.20	0.003	0.732	0.274
NI	1.43	0.17	1.27	0.15	0.500	0.677	0.779

[a] p(F) < 0.05 indicates a statistically significant effect;
[b] analysis only with clones that comprised the particular inflorescence.

Table 3. Comparison of the parental clones in Experiment 2, NT8 and LT7. Presented are means, standard errors (SE), and significance probabilities for clone, block, and clone × block effect. Abbreviations are explained in Table 1.

Trait	NT8		LT7		Source of variation		
					Clone	Block	C × B
	Mean	SE	Mean	SE	p(F)[a]	p(F)[a]	p(F)[a]
Agronomic traits:							
SN	1.22	0.15	1.14	0.10	0.582	0.448	0.485
TNT	4.27	0.59	9.57	1.35	0.001	0.011	0.076
TNC1	1.45	0.25	4.21	0.70	0.001	0.147	0.029
TNC2	1.73	0.54	4.71	0.83	0.007	0.019	0.555
TNC3	1.09	0.31	0.64	0.20	0.207	0.070	0.348
TNC4	0.00	0.00	0.00	0.00	-	-	-
TND	3.50	0.82	3.57	0.40	0.824	0.109	0.309
TWT	297	68.5	454	68.4	0.142	0.048	0.579
TWC1	11.2	2.80	41.8	7.77	0.002	0.326	0.161
TWC2	115	43.8	315	56.5	0.013	0.024	0.827
TWC3	171	55.4	96.6	28.5	0.196	0.104	0.424
TWC4	0	0	0	0	-	-	-
TSD	4.10	0.75	4.14	0.44	0.771	0.146	0.509
MT	122	1.65	120	2.35	0.567	0.551	0.300
TSG	3.57	0.37	2.56	0.29	0.116	0.924	0.056
TDE	8.14	0.40	3.22	0.22	0.000	0.959	0.180
TGA	1.52	0.19	8.87	0.78	0.000	0.979	0.850
Morphological traits:							
HT	54.2	8.98	75.5	7.95	0.054	0.336	0.171
NNT	18.4	2.75	21.6	1.47	0.405	0.169	0.791
ANL	3.25	0.93	3.32	0.24	0.694	0.843	0.164
LS	1.22	0.15	2.08	0.08	0.000	0.972	0.279
LLS	6.33	0.33	5.00	0.00	0.000	0.158	0.158
DV	3.89	0.35	5.67	0.28	0.003	0.915	0.686
WM	5.22	0.22	5.50	0.36	0.564	0.730	0.919
CAF	5.00	0.58	1.46	0.24	0.000	0.627	0.826
CBF	1.22	0.22	1.31	0.75	0.690	0.296	0.829
CAI	4.56	0.44	1.77	0.43	0.001	0.976	0.694

[a] $p(F) < 0.05$ indicates a statistically significant effect.

Total tuber weight and tuber weights in different size classes (TWT, TWC1-4) It is obvious that total tuber weight is an important trait in the potato crop. Also the size classes are important, as each one represents both a separate economical and a physiological group. Although a size classification is by definition arbitrary, we have chosen representable classes. Size class 1 contains small tubers, which potentially can grow out to a marketable size. Size class 2 contains seed tubers, which are an

Table 4. Analysis of Cara × LT7 progeny clones in Experiment 1. Presented are means, standard errors (SE), significance probabilities for clone and block effect, proportion of total variation accounted for by the statistical model used (R^2), normality of the frequency distribution of means before and after transformation to common logarithm. Abbreviations are explained in Table 1.

Trait	Population Mean	SE	Progeny Clone p(F)[a]	Block p(F)[a]	R^2	Normal distribution p(W)[b]	Normal distribution $\log_{10}$ p(W)[b]
Agronomic traits:							
TDM	3.80	0.07	0.000	0.152	0.73	0.000	0.000
SN	1.21	0.02	0.002	0.158	0.43	0.000	0.000
TNT [c]	15.7	0.38	0.000	0.953	0.56	0.000	0.899
TNC1 [c]	5.56	0.29	0.000	0.228	0.54	0.000	0.402
TNC2	7.98	0.21	0.000	0.706	0.54	0.001	0.000
TNC3	2.08	0.09	0.000	0.155	0.60	0.000	0.000
TNC4	0.07	0.02	0.000	0.003	0.52	0.000	0.000
TND	4.47	0.05	0.000	0.418	0.55	0.015	0.000
TWT	996	14.9	0.000	0.000	0.66	0.946	0.000
TWC1 [c]	58.7	3.62	0.000	0.303	0.50	0.000	0.893
TWC2	552	14.3	0.000	0.442	0.53	0.005	0.000
TWC3	367	17.2	0.000	0.069	0.62	0.000	0.018
TWC4	25.4	6.40	0.000	0.001	0.53	0.000	0.002
TSD	5.11	0.06	0.000	0.445	0.54	0.000	0.000
MT	130	0.46	0.000	0.003	0.59	0.000	0.000
TSG	2.34	0.09	0.000	0.000	0.62	0.000	0.000
TDE	3.34	0.08	0.000	0.329	0.66	0.000	0.000
TGA [c]	19.0	0.75	0.000	0.000	0.73	0.000	0.395
Morphological traits:							
HT	92.3	1.05	0.000	0.243	0.65	0.877	0.335
NNT	29.6	0.19	0.000	0.252	0.58	0.022	0.000
NNI1 [d]	22.4	0.20	0.000	0.559	0.68	0.864	0.866
NNI2 [d]	27.3	0.25	0.000	0.890	0.86	0.005	0.006
NNI3 [d]	29.8	0.48	0.333	0.316	0.99	0.017	0.050
ANL	3.14	0.03	0.000	0.634	0.71	0.466	0.844
ST	4.45	0.06	0.000	0.096	0.48	0.000	0.000
LS	5.02	0.06	0.000	0.257	0.55	0.000	0.000
LC	4.93	0.05	0.000	0.010	0.52	0.000	0.000
LLN	5.11	0.04	0.000	0.064	0.46	0.000	0.000
LLS	5.21	0.06	0.000	0.091	0.58	0.000	0.000
LSH	1.90	0.02	0.000	0.809	0.60	0.000	0.000
DV	3.27	0.04	0.000	0.054	0.56	0.000	0.000
WM	3.78	0.08	0.000	0.000	0.66	0.000	0.000
CAF	2.10	0.14	0.000	0.002	0.68	0.000	0.000 [e]
CBF	0.86	0.10	0.000	0.067	0.68	0.000	0.000 [e]
CAI	1.83	0.12	0.000	0.000	0.62	0.000	0.000 [e]
NLS	4.55	0.10	0.000	0.000	0.64	0.297	0.000
NI	1.21	0.04	0.002	0.983	0.51	0.000	0.000 [e]

[a] p(F) < 0.05 indicates a statistically significant effect; [b] p(W) ≥ 0.05 indicates a normal distribution; [c] analysis of variance carried out on values transformed to a common logarithm; [d] analysis only with clones that comprised the particular inflorescence;

[e] $\log_{10}(x+1)$, as the value zero (0) has been used as well.

Table 5. Analysis of NT8 × LT7 progeny clones in Experiment 2. Presented are means, standard errors (SE), significance probabilities for clone and block effect, proportion of total variation accounted for by the statistical model used (R^2), normality of the frequency distribution of means before and after transformation to common logarithm. Abbreviations are explained in Table 1.

Trait	Population Mean	SE	Progeny Clone p(F) [a]	Block p(F) [a]	R^2	Normal distribution p(W) [b]	Normal distribution $\log_{10}$ p(W) [b]
Agronomic traits:							
SN	1.16	0.02	0.001	0.145	0.47	0.000	0.000
TNT	13.2	0.53	0.000	0.096	0.66	0.000	0.000
TNC1	5.62	0.45	0.000	0.004	0.77	0.000	0.316
TNC2	6.87	0.21	0.001	0.829	0.43	0.145	0.000
TNC3	0.67	0.05	0.003	0.059	0.43	0.000	0.000
TNC4	0.01	0.01	0.000	0.760	0.54	0.000	0.000
TND	3.48	0.08	0.000	0.073	0.49	0.004	0.001
TWT	581	15.4	0.000	0.753	0.49	0.198	0.000
TWC1 [c]	66.0	5.27	0.030	0.596	0.70	0.000	0.593
TWC2	408	12.1	0.004	0.919	0.42	0.808	0.000
TWC3	104	7.66	0.000	0.022	0.46	0.000	0.030
TWC4	2.41	1.42	0.000	0.684	0.53	0.000	0.000
TSD	3.83	0.08	0.000	0.092	0.47	0.003	0.000
MT	120	0.28	0.000	0.052	0.18	0.119	0.148
TSG	2.65	0.09	0.029	0.489	0.52	0.000	0.000
TDE	3.77	0.12	0.000	0.935	0.58	0.000	0.000
TGA [c]	4.21	0.18	0.000	0.646	0.70	0.000	0.352
Morphological traits:							
HT	65.1	1.28	0.000	0.587	0.50	0.435	0.000
NNT	22.1	0.25	0.000	0.539	0.49	0.021	0.000
ANL	2.88	0.04	0.000	0.756	0.52	0.399	0.000
LS	1.98	0.03	0.000	0.383	0.53	0.000	0.000
LLS	5.08	0.05	0.000	0.257	0.59	0.000	0.000
DV	5.17	0.06	0.000	0.141	0.56	0.000	0.000
WM	5.33	0.08	0.000	0.649	0.57	0.000	0.000
CAF	2.38	0.11	0.000	0.784	0.57	0.000	0.000
CBF	1.25	0.05	0.000	0.563	0.59	0.000	0.000
CAI	2.85	0.13	0.000	0.299	0.49	0.000	0.000

[a] p(F) < 0.05 indicates a statistically significant effect;
[b] p(W) ≥ 0.05 indicates a normal distribution;
[c] analysis of variance carried out on values transformed to a common logarithm.

important economical product. Size class 3 contains tubers that are fit for the consumer market. Size class 4 contains oversized tubers, which are too big for most ware markets, but which are appropriate for the processing industry.

In Experiment 1, the parental clone Cara had a significantly higher total tuber weight than LT7 (Table 2). This might be expected, as Cara is a cultivated cultivar that reflects the extensive effort of breeders to increase yield. In size class 4, Cara had a higher tuber weight than LT7 as well, but was not significantly different from LT7 in size classes 1 and 3, and was significantly lower in size class 2. Neither parental clone contained tubers in size class 4 in Experiment 2, but some of the progeny had. This shows that tuber size of progeny clones can differ considerably from the parental clones. Also in Experiment 2, the parental clones were not significantly different for total tuber weight, although in size classes 1 and 2, LT7 had a significantly higher tuber weight than NT8 (Table 3). We conclude that differences in total tuber weight between genotypes do not necessarily reflect these differences in each particular size class.

Both in Experiment 1 and in Experiment 2, total tuber weight had a normal frequency distribution. Also in both experiments, in size class 1, tuber weight was normally distributed after common logarithmic transformation, which suggests dominance for low frequency of minitubers. In Experiment 1, the frequency distribution of size class 2 had an almost normal distribution, i.e. slightly skewed towards lower values, and normally distributed in Experiment 2. In both experiments, tuber weight of size class 2 was the main contributor of total tuber weight (Tables 4 and 5), whereas total tuber weight was distributed normally. Both total tuber weight and the particular size classes were distributed continuously. The mid-parent values were not significantly different for neither total tuber weight nor for any of the size classes. These results suggest that both total tuber weight and its size-components are genetically controlled in an additive way and that several genes are involved. This agrees with the results of Kreike *et al.* (1996), who detected a minor QTL involved in tuber yield. Schäfer-Pregl *et al.* (1998) identified eight putative QTLs for tuber yield in a cross of dihaploid *S. tuberosum* and a diploid interspecific hybrid between *S. tuberosum* and *S. chacoense*.

Broad sense heritability of total tuber weight was moderate to high (Table 7) and within the range of values reported by Chaudhary & Sharma (1984), Gopal *et al.* (1994), and Du Plooy *et al.* (1996). Heritability estimates of tuber weight in size class 1 were remarkably high. In the other size classes, heritability estimates varied considerably between the two experiments.

Tuber number distribution and tuber size distribution (TND, TSD) Besides tuber yield,

the value of the potato crop depends on specific size grades, each commanding a different price per tonne (Burstall *et al.*, 1986). The farmer's objective is to achieve the maximum yield in a predefined size range e.g. for seed or for ware markets (Marshall & Thompson, 1986). A uniform tuber size distribution is an important characteristic of potato cultivars (Struik & Wiersema, 1999).

In both experiments, parental clones were not significantly different, neither for TND nor for TSD. Also in both experiments, scores for these two characteristics varied among the progenies ranging from 2 to 7. Distinct differences in number and size distribution are mainly determined by their earliness, intensity of tuber set, and the characteristic relative variability of tuber size (Struik & Wiersema, 1999). The mid-parent values for scores of both TND and TSD appeared to be significantly higher than the respective progeny means. Thus, both parental clones having a similar frequency distribution, the progeny tends to shift towards a frequency distribution with a higher proportion of smaller tubers. This suggests that a distribution towards smaller tubers is dominant. Besides genetic factors, crop husbandry, such as plant density, seed size and harvest date, can influence tuber size distribution (Struik *et al.*, 1990). On a plant level, tuber size distribution depends for a great part on stolon-related characteristics, such as number of stolons, time of stolon formation, number of tuber incipients and place on stolon where tubers are being formed (Struik *et al.*, 1991). Moreover, several tuber-related characteristics have an effect on tuber size distribution, such as time of tuber formation and growth rate of individual tubers (Wurr, 1977; Struik *et al.*, 1991). As TSD is composed of tuber weight and tuber number, each being controlled by many genes, tuber size distribution itself may also have a complex genetic control. Recent work from Struik *et al.* (1999) showed indeed on a molecular level that processes related to tuber formation involve specific expression of numerous cDNA fragments.

Tuber yield was correlated to TSD. Correlation coefficient was 0.32 in Experiment 1 and 0.50 in Experiment 2. This means that with higher tuber yield, TSD shifted towards higher scores, i.e. more tubers in higher size classes and/or less tubers in lower size classes. This agrees with Struik *et al.* (1991), who reported that when yield increases, the average tuber size increases and the range of sizes increases.

Comparing the two experiments, both tuber weight and tuber number of size class 1 were not significantly different (Tables 4 and 5). In Experiment 1, both tuber weight and tuber number had higher values than in Experiment 2 in all other size classes. This is reflected in higher values in Experiment 1 of both tuber number distribution (TND) and tuber size distribution (TSD) than in Experiment 2, which is visualised in Figure 3.

Mean tuber weight per size class Comparing average tuber weight per size class

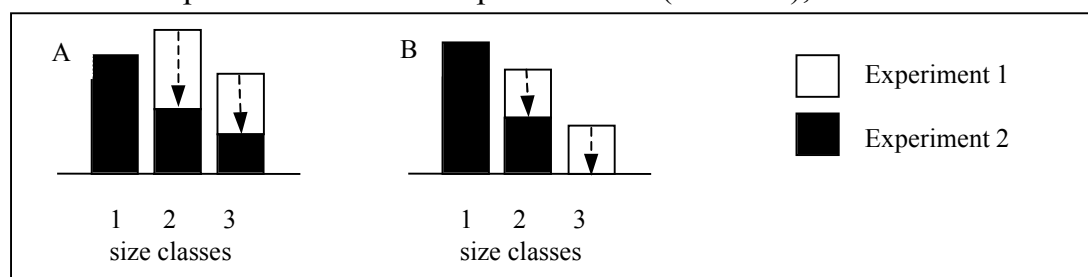between Experiment 1 and Experiment 2 (Table 6), shows that no significant

Figure 3. Two arbitrary examples for the comparison of tuber size distribution or tuber number distribution of Experiment 1 and Experiment 2. There were no differences between both experiments in values for size class 1. However, values for size classes 2 and 3 were significantly lower in Experiment 2. In example A, the score for distribution is 5 in Experiment 1 and score 4 in Experiment 2, because of lower frequencies in size classes 2 and 3. In example B, the score is 4 in Experiment 1 and score 2 in Experiment 2 because of lower frequencies in size class 2 and absence of size class 3.

Table 6. Comparison of progeny clones of Experiment 1 (Cara × LT7) and Experiment 2 (NT8 × LT7) of mean tuber weight per size class. Presented are means and standard errors (SE). Abbreviations are explained in Table 1.

	Cara × LT7		NT8 × LT7	
	Mean	SE	Mean	SE
TWT/TNT	73.2	1.70	47.5	1.30
TWC1/TNC1	10.3	0.26	11.5	0.25
TWC2/TNC2	70.9	0.85	60.7	1.00
TWC3/TNC3	173	2.2	151	2.8
TWC4/TNC4	341	10.7	284	8.3

difference was observed for size class 1. However, significantly higher average tuber weights were recorded in Experiment 1 in size classes 2, 3, and 4. This suggests that despite a higher tuber number, a remarkably higher total tuber weight in the larger size classes in Experiment 1 resulted in a higher average weight per tuber than in Experiment 2.

Both experiments had LT7 as mutual parent. Therefore, the differences between the two experiments can – at least partly – be contributed to the differences between Cara and NT8 as parental clone. This agrees with the results of the parental clones. Compared to LT7 in the respective experiments, Cara had a tendency towards higher tuber weights (Table 2) and NT8 had a tendency towards lower tuber weights (Table

3).

Struik *et al*. (1991) reported that the yield of larger tubers is closely related to total tuber yield. This is reflected in Experiment 1, compared to Experiment 2, by higher average scores in the progeny for tuber size distribution (Tables 4 and 5).

Maturity of the foliage (MT) Maturity in conjunction with date of planting determines the length of the growing season and is therefore a major agronomical trait. The longer the crop cycle, the more radiation can be intercepted, the higher is the yield (Allen & Scott, 1992). Compared with late cultivars, early potato cultivars divert a larger part of the available assimilates to the tubers early in the growing season, leading to shorter growing periods and lower yields (Kooman & Rabbinge, 1996; Haverkort & Kooman, 1997). Thus, in principle, late cultivars are expected to have greater yields (Howard, 1963) and are therefore desired (Ewing & Struik, 1992). However, the fitness of a certain cultivar to be grown in given climatic environment is for a great deal dictated by its earliness. Early cultivars can be necessary in regions where the growing season is short due to unfavourable circumstances during parts of the season. This is especially true for areas with high temperatures, late season drought or killing night frosts. In areas with very low temperatures, frost tolerance is favoured rather then frost avoidance by using frost tolerant species in breeding programmes (Midmore, 1992). Regions, where climate permits two growing seasons per year or year-round cultivation, earliness in conjunction with a short period of dormancy in storage is desirable (International Potato Center, 1984; Allen *et al*., 1992).

As expected, there was no difference in maturity between the parental clones, as all clones used are late maturing under Israeli conditions. In the Catalogue of Potato Varieties, Scotland, 1990, Cara has been described as very late. In both experiments, the progeny mean was not significantly different from the mid-parent value. In Experiment 1, which contained Cara as parental clone, the progeny was skewed towards late maturity, probably as a result of genes controlling lateness from Cara. In Experiment 2, the progeny was normally distributed. These results agree with those of Howard (1970), who described crosses between early cultivars and a range of cultivars from early to late, where early × early gave only 61% earlies, and early × late gave only 18% earlies. He concluded that time of maturity depends upon a number of genes. Ross (1986) also reported that maturity is polygenetically determined, but our results seem not to agree with his conclusion that earliness tends to be dominant to late maturity. Recent work of Oberhagemann *et al*. (1999) reported two major QTLs associated with foliage maturity. One of them was closely linked to a major QTL for late blight resistance.

Broad sense heritability was moderate to low (resp. 0.33 and 0.17; Table 7),

although other authors reported much higher values of 0.62 (Lynch & Kozub, 1988) and 0.58 (Tai & Young, 1984).

Secondary growth of tubers (TSG) Secondary growth of tubers is a tuber disorder that can be considered as an interruption in induction to tuberize once tubers have started to form. Environmental factors that influence the expression of secondary growth are the absence of mechanical resistance during tuber initiation, drought and heat stress, or sudden increases in nitrogen fertiliser during the period of tuber growth (Ewing & Struik, 1992).

In Experiment 1, neither Cara nor LT7 showed severe expression of secondary growth of tubers and they were not significantly different. In Experiment 2, NT8 and LT7 had somewhat higher scores, but were not significantly different either. In both experiments the mid-parent value was not significantly different from the progeny mean. In Experiment 1, there was a significant clone-effect on secondary growth (Table 4), which confirms findings reported by Howard (1963) and Ewing & Struik (1992) that the degree of susceptibility to secondary growth is genetically controlled.

Frequency distributions were skewed towards low scores, where only few of the progeny had much higher scores than the parental clones. We suggest that severe expression of secondary growth is recessive. Heritability was moderate to low (Table 7), which can be explained by the large influence of environmental factors to second growth.

Depth of eyes (TDE) Potatoes with shallow eyes are desirable both for consumers and for the processing industry. Deep eyes cause a considerable peeling loss. Therefore, eye depth is an important breeding objective (Caligari, 1992a).

Cara and LT7 had both shallow eyes and were not significantly different. Scores obtained in this study correspond with scores reported respectively in the Scottish catalogue of potato varieties (1990) for Cara and by the International Potato Center (CIP) in the list of potato varieties and advanced cultivars (1992) for LT7. The mid-parent value was not significantly different from the progeny mean (Tables 2 and 4). The frequency distribution for depth of eyes was skewed towards lower scores, although the progeny included also clones with deep (score 7) and very deep eyes (score 9). This is in agreement with Howard (1963) that on the whole, crosses between two varieties, both of which have shallow eyes, produce seedlings that also have shallow eyes. The parental clones NT8 and LT7 were significantly different, where NT8 had deep to very deep eyes and LT7 had shallow eyes. Deep eyes are one of the characteristic negative traits of neotuberosum clones (Mendoza, 1989; Bradshaw & Mackay, 1994), i.e. the breeding source of NT8.

The progeny of Experiment 2 was, compared to Experiment 1, more evenly

Table 7. Comparison of broad sense heritabilities estimated in cross Cara × LT7 (Experiment 1) and cross NT8 × LT7 (Experiment 2) with estimates reported elsewhere. Abbreviations are explained in Table 1.

Trait	Cara × LT7 h^2_{BS}	NT8 × LT7 h^2_{BS}	Reported h^2_{BS}	Reference	Experimental design
Agronomic traits:					
TDM	0.58	n.a.	0.57	Thompson *et al.* (1980)	4 diploid progeny sets
SN	0.13	0.16	n.a.		
TNT	0.44 [a]	0.48	0.67	Chaudhary & Sharma (1984)	339 F_1C_2 clones
			0.30	Gopal *et al.* (1994)	135 F_1C_3 clones
			0.64	Du Plooy *et al.* (1996)	14 clones, 2 locations, 3 planting dates
TNC1	0.38	0.65	n.a.		
TNC2	0.29	0.14	n.a.		
TNC3	0.38	0.13	n.a.		
TNC4	0.04	0.30	n.a.		
TND	0.29	0.20	n.a.		
TWT	0.45	0.24	0.59	Chaudhary & Sharma (1984)	339 F_1C_2 clones
			0.33	Gopal *et al.* (1994)	135 F_1C_3 clones
			0.55	Du Plooy *et al.* (1996)	14 clones, 2 locations, 3 planting dates
TWC1	0.34	0.57	n.a.		
TWC2	0.29	0.12	n.a.		
TWC3	0.41	0.17	n.a.		
TWC4	0.02	0.28	n.a.		
TSD	0.30	0.18	n.a.		
MT	0.33	0.17	0.62	Lynch & Kozub (1988)	9 clones, 3 years, 5 locations
			0.58	Tai & Young (1984)	20 clones, 3 years
TSG	0.24	0.12	n.a.		
TDE	0.36	0.24	n.a.		
TGA	0.54 [a]	0.50 [a]	0.86-0.89	Sanford & Sinden (1972)	10 crosses, 2 years
			0.25-0.26	Ross *et al.* (1978)	3 clones, 4 years, 5 locations
Morphological traits:					
HT	0.42	0.23	0.58	Tai & Young (1984)	20 clones, 3 years
			0.63	Yildirim & Çaliṣkan (1985)	11 clones, 2 year, 3 locations
			0.45-0.47	Šimko *et al.* (1999)	155 F_1 clones, 2 growing conditions
NNT	0.31	0.20	n.a.		
NNI1 [b]	0.37	n.a.	n.a.		
NNI2 [b]	0.58	n.a.	n.a.		
NNI3 [b]	0.55	n.a.	n.a.		
ANL	0.53	0.24	n.a.		
ST	0.20	n.a.	n.a.		
LS	0.29	0.26	n.a.		
LC	0.25	n.a.	n.a.		
LLN	0.18	n.a.	n.a.		
LLS	0.33	0.34	n.a.		
LSH	0.38	n.a.	n.a.		
DV	0.31	0.30	n.a.		
WM	0.43	0.31	n.a.		
CAF	0.49	0.30	n.a.		
CBF	0.49	0.34	n.a.		
CAI	0.38	0.18	n.a.		
NLS	0.40	n.a.	n.a.		
NI	0.16	n.a.	n.a.		

[a] Broad sense heritability estimated after transformation to common logarithm;

[b] Analysis only with clones that comprised the particular inflorescence;
n.a. not available.

distributed among the scores, containing more high scores. This agrees with Ross (1986) who reported that a parent with deep eyes always shifts the progeny towards deeper eyes. The progeny mean of Experiment 2, however, was not significantly different from Experiment 1 (Tables 4 and 5). This suggests that shallow eyes are dominant over deep eyes, which is in agreement with results of other authors, as reported by Oritz & Huamán (1994). Broad sense heritability estimates were moderate, which corresponds with the observation that large cultivar differences exist (Cutter, 1992; B. Bar-Tel, pers. comm., 2000).

Morphological traits

Plant height (HT) The growth habit of each individual stem is determinate: it produces leaves and completes its development with the development of a terminal inflorescence. Continuation of shoot growth from lateral buds gives rise to more levels of growth, with corresponding inflorescences. In conditions that allow shoot growth to continue, inflorescence production of the shoot system is theoretically unlimited (Almekinders, 1995).

Plant height of the parental clones Cara and LT7 was very similar (Table 2) and progeny mean was close to the mid-parent value. The parental clones NT8 and LT7 were significantly different, whereas NT8 had shorter plants than LT7, and the progeny mean (65.1 cm) was not significantly different from the mid-parent value (64.9 cm). In Experiment 1, the progeny mean was 92.3 ± 1.1 cm (n = 211) (Table 4). Calculating mean plant height using only plants that developed at least one inflorescence, progeny mean was 94.5 ± 1.1 cm (n = 193), whereas mean plant height for plants that did not develop any inflorescence was 86.4 ± 2.0 cm (n = 82). When a progeny clone had replicates both with and without producing an inflorescence, each one was taken into calculation accordingly, i.e. that progeny clone was used twice, but each single plant was used only once.

Heritability was moderate to high (Table 7), which shows a strong genetic control of this characteristic. Environmental factors such as stem density, temperature and photoperiod influence plant height as well (Allen & Wurr, 1992; J. van Dam, unpublished). Plant height had a normal frequency distribution in both experiments, and ranged from 40-140 cm (Experiment 1) and 30-120 cm (Experiment 2), respectively. This suggests that maximum plant height is inherited in an additive way and that several genes are involved. These results agree with recent work of Šimko *et al*. (1999), who detected for plant height four major QTLs under *in vitro* conditions and three major QTLs under greenhouse conditions in a backcross of (diploid *S.*

41

tuberosum × *S. berthaultii*) × *S. tuberosum*.

Number of nodes on main stem (NNT, NNI1-3) The number of nodes on the main stem is equal to the number of leaves produced, which is a measure of plant development (Firman *et al.*, 1991; Almekinders, 1995). The extent of leaf appearance above the first inflorescence is taken as a measure of the degree of indeterminacy in the main shoot and is influenced by temperature and photoperiod (Allen & Scott, 1992; Almekinders, 1995). Cultivars show a wide range in this characteristic, ranging from complete indeterminacy to almost complete determinacy in some early cultivars.

In Experiment 1, Cara had a significantly higher total number of nodes than LT7, but not for number of nodes till respectively first and second inflorescence (Table 2). Both Cara and LT7 did not develop a third inflorescence. In Experiment 2, NT8 and LT7 were not significantly different for total number of nodes (Table 3).

Significant differences were observed among the progeny for total number of nodes and for number of nodes till first and second inflorescence, respectively. This agrees with results of Firman *et al.* (1991) and Allen *et al.* (1992), who reported cultivar differences for the total number of nodes, especially the number of nodes till the first inflorescence. Other factors affecting the number of nodes are physiological age of the tubers, sprout length at planting, photoperiod and the duration of cold storage (Firman *et al.*, 1991). The mid-parent value of total number of nodes did not differ significantly from the progeny mean. In Experiment 1, the mid-parent value was also not significantly different from the progeny mean for node number till both first and second inflorescence. By developing a third inflorescence, 10% of the progeny was more indeterminate than the parental clones. The progeny of Experiment 1 was nearly normally distributed for total number of nodes, whereas the progeny of Experiment 2 was normally distributed for this trait (Tables 4 and 5).

Most plants developed at least one inflorescence. However, 12 progeny clones did not develop inflorescences in any replicate. For those clones that flowered, the progeny was normally distributed for number of nodes till first inflorescence (n = 200), and almost normally distributed for number of nodes till second inflorescence – i.e. slightly skewed towards lower values – (n = 130). For the number of nodes till third inflorescence, which was absent in both parental clones, the progeny was normally distributed after transformation to common logarithm (n = 21). It seems that increased inflorescence production, i.e. a stronger degree of indeterminacy, is concomitant with a low number of nodes among those flowering.

The broad sense heritability was intermediate for total number of nodes and medium to high for the node number till the respective inflorescence. Total number of nodes on the main stem was inherited in an additive way and we suggest that several genes are involved.

Analysis of variance within the progenies of each of the two experiments showed that the total number of nodes was highly significantly related to total tuber weight (Exp. 1: p(F) = 2.6×10^{-27}, R^2 = 0.20; Exp. 2: p(F) = 4.1×10^{-55}, R^2 = 0.40).

Average node length (ANL) The average node length was calculated as plant height divided by total number of nodes. In both experiments, parental clones were not significantly different, although in Experiment 1, Cara had a significantly higher total number of nodes than LT7. Also the mid-parent values were not significantly different from progeny mean in both experiments. Frequency distributions of average node length were normal in both experiments. This was to be expected, as in both experiments plant height was normally distributed and the total number of nodes was almost normally distributed, i.e. only slightly skewed towards high number of nodes. We suggest that the average node length is inherited in an additive way, and that several genes are involved.

Heritability estimates were moderate to high (Table 7). It appeared that heritability estimates of average node length were higher than the two elements it is composed of: plant height and total number of nodes.

Stem thickness (ST) Cara and LT7 were not significantly different and the mid-parent value was not significantly different from the progeny mean (Tables 2 and 4). We suggest that stem thickness is inherited in an additive way. Broad sense heritability estimate was moderate (Table 7).

Leaf size (LS) Leaf size is one of the components of leaf area index (LAI), which in conjunction with leaf area duration (LAD) determines the amount of irradiation that can be intercepted by the crop during its growth season (Allen & Scott, 1992). A drawing of a typical potato leaf is presented in Figure 4.

The parental clones of Experiment 1, Cara and LT7, were quite similar and not significantly different (Table 2). In Experiment 2, NT8 had a significantly smaller leaf than LT7, although their scores were not much different in absolute values (Table 3). Leaf size is consistent over genotypes (UPOV, 1986). The progeny of Experiment 2 had a limited range of sizes, only from 1 (very small) to 3 (small). This may be explained by parental clone NT8, which had very small leaves (Table 3). Genes that cause the development of very small leaves seem to be dominant and reduce progeny mean.

Leaf size is moderately heritable (Table 7), with h^2_{BS}-values of respectively 0.29 and 0.26. No block effect was observed in both experiments, neither between parental clones (Tables 2 and 3) nor within the progeny (Tables 4 and 5), which indicates a

strong genetical control of leaf size, rather than influenced by environmental factors.
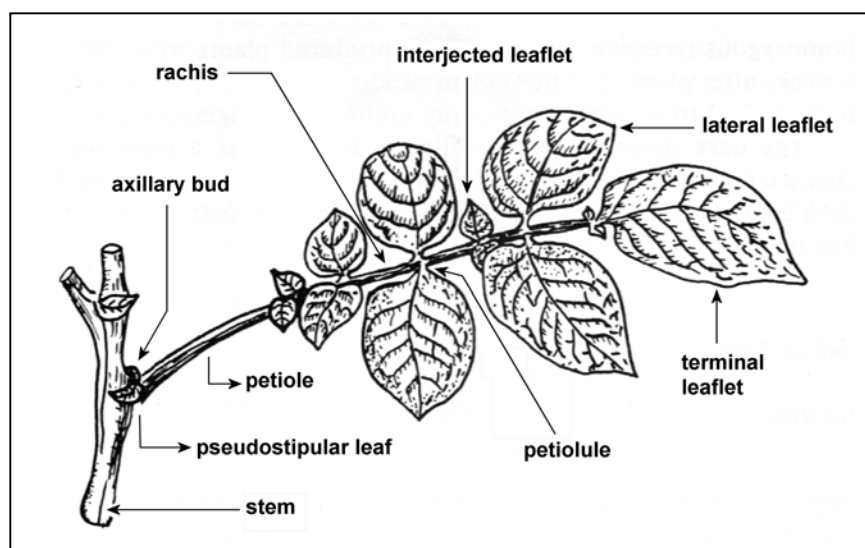
Figure 4. Leaf of the potato plant. With kind permission from CABI Publishing. Taken from Potato Genetics (1994) edited by J.E. Bradshaw & G.R. Mackay.

However, high air temperatures, short daylength, and temperature × daylength interaction reduce leaf size in a single clone (Bodlaender, 1963; Sale, 1973; Struik & Ewing, 1995). Nitrogen supply increases leaf size (Vos & Biemond, 1992; Vos & Van der Putten, 2001). However, both experiments described in this study were conducted under similar environmental and experimental conditions, which diminishes temperature, daylength and nitrogen supply as an explanation for differences observed between the two experiments.

Leaf compactness (LC) This trait was observed in Experiment 1. Leaf compactness is the relative amount of leaf blade covering the surface of the leaf contour. This characteristic is composed of the length of the rachis, number of leaflets, and the size of the leaflets.

Cara had a significantly more compact leaf than LT7 (Table 2). The mid-parent value was not statistically different from the progeny mean. We concluded that leaf compactness is inherited in an additive way. The broad sense heritability was moderate (0.25), which justifies the use of this compound characteristic for the description of potato cultivars for plant breeders' rights (UPOV, 1986).

Number of leaflets per leaf (LLN) This trait was observed in Experiment 1. The number of leaflets per leaf is a morphological characteristic that varies among cultivars (Cutter, 1992).

The parental clones were not significantly different (Table 2). The mid-parent value

was significantly higher than the progeny mean. We suggest that a lower number of leaflets is dominant over a large number of leaflets. This is in agreement with results of Kumikura, cited by Oritz & Huamán (1994), who suggested that one dominant gene was responsible for less than three pairs of leaflets.

Terminal leaflet size (LLS) In Experiment 1, the parental clones Cara and LT7 were not significantly different in terminal leaflet size (Table 2). In Experiment 2, NT8 had a significantly larger terminal leaflet than LT7 (Table 3). No block effect was observed in both experiments, neither between parental clones (Tables 2 and 3) nor within the progeny (Tables 4 and 5), which suggests that terminal leaflet size is genetically controlled, rather than influenced by environmental factors. In both experiments, the mid-parent value was not significantly different from the progeny mean. Terminal leaflet size seems to be genetically controlled in an additive way. Terminal leaflet size is moderately heritable (Table 7), with h^2_{BS}-values of, respectively, 0.33 and 0.34.

In Experiment 1, leaf size (LS) and terminal leaflet size (LLS) were highly correlated (Pearson product-moment correlation = 0.52). However, in Experiment 2, these traits were not correlated (-0.08), what may be due to the small range of scores for leaf size in that population. Data of 27 unrelated cultivars, which were described after application for plant breeders' rights in Israel, show that leaf size (LS) and terminal leaflet size (LLS) had a high correlation (0.62). However, few of these cultivars had small leaves and large terminal leaflets or *vice versa*. Likewise in our study, parental clone NT8 had very small leaves and medium to large terminal leaflets (Table 3).

Terminal leaflet shape (LSH) This trait was observed in Experiment 1. Scores of Cara and LT7 were equal (both score 2) and the mid-parent value was not significantly different from the progeny mean. Most progeny clones (85%) had medium sized terminal leaflets, whereas 12% had broad leaflets and 3% had narrow leaflets. The high estimate of broad sense heritability (0.38, Table 7) and the absence of a block effect, both between the parental clones and within the progeny, suggest a strong genetical control of terminal leaflet shape.

Terminal leaflet shape was not correlated with terminal leaflet size (Pearson product-moment correlation = -0.04).

Vein depth (DV) Larger veins have protrusions on the underside of the leaf (Raven *et al.*, 1986). On the upper side of the potato leaf, opposite to these protrusions, these veins have intrusions. Their depth is referred to as vein depth.

Both parental clones of Experiment 1, Cara and LT7, had shallow veins and their

scores were similar (Table 2). NT8 however, had much shallower veins than LT7 (Table 3). In both experiments, the mid-parent value was close to the progeny mean. The heritability of vein depth was moderate (Table 7).

For the International Union for the Protection of New Varieties of Plants (UPOV, 1986) a score-scale from 1 (very low) to 9 (very high) is being used. A minimum difference in score of 2 is commonly being considered as distinct between two plants (B. Bar-Tel, pers. comm.). In both experiments, only 7% of the progeny clones differed more than a score of 2 from the progeny mean, suggesting that vein depth is genetically regulated in an additive way. This was confirmed by recent findings where two alleles were identified to be associated with shallow veins in the NT8 × LT7 progeny (J. van Dam, unpublished).

In the progenies of both experiments, a significant clone-effect was observed. Neither within the progeny clones nor between the parental clones a significantly difference for block-effect was observed, which suggests that vein depth is not influenced by the environment, at least not under the conditions in which these experiments were conducted.

Waviness of margin (WM) The margin of the leaflet blade can be smooth or wavy. Artschwager (1918) described the course of the veins in the leaves as acrodromous, i.e. strongly developed secondary veins running in convergent arches towards the apex. These secondary veins are small vascular bundles, which are the continuation of adaxial wings of the rachis (McCauley & Evert, 1988). It might be that the waviness of the leaflet margin is caused by abundant vein tissue in the outer leaflet blade.

In both experiments, the parental clones were not significantly different (Tables 2 and 3). The mid-parent values were similar to the progeny means. Also in both experiments, scores of the progeny were distributed widely around the parental scores. The broad sense heritability was moderate (Table 7). The waviness of the leaf margin, i.e. the amount of vein tissue in the outer leaflet blade, seems to be genetically controlled in an additive way.

Coalescence (CAF, CBF, CAI) The potato leaf is pinnately compound and has small folioles between the pinnae (Cutter, 1992). Coalescence in potato leaves is when at least two leaflets are fused to some extent. The phenomenon resembles lobed terminal leaflets of juvenile potato leaves, which apparently arise from fusion or lack of separation of a lateral leaflet and the terminal one during development (McCauley & Evert, 1988). Fused terminal leaflets in adult potato leaves were often found in some chromosome-doubled dihaploids (De Maine, 1985). Coalescence can occur between the terminal and one or more lateral leaflets (CAF), or between two lateral leaflets

(CBF). The frequency of occurrence for each of these two forms of coalescence was observed, as a measure of how many leaves per plant are affected. Especially with CAF, a difference in the degree of coalescence could be distinguished (CAI), which is the 'severeness' of the fusion of the leaflets.

The frequency of coalescence between terminal and lateral leaflets (CAF) for parental clones in Experiment 1, Cara and LT7, was low and very similar (Table 2). In Experiment 2, NT8 had a significantly higher score for CAF than LT7. In both experiments the mid-parent value had a score close to the progeny mean and the frequency distribution of both experiments were strongly skewed towards lower values. However, in both experiments high to very high frequency of coalescence was recorded for a few progeny clones. Broad sense heritability was medium to high (Table 7).

The frequency of coalescence between two lateral leaflets (CBF) was very similar to CAF in both experiments. The mid-parent values were close to the progeny mean and frequency distributions were skewed towards lower values. The two forms of coalescence appeared to be independent characteristics. This was demonstrated in Experiment 1 by parental clone Cara, where CAF-coalescence was observed, but CBF-coalescence not (Table 2).

In Experiment 1, LT7 had significantly higher scores than Cara for the intensity of coalescence between a lateral and the terminal leaflet (CAI; i.e. the 'severeness' of the fusion of the leaflets). In Experiment 2, NT8 and LT7 were not significantly different. For CAI, like for CAF and CBF, the mid-parent value was close to the progeny mean and frequency distributions of both experiments were strongly skewed towards lower values.

A marked difference between the two experiments was that plants with no coalescence, value '0' (zero), occurred only in Experiment 1, but not in Experiment 2. Probabilities for CAF, when calculated in Experiment 1 for those observations with at least very few coalescence (scores ≥ 1) and omitting those clones with no coalescence at all, was lower than values shown in Table 4, but still statistically significant (p(F) = 0.02, R^2 = 0.58). The heritability, however, was then much lower and more similar to values obtained in Experiment 2 where no '0' (zero) was observed. Absence and presence of this type of coalescence was calculated by means of Pearson-χ^2, assigning '0' (zero) for absence and '1' (one) for any amount of coalescence. Probability of χ^2 was < 0.001; R^2 = 0.63. This suggests that the degree of coalescence is less pronounced than absence or presence of it.

The moderate to high values for broad sense heritability show that the phenomenon of coalescence in potato leaves is not a physiological disorder caused by environmental influences. The strong skewness towards low frequencies of all forms

of coalescence described shows that 'low frequency' is dominant over 'high frequency'.

Necrotic leaf spots (NLS) The phenomenon of necrotic leaf spots, a physiological disorder resulting in dark brown spots, usually 2-5 mm in diameter, on the leaf blade. Necrotic leaf spots were only detected in Experiment 1. In Experiment 2, no necrotic leaf spots were detected. LT7, which was grown in both experiments, was moderately affected in Experiment 1, but was not affected at all in Experiment 2. We assume that environmental conditions cause the appearance of this disorder. LT7 was significantly more prone to necrotic leaf spots than Cara. Both the parental clones and the progeny mean had a moderate score and the progeny was normally distributed. Within the progeny, all scores from 1 to 9 were present. Heritability was moderately high (Table 7). It seems that susceptibility for necrotic leaf spot is inherited in an additive way.

Number of inflorescences (NI) This trait was observed in Experiment 1. A relevant characteristic for the production of TPS (true potato seed) is the number of inflorescences per plant (Almekinders, 1995). When more inflorescences are produced, more flowers, and thus more berries can be produced.

Cara and LT7 were not significantly different in number of inflorescences. The mid-parent value was not significantly different from the progeny mean. Most of the progeny developed at least one inflorescence. However, 12 progeny clones did not develop an inflorescence in any replicate. Neither Cara nor LT7 produced a third level inflorescence. However, in 21 progeny clones a third level inflorescence was observed. Accordingly, significant differences were observed among the progenies. In agreement with these results, Struik & Wiersema (1999) reported that the number of inflorescences strongly depends on cultivar or genotype, although environmental conditions, type and age of the planting material used and crop husbandry do influence the number of inflorescences as well. Nevertheless influence of the genotype, broad sense heritability estimate was low (Table 7).

CHAPTER 4

Identification of epistatic interaction affecting glycoalkaloid content in tubers of tetraploid potato (*Solanum tuberosum* L.)[*]

Jacob van Dam[1], Ilan Levin[1], Paul C. Struik[2] & David Levy[1]

[1] Institute of Field and Garden Crops, The Volcani Center, POB 6, Bet Dagan 50250, Israel

[2] Department of Plant Sciences, Crop and Weed Ecology Group, Wageningen University, Haarweg 333, 6709 RZ Wageningen, The Netherlands

Abstract

Glycoalkaloids are secondary metabolites which are characterised by an undesirable taste, and known to be toxic when consumed in large quantities. Their content in potato tubers should therefore be selected against and DNA markers can significantly facilitate such process. This study was designed to identify DNA markers associated with the total content of glycoalkaloids (TGA) in the potato tuber. Two segregating populations were constructed from tetraploid potato clones, which were characterised by divergent TGA content. The two populations had a common high-TGA parent, LT7. 342 Inter-Simple Sequence Repeat (ISSR) single primers or two such primer combinations were used to amplify random PCR products. Parental clones of both populations were used to identify polymorphic PCR products for further linkage analyses with TGA-content. In these analyses, two single ISSR markers were found significantly associated with TGA-content. A multiple regression analysis was also carried out using a 'stepwise' procedure. In this analysis TGA-content was the dependent variable whereas the polymorphic PCR products and all possible two-way interactions among them were the independent variables. The resulting best model consisted of an interaction between two loci in addition to a single locus effect. This interaction suggests that the expression of TGA-content is partially modulated by two interacting loci.

Key words: Gene interaction, genetic background, glycoalkaloids, ISSR, polyploidy, secondary metabolites

Introduction

Breeding potato cultivars adapted to hot and dry climates is considered to be one of the main objectives of modern potato breeding programmes (Caligari, 1992a). To obtain cultivars adjusted to such conditions, wild potato germplasm is widely being used as a source for genes that encode elements of heat-tolerance. Several members of this wild germplasm are also characterised by naturally high levels of secondary metabolites (Kutchan, 1995). For instance, introgressions of wild potato germplasm are often associated with an increase in total glycoalkaloids (TGA) in the potato tubers. Examples are: *Solanum vernei* which is a source for genes encoding resistance to potato cyst nematodes (Van Gelder & Scheffer, 1991) and *Solanum chacoense* which harbors genes encoding for heat-tolerance (Veilleux *et al.*, 1997; Levy *et al.*, 2001). High levels of TGA cause an undesirable taste and are considered toxic to humans (Storey & Davies, 1992). Therefore, levels of TGA should be included as a major selection criterion in introgression programmes utilising species containing high TGA-levels as donors of genes.

The genetic control of the metabolism of TGA in the tubers of tetraploid potato is complex due to the possible involvement of four alleles at each locus (Tai, 1982). Consequently, each locus has the potential for several first-, second-, and third-order allelic interactions (Bonierbale *et al.*, 1993). Moreover, in the most extreme case, eight different alleles may independently segregate in populations, resulting from crosses between two tetraploid parents. This implies that even a single locus can display a continuous variation (Meyer *et al.*, 1998) and therefore most research has been carried out using diploid populations. Bradshaw *et al.* (1998), however, pointed out that the avoidance of the complexity of tetrasomic inheritance by using haploids or diploids in the genetic analyses followed by polyploidisation is too time consuming. Moreover, the performance of genes at a monoploid or diploid level does not always reflect their performance in tetraploids. This is especially true for traits that are regulated by several genes.

The biochemical pathways of glycoalkaloid synthesis in tubers resemble those in leaves, as all glycoalkaloids follow the general pathway of the steroid biosynthesis (Van Gelder, 1989). Foliar glycoalkaloids in potato can be accumulated via several different pathways. Two major quantitative trait loci (QTL) and six additional QTLs were described for foliar glycoalkaloids in *S. tuberosum* × *S. berthaultii* progenies, accounting for 10% to 22% of the explained variation. The QTLs represent distinct structural and/or regulatory genes for the biosynthetic pathway of solasodine or solanidine in leaves (Yencho *et al.*, 1998). These two chemical compounds are main steps in the biosynthesis of TGAs (Van Gelder, 1989). Ronning *et al.* (1999) reported

two molecular markers associated with foliar glycoalkaloids in *S. chacoense* × *S. chacoense* crosses. One of these markers was localized near one of the QTLs reported by Yencho *et al*. (1998). Ronning *et al*. (1999) suggested that two or more major genes, which determine the accumulation of glycoalkaloids, might exist in that region of the genome. We have previously estimated, using classical quantitative analyses, that a minimum number of 3 to 7 genes may contribute to TGA-content in the tubers (Van Dam *et al*., 1999).

In the present study, two tetraploid populations were especially designed for the identification of DNA markers associated with TGA-content in the tubers. These populations were extensively characterised elsewhere (Van Dam *et al*., 1999). This study is, to our knowledge, the first to demonstrate the involvement of an interaction between two DNA loci in the control of glycoalkaloid metabolism in potato tubers.

Materials and methods

Plant material and culture

Two tetraploid segregating potato populations were constructed. Parental clones were chosen on the basis of their known divergent tuber TGA-content and genetic background. One population resulted in a cross between the clones Cara and LT7, and the second population was a cross between the clones NT8 and LT7 (Van Dam *et al*., 1999). The shared male parent LT7 is characterised by a high TGA-content in the tubers (Dimenstein *et al*., 1997), whereas both female parents Cara and NT8 are characterised by a low TGA-content in the tubers (Dimenstein *et al*., 1997; Levy *et al*., 1995). LT7 is a clone from the International Potato Center (CIP), Peru (International Potato Center, 1982), Cara is an Irish cultivar from the Oak Park Research Center, and NT8 is a neotuberosum clone selected from true potato seed (TPS) received from the Cornell University, USA. The clones Cara, LT7 and NT8 are unrelated, late maturing and have fair yields under Israeli climatic conditions (Levy *et al*., 1991; K.M. Paddock, pers. comm.).

Experimental design and quantification of tuber TGA-content

Each of the two populations was grown and evaluated in separate experiments. The experimental design was a complete randomised block design with three blocks containing one replicate of each progeny clone of both segregating populations. Also, five replicates of each parental clone per block were included in each experiment.

Total glycoalkaloid (TGA) content in the tubers was assessed per plant. Plant cultivation and the assessment of TGA-content were described in detail by Van Dam *et al*. (1999).

Table 1. ISSR primers which produced polymorphic bands associated with TGA content in the tubers, either as single primer ([A]) or as primer combination ([B+C]).

Code	Primer sequence (5' → 3')	Size (bp)
[A]	CTCTCTCTCTCTCTCCCG	18
[B]	TCTCTCTCTCTCTCCCCG	18
[C]	TCTCTCTCTCTCTCCGGT	18

DNA extraction

Apical leaves from individual plants of parental and progeny clones were sampled and immediately frozen in liquid nitrogen. Genomic DNA was extracted according to the miniprep protocol of Fulton *et al*. (1995).

PCR primers

Three hundred forty two single Inter Simple Sequence Repeats (ISSR) primers or two such ISSR primer combinations were used in the PCR reactions to amplify random PCR products in the potato genome. One hundred seventy one of these single ISSR primers or primer combinations were polymorphic between the parental clones. Most of the ISSR primers were based on (TC) dinucleotide repeats which appeared to amplify many DNA targets in both the potato and tomato genome. The ISSR primers differed from each other by different specific DNA sequences at their 3' end. Three of the primers used (Table 1) appeared to show polymorphic PCR products associated with TGA-content in the tubers.

PCR reaction

The amplification reaction of ISSR sequences (25 µl final volume) was performed with 40 ng template DNA, 25 mM TAPS (pH = 9.3 at 25 °C), 50 mM KCl, 2.5 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.2 mM of each of the four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP and dTTP), 20 ng of a single primer or 10 ng of each of two primers when combinations of two primers were used, and 1 U of thermostable *Taq* DNA polymerase (Supernova *Taq* polymerase, Madi Ltd, Rishon LeZion, Israel). Reactions were carried out in an automated thermocycler (MJ Research Inc, Watertown, Mass., USA). Initial incubation was at 94 °C for 1.5 min, followed by 34 cycles of denaturation at 94 °C for 1 min, annealing at 40 °C for 1 min and polymerisation at 72 °C for 1 min. A final polymerisation reaction at 72 °C was carried out for 7 min after the above-mentioned cycles were completed. The amplification products were visualised by electrophoresis in 1.5% agarose gels. The PCR products were detected by staining with ethidium bromide.

Table 2. Average, lowest and highest values of TGA-content (mg/100 g FW) of the whole NT8 × LT7 population and the sample used.

Population	Number of progeny clones	Average TGA-content (mg / 100 g FW)	Lowest value (mg / 100 g FW)	Highest value (mg / 100 g FW)
Whole population	180	4.2	0.8	15.2
Sample	94	3.8	1.0	12.2

Identification of polymorphic PCR products

Initial identification of polymorphic PCR products was carried out in PCR reactions using DNA templates extracted from the parental clones of both segregating populations. For further analyses only PCR products amplified exclusively in either LT7 (i.e. the parent characterised by high TGA content) or in both Cara and NT8 (i.e. the parents characterised by low TGA content) were used. These polymorphic PCR products were further analysed for association with TGA-content in about half of the segregating population resulting from the cross NT8 × LT7 (Table 2). In these analyses only templates showing clear amplification patterns were included.

Data analysis

Linkage analyses among DNA markers were carried out using the MapMaker analysis program (Lander *et al*., 1987). Association between TGA-content in the tubers and the DNA markers was carried out using the JMP Statistical Package (SAS Institute version 3.5.1). Initially, one-way ANOVA analyses were carried out for each marker separately. In addition, a 'stepwise' procedure for multiple regression analysis (probability to leave or to enter the model = 0.05; direction = mixed) was carried out. The dependent variable in the regression model was tuber TGA-content whereas the independent variables included all polymorphic ISSR markers and all possible two-way interactions among them.

Results

Two single ISSR markers were found significantly associated with TGA-content, when analysed using the one-way ANOVA procedure (Table 3). Effects of the two markers were characterised by a low coefficient of determination (R^2), suggesting that each marker explained only a relative small portion of the total model variance. When both markers were included in a single model (Table 3), both markers were statistically significant, and the coefficient of determination increased to a value (11%)

Table 3. Results of associations between ISSR markers and TGA content in the tubers (mg/100 g FW). Presented are one-way ANOVA, a stepwise analysis without interactions, and a stepwise analysis including a two-way interaction between two loci, respectively.

Statistical analysis	Model	R^2	LOD score
One way ANOVA	[A] [a]	5.4 %	3<LOD<4
One way ANOVA	[B+C] [a]	3.4 %	2<LOD<3
Stepwise, no interaction	[A] [a]	11 %	4<LOD<5
	[B+C] [a]		3<LOD<4
Stepwise, with interaction	[B+C] [a]	21 %	12<LOD<13
	[A]*[B+C] [b]		10<LOD<11

[a] [A] and [B+C] indicate ISSR PCR products generated by primer A and primer combination B+C, respectively (Table 1).

[b] [A]*[B+C] indicates an interaction between loci represented by PCR products generated by the single primer A and the primer combination B+C presented in Table 1.

which was slightly more than the value obtained when combining R^2 values obtained in the single one-way ANOVA models (8.9%). Segregation of the clones used for the identification of polymorphic PCR products (Cara, NT8, LT7) and part of the progeny clones of the cross NT8 × LT7, using primer [A], are shown in Figure 1.

The best statistical model, constructed to characterise the association between ISSR markers and TGA-content in the tubers, was derived from the 'stepwise' procedure. The model was comprised of the ISSR marker [B+C] and a two-way interaction between loci represented by markers [A] and [B+C] (Table 3). Marker [A] appeared not to be a significant effect (p(F) = 0.7451), and was therefore excluded from this model.

The inclusion of the interaction between two loci, as presented in Table 3, caused a remarkable increase in R^2 from 11% to 21%. The quantitative effect on TGA-content of each of the components of the models is presented in Table 4.
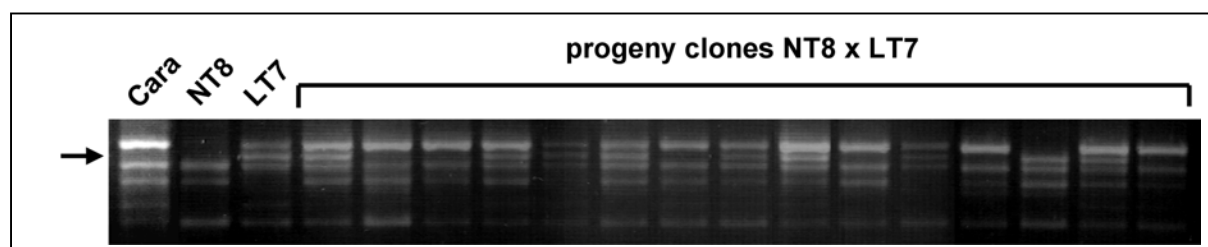

Figure 1. Segregation of the clones used for the identification of polymorphic PCR products (Cara, NT8, LT7) and part of the progeny clones of cross NT8 × LT7, using primer [A]. The PCR product associated with TGA-content is indicated with an arrow.

Table 4. Average TGA-content in the tubers (mg/100 g FW) ± standard error; (a) for each of the ISSR markers separately and (b) demonstrating the interaction between the marker bands. n = number of progeny clones.

(a)

Marker	No PCR product	With PCR product
[A] [a]	4.39 ± 0.48 n = 33	3.43 ± 0.18 n = 69
[B+C] [a]	3.48 ± 0.23 n = 50	4.02 ± 0.36 n = 47

[a] [A], [B+C] indicate ISSR PCR products generated by primer A and primer combination B+C, respectively (Table 1).

(b)

		[B+C] [a]	
		No PCR product	With PCR product
[A] [a]	No PCR product	3.54 ± 0.36 n = 19	5.94 ± 1.07 n = 12
	With PCR product	3.47 ± 0.31 n = 30	3.39 ± 0.25 n = 33

[a] [A], [B+C] indicate ISSR PCR products generated by primer A and primer combination B+C, respectively (Table 1).

Both markers included in the final stepwise model originated from the parental clone LT7, which is characterised by high TGA-content. NT8 and LT7 are characterised by average TGA-content (± SE) of 1.54 (± 0.19) and 8.87 (± 0.78), respectively. Table 4a shows that each PCR product had a different effect on TGA-content: [A] is associated with low TGA-content and [B+C] is associated with high TGA-content.

Table 4b shows that specific combinations of the loci represented by the ISSR marker [A] and [B+C] resulted in a significant increase in TGA-content. The expression of TGA-content when marker [B+C] is present depends on either presence or absence of the allele at the locus represented by marker [A].

Discussion

Total glycoalkaloid content in potato tubers is an important commercial trait. DNA markers could potentially enable the identification and selection of genetic material at a relatively early stage of crop growth, and thus speed up the development of new varieties. Levin *et al.* (2000) reported the identification of ISSR markers, similar to

those used in this study, highly associated with fructose to glucose ratio in the mature tomato fruit. ISSR-PCR primers were also reported to yield reproducible results with sufficient polymorphism in tetraploid potato (Prevost & Wilkinson, 1999; McGregor *et al.*, 2000).

Polymorphic ISSR markers usually display a dominant mode of inheritance in segregating populations, similarly to Random Amplified Polymorphic DNA (RAPD) markers (Ziętkiewicz *et al.*, 1994; Staub & Serquen, 1996). In tetraploid species, where more than two different alleles may reside at a given locus and where a polymorphic ISSR PCR product may represent two or more alleles that are divergent in their inter-primers sequence, clear interpretation of allelic relationships can not be easily established. A MapMaker linkage analysis carried out on all of the polymorphic ISSR products recorded in this study revealed that none of these were statistically associated with any of the others. Based on this analysis, we chose to refer to different PCR products generated by different primers as alleles at different loci, although unambiguous allelic relationships could not possibly be finally determined.

Combined models, consisting of main effects and interactions, can reveal genetic control, such as epistasis, which otherwise would have been overlooked. In many studies, interaction among loci is not taken into account, also in cases where multiple markers are recorded and analysed (Bonierbale *et al.*, 1993; Rouppe van der Voort *et al.*, 1998; Oberhagemann *et al.*, 1999). Our results (Table 3) clearly show the involvement of an epistatic interaction between alleles in the expression of TGA-content. The inclusion of a two-way interaction between two ISSR markers in the regression model almost doubled the percentage of variance accounted for from 11% to 21%. A similar increase in the percentage of variance accounted for by including two-way interactions was also reported in diploid potato by Freyre *et al.* (1994) for tuber dormancy.

The involvement of epistasis in the expression of traits has been demonstrated in various diploid and polyploid crops and for a wide variety of characteristics. A less-than-additive epistatic interaction among genes controlling correlated yield-associated traits was shown in tomatoes (Eshed & Zamir, 1996). Epistatic effects were also reported in crops such as rice, triploid and tetraploid *Musa* spp., diploid and autotetraploid alfalfa, and *Arabidopsis*. Disease resistance, yield components and flowering-time were among the divers characteristics affected by epistatic effects (Wu *et al.*, 1995; Craenen & Ortiz, 1996; Tenkouano *et al.*, 1999; Kidwell *et al.*, 1994; Bingham *et al.*, 1994; Coupland, 1995). Epistatic interactions have also been reported in potato for several traits using DNA marker technology. In a cross between *Solanum* species, QTLs and epistatic interaction between two loci were reported for the development of the root system (Kreike *et al.*, 1996). Šimko *et al.* (1999) reported both

main-effect QTLs and QTLs identified only through interactions for both plant height and tuberisation earliness in diploid potato.

In this study, the vast genetic variation between the parental lines created a segregating population that facilitated the identification of interaction between specific alleles. Epistatic interaction may explain the values of TGA-content that are beyond those of the parental clones, as reported by Van Dam *et al.* (1999). By using Inter-Simple Sequence Repeat (ISSR) primers, we have demonstrated a more-than-additive effect in the genetic control of glycoalkaloid synthesis in tetraploid potato tubers. Such interactions have the potential of extreme genetic expression, which may also be useful in breeding for economically important quantitative traits such as yield and quality. To the best of our knowledge no earlier reports have been made in potato of such interactions for traits related to glycoalkaloid synthesis.

Van Dam *et al.* (1999) reported broad sense heritability estimates for TGA-content ranging from 0.50 to 0.54 with a minimum number of 3 to 7 genes that contribute to such estimate. Using the same populations we managed, in this study, to account for 21% of the variation in TGA content using two ISSR markers, leaving us with about 29% of the variation yet to be accounted for. This remaining variation could be either attributed to the distances between the markers identified in this study and the genes that encode TGA content, or to loci affecting TGA content that are yet to be identified.

CHAPTER 5

Genetic analysis of vein depth in tetraploid potato (*Solanum tuberosum* L.) and its potential for studies of tetrasomic inheritance[*]

J. van Dam[1], P.C. Struik[2] and D. Levy[1]

[1] Institute of Field and Garden Crops, The Volcani Center, POB 6, Bet Dagan 50250, Israel
[2] Department of Plant Sciences, Crop and Weed Ecology Group, Wageningen University, Haarweg 333, 6709 RZ Wageningen, The Netherlands

Abstract

A single copy DNA marker for a morphological leaf character, vein depth, was generated in a study of the association of molecular markers with morphological characteristics in a segregating population of tetraploid potatoes (*Solanum tuberosum* L). Inter-Simple-Sequence Repeats (ISSR) were used to generate random PCR products followed by the design of a Cleaved Amplified Polymorphic Sequence (CAPS) marker. A multiple regression analysis showed that the best model consisted of the ISSR-PCR product and a CAPS-PCR product. The results suggest that these products represent separate alleles at the same DNA locus. Vein depth is apparently controlled by one or several genes that modulates the lamina expansion, either during cell division or during cell elongation. Vein depth is shown to be regulated in an additive way and hence allelic differences are supposedly accountable for small differences in lamina growth. In this study, the feasible generation of a single copy DNA marker in tetraploids and its possible use in the study of tetrasomic inheritance are demonstrated.

Keywords: Plant morphology, tetraploid inheritance, PCR, ISSR, CAPS, leaf morphogenesis

Introduction

Genetics of tetraploid potato is complex due to the possible involvement of four alleles at each locus (Tai, 1982). Potentially, eight different alleles could segregate independently when crossing two tetraploid parents. This implies that even a single locus can display a continuous variation (Meyer *et al.*, 1998). Therefore most genetic research in potatoes has been carried out using diploid populations. Bradshaw *et al.* (1998), however, pointed out that using haploids and diploids for the genetic analyses followed by polyploidisation is time consuming. Moreover, the performance of genes at a monoploid or diploid level does not always reflect their performance at the tetraploid level. Hutten *et al.* (1995) reported significantly lower yields and significantly higher under water weights in diploid progenies than in tetraploid progenies. Therefore, genetic studies at the tetraploid level are important. The understanding of the genetic control is particularly difficult for traits that display continuous variation, such as tuber dormancy, tuber weight, and tuber number (Bradshaw *et al.*, 1995), while the study of discontinuous traits controlled by a single gene or a few genes is feasible. In the present study, vein depth is shown to be such a characteristic that may therefore serve as a model to elucidate heritability in tetraploid potato.

Leaf veins are the continuation of the vascular system of the stem, having a transport function for water, minerals and assimilates. The larger veins have protrusions on the abaxial side of the leaflet (Raven *et al.*, 1986). Visually, vein depth is the depth of the intrusions on the adaxial side of the leaflet blade (Figure 1), opposite to the
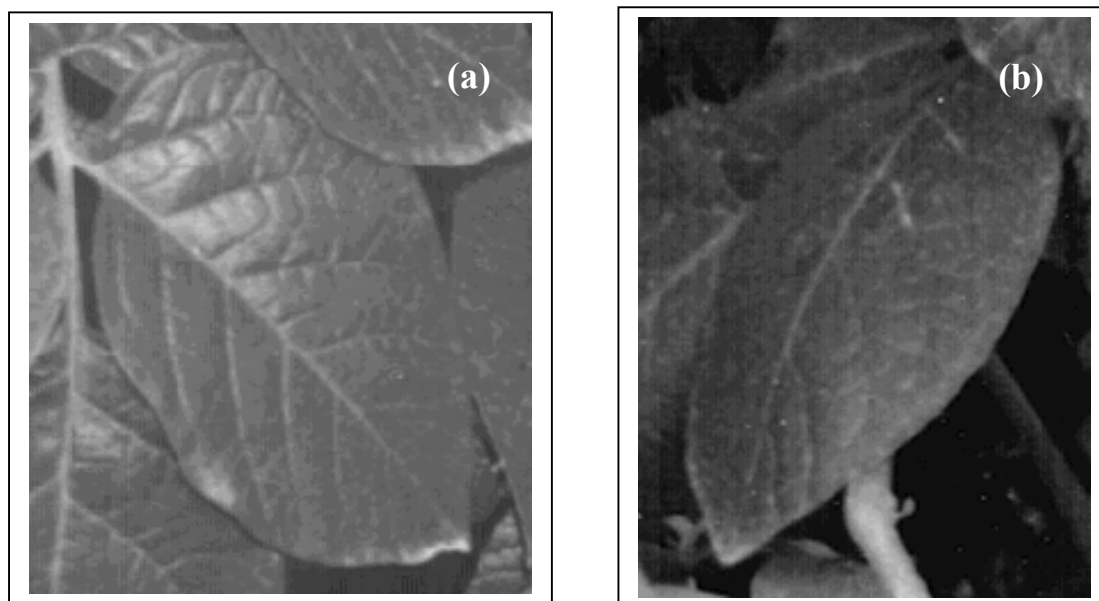

Figure 1. (a) Potato leaflet with deep veins, showing bulging on the adaxial side of the leaflet blade. (b) Potato leaflet with shallow veins, with a smooth surface in between the veins.

aforementioned protrusions where veins are positioned. Morphologically, however, vein depth is the relative amount of leaf tissue in the area in between the midrib and secondary veins of the leaflets. Thus, deep veins have an abundant amount of leaflet blade tissue, causing bulging in between the veins. This morphological trait is commonly used for visual distinction among varieties and is part of the formal description of any cultivar registered for plant breeders' rights (UPOV, 1986).

Vein depth is the reflection of a series of morphogenetic events related to leaf development. All processes in leaf development are accurately controlled by specific genes (Tsukaya, 1995). Three phases are distinguished in the development of a leaf: (1) initiation, (2) primary morphogenesis, and (3) expansion and secondary morphogenesis (Dengler & Tsukaya, 2001). A key issue in leaf morphogenetics is meristematic growth (Veit & Foster, 2002). The manifestation of vein depth takes place during the expansion phase of the lamina. Therefore, vein depth is expected to be controlled by a gene or a few genes that control(s) meristematic growth during lamina expansion.

In a tetraploid segregating population, designed for the identification of a molecular marker for total glycoalkaloid (TGA) content in the tubers, vein depth of the leaflets was assessed, among many other morphological and agronomical characteristics (Van Dam *et al.*, 1999). In this experiment, DNA marker association was found with vein depth. It was also shown that vein depth had a broad sense heritability of 0.30 - 0.31 (Van Dam *et al.*, 1999).

In the present study, we have cloned the PCR product associated with vein depth and subsequently synthesised a Cleaved Amplified Polymorphic Sequence (CAPS) marker, which was also associated with vein depth. Having a single copy DNA marker for vein depth, we found this characteristic appropriate to use as a model for tetrasomic inheritance in tetraploid potato, and thus to show the feasibility of generating molecular markers at this ploidy level.

In other crops, genetic models are being constructed as well for the inheritance of morphological characteristics. Many of these genetic models are related to leaf formation of compound or lobed leaves, such as in chickpea, tomato and cotton (Fagerberg *et al.*, 1990; Hareven *et al.*, 1996; Jiang, 2000).

In this chapter, we present a genetic marker for vein depth in tetraploid potato. To understand the biological relevance of the alleles detected, we discuss our findings within the framework of the morphogenesis of the leaf.

Materials and methods

Plant material

A segregating potato population was constructed with the tetraploid clones NT8 and

LT7 as parents. NT8 is a neotuberosum clone developed in Israel from true potato seed received from Cornell University, USA, and is characterised by shallow leaf veins. LT7 is a clone from the International Potato Center (CIP), Peru (International Potato Center, 1982), and is characterised by medium to deep leaf veins. NT8 and LT7 are unrelated clones, late maturing and have fair yields under Israeli climatic conditions (Levy *et al.*, 1991; K.M. Paddock, pers. comm., 1998). LT7 had significantly deeper veins than NT8 ($p(F) = 0.003$; $R^2 = 0.48$), and among the progeny, a significant clone-effect for vein depth was observed ($p(F) < 0.001$; $R^2 = 0.56$) (Van Dam *et al.*, 1999).

Experimental design

A complete randomised block design with three blocks containing one replicate of each progeny clone was constructed. Five replicates of each parental clone per block were included.

The morphological trait of vein depth was visually assessed by assigning a score on a scale from 1 (very shallow) to 9 (very deep), according to recommendations from UPOV (International Union for the Protection of New Varieties of Plants). Plant cultivation was described in detail by Van Dam *et al.* (1999).

DNA extraction

Apical leaves from individual plants of parental and progeny clones were sampled and immediately frozen in liquid nitrogen. Genomic DNA was extracted according to the miniprep protocol of Fulton *et al.* (1995).

PCR primers

Three hundred forty two single Inter Simple Sequence Repeats (ISSR) primers or two ISSR primer combinations were used in the PCR reactions to amplify random PCR products in the potato genome. One hundred seventy one of these single ISSR primers or primer combinations showed polymorphic bands between the parental clones. Most of the ISSR primers were based on (TC) dinucleotide repeats which appeared to amplify many DNA targets in both the potato and tomato genome. The ISSR primers differed from each other by different specific DNA sequences at their 3' end. One PCR product, generated by ISSR primer combination MS634 (TCTCTCTCTCTCTCCCCG) and MS842 (TCTCTCTCTCTCTCCGGT) appeared to be associated with vein depth.

ISSR - PCR reaction

The amplification reaction of ISSR sequences (25 µl final volume) was performed with 40 ng template DNA, 25 mM TAPS (pH = 9.3 at 25 °C), 50 mM KCl, 2.5 mM

MgCl$_2$, 1 mM β-mercaptoethanol, 0.2 mM of each of the four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP and dTTP), 20 ng of a single primer or 10 ng of each of two primers when combinations of two primers were used, and 1 U of thermostable Taq DNA polymerase Supernova Taq polymerase (Madi Ltd, Rishon LeZion, Israel). Reactions were carried out in an automated thermocycler (MJ Research Inc, Watertown, Mass., USA). Initial incubation was at 94 °C for 1.5 min, followed by 34 cycles of denaturation at 94 °C for 1 min, annealing at 40 °C for 1 min and polymerisation at 72 °C for 1 min. A final polymerisation reaction at 72 °C was carried out for 7 min after the above mentioned cycles were completed. The amplification products were visualised by electrophoresis in 1.5% agarose gels. The PCR products were detected by staining with ethidium bromide.

DNA cloning and sequencing

The PCR product associated with vein depth was excised from an agarose gel and purified with the Gene Clean II-kit (Bio 101, La Jolla, CA, USA). The PCR band was cloned into a pGEM-T Easy vector using the pGEM-T Easy Vector System (Promega, Madison, WI, USA). DNA clones were extracted with QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany). The DNA clone generated by the MS634 + MS842 primer combination was sequenced with an automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequence analysis and locus-specific primer design for the clone produced by primer combination MS634 + MS842 were carried out with Clone Works, clone design system 1.94 (Anteater Software Corp., Los Angeles, CA, USA).

CAPS – PCR reaction and restriction

PCR was performed with 40 ng template DNA, 25 mM TAPS (pH = 9.3 at 25 °C), 50 mM KCl, 2.5 mM MgCl$_2$, 1 mM β-mercaptoethanol, 0.2 mM of each of the four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP and dTTP), 10 ng of the forward and reverse primers and 1 U of thermostable Taq DNA polymerase Supernova Taq polymerase (Madi Ltd, Rishon LeZion, Israel). PCR amplification was carried out in an automated thermocycler (MJ Research Inc, Watertown, MA, USA) programmed for 1.5 min preheating at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C for the denaturation, annealing and extension steps, respectively. There was a final incubation for 7 min at 72 °C. The PCR products were analyzed on 1.5% agarose gels stained with ethidium bromide to check that single fragments had been amplified. Amplified fragments were digested with 17 restriction enzymes to detect CAPS and the products were resolved by electrophoresis on 1.5% gels.

Data analysis

Linkage analyses among DNA markers were carried out using the MapMaker analysis program (Lander *et al.,* 1987). Association between vein depth and the DNA markers was carried out using the JMP Statistical Package (SAS Institute version 3.5.1, 1995). In order to evaluate the linkage between the markers and vein depth of the leaflets, we applied a 'stepwise' procedure for multiple regression analysis (probability to leave or to enter the model = 0.05; direction = mixed) to data obtained from the segregating population. The independent variable in the regression model was vein depth whereas the dependent variables included all polymorphic CAPS markers, the PCR product associated with vein depth (ISSR primer combination MS634 and MS842), and all possible two-way interactions among them.

Results

Three hundred forty two single ISSR primers or two ISSR primer combinations were screened in PCR reactions with DNA extracted from the parental clones. One hundred seventy one of these single ISSR primers or primer combinations were polymorphic between the parental clones. To evaluate the linkage between each of these segregating bands and the trait of vein depth, the clearest polymorphic bands were selected to be analyzed on half of the progeny clones. According to this analysis, one primer combination was associated with vein depth (p(F) = 0.0045; R^2 = 0.06). The PCR fragment produced by primer combination MS634 and MS842 was coupled to shallow vein depth.

```
TCTCTCTCTC TCTCCGGTGG TAGCCAGAGG CATAACATAA CTGGCCGGGA CATTTGCATC GGGAACCTCA
GAAGCCTCTA CTGACTCAAA CAGTGAAGTA AAGTCTGTGG ACTTCAGCTG GTCCACATCC TTCCTCAACT
CCGAAACCTT AGCTTTCAAA GATGTAACCT CAATAGTAAT CCCCTGTTGA CTCTCACAAG TCTCGACTCT
CACCGTGAGA GCATCAATAG ATGTTCGGAG GGGTGTTAAA GCAGCAGATA TGGCTCGCTT AATCATCCAA
GGCACCTCAG ACTCTAGCCG GGAAGCTCGC ATATCTGCAG AATAGACTAG ATGCCCCATC TTGATGAGCA
TGTCCTGAGT AATCAAAGTC CTCGAGGCAT TGGTACCTGA AGTAGAAGCG TGGGAAGTAG TGGTAGAACC
TGGGGCTGGG AAGGAGTGGA AGTGGATGTA CTTGAAGGTT CGAATGCTGG AGTAGGCAAC GCTACCTCTA
TAGGTATAGA CTCAACATCA ACATTTGGGA TGTATACACC GGAGCTGCTC TCTTCGTACC CACCTTATCC
CGTGTGTACT CGGCCTCAAT ACGCCAGATG TCTATGGAGA ATGTGGGTGT AACCTCAATG TACCTCTTAT
CGTAAAGAGA AACTCCAACT CTTCAACATA ACTTCGTAAT AAGCACTGGG AAATGAAGGG ATGTCTGGCG
TTGCTTGGCC TTTATTACCA TCTCCTGCTC AATGATTAGT CCCAAATTGA TGCTCTTTCG GGCTATGATT
GATTCGAGAC AGGCCACCTT TGGGTGGCAG AGGATGGACT CATTTAGTGA GGGCATGATA GAGCTGCTAA
TGAACCCAAA CCAGTACCGC ACTACTGTAA TCAAGTCCTT CTTCTCAATC GAAACTCCAG CCTCGATCCA
CCTCGAGGTG GTATAAAAAA TACGGGGAGA GAGAGAGAGA
```

Figure 2. Nucleotide sequence of the PCR fragment, generated with primers MS634 + MS842, associated with vein depth. The size of the fragment is 950 bp. Primer annealing sites are shown in italics. Designed CAPS primers (DV1 forward and reverse) are shown in underlined italics.

The band was subsequently excised from a gel, cloned, and sequenced as outlined in section Materials and Methods. The nucleotide sequence of the PCR fragment coupled to vein depth had a length of 950 bp (Figure 2). To generate a locus-specific PCR marker for vein depth, we designed two CAPS primers (forward and reverse) complementary tot the 950 bp insert and designated the marker DV1 (Figure 2).

The best statistical model, constructed to explain the association between DNA markers and vein depth, was derived from the 'stepwise' procedure. The model was comprised of a band of the PCR fragment obtained with CAPS marker DV1 digested with restriction enzyme Hpa II, and the 'original' band generated by ISSR-primers MS634 and MS842 (Table 1).

The model presented in Table 1, increases the explained variation considerably, compared to a model which consists only of the data obtained from the ISSR marker (R^2 values from 0.06 to 0.13). By including the CAPS marker to the model, the significance of the ISSR marker was increased a tenfold.

The parental clones of the population were significantly different (Table 2). In addition, besides being statistically different, also according to standards of the International Union for the Protection of New Varieties of Plants (UPOV, 1986), the presented values in Table 2 are considered as 'distinct'. A minimum difference in score of 2 of the rounded values (4 vs. 6; Table 2) is commonly being considered as distinct between two plants (B. Bar-Tel, pers. comm., 1995).

Table 1. Results of the association between DNA markers and vein depth. Presented is the best model ($R^2 = 0.13$), obtained by using stepwise regression.

Source	DF	Prob > F
Block	2	0.8009
MS634 + MS842	1	0.0005
DV1, Hpa II	1	0.0080

Table 2. Mean scores and standard errors (S.E.) for vein depth of parental clones LT7 and NT8 and their tetraploid crossing population. Indicated are absence or presence in the parental clones of the PCR product generated by marker DV1.

Parent	No. individuals	Mean	S.E.	Marker DV1
LT7	15	5.67	0.28	present
NT8	15	3.89	0.35	absent
Population	174	5.17	0.06	

Table 3. Mean scores and standard errors (S.E.) for vein depth of individuals of the population. PCR product, generated by ISSR primers MS634 + MS842, and PCR product generated by CAPS marker DV1 digested with enzyme Hpa II, related to leaflet vein depth.

MS634 + MS842	DV1, Hpa II	No. individuals	Mean	S.E.
absent	absent	4	6.17	0.79
absent	present	3	5.78	0.40
present	absent	25	5.25	0.14
present	present	17	4.73	0.18

For those individuals of the population where marker data were available both for the ISSR marker MS634 + MS842 and CAPS marker DV1, mean scores and standard errors were calculated for vein depth (Table 3).

Both the PCR fragment generated with the ISSR primers and the PCR fragment generated with CAPS marker DV1 (Table 3) were associated with shallow vein depth, whereas absence of both PCR fragments was associated with deep vein depth. Occurrence of only one of the PCR products was associated with an intermediate vein depth.

Discussion

Analysis of the ISSR marker data showed statistically significant association of leaflet vein depth with one of the primer combinations (MS634 and MS842). CAPS marker DV1, designed according to the DNA sequence of the PCR fragment generated with this ISSR primer combination, was digested with enzyme Hpa II. Five separate PCR products were generated at the parental clones: one PCR product at NT8 and four PCR products at LT7. The band associated with leaflet vein depth was present in LT7.

Statistical analysis showed that marker DV1 increased statistical significance to the model based on the ISSR marker considerably, and did not just replace what was detected with the ISSR marker (Table 1). This suggests that both PCR fragments represent two separate alleles which are associated with vein depth. In the progeny, both the PCR product generated with ISSR primers MS634 and MS842 and the one generated with CAPS marker DV1, are associated with shallow vein depth (Table 3). However, values of the parental clones (Table 2) are opposite. This suggests that more alleles, which could not be detected yet, are involved in the trait of vein depth.

To understand the biological relevance of the alleles detected, understanding of the morphogenesis of the leaf is an indispensable prerequisite, especially the expansion of the lamina. Plant morphology has the potential to provide the context for molecular genetic investigations, especially those concerned with leaf morphogenesis (Kaplan,

2001). Leaves of green plants vary widely in morphology. However, the underlying cell types and structures observed in leaves of different species are remarkably similar (Hall & Langdale, 1996).

When leaves are formed, first the main veins (midrib and primary lateral veins) develop (Nelson & Dengler, 1997). In potato, lateral veins are similar to the midrib in anatomy and morphological structure (Artschwager, 1918). Commonly, six to seven vein orders are found in the potato leaf. The first three orders are associated with rib tissue for all or most of their length, and are thus major veins (McCauley & Evert, 1988). Then the lamina starts to expand, which depends on the division and the elongation of cells (Tsukaya, 1995; Nelson & Dengler, 1997). Lamina expansion consists of a diffuse pattern of regulated cell division without any clear temporal or spatial pattern. This pattern of cell division has led to the designation of the lamina in this stage as a 'plate meristem' (Steeves & Sussex, 1989; Veit & Foster, 2002). Thereafter the small veins develop. Secondary vein formation is described as progressive, as the amount of small veins depends on the size of the developing lamina (Nelson & Dengler, 1997; Kull, 1999). The development of small veins is a process of self-organisation. This can be described as a fractal, i.e., a reiteration of the same pattern on different scales, what can be applied in developmental models (Kull & Herbig, 1995). Genes that act to determine the various parts of the leaf, such as blade, margin and vasculature and tissue systems seem to be similar in simple leaves as in leaflets of compound (dissected) leaves, so that each leaflet resembles a simple leaf in its development and histogenesis (Esau, 1965; Steeves & Sussex, 1989; Goliber *et al.*, 1999).

Much of the diversity of leaf shape in nature arises from variation in the amount of expansion within the plane of the lamina. Allometric analyses of leaf expansion demonstrate that this may occur either during the initiation of the lamina or much later, as the lamina expands (Poethig, 1997). The majority of cells in a leaf are formed after expansion of the lamina has begun (Dengler & Tsukaya, 2001). Studies of *Xantium* and tobacco showed that the relative growth rates for expansion of both the lamina and the midrib differs among various regions of the leaf (Maksymowych, 1973; Poethig & Sussex, 1985a). Bulging of the interveinal regions can thus develop as a result of a higher growth rate of the lamina regions than of the main vascular tissues. Although preliminary observations indicate that the pattern of cell division is closely correlated with the dynamics of leaf growth, there is no evidence that cell division directly controls this pattern. Contiguous cells in the lamina can have the same relative rate of expansion while having dramatically different rates of cell division (Poethig & Sussex, 1985b). However, the control of the distribution or of the number of cells in the leaf blade must also participate in the regulation of leaf expansion (Tsukaya, 1995).

Insight in genetic control of lamina expansion has been gained by means of the analysis of mutants. In *Arabidopsis*, three mutants have been identified that are directly related to reduced cell elongation in the lamina: *angustifolia* (width direction), *rotundifolia3* (length direction), and *acaulis1* (all directions) (Tsuge *et al.*, 1996; Tsukaya *et al.*, 1993). Likewise, mutants have been identified that do develop major veins, but which hardly develop a lamina or not at all, such as *wiry* mutants in tomato, the *lam-1* mutation in tobacco and the *phantastica* mutant in snapdragon (Kessler *et al.*, 2001; McHale, 1992; Waites & Hudson, 1995). It is reasonable to assume that vein depth, being actually the amount of leaf tissue (lamina) in the area in between the main veins of the leaflets, is controlled by a gene that controls the lamina expansion. This implies that the alleles identified in this study, account for a more or lesser extent of lamina growth, which can be caused by either more cell division during leaf ontogeny or more cell elongation in a later stage.

Both in the population described in this study and in a Cara × LT7 population, vein depth was significantly different among progeny clones, and no environmental influence was observed. Moreover, we found that vein depth in the potato leaflet is genetically regulated in an additive way (J. Van Dam, unpublished). Each gene or combination of genes, and within each gene each combination of alleles during leaflet development is accountable for each 'pre-programmed' step. In case of genetic additivity, allelic differences are supposedly accountable for a more or lesser extent of small differences in morphology. This means for vein depth a more or lesser extent of lamina growth in the interveinal region. This reasoning is supported by an extensive genetic study of leaf morphology it tetraploid cotton. QTL analysis suggested that some alleles increase and others decrease many small leaf attributes, such as leaf length, width, and sublobe angle (Jiang *et al.*, 2000).

So far, DNA markers have been obtained in tetraploid potato by using RFLP (Restriction Fragment Length Polymorphism) or AFLP (Amplified Fragment Length Polymorphism) marker systems (e.g. Bendahmane *et al.*, 1997; Bradshaw *et al.*, 1998; Li *et al.*, 1998; Meyer *et al.*, 1998). RFLP needs high quantities of DNA and AFLP needs high operational and financial investment (Karp *et al.*, 1997). In our study, we used Inter-Simple Sequence Repeats (ISSR), which needs small amounts of DNA and which is a relative inexpensive marker system. ISSR was reported to give reproducible results with sufficient polymorphism in tetraploid potato (Prevost & Wilkinson, 1999; McGregor *et al.*, 2000). The single copy DNA marker for vein depth found in this study, demonstrates the feasibility of generating this type of molecular markers in tetraploids and their possible use in studies of tetrasomic inheritance.

CHAPTER 6

Background parental clones

Two segregating resource populations were constructed for the research described in this thesis. One population consisted of the cross Cara × LT7, and a second population of the cross NT8 × LT7, so the two crosses had one parent in common. All parental clones used were tetraploid. The choice of these clones was firstly on the basis of their known divergent content of total glycoalkaloid (TGA) in the tubers (Dimenstein *et al.*, 1997). LT7 is characterised by a high TGA-content in the tubers, whereas both Cara and NT8 are characterised by a low TGA-content in the tubers (Levy *et al.*, 1995; Dimenstein *et al.*, 1997). Secondly, these clones were chosen because of their similar performance under Israeli conditions, being late maturing and having fair yields. Thirdly, LT7, Cara and NT8 are unrelated clones (Levy *et al.*, 1991; K.M. Paddock, pers. comm.). Besides this general comparison of the parental clones, specific information is presented in this chapter on the parental clones used. The choice of the parental clones determines the potential of their offspring, both for each characteristic individually and for a combined, arbitrary set of traits.

LT7

LT7 is a clone from the International Potato Center (CIP), Peru. The advanced breeding clone LT7 (CIP accession number 378017.2) was bred in 1978 as part of the tropical lowland breeding programme at CIP, and was distributed to national breeding programmes in 1983 (International Potato Center, 1982). The programme was aimed at the development of genetic materials with tolerance to heat under both arid and humid conditions. To complement heat tolerance, emphasis was put on resistance to viruses, particularly PVY and PVX and increased levels of quantitative resistance to PLRV. In addition to Tuberosum clones, a range of native cultivated species (such as *S. phureja*, *S. stenotomum*, *S. ajanhuiri*, and *S. tuberosum* subsp. *andigena*) and wild species (such as *S. sparsipilum*, *S. chacoense*, *S. microdontum*, *S. demissum*, *S. acaule*, and *S. bulbocastanum*) were used in developing the tropical lowlands population. Despite the use of primitive germplasm, the lowland tropic population with virus resistance has the phenotypic aspects of the subspecies Tuberosum (M. Bonierbale, pers. comm.). The breeding philosophy of germplasm enhancement of tropical populations at CIP is presented in Figure 1.
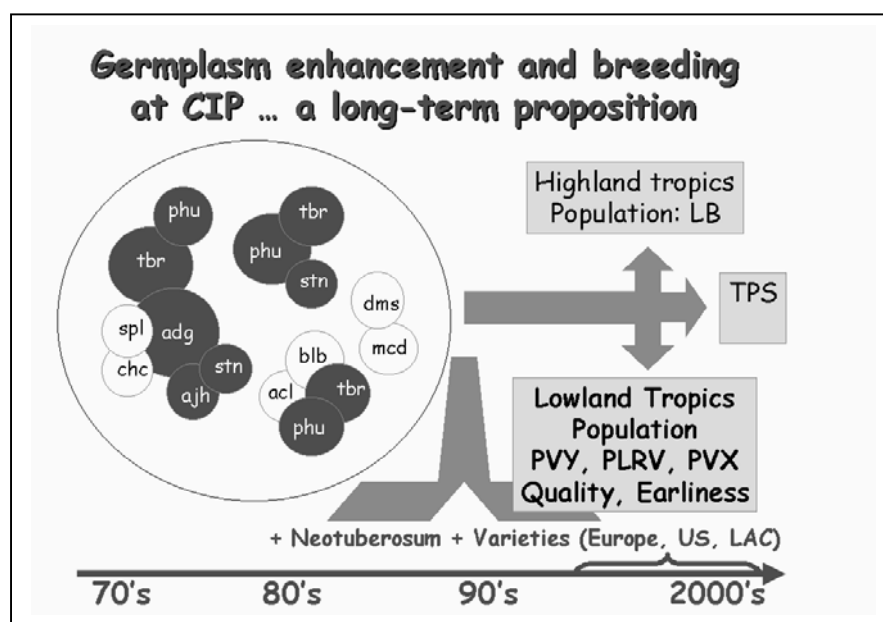
Figure 1. Breeding philosophy of germplasm enhancement of tropical populations at CIP. The large circle symbolises the gene pool of native cultivated species (dark circles) and wild species (light circles) used. In addition, European, US and Latin American cultivars, and Neotuberosum clones have been used. The breeding programmes aimed to produce advanced breeding material for the highland tropics, the lowland tropics, where LT7 is a product from, and the production of true potato seed (TPS). Reproduced with kind permission from CIP.

The pedigree of LT7 is listed in the CIP records as 'ASC-77.055 × ?'. The '?' probably refers to bulk pollen, as mentioned in a comment in the description of LT7 of the CIP records: 'TPS male parent'. It is assumed that the pedigree includes *S. demissum*, Andigena and Neotuberosum, in addition to Tuberosum (M. Bonierbale, pers. comm.). In the CIP records, LT7 is described as adapted to a warm, tropical climate, and drought resistant. LT7 is characterised as an outstanding progenitor transmitting high yield and tuber uniformity to its progenies (International Potato Center, 1982).

NT8

NT8 is a Neotuberosum clone, selected in Israel from true seed (S 375) received from Cornell University, Ithaca, NY, USA. The seed was obtained in 1977 from the Cornell Andigena Improvement Program. This breeding programme aimed to introduce Andigena-material into Tuberosum, to broaden its genetic base. In a long-term programme at Cornell University, breeding was carried out by alternating controlled or bulk pollination, and open pollination. The breeding programme was extensively described by Plaisted (1980). The pedigree of NT8 is presented in Figure 2.
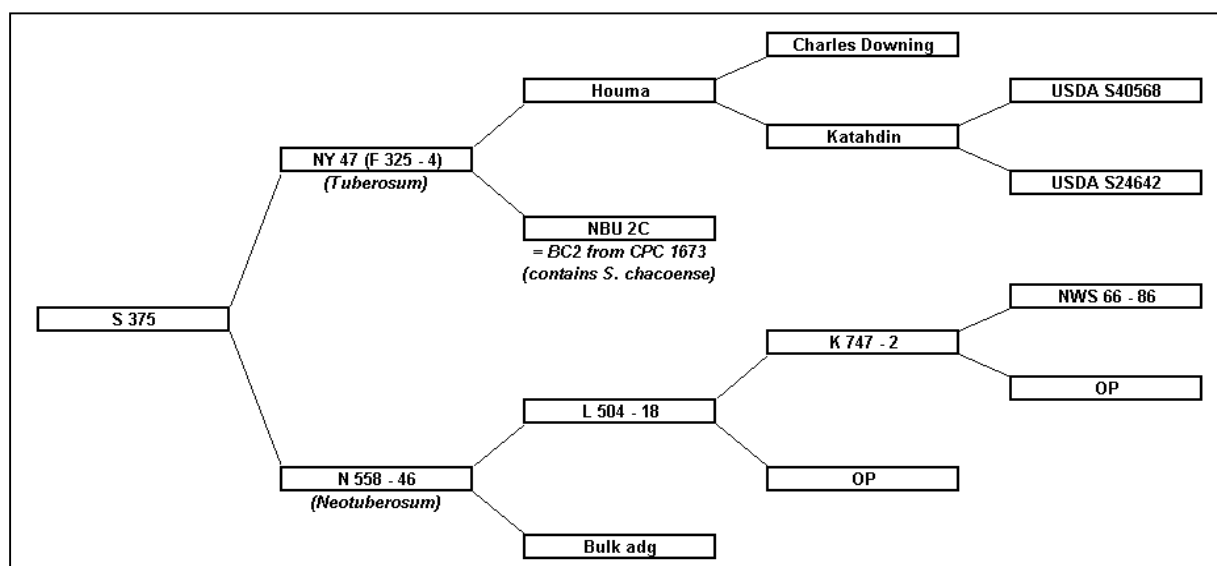
70

Figure 2. Pedigree of NT8, which is a clone of true seed (S 375). Information was kindly provided by Mr. K.M. Paddock from Cornell University or obtained from the Potato Variety Inventory of the Potato Association of America (http://www.ume.maine.edu/PAA/PVI.htm) (Chase, 2002). OP means open pollination. CPC 1673 is an accession of the Commonwealth Potato Collection, which is a collection of seeds from the Bolivian Andigena cultivar Polo (Plaisted & Hoopes, 1989). NWS 66 refers to bulk seed that Cornell University obtained from N.W. Simmonds in 1966 from one or two cycles of selection from CPC collection of Andigena (K.M. Paddock, pers. comm.).

Cara

Cara is a cultivar bred at An Foras Taluntais, Oak Park Research Station, Carlow, Ireland. It was published on the Irish National List in 1973 (Stegemann & Schnick, 1985). The parental clones are Ulster Glade, a cultivar from Northern Ireland, and A25/19, which is probably an Andigena breeding line of the research station (Stegemann & Schnick, 1985). The pedigree of Cara, as far as information was published on internet at the Potato Pedigree Database (http://www.dwp.wageningen-ur.nl/pv/query.asp) (Hutten & Van Berloo, 2001) is presented in Figure 3. Cara has been grown in Israel for many years. In 2001, ca. 360 tonnes of this cultivar were produced and it was sold on the local market and exported to Europe (Y. Peleg, pers. comm.).

Cluster analysis

As stated above, LT7, Cara and NT8 are unrelated clones. Using the data obtained in the ISSR-PCR analyses, a cluster analysis of the three parental clones was carried out.
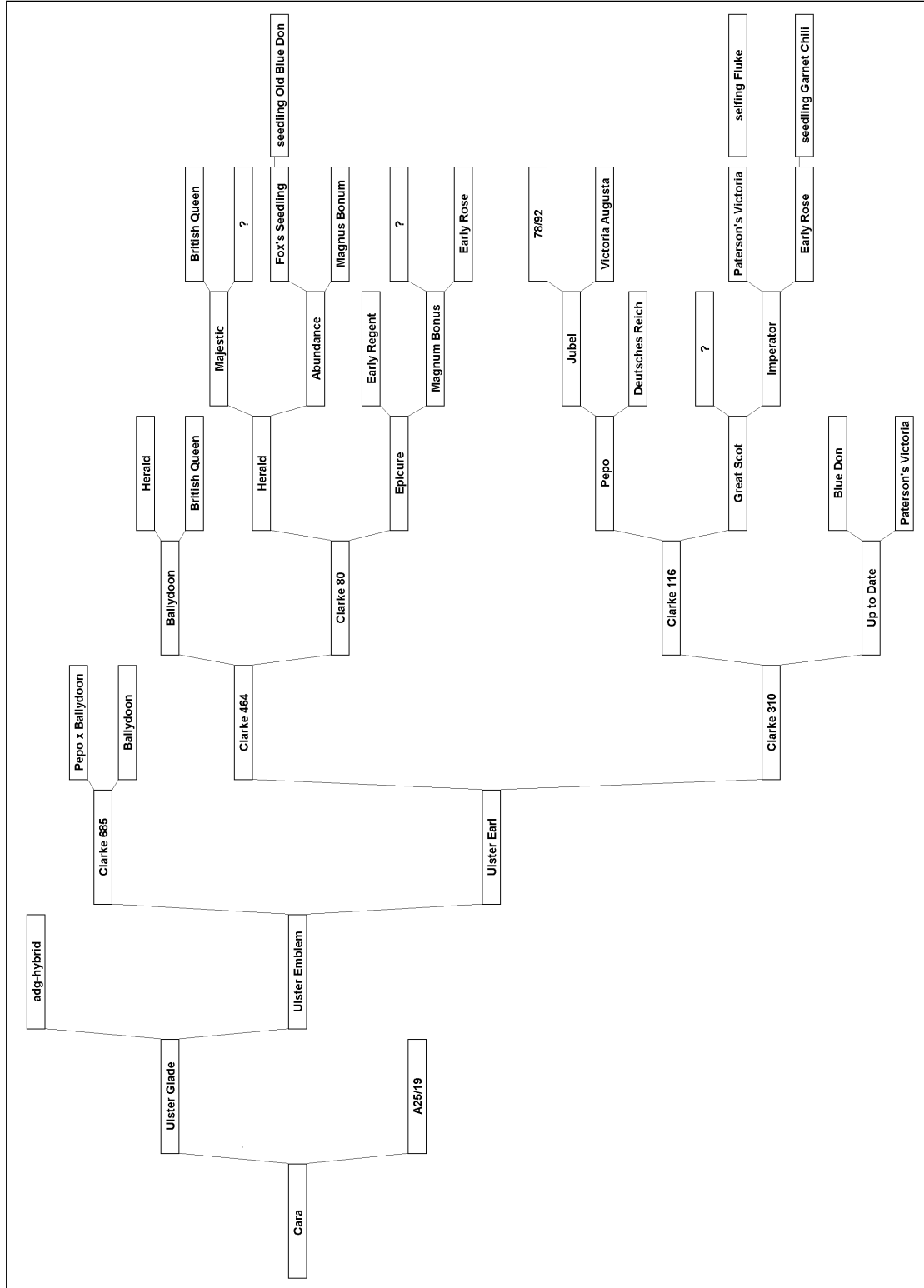
71

Figure 3. Pedigree of Cara. Information was retrieved from the Potato Pedigree Database. Synonym for Cara is Oak Park Beauty. British Queen is the synonym for Up-to-Date in the USA. A question mark means that no record is known.
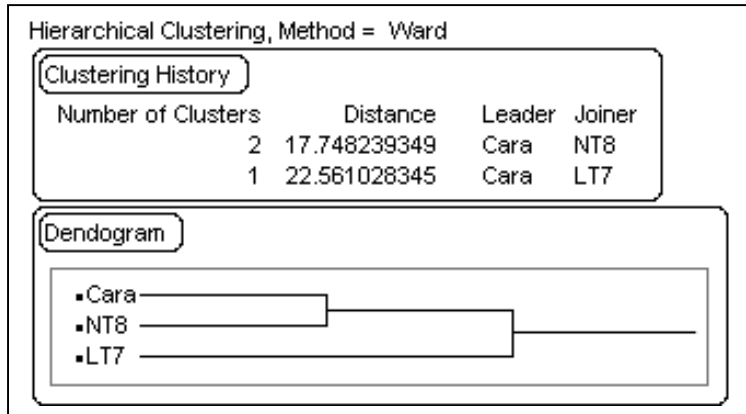
Figure 4. Cluster analysis of the parental clones, using all polymorphic ISSR PCR-products obtained.

This analysis was carried out using a total of 412 polymorphic ISSR PCR-products. The resulting cluster analysis is presented in Figure 4.

Ward's Method distance is the Anova sum of squares between the two clusters summed over all the variables. The cluster analysis demonstrates an estimation of the relative genetic distance among the three parental clones. Cara is a Tuberosum clone and NT8 is a Neotuberosum clone. Especially LT7 is genetically more distant from Cara than NT8, which is likely, as many wild species are used in its pedigree.

CHAPTER 7

General discussion

The main objective of this study was to develop DNA markers associated with total glycoalkaloid (TGA) content in the potato tubers. For this purpose two tetraploid resource populations segregating for TGA-content were constructed and analysed in two consecutive experiments. The populations were characterised for the genetic control of total glycoalkaloid content in the tubers. Although tuber glycoalkaloid content was the most important trait under study, additional agronomic and morphological traits were also recorded and analysed. These additional traits were used to characterise the phenotypic and genetic architectures of the two segregating populations used in this study.

Experimental design

The experimental design of the two experiments conducted for this study was especially constructed to eliminate environmental factors that could bias the results. Blocks were designed and each progeny clone was planted in replicates. Both experiments consisted of a progeny of around 200 individuals in three replicates, which provided a resource population of a good size. The replicates per progeny clone were tubers of the same mother plant. Hence, there was by definition no genetic variation among the replicates. All variation observed was thus environmental. We have chosen populations with clonal replicates rather than larger populations without replicates. Using clonal replicates enabled us to eliminate the environmental effects in statistical analyses when calculating clonal variation and genetic correlation between tuber TGA-content and all other characteristics assessed.

Genetic characterisation of the two populations

Glycoalkaloids are natural substances that are synthesised in many plant organs of the potato plant, among which in the tubers. At low concentrations, glycoalkaloids enhance potato flavour, but at higher contents they impart bitterness and a burning sensation in the mouth and throat (Clayton & Percival, 2000). The synthesis of TGA is enhanced by several environmentally induced stress conditions. The influence of environmental factors on total glycolkaloids has been thoroughly studied. Among the factors influencing glycoalkaloid levels are: temperature, frost and hail damage to

foliage, and exposure of tubers to light. In addition, crop maturity, tuber damage and prolonged storage may also affect TGA-production in the potato tuber (Storey & Davis, 1992).

Besides the influences of tuber development and of environmental factors, attempts to characterise the genetic control of TGA-content in the tubers were carried out as well. There is a large variation among cultivars in their ability to produce tubers with high TGA-contents and this characteristic is heritable. The inheritance of glycoalkaloids in potato tubers was first studied in the early 1970s by Sanford & Sinden (1972). Since then, additional studies have been conducted on the genetics of glycolkaloids, covering tetraploids and diploids. Studies on the genetic inheritance of TGA-content are obviously important for breeding purposes. Such studies are also important in order to predict the ability to tag informative DNA markers associated with TGA-content in potato and the ability to improve classical breeding by marker assisted selection (MAS).

The purpose of breeding is to obtain cultivars that perform well in a diversity of characteristics and are commercially successful. The breeding objective is a function of crop characteristics under a set of growth conditions, such as yield, tuber number, tuber size distribution, tuber quality traits, disease resistances, and of market demands. Therefore, all economically important traits are to be taken into account when defining the breeding objectives. In the present study, besides TGA-content in the tubers, other agronomical traits were assessed as well, such as tuber dormancy, tuber weight and tuber number. Several traits were calculated based on the data obtained, such as tuber size distribution and tuber number distribution.

Newly bred cultivars can be submitted for plant breeders' rights, which provide legal protection against unauthorized use of products of breeding efforts, and which is part of the protection of intellectual property. Companies or individuals owning these rights may generate royalties from cultivars protected by plant breeders' rights. For the purpose of plant breeders' rights, morphological characteristics form an excellent basis for distinction among cultivars. Agronomic traits are not used for cultivar description, as these are heavily influenced by environmental factors such as climate, weather conditions and farmers' cultivation practices. In the present study, a description of morphological characteristics of the two populations designed for this work was therefore made.

Genetic control of TGA-content in the tubers

Following the first screenhouse experiment and assessment of TGA-content in the tubers, prior to the beginning of the DNA marker analysis, we estimated the minimum

number of genes contributing to the TGA-content to be between 3 and 7. From the relatively low number of genes involved and relatively high broad sense heritability estimates (0.54 and 0.50) found in this study, we concluded that the populations used were suitable for DNA marker analysis for TGA-content in the tubers.

The search for DNA markers associated with TGA-content in the tubers, was carried out using the resource population from the second screenhouse experiment. We were able to identify two single ISSR markers that were significantly associated with TGA-content. These markers explained 5.4% and 3.4% of the total variation, respectively. These relatively low percentages suggest the involvement of minor genetic factors. Following a multiple regression analysis that included all PCR products and all possible two-way interactions among them, we were able to explain 21% of the total variation. The resulting best model consisted of a single ISSR product and an interaction between two loci represented by PCR products. A similar increase in the percentage of variance accounted for by including two-way interactions was also reported in diploid potato by Freyre *et al*. (1994) for tuber dormancy.

Intentionally we prefer to use the term PCR product instead of allele or gene. The results show clear genetic factors, as the statistical significance of the associations found was high. However, due to tetraploidity of potato and the ISSR marker system used, there is no way to determine whether the two genetic factors found are two alleles of the same gene, or two genetic factors of two different genes.

Integrating the three aforementioned points: estimation of the minimum number of genes contributing to the TGA-content, the best statistical model associating PCR products with TGA-content, and the denomination of the genetic factors as PCR products gives some room to philosophise. One PCR product and one interaction between two PCR products explained 21% of the total variation. These PCR products could be either alleles of a single gene or two different genes. Extrapolation of the case that there are two alleles of one gene, supposing all other genes related to TGA-content in the tubers function similar to the one presented here, leads to the conclusion that two or three genes control TGA-content in the tubers. Extrapolation of the case that these PCR products are different genes, leads to the conclusion that four or five genes control TGA-content in the tubers. Thus, the estimate of the minimum number of genes contributing to TGA-content of a value between 3 and 7 seems to be realistic. Obviously, this extrapolation is somewhat tentative. Theoretically, the remaining total variation to be accounted for might be one single major gene or numerous genes. Nevertheless, one major gene is less likely than the contribution of several genes to control tuber TGA-content, due to the quantitative nature of the characteristic. This was demonstrated with the normal frequency distribution of mean TGA-content observed in the progeny population after transformation to a common logarithm.

We were able to account for 21% of the total variation of TGA-content in the tubers, based on the DNA marker association identified in cross NT8 × LT7. The broad sense heritability estimated for TGA-content in the tubers in this cross was 0.50, which suggests that another 29% of the total genetic variation is yet to be explained. This result implies that other genes and/or alleles are involved and still need to be identified.

DNA marker associations

The population genetics of TGA-content in the two populations used was described as an important step in the identification of DNA markers associated with this trait. Association between TGA-content and polymorphic PCR products, generated by Inter Simple Sequence Repeats (ISSR) primers was demonstrated. Probably the most remarkable feature of the detected association is the epistatic interaction between two loci. The awareness of the occurrence of this type of genetic control is increasing in recent years, and this type was revealed recently in several crops for various traits.

In the search for DNA markers associated with TGA-content in the tubers, data were obtained from the numerous polymorphic PCR products generated. Besides tuber TGA, over 30 different traits were assessed. The same statistical approach, which was used to identify association between tuber TGA and the polymorphic PCR products, was applied to all other traits assessed. As a result of this analysis, a DNA marker associated with the morphological characteristic of vein depth of the leaflets was detected. This characteristic is the relative amount of leaf tissue in the area in between the midrib and secondary veins of the leaflets.

PCR products associated with tuber TGA-content

Our data confirmed that the parental clones in both experiments had TGA-values towards the extremes of the frequency distribution of the progeny, as expected. The common parent in both experiments, LT7, was high in TGA-content in the tubers. Hence, we assumed that LT7 should be the donor of genes related to tuber TGA-content. This seemed more likely than genes contributing to low TGA-content from Cara and NT8. Therefore, we assumed that a PCR product associated with high TGA-content should be present in LT7 only, and should not be present in either Cara or NT8.

In the original concept of the research it was planned both to extract DNA and to determine TGA-content in the tubers from all clones in both experiments. The idea was to search for DNA markers related to TGA-content in the tubers in the first

population (Cara × LT7), and to check the results in the second population (NT8 × LT7). However, during the first experiment we were not able to extract DNA of good quality or in a sufficient quantity. During the second experiment we had learned to overcome the technical difficulties, and so we extracted the required high quality DNA.

Polymorphic PCR products were tested for association with tuber TGA-content in a random sample of 20 individuals of the NT8 × LT7 progeny. When these preliminary results showed sufficient statistical significance, the number of individuals was increased. After each increase in number of individuals tested, statistical significance of the association between PCR product and TGA-content was calculated. When the statistical significance did not increase, the PCR product was discarded as putative DNA marker.

Cloning of PCR products

Cloning of the PCR product associated with the TGA-content in the tubers is required for designing a codominant single copy DNA marker. After excising the PCR product from the agarose gel and its purification, cloning is a standard procedure. We attempted cloning of the PCR product associated with TGA-content in the tubers over numerous times, but without success. Therefore, we concluded that this PCR product is not clonable. The most common explanation of such cloning failure is that the insert encodes a product that is toxic to the bacteria. On the other hand, the PCR product associated with vein depth was cloned without difficulties.

Some additional comments on the genetics of TGA-content

It is assumed that genes that can induce high levels of TGA in the tubers originate from clone LT7. However, it should be noted that *all* clones, including those low in TGA-content in the tubers, do produce glycoalkaloids to some extent. This is important because we found that one of the factors involved in TGA-content is a genetic interaction. Thus, theoretically, a clone with low TGA-content can contribute considerably to the TGA-content of its progeny. *Vice versa* it is known that clones with high levels of tuber-TGA can produce progeny clones low in TGA-content. The 'classic' example is the cultivar Lenape, which was removed from commerce due to its high TGA-content in the tubers. Nevertheless, Lenape was the male parent of the cultivar Atlantic, which did not reach excessive levels of TGA in repeated tests (Sinden *et al*., 1984). Atlantic was listed in the top ten ranking U.S. potato cultivars in the 1984 certified seed crop (Plaisted & Hoopes, 1989). The new Israeli cultivar Zohar

had LT7 as male parent. Similar to Atlantic, Zohar remained under the safety threshold for TGA-content in the tubers in repeated tests (Levy *et al.*, 2001). The fact that a parental clone which is high in TGA-content produces progeny clones low in TGA-content may be explained by splitting up of interacting genes that account for high levels of TGA, eliminating the epistatic effect and resulting in genotypes low in glycoalkaloids.

We surmise that additional genes and/or alleles beyond those identified in the populations used are involved in the control of TGA-content in the tubers, as some wild potato species produce tubers with TGA-levels that exceed considerably the levels of TGA detected in this study.

The nature of the research programme

Fundamental research projects on the genetic analysis of potato tend to be defined by strictly and narrowly defined research goals. Experiments like the ones carried out for this thesis are usually of considerable size. In many instances, only a few closely related traits are measured as well besides the main characteristics in this kind of experiments. This might be due to lack of time or because of lack of interest. Although understandable, it is nevertheless regretful that seemingly 'not-so-important' characteristics are left out. In the experiments carried out for this thesis, the assessment of TGA-content in the tubers was the main objective. However, besides that specific characteristic, a considerable number of other traits was assessed as well. From a scientific point of view, all knowledge generated can be valuable. By observing a broad variety of traits, comprehensive data sets can be built. Analysis of these data allowed us to reveal new and unexpected insights.

One of the morphological observations made was leaflet vein depth. This characteristic is a standard observation for the description of cultivars submitted for plant breeders' rights. It should be noted that this characteristic is only used by experts for the description of cultivars, but not by breeders as a breeding objective. Combining vein depth data with the data of the molecular markers collected already for TGA-content, lead to the identification of a Cleaved Amplified Polymorphic Sequence (CAPS) marker associated with vein depth. This marker appeared to have relevance to leaf development, and was associated with the relative amount of leaf tissue in the area in between the midrib and secondary veins of the leaflets. Having a single copy DNA marker for vein depth, we found this characteristic appropriate to use as a model for tetrasomic inheritance in tetraploid potato, and thus to show the feasibility of generating molecular markers at this ploidy level.

Prospects of the use of DNA markers for breeding potatoes for warm climates

Until a few decades ago, most world potato production was concentrated in Europe. The potato has rapidly migrated into tropical and subtropical areas where it is grown extensively (International Potato Center, 1984). In the global distribution of potato area, two main peaks can be distinguished in the distribution by latitude. One peak is between 45 °N and 57 °N, which decreased considerably between 1950 and 1998. The second peak is between 23 °N and 34 °N, which mainly represents production zones in the subtropical lowlands, coinciding with the areas in the world showing the highest population densities (Hijmans, 2001). More than 40% of the world's potatoes are already grown in developing countries and that proportion is expected to increase (Collins, 2000). The growth rate for the production of potatoes in developing countries was calculated to be 2.7% annually from 1993 to 2020, which is noticeably higher than for any other crop (Scott *et al*., 2000). Hence, the need for potatoes adapted to warm climates is expected to increase.

The genetic base for modern cultivar development is narrow. Because of its complex genetics, broadening of the genetic base of the cultivated potato has progressed very slowly. Breeders responsible for developing new cultivars generally do not have the time or resources to systematically transfer the many useful characteristics from the wild species or primitive varieties to 4× Tuberosum parents (Pavek & Corsini, 2001). Besides resistance to pests and diseases, there is a need for breeding for increased size of tubers, long dormancy period, and elimination of increase in glycoalkaloids caused by exposure to light during the shelf life. Improving processing qualities of potato will become possible if the breeding strategy involves an appropriate blend of Mendelian and molecular breeding (Swaminathan, 2000).

Inheritance studies and DNA marker association analysis on the tetraploid level, as carried out for this thesis, were shown to be useful. Although data at a diploid or at a monoploid level may be easier to analyse, it was demonstrated that working at the tetraploid level could reveal unique genetic action. Moreover, progress has been made in the development of a DNA marker associated with TGA-content in potato tubers. When the development of such DNA marker will be completed, it may be valuable in programmes which include the introgression of genes from wild species while discarding genotypes high in TGA-content, contributing considerably to breeding potatoes adapted to warm climates.

References

Allen E.J. and Scott R.K., 1992. Principles of agronomy and their application in the potato industry. *In: P. Harris [Ed.], The potato crop. 2nd ed. Chapman & Hall, London*, p. 816-881.

Allen E.J. and Wurr D.C.E., 1992. Plant density. *In: P. Harris [Ed.], The potato crop. 2nd ed. Chapman & Hall, London*, p. 292-333.

Allen E.J., O'Brien P.J. and Firman D., 1992. Seed tuber production and management. *In: P. Harris [Ed.], The potato crop. 2nd ed. Chapman & Hall, London*, p. 247-291.

Almekinders C.J.M., 1995. *On flowering and botanical seed production in potato (*Solanum tuberosum *L.)*. Wageningen Agricultural University, Wageningen, 133p.

Arazi J., 1990. *Effect of environmental factors on developmental stages and accumulation of yield in potato plants*. M.Sc. Thesis [in Hebrew]. Hebrew University of Jerusalem, Rehovot, 93p.

Artschwager E.F., 1918. Anatomy of the potato plant, with special reference to the ontogeny of the vascular system. *Journal of Agricultural Research* 14: 221-252.

Basu P.S. and Minhas J.S., 1991. Heat tolerance and assimilate transport in different potato genotypes. *Journal of Experimental Botany* 42: 861-866.

Ben David E., 1999. *Fresh potatoes*. M.Sc. thesis [in Hebrew]. Hebrew University of Jerusalem, Rehovoth, 61p.

Bendahmane A., Kanyuka K. and Baulcombe D.C., 1997. High-resolution genetical and physical mapping of the Rx gene for extreme resistance to potato virus X in tetraploid potato. *Theoretical and Applied Genetics* 95: 153-162.

Ben Khedher M. and Ewing E.E., 1985. Growth analyses of eleven potato cultivars grown in the greenhouse under long photoperiods with and without heat stress. *American Potato Journal* 62: 537-554.

Beukema H.P. and Van der Zaag D.E., 1990. *Introduction to potato production. Pudoc, Wageningen,* 208p.

Bingham E.T., Groose R.W., Woodfield D.R. and Kidwell K.K., 1994. Complementary gene interactions in alfalfa are greater in autotetraploids than diploids. *Crop Science* 34: 823-829.

Bodlaender K.B.A., 1963. Influence of temperature, radiation and photoperiod on development and yield. *In: J.D. Ivins and F.L. Milthorpe [Eds.], The growth of the potato. Butterworths, London,* p. 199-210.

Bogucki S. and Nelson D.C., 1980. Length of dormancy and sprouting characteristics of ten potato cultivars. *American Potato Journal* 57: 151-157.

Bonierbale M.W., Plaisted R.L. and Tanksley S.D., 1993. A test of the maximum

heterozygosity hypothesis using molecular markers in tetraploid potatoes. *Theoretical and Applied Genetics* 86: 481-491.

Bradshaw J.E., 1994. Theory for locating quantitative trait loci. *In: J.E. Bradshaw and G.R. Mackay [Eds.], Potato Genetics. CAB International, Wallingford,* p. 101-107.

Bradshaw J.E. and Mackay G.R., 1994. *Potato genetics. CAB International, Wallingford,* 552p.

Bradshaw J.E. and Mackay G.R., 1994. Breeding strategies for clonal propagated potatoes. *In: J.E. Bradshaw and G.R. Mackay [Eds.], Potato Genetics. CAB International, Wallingford,* p. 467-497.

Bradshaw J.E., Stewart H.E., Wastie R.L., Dale M.F.B. and Phillips M.S., 1995. Use of the seedling progeny tests for genetical studies as part of a potato (*Solanum tuberosum* subsp. *tuberosum*) breeding programme. *Theoretical and Applied Genetics* 90: 899-905.

Bradshaw J.E., Hackett C.A., Meyer R.C., Milbourne D., McNicol J.W., Phillips M.S. and Waugh R., 1998. Identification of AFLP and SSR markers associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker-assisted selection. *Theoretical and Applied Genetics* 97: 202-210.

Burstall L., Thomas M.N. and Allen E.J., 1986. Improving upon saleable yield. *Potato World* 3: 10-12.

Buso J.A., Boiteux L.S. and Peloquin S.J., 2000. Heterotic effects for yield and tuber solids and type of gene action for five traits in 4x potato families derived from interploid (4x-2x) crosses. *Plant Breeding* 119: 111-117.

Caligari P.D.S., 1992a. Breeding new varieties. *In: P. Harris [Ed.], The potato crop. 2nd ed. Chapman & Hall, London,* p. 334-372.

Caligari P.D.S., 1992b. Development and introduction of potato varieties. *In: L. Tsror, A. Nachmias and D. Van der Zaag [eds.]. Proceedings of the symposium on advanced potato production in hot climates. 24-28 May 1992, Nahal-Oz, Israel,* p. 1-2.

Chase R.W., 2002. Potato variety inventory. http://www.ume.maine.edu/PAA/PVI.htm

Chaudhary S.K. and Sharma S.K., 1984. Genetic variability for yield and its components in potato breeding material. *Indian Journal of Agricultural Sciences* 54: 941-942.

Clayton R. and Percival G., 2000. Glycoalkaloids in potato tubers - a cause for concern? *In: Proceedings of the fourth World Potato Congress, Amsterdam, The Netherlands, 4-6 September 2000,* p. 170-173.

Collins W., 2000. Research strategies for potatoes: a global approach. *In: Proceedings of the fourth World Potato Congress, Amsterdam, The Netherlands, 4-6 September*

2000, p. 209-214.

Coupland G., 1995. Genetic and environmental control of flowering time in *Arabidopsis. Trends in Genetics* 11: 393-397.

Craenen K. and Ortiz R., 1996. Effect of the black sigatoka resistance locus bs1 and ploidy level on fruit and bunch traits of plantain-banana hybrids. *Euphytica* 97: 97-101.

Cutter E.G., 1992. Structure and development of the potato plant. *In: P. Harris [Ed.], The potato crop. 2nd ed. Chapman & Hall, London*, p. 65-161.

Dale M.F.B. and Mackay G.R., 1994. Inheritance of table and processing quality. *In: J.E. Bradshaw and G.R. Mackay [Eds.], Potato Genetics. CAB International, Wallingford*, p. 285-315.

De Maine M.J., 1985. Shoot structure and morphology of dihaploid potatoes, their chromosome-doubled derivatives and parthenogenetic tetraploid parents. *Potato Research* 28: 403-413.

Dengler N.G. and Tsukaya H., 2001. Leaf morphogenesis in dicotyledons: current issues. *International Journal of Plant Science* 162: 459-464.

Dimenstein L., Lisker N., Kedar N. and Levy D., 1997. Changes in the content of steroidal glycoalkaloids in potato tubers grown in the field and in the greenhouse under different conditions of light, temperature and daylength. *Physiological and Molecular Plant Pathology* 50: 391-402.

Du Plooy F.I., Eisenberg B.E., Vorster H.J., Roux C.Z. and Geerthsen J.M.P., 1996. Study of the inheritance of some agronomic traits in a potato breeding population. *Journal of the South African Society of Horticultural Science* 6: 20-22.

Dudley J.W. and Moll R.H., 1969. Interpretation and use of estimates of heritability and genetic variances in potato breeding. *Crop Science* 9: 257-262.

Esau K., 1965. *Plant anatomy*. John Wiley and Sons, New York.

Eshed Y. and Zamir D., 1996. Less-than-additive epistatic interactions of quantitative trait loci in tomato. *Genetics* 143: 1807-1817.

Ewing E.E. and Struik P.C., 1992. Tuber formation in potato: induction, initiation, and growth. *Horticultural Reviews* 14: 89-198.

Fagerberg W.R., Davis T.M. and Matthews L.J., 1990. Comparison of tetraploid and single gene-induced gigas variants in chickpea (*Cicer arietinum*). II. Morphological and anatomical leaf characters. *American Journal of Botany* 77: 300-304.

Fahem M. and Haverkort A.J., 1988. Comparison of the growth of potato crops in autumn and spring in North Africa. *Potato Research* 31: 557-568.

Falconer D.S., 1989. *Introduction to quantitative genetics*. Third Edition. Longman Scientific & Technical, Burnt Mill, 438p.

FAO, 2001. *FAO production yearbook 1999. Vol. 53*. Food and Agricultural Organi

zation of the United Nations, Rome.

Firman D.M., O'Brien P.J. and Allen E.A., 1991. Leaf and flower initiation in potato (*Solanum tuberosum*) sprouts and stems in relation to number of nodes and tuber initiation. *Journal of Agricultural Science, Cambridge* 117: 61-74.

Freyre R., Warnke S., Sosinski B. and Douches D.S., 1994. Quantitative trait locus analysis of tuber dormancy in diploid potato (*Solanum* spp.). *Theoretical and Applied Genetics* 89: 474-480.

Fulton M.T., Chunwongse J. and Tanksley S.D., 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Molecular Biology Reporter* 13: 207-209.

Gawronska H., Thornton M.K. and Dwelle R.B., 1992. Influence of heat stress on dry matter production and photoassimilate partitioning by four potato clones. *American Potato Journal* 69: 653-665.

Goliber T., Kessler S., Chen J., Bharathan G. and Sinha N., 1999. Genetic, molecular, and morphological analysis of compound leaf development. *Current Topics in Developmental Biology* 43: 259-290.

Golmirzaie A.M., Malagamba P. and Pallais N., 1994. Breeding potatoes based on true seed propagation. *In: J.E. Bradshaw and G.R. Mackay [Eds.], Potato Genetics. CAB International, Wallingford*, p. 499-513.

Gopal J., Gaur P.C. and Rana M.S., 1994. Heritability, and intra- and inter-generation associations between tuber yield and its components in potato (*Solanum tuberosum* L.). *Plant Breeding* 112: 80-83.

Hall L.N. and Langdale J.A., 1996. Tansley Review No. 88: Molecular genetics of cellular differentiation in leaves. *New Phytologist* 132: 533-553.

Hareven D., Gutfinger T., Parnis A., Eshed Y. and Lifschitz E., 1996. The making of a compound leaf: Genetic manipulation of leaf architecture in tomato. *Cell* 84: 735-744.

Haverkort A.J. and Kooman P.L., 1997. The use of system analysis and modelling of growth and development in potato ideotyping under conditions affecting yield. *Euphytica* 94: 191-200.

Hawkes J.G., 1992. Biosystematics of the potato. *In: P. Harris [Ed.], The potato crop. 2nd ed. Chapman & Hall, London*, p. 13-64.

Hijmans R.J., 2001. Global distribution of the potato crop. *American Journal of Potato Research* 78: 403-412.

Howard H.W., 1963. The significance of breeding in improving quality and yield. *In: J.D. Ivins and F.L. Milthorpe [Eds.], The growth of the potato. London, Butterworths*, p. 292-302.

Howard H.W., 1970. *Genetics of the potato*. Logos Press, London, 126p.

Hutten R.C.B. and Van Berloo R., 2001. An online potato pedigree database. URL: http://www.dwp.wageningen-ur.nl/pv/query.asp

Hutten R.C.B., Schippers M.G.M., Hermsen J.G.Th. and Jacobsen E., 1995. Comparative performance of diploid and tetraploid progenies from 2x.2x crosses in potato. *Euphytica* 81: 187-192.

International Potato Center, 1982. *Annual Report CIP 1982*. International Potato Center, Lima, 146p.

International Potato Center, 1984. *Potatoes for the developing world*. International Potato Center (CIP), Lima, 150p.

Jiang C., Wright R.J., Woo S.S., DelMonte T.A. and Paterson A.H., 2000. QTL analysis of leaf morphology in tetraploid *Gossypium* (cotton). *Theoretical and Applied Genetics* 100: 409-418.

Kaplan D.R., 2001. Fundamental concepts of leaf morphology and morphogenesis: a contribution to the interpretation of molecular genetic mutants. *International Journal of Plant Science* 162: 465-474.

Karp A., Kresovich S., Bhat K.V., Ayad W.G. and Hodgkin T., 1997. *Molecular tools in plant genetic resources conservations: a guide to the technologies. IPGRI Technical Bulletin no. 2*. International Plant Genetic Resources Institute, Rome, 47p.

Kehoe H.W., 1992. Comments on: Potatoes in hot climates - could we do more? *In: L. Tsror, A. Nachmias and D. Van der Zaag [eds.], Proceedings of the symposium on advanced potato production in hot climates. 24-28 May 1992, Nahal-Oz, Israel*: p. 8.

Kessler S., Kim M., Pham T., Weber N. and Sinha N., 2001. Mutations altering leaf morphology. *International Journal of Plant Science* 162: 475-492.

Kidwell K.K., Bingham E.T., Woodfield D.R. and Osborn T.C., 1994. Relationships among genetic distance, forage yield and heterozygosity in isogenic diploid and tetraploid alfalfa populations. *Theoretical and Applied Genetics* 89: 323-328.

Kooman P.L. and Rabbinge R., 1996. An analysis of the relation between dry matter allocation to the tuber and earliness of a potato crop. *Annals of Botany* 77: 235-242.

Kooman P.L., Fahem M., Tegera P. and Haverkort A.J., 1996. Effects of climate on different potato genotypes 2. Dry matter allocation and duration of the growth cycle. *European Journal of Agronomy* 5: 207-217.

Krauss A. and Marschner H., 1984. Growth rate and corbohydrate metabolism of potato tubers exposed to high temperatures. *Potato Research* 27: 297-303.

Kreike C.M., Kok-Westeneng A.A., Vinke J.H. and Stiekema W.J., 1996. Mapping of QTLs involved in nematode resistance, tuber yield and root development in *Solanum* sp.. *Theoretical and Applied Genetics* 92: 463-470.

References

Kull U., 1999. Zur Evolution der Adernetze von Blattern, insbesondere der Angiospermen. *Profil* 16: 35-48.

Kull U. and Herbig A., 1995. Das Blattadersystem der Angiospermen: Form und Evolution. *Naturwissenschaften* 82: 441-451.

Kutchan T.M., 1995. Alkaloid biosynthesis - The basis for metabolic engineering of medical plants. *The Plant Cell* 7: 1059-1070.

Lande R., 1981. The minimum number of genes contributing to quantitative variation between and within populations. *Genetics* 99: 541-553.

Lander E.S., Green P., Abrahamson J., Barlow A., Daly M.J., Lincoln S.E. and Newburg L., 1987. MapMaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1: 174-181.

Levin I., Gilboa N., Yeselson E. and Shen S., 2000. Fgr, a major locus that modulates the fructose to glucose ratio in mature tomato fruits. *Theoretical and Applied Genetics* 100: 256-262.

Levy D., 1983a. Varietal differences in the response of potatoes to repeated short periods of water stress in hot climates. 1. Turgor maintenance and stomatal behaviour. *Potato Research* 26: 303-313.

Levy D., 1983b. Varietal differences in the response of potatoes to repeated short periods of water stress in hot climates. 2. Tuber yield and dry matter accumulation and other tuber properties. *Potato Research* 26: 315-321.

Levy D., 1984. Cultivated *Solanum tuberosum* L. as a source for the selection of cultivars adapted to hot climates. *Tropical Agriculture (Trinidad)* 61: 167-170.

Levy D., 1986. Genotypic variation in the response of potatoes (*Solanum tuberosum* L.) to high ambient temperatures and water deficit. *Field Crops Research* 15: 85-96.

Levy D., 2000. Achievements in potato breeding in diverse climates. *In: S.M. Paul Khurana, G.S. Shekhawat, B.P. Singh and S.K. Pandey [Eds.], Potato: global research and development. Proceedings of the Global Conference on Potato. New Delhi (India), 6-11 December 1999. Volume 1*, p. 122-128.

Levy D., Kastenbaum E. and Itzhak Y., 1991. Evaluation of parents and selection for heat tolerance in the early generations of a potato (*Solanum tuberosum* L.) breeding program. *Theoretical and Applied Genetics* 82: 130-136.

Levy D., Gamliel B., Warshavsky S. and Shoham C., 1992. Potato. *In: Y. Arnon [Ed.], The encyclopaedia of agriculture. Volume 6*, p. 3-59.

Levy D., Meiri A., Carmi A. and Nachmias A., 1995. *Improvement of potato productivity in developing countries. Research and Development project between the Netherlands and Israel. Report for 1994-1995*. The Volcani Center, Bet Dagan.

Levy D., Itzhak Y., Fogelman E., Margalit E. and Veilleux R.E., 2001. Ori, Idit, Zohar

and Zahov: tablestock and chipstock cultivars bred for adaptation to Israel. *American Journal of Potato Research* 78: 167-173.

Li X., Van Eck H.J., Rouppe van der Voort J.N.A.M., Huigen D.J., Stam P. and Jacobsen E., 1998. Autotetraploids and genetic mapping using common AFLP markers: the R2 allele conferring resistance of *Phytophthora infestans* mapped on potato chromosome chromosome 4. *Theoretical and Applied Genetics* 96: 1121-1128.

Lynch D.R. and Kozub G.C., 1988. An analysis of the response of nine potato genotypes to five prairie environments. *Canadian Journal of Plant Science* 68: 1219-1228.

Lynch D.R., Kozub G.C. and Kawchuk L.M., 2001. The relationship between yield, mainstem number, and tuber number in five maincrop and two early-maturing cultivars. *American Journal of Potato Research* 78: 83-90.

Maksymowych, R., 1973. *Analysis of leaf development*. Cambridge University Press, Cambridge.

Marinus J. and Bodlaender K.B.A., 1975. Response of some potato varieties to temperature. *Potato Research* 18: 189-204.

Marshall B. and Thompson R., 1986. Tuber-size distribution. *Potato Research* 29: 261-262.

McCauley M.M. and Evert R.F., 1988. Morphology and vasculature of the leaf of potato (*Solanum tuberosum*). *American Journal of Botany* 75: 377-390.

McGregor C.E., Lambert C.A., Greyling M.M., Louw J.H. and Warnich L., 2000. A comparitive assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. *Euphytica* 113: 135-144.

McHale N.A., 1992. A nuclear mutation blocking initiation of the lamina in leaves of *Nicotiana sylvestris*. *Planta* 186: 355-360.

Medina T.B., Fogelman E., Chani E., Miller A.R., Levin I., Levy D. and Veilleux R.E., 2002. Identification of molecular markers associated with leptine in reciprocal backcross families of diploid potato. *Theoretical and Applied Genetics*: accepted.

Mendoza H.A., 1989. Population breeding as a tool for germplasm enhancement. *American Potato Journal* 66: 639-653.

Menzel C.M., 1985. The control of storage organ formation in potato and other species: a review. Part 1. *Field Crops Abstracts* 38: 527-537.

Meyer R.C., Milbourne D., Hackett C.A., Bradshaw J.E., McNicol J.W. and Waugh R., 1998. Linkage analysis in tetraploid potato and association of markers with quantitative resistance to late blight (*Phytophthora infestans*). *Molecular and General Genetics* 259: 150-160.

References

Midmore D.J., 1984. Potato (*Solanum* spp.) in the hot tropics. I. Soil temperature effects on emergence, plant development and yield. *Field Crops Research* 8: 255-271.

Midmore D.J., 1992. Potato production in the tropics. *In: P. Harris [Ed.], The potato crop. 2nd ed. Chapman & Hall, London*, p. 728-793.

Midmore D.J. and Prange R.K., 1991. Sources of heat tolerance amongst potato cultivars, breeding lines, and *Solanum* species. *Euphytica* 55: 235-245.

Midmore D.J. and Prange R.K., 1992. Growth responses of two *Solanum* species to contrasting temperatures and irradiance levels: relations to photosynthesis, dark respiration and chlorophyll fluorescence. *Annals of Botany* 69: 13-20.

Mohabir G. and John P., 1988. Effect of temperature on starch synthesis in potato tuber tissue and amyloplasts. *Plant Physiology* 88: 1222-1228.

Morgan M.R.A., Coxon D.T., Bramham S., Chan H.W.S., Van Gelder W.M.J. and Allison M.J., 1985. Determination of glycoalkaloid content of potato tubers by three methods including enzyme-linked immunosorbent assay. *Journal of the Science of Food and Agriculture* 36: 282-288.

Murti G.S.R., Singh M., Saha S.N. and Banerjee V.N., 1976. Effects of night temperatures in the pre- and post-tuber initiation phases on the development of potato under short days. *Indian Journal of Agricultural Sciences* 46: 65-73.

Nelson T. and Dengler N., 1997. Leaf vascular pattern formation. *The Plant Cell* 9: 1121-1135.

Oberhagemann P., Chatot-Balandras C., Schäfer-Pregl R., Wegener D., Palomino C., Salamini F., Bonnel E. and Gebhardt C., 1999. A genetic analysis of quantitative resistance to late blight in potato: towards marker-assisted selection. *Molecular Breeding* 5: 399-415.

Ortiz R. and Huamán Z., 1994. Inheritance of morphological and tuber characteristics. *In: J.E. Bradshaw and G.R. Mackay [Eds.], Potato Genetics. CAB International, Wallingford*, p. 263-283.

Pavek J.J. and Corsini D.L., 2001. Utilization of potato genetic resources in variety development. *American Journal of Potato Research* 78: 433-441.

Peleg Y., 2001. *Summary of potato season of the year 2000* [in Hebrew]. Israel Vegetable Board, Tel Aviv, 97p.

Plaisted R.L., 1980. Progress and future plans for the use of Neo-tuberosum populations. *In: Utilization of the genetic recources of the potato III, Report of the planning conference 1980, CIP, Lima*, p. 65-79.

Plaisted R.L. and Hoopes R.W., 1989. The past record and future prospects for the use of exotic potato germplasm. *American Potato Journal* 66: 603-627.

Poethig R.S., 1997. Leaf morphogenesis in flowering plants. *The Plant Cell* 9: 1077-

1087.

Poethig R.S. and Sussex I.M., 1985a. The developmental morphology and growth dynamics of the tobacco leaf. *Planta* 165: 158-169.

Poethig R.S. and Sussex I.M., 1985b. The cellular parameters of the leaf development in tobacco: a clonal analysis. *Planta* 165: 170-184.

Prange R.K., McRae K.B., Midmore D.J. and Deng R., 1990. Reduction of potato growth at high temperature: role of photosynthesis and dark respiration. *American Journal of Botany* 67: 357-369.

Prevost A. and Wilkinson M.J., 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics* 98: 107-112.

Raven P.H., Evert R.F. and Eichhorn S.E., 1986. *Biology of plants*. Fourth Edition. *Worth Publishers, New York,* 775p.

Reynolds M.P. and Ewing E.E., 1989. Effects of high air and soil temperature stress on growth and tuberization in *Solanum tuberosum*. *Annals of Botany* 64: 241-247.

Ronning C.M., Stommel J.R., Kowalski S.P., Sanford L.L., Kobayashi R.S. and Pineada O., 1999. Identification of molecular markers associated with leptine production in a population of *Solanum chacoense* Bitter. *Theoretical and Applied Genetics* 98: 39-46.

Ross H., 1966. The use of wild *Solanum* species in German potato breeding of the past and today. *American Potato Journal* 43: 63-80.

Ross H., 1986. Potato breeding: problems and perspectives. *Fortschritte der Pflanzenzuchtung No 13*, 132p.

Ross H., Pasemann P. and Nitzsche W., 1978. Der Glycoalkaloidgehalt von Kartoffel-sorten in seiner Abhängigkeit von Anbauort und -jahr und seiner Beziehung zum Geschmack. *Zeitschrift fuer Pflanzenzuechtung* 80: 64-79.

Rouppe van der Voort J., Lindeman W., Folkertsma R., Hutten R., Overmars H., Van der Vossen E., Jacobsen E. and Bakker J., 1998. A QTL for broad-spectrum resistance to cyst nematode species (*Globodera* spp.) maps to a resistance gene cluster in potato. *Theoretical and Applied Genetics* 96: 654-661.

Rozenblatt U., 1946. History of the potato in Erets Yisrael. *In: M. Gershenzon, History of the potato* [in Hebrew]. *Publishing House Hakibutz Hameuchad, Ein Charod,* p. 131-137.

Salaman R.N., 1928. *Agricultural leaflets. Series VI. Staple Crops No 4. "First early" potatoes for the British market.* s.n., s.l., 16p.

Sale P.J.M., 1973. Productivity of vegetable crops in a region of high solar input. I. Growth and development of the potato (*Solanum tuberosum* L.). *Australian Journal of Agricultural Research* 24: 733-749.

References

Sanford L.L. and Sinden S.S, 1972. Inheritance of potato glycoalkaloids. *American Potato Journal* 49: 209-217.

SAS Institute, 1995. *JMP Statistical Discovery Software, Version 3.1.5*. SAS Institute Inc., Cary, NC.

Schäfer-Pregl R., Ritter E., Concilio L., Hesselbach J., Lovatti L., Walkemeier B., Thelen H., Salamini F. and Gebhardt C., 1998. Analysis for quantitative trait loci (QTLs) and quantitative trait alleles (QTAs) for potato tuber yield and starch content. *Theoretical and Applied Genetics* 97: 834-846.

Scott G.J., Best R., Rosegrant M. and Bokanga M., 2000. *Roots and tubers in the global food system: A vision statement to the year 2020.* A co-publication of the International Potato Center (CIP), Centro Internacional de Agricultura Tropical (CIAT), International Food Policy Research Institute (IFPRI), International Institute of Tropical Agriculture (IITA), and International Plant Genetic Resources Institute (IPGRI), Lima, 111p.

Šimko I., Vreugdenhil D., Jung C.S. and May G.D., 1999. Similarities of QTLs detected for *in vitro* and greenhouse development of potato plants. *Molecular Breeding* 5: 417-428.

Sinden S.L., Sanford L.L. and Webb R.E., 1984. Genetic and environmental control of potato glycoalkaloids. *American Potato Journal* 61: 141-156.

Snyder R.G. and Ewing E.E., 1989. Interactive effects of temperature, photoperiod, and cultivar on tuberization of potato cuttings. *HortScience* 24: 336-338.

Staub J.E. and Serquen F.C., 1996. Genetic markers, map construction, and their application in plant breeding. *HortScience* 31: 729-741.

Steeves T.A. and Sussex I.M., 1989. *Patterns in plant development. Cambridge University Press, Cambridge*.

Stegemann H. and Schnick D., 1985. *Index 1985 of European potato varieties. National registers, characteristics, genetic data*. Biologische Bundesanstalt fuer Land- und Forstwirtschaft, Berlin, 128p.

Storey R.M.J. and Davies H.V., 1992. Tuber quality. *In: P. Harris [Ed.], The potato crop. 2nd ed. Chapman & Hall, London*, p. 507-569.

Struik P.C. and Van Voorst G., 1986. Effects of drought on the initiation, yield, and size distribution of tubers of *Solanum tuberosum* L. cv. Bintje. *Potato Research* 29: 487-500.

Struik P.C. and Ewing E.E., 1995. Crop physiology of potato (*Solanum tuberosum*): responses to photoperiod and temperature relevant to crop modelling. *In: A.J. Haverkort and D.K.L. MacKerron [Eds.], Potato ecology and modelling of crops under conditions limiting growth. Kluwer Academic Publishers, Dordrecht, The Netherlands*, p. 19-40.

Struik P.C. and Wiersema S.G., 1999. *Seed potato technology. Wageningen Pers, Wageningen,* 383p.

Struik P.C., Haverkort A.J., Vreugdenhil D., Bus C.B. and Dankert R., 1990. Manipulation of tuber-size distribution of a potato crop. *Potato Research* 33: 417-432.

Struik P.C., Vreugdenhil D., Haverkort A.J., Bus C.B. and Dankert R., 1991. Possible mechanisms in size hierarchy among tubers on one stem of a potato (*Solanum tuberosum* L.) plant. *Potato Research* 34: 187-203.

Struik P.C., Vreugdenhil D., Van Eck H.J., Bachem C.W. and Visser R.G.F., 1999. Physiological and genetic control of tuber formation. *Potato Research* 42: 313-331.

Swaminathan M.S., 2000. International developments: five point action plan for strengthening potato's role in global food security. *In: Proceedings of the fourth World Potato Congress, Amsterdam, The Netherlands, 4-6 September 2000*, p. 167-169.

Tai G.C.C., 1982. Estimation of double reduction and genetic parameters of auto-tetraploids. *Heredity* 49: 63-70.

Tai G.C.C. and Young D.A., 1984. Early generation selection for important agronomic characteristics in a potato breeding population. *American Potato Journal* 61: 419-434.

Tenkouano A., Crouch J.H., Crouch H.K., Vuylsteke D. and Ortiz R., 1999. Comparison of DNA marker and pedigree-based methods of genetic analysis in plantain and banana (*Musa* spp.) clones. II. Predicting hybrid performance. *Theoretical and Applied Genetics* 98: 69-75.

Thompson P.G., Haynes F.L. and Moll R.H., 1980. Estimation of genetic variance components and heritability for tuber dormancy in diploid potatoes. *American Potato Journal* 57: 39-46.

Tsuge T., Tsukaya H. and Uchimiya H., 1996. Two independent and polarized processes of cell elongation regulate leaf blade expansion in *Arabidopsis thaliana* (L.) Heynh.. *Development* 122: 1589-1600.

Tsukaya H., 1995. Developmental genetics of leaf morphogenesis in dicotyledonous plants. *Journal of Plant Research* 108: 407-416.

Tsukaya H., Naito S., Redei G.P. and Komeda Y., 1993. A new class of mutation in *Arabidopsis thaliana*, *acaulis1*, affecting the development of both inflorescences and leaves. *Development* 118: 751-764.

UPOV, 1986. *Guidelines for the conduct of tests for distinctness, homogeneity and stability. TG/23/5. Potato (*Solanum tuberosum *L.)*. International Union for the Protection of New Varieties of Plants (UPOV), Geneva, 27p.

Valkonen J.P.T., Keskitalo M., Vasara T. and Pietila L., 1996. Potato glycoalkaloids: a

burden or a blessing? *Critical Reviews in Plant Science* 15: 1-20.

Van Dam J., Kooman P.L. and Struik P.C., 1996. Effects of temperature and photoperiod on early growth and final number of tubers in potato (*Solanum tuberosum* L.). *Potato Research* 39: 51-62.

Van Dam J., Levin I., Struik P.C. and Levy D., 1999. Genetic characterisation of tetraploid potato (*Solanum tuberosum* L.) emphasising genetic control of total glycoalkaloid content in the tubers. *Euphytica* 110: 67-76.

Van den Berg J.H., Ewing E., Plaisted R.L., McMurry S., Bonierbale M.W., 1996. QTL analysis of potato dormancy. *Theoretical and Applied Genetics* 93: 317-324.

Van Gelder W.M.J., 1989. *Steroidal glycoalkaloids in Solanum species: consequences for potato breeding and food safety*. Wageningen Agricultural University, Wageningen, 184p.

Van Gelder W.M.J. and Scheffer J.J.C., 1991. Transmission of steroidal glycoalkaloids from *Solanum vernei* to the cultivated potato. *Phytochemistry* 30: 165-168.

Van Ittersum M.K., 1992. *Dormancy and growth vigour of seed potatoes*. Wageningen Agricultural University, Wageningen, 187p.

Veilleux R.E., Paz M.M. and Levy D., 1997. Potato germplasm development for warm climates: genetic enhancement of tolerant to heat stress. *Euphytica* 98: 83-92.

Veit B. and Foster T., 2002. Axial patterning and meristimatic growth in leaves. *In: M.T. McManus and B.E. Veit [Eds.], Meristimatic tissues in plant growth and development, Sheffield Academic Press, Sheffield,* p. 213-253.

Vos J. and Biemond H., 1992. Effects of nitrogen on the development and growth of the potato plant. I. Leaf appearance, expansion growth, life spans of leaves and stem branching. *Annals of Botany* 70: 27-35.

Vos J. and Van der Putten P.E.L., 2001. Effects of partial shading of the potato plant on photosynthesis of treated leaves, leaf area expansion and allocation of nitrogen and dry matter in component plant parts. *European Journal of Agronomy* 14: 209-220.

Waites R. and Hudson A., 1995. Phantastica: a gene required for dorsoventrality of leaves in *Antirrhinum majus*. *Development* 121: 2143-2154.

Wenzel G., Braun A., Mohler V. and Frei U., 2000. Genetical markers speed up the breeding process. *In: Proceedings of the fourth World Potato Congress, Amsterdam, The Netherlands, 4-6 September 2000*, p. 41.

Wolf M.J. and Duggar B.M., 1946. Estimation and physiological role of solanine in the potato. *Journal of Agricultural Research* 73: 1-32.

Woolfe J.A., 1987. *The potato in the human diet. Press syndicate of the University of Cambridge, Cambridge,* 231p.

Wu P., Zhang G., Huang H. and Ladha J.K., 1995. Non-allelic interaction conditioning

spikelet sterility in an F2 population of indica/japonica cross in rice. *Theoretical and Applied Genetics* 91: 825-829.

Wurr, D.C.E., 1977. Some observations of patterns of tuber formation and growth in the potato. *Potato Research* 20: 63-75.

Yencho G.C., Kowalski S.P., Kobayashi R.S., Sinden S.L., Bonierbale M.W. and Deahl K.L., 1998. QTL mapping of foliar glycoalkaloid aglycones in *Solanum tuberosum* × *S. berthaultii* potato progenies: quantitative variation and plant secondary metabolism. *Theoretical and Applied Genetics* 97: 563-574.

Yildirim M.B. and Çalişkan C.F., 1985. Genotype × environment interactions in potato (*Solanum tuberosum* L.). *American Potato Journal* 62: 371-375.

Ziętkiewicz E., Rafalski A. and Labuda D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.

Summary

Glycoalkaloids are secondary metabolites that are characterised by an undesirable taste, and which are known to be toxic when consumed in large quantities. Some wild potato germplasms that are used for the introgression of genes encoding heat tolerance contain high concentrations of glycoalkaloids in the tubers. Breeding and selection should therefore take place to lower the glycoalkaloid content in potato tubers and DNA markers can significantly facilitate the selection process.

This study was designed to identify DNA markers associated with the total content of glycoalkaloids (TGA) in the potato tuber. Two segregating resource populations were constructed, one of 216 clones using the tetraploid clones Cara and LT7 as parents and one of 176 clones using the tetraploid parents NT8 and LT7. The two populations were characterised by divergent TGA-content of the parental clones, where LT7 was the mutual male parent with high TGA-content, and both Cara and NT8 the parental clones with low TGA-content. The populations were used, in replicates, to estimate genetic parameters for total glycoalkaloid (TGA) content in the tubers.

Besides TGA-content in the tubers, over thirty other agronomic and morphological traits were assessed for genetic characterisation of the two populations used. Among agronomic traits assessed were tuber dormancy, tuber weight and tuber number. Several traits were calculated based on data obtained, such as tuber size distribution and tuber number distribution. Morphological characteristics assessed included plant height, number of nodes on the main stem, and terminal leaflet shape.

In both populations, the parental clones differed markedly in TGA-content and the progeny population was normally distributed for this trait after logarithmic transformation. Broad sense heritability estimates for TGA-content were 0.54 in Cara × LT7 and 0.50 in NT8 × LT7 and the trait proved to be inherited in a non-dominant manner. The minimum number of genes contributing to TGA-content was estimated to be between 3 and 7. Broad sense heritability was also estimated for all other traits assessed. Heritability estimates that were similar in both populations ranged from 0.1 for the number of main stems to 0.5 for total tuber glycoalkaloid content. In both populations, most heritabilities estimated were about 0.3. Main agronomic traits, such as tuber dormancy, tuber weight, and maturity, appeared to be controlled by several additive genes. None of the traits recorded in this study showed a statistically significant genetic association with TGA-content. This suggests that tuber TGA-content may be genetically modulated without any significant adverse effects on other agronomic traits. The genetic parameters estimated in this study indicate that these

Summary

populations are suitable for DNA-marker analysis for TGA-content in tubers.

Three hundred forty two Inter-Simple Sequence Repeat (ISSR) single primers or combinations of two of such primers were used to amplify random PCR products. Parental clones of both populations were used to identify polymorphic PCR products for further linkage analyses with TGA-content. In these analyses, two single ISSR markers were found to be significantly associated with TGA-content. A multiple regression analysis was also carried out using a 'stepwise' procedure. In this analysis TGA-content was the dependent variable whereas the polymorphic PCR products and all possible two-way interactions among them were the independent variables. The resulting best model consisted of an interaction between two loci in addition to a single locus effect. This interaction suggests that the expression of TGA-content is partially modulated by two interacting loci.

A single copy DNA marker for a morphological characteristic, leaflet vein depth, was generated using two ISSR-primers to generate random PCR products followed by the design of a Cleaved Amplified Polymorphic Sequence (CAPS) marker. A multiple regression analysis showed that the best model consisted of the ISSR-PCR product and a CAPS-PCR product. The results suggest that these products represent separate alleles at the same DNA locus. Vein depth is apparently controlled by one or several genes that modulates the lamina expansion, either during cell division or during cell elongation. Vein depth is shown to be regulated in an additive way and hence allelic differences are supposedly accountable for small differences in lamina growth. The feasible generation of a single copy DNA marker in tetraploids and its possible use in the study of tetrasomic inheritance are thereby demonstrated.

Samenvatting

Glycoalkaloïden zijn secundaire metabolieten die worden gekenmerkt door een onaangename, bittere smaak en die toxisch zijn wanneer ze in grote hoeveelheden worden genuttigd. Sommig genetisch materiaal van wilde aardappelsoorten dat wordt gebruikt bij het inkruisen van hittetolerantie, bevat hoge concentraties glycoalkaloïden in de knollen. Veredeling en selectie zijn daarom noodzakelijk om het gehalte aan glycoalkaloïden in aardappelknollen te verlagen. DNA-merkers kunnen het selectieproces aanzienlijk vergemakkelijken.

Deze studie werd opgezet om DNA-merkers te identificeren die geassocieerd zijn met het totale gehalte aan glycoalkaloïden (TGA) in aardappelknollen. Twee uitsplitsende uitgangspopulaties werden gemaakt: één van 216 klonen die Cara en LT7 als tetraploïde ouderklonen had en één van 176 klonen die NT8 en LT7 als tetraploïde ouderklonen had. Beide populaties werden gekenmerkt door een uiteenlopend TGA-gehalte van de ouderklonen. Ze hadden LT7 als mannelijke ouder met een hoog TGA-gehalte gemeen. De andere, vrouwelijke, ouder was Cara of NT8, beide met lage TGA-gehalten. De gebruikte populaties werden in herhalingen opgekweekt om genetische parameters te schatten voor het gehalte aan glycoalkaloïden (TGA) in de knollen.

Naast het TGA-gehalte in de knollen werden meer dan dertig andere agronomische en morfologische eigenschappen gemeten teneinde de twee gebruikte populaties genetisch te karakteriseren. Onder de gemeten agronomische eigenschappen waren o.a. kiemrust, knolgewicht en knolaantal. Enkele eigenschappen werden berekend op basis van de verkregen data, zoals knolgrootteverdeling en knolaantalverdeling. Gemeten morfologische eigenschappen waren o.a. planthoogte, aantal knopen op de hoofdstengel en de vorm van het terminale blaadje.

In beide populaties verschilden de ouderklonen aanzienlijk in TGA-gehalte. Na logarithmische transformatie was de frequentieverdeling van de nakomelingen normaal voor deze eigenschap. De geschatte erfelijkheidsgraad in brede zin voor het TGA-gehalte was 0,54 in Cara × LT7 en 0,50 in NT8 × LT7; het TGA-gehalte bleek niet dominant te worden vererfd. Het minimum aantal genen dat bijdraagt aan het TGA-gehalte werd geschat op een aantal tussen 3 en 7. De erfelijkheidsgraad in brede zin werd ook geschat voor alle andere eigenschappen die gemeten werden. De schattingen van de erfelijkheidsgraad waren vergelijkbaar voor beide populaties, met waarden van 0,1 voor het totaal aantal stengels tot 0,5 voor totaalgehalte aan glycoalkaloïden. In beide populaties lagen de meeste schattingen voor de erfelijkheidsgraad rond de 0,3. Belangrijke agronomische eigenschappen, zoals kiemrust, knolgewicht en

vroegheid bleken gecontroleerd te worden door enkele additieve genen. Geen van de gemeten eigenschappen was genetisch en statistisch significant geassocieerd met het TGA-gehalte. Dit doet vermoeden dat het TGA-gehalte genetisch kan worden aangepast, zonder dat een significant negatief effect op andere agronomische eigenschappen optreedt. De in deze studie geschatte genetische parameters wijzen erop dat de gebruikte populaties geschikt zijn voor DNA-merkeranalyse voor het TGA-gehalte in de knollen.

Driehonderdtweeënveertig Inter-Simple Sequence Repeat (ISSR) enkelvoudige primers of twee van dergelijke primer-combinaties werden gebruikt voor de vermeerdering van 'random' PCR-producten. De ouderklonen van beide populaties werden gebruikt voor de identificatie van polymorfe PCR-producten voor verdere analyses van de koppeling met het TGA-gehalte. In deze analyses werden twee enkelvoudige ISSR-merkers gevonden die significant geassocieerd waren met het TGA-gehalte. Een meervoudige regressie-analyse werd uitgevoerd m.b.v. de 'stapsgewijze' procedure. Het TGA-gehalte was de afhankelijke variabele in deze analyse en de polymorfe PCR-producten en alle mogelijke tweeweginteracties daartussen waren de onafhankelijke variabelen. Het resulterende beste model bestond uit een interactie tussen twee loci, boven een effect van een enkelvoudige locus. Deze interactie suggereert dat de expressie van het TGA-gehalte gedeeltelijk wordt beïnvloed door twee loci met hun interactie.

Een 'single copy' DNA-merker werd gegenereerd voor een morfologische eigenschap, de nerfdiepte van het blaadje, met twee ISSR-primers. Random PCR-producten werden gegenereerd, gevolgd door het ontwerpen van een Cleaved Amplified Polymorphic Sequence (CAPS) merker. Een meervoudige regressie-analyse toonde aan dat het beste model bestond uit het ISSR-PCR-product en een CAPS-PCR-product. De resultaten suggereren dat deze producten verschillende allelen op dezelfde locus vertegenwoordigen. Nerfdiepte wordt klaarblijkelijk gecontroleerd door één of meerdere genen die de oppervlaktetoename van de bladschijf reguleren, hetzij tijdens de celdeling, hetzij tijdens de celstrekking. Nerfdiepte bleek additief gereguleerd te worden; allelverschillen zijn derhalve vermoedelijk verantwoordelijk voor kleine verschillen in de groei van de bladschijf. Het genereren van een 'single copy' DNA-merker in tetraploïden en het mogelijke gebruik ervan in de studie van tetrasome overerving zijn hiermee aangetoond.

Curriculum Vitae

Jacob van Dam was born in Castricum, The Netherlands, on 14 June 1969. After obtaining the Atheneum-B diploma at the Maimonides Lyceum in Amsterdam, he started his studies in Tropical Crop Science at the Wageningen Agricultural University in 1988. In 1992, he did a seven months practical training period at the Agricultural Research Organization The Volcani Center, Bet Dagan, Israel. In 1994 he graduated with specializations in Plant Physiology, Crop Physiology, and Crop Modelling. Immediately after his graduation he immigrated to Israel. From 1994 till 2000 he performed research for his PhD-thesis at ARO The Volcani Center, and he had concurrently a position as examiner at the Testing Unit for the Israeli Plant Breeders' Rights. Since February 2002 he has a Postdoc position at the Department of Biotechnology of the Tel Aviv University. The research deals with the cell division cycle and pre-mRNA splicing of budding yeast. He is married and has 3 children.