The life cycle of the potato (*Solanum tuberosum* L.): From crop physiology to genetics

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Propositions

I

Within a potato population with a large variation in physiological behaviour, dormancy and physiological ageing do not have a determinant influence on the onset of tuberization and the duration of the plant cycle.
This thesis

II

Tuber initiation should be regarded as only one of the several developmental events taking place during the life cycle of the potato and not as the all determining factor.
This thesis

III

The potato is very plastic in the chronological order and duration of overlap of the different physiological phenomena.
This thesis

IV

The availability of plant populations highly diverse for several traits related to major developmental processes is the key factor for a successful integration of crop physiology and genetics.

V

"Geneticists and physiologists will need to work together to turn the abstractions of molecular marker maps into real biology and agronomy". Jones et al., 1997. New Phytologist 137: 165-177.

VI

In the path to discovery, inspiration and perseverance are our best companions. Many of those that failed in this quest, as Thomas Edison said, did not realize how close there were to success when they gave up.

VII

In research, as in many other human activities, team work is the basis of efficiency and success. A true leader is the one that encourages and treats each person as he/she could be and sooner than later, this person will become what he/she should be and even more!

VIII

For someone that can not accept other reality than his/her own, showing that "the pig is black because you hold the hairs in your hand" is not enough.
From Venezuelan folklore

IX

The most basic biological need is the nourishment of our body every single day. However, very little "social appreciation" is given to the people making this miracle possible.

These propositions are part of the thesis "The life cycle of the potato (*Solanum tuberosum* L.): from crop physiology to genetics", by Blanca Carolina Celis-Gamboa. September 9, 2002.

Contents

To my parents, in loving memory

To my children, Patricia and Alexander

1

General introduction

Importance of potato in the world

The cultivated potato, *Solanum tuberosum* L. originated from the Andean region of South America. Since its introduction into Europe in 1570, potato has been spread globally; nowadays it is the fourth most important crop in food production in the world, after maize, rice and wheat (www. FAO.com). Potato is a rich source of energy, with a starch content that accounts for 80% of the tuber's dry weight and with a high content of high quality protein and vitamin C (Scott et al., 2000). Potato yields on average more food, energy and protein per unit of land than cereals (Horton, 1988). The production of protein per unit area is second only to soybean (Tarn et al. 1992), and because of the high lysine content, it can complement cereal-based diets deficient in this amino acid (Scott et al, 2000). Furthermore, due to the crop's agronomical plasticity, it is grown in more countries and agro-ecological zones than any other crop.

Based on a projection of human population growth rate and some economical parameters such as increase in household income and a more intensive participation of women in the labour force, it is estimated that potato production worldwide will increase from 288.3 in 1993 to 403.5 millions metric tons per year by the year 2020 (Scott et al., 2000). In developing countries the production of potato will increase from 94.3 to 194.0 millions metric tons per year. China, India and regions including South Asia, West Asia/North Africa and Sub-Saharan Africa are expected to have the highest increase in potato production (Scott et al., 2000).

In potato breeding, yield potential, tuber quality traits, range of adaptation, and disease and pest resistances are the most important criteria for selection. The range of adaptation (or degree of stability in performance over a wide range of environments) is particularly difficult to breed for as the physiology of potato shows a very strong influence of genotype x environment interactions.

A better understanding of the physiological and genetic regulation of tuber formation will facilitate the breeding of varieties able to perform well under specific sets of conditions. This is particularly true for environmental circumstances that are considered unfavourable for potato, but where the urgent need for a highly productive crop with high nutritional value makes potato cultivation desirable, such as in the lowland tropics.

The morphology of the potato plant

Above-ground development

Generally, the potato crop is propagated vegetatively using tubers (seed tubers), from

which several main stems originate. Thus, a potato plant consists of a variable number of main stems, which will exhibit a variable degree of branching depending on genotype, physiological age of the mother tuber and environmental conditions. In general, each main stem will grow and terminate in an inflorescence, which can abort or develop fully into berries with true sexual seeds. Once the main stem has an inflorescence, vegetative growth continues through the development of axillary buds below the inflorescence, which will grow into a second-order stem. This second-order stem also produces leaves and an inflorescence and, perhaps, an axillary bud below this secondary order inflorescence will develop and flower. From the resulting tertiary stem, a fourth-order stem can develop and so on (Almekinders and Struik, 1996). The degree of stem branching determines the total amount of leaf area, the duration of the plant cycle, the number of stolons and tubers and the number of inflorescences, flowers and true seeds per plant.

Based on the stem structure of the plant, potato genotypes are classified as determined and undetermined. Genotypes with the determinate plant growth habit are characterised by a short plant, with a limited production of new orders of stems after development of an inflorescence on the main stem and a short life cycle. Plants with the indeterminate growth habit, on the other hand, produce several orders of stems, resulting in a prolonged flowering period and a long life cycle.

Potato, as many other annual plants, is considered a plant of monocarpic habit, since some time after tuber initiation and usually after or towards the end of the flowering period, the whole plant will undergo a process of senescence. In potato, very limited information exists on the process of senescence, other than that it is apparently hastened by tuber formation and by certain environmental conditions such as heat and water stress.

Below-ground development

Botanically, potato tubers are greatly shortened and thickened stems that bear scale leaves, each one with a bud in its axil (Ewing and Struik, 1992). Tuber formation is a very complex set of developmental events leading to the production of harvestable tubers; it includes several sequential steps, which are illustrated in Figure 1.

a. Stolon initiation; stolons are diageotropic stems usually arisen from the underground nodes of the main stems. They have long internodes and the apical region has a characteristic curved shape or "hook".

b. Stolon growth; elongation and branching of the stolons under favourable conditions i.e. long days and high temperatures.

c. cessation of stolon growth;

d. tuber induction and initiation; the first visual manifestation of tuber induction is the incipient swelling of the sub-apical region of the stolon. When this swelling reaches about twice the diameter of the subtending stolon, it is considered a tuber incipient. At this point, it is common to say that tuber initiation has begun.
e. Tuber growth (bulking); tuber incipients reaching at least 20 mm in diameter are considered growing tubers. Tubers will increase in size and weight due to cell division, cell elongation and starch and protein deposition.
f. Tuber maturity; at the end of the plant cycle, the tuber's skin will set and suberize. In addition, since their initiation until harvest, tubers undergo a process of increasing dormancy, reaching a maximum at the end of the plant cycle or at harvest after haulm removal, or shortly thereafter.

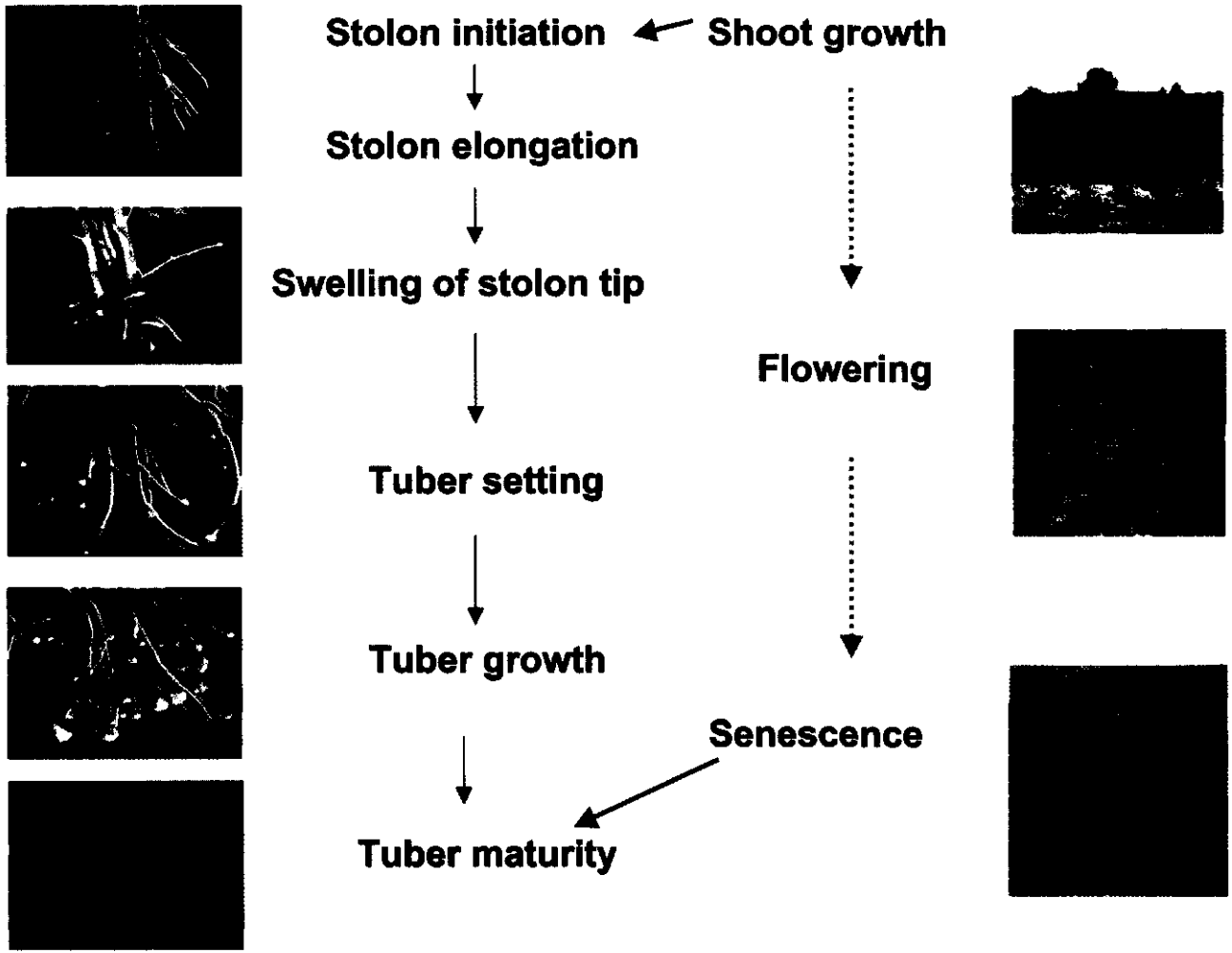
The physiology of tuber formation

Tuber formation from a crop physiological perspective

An impressive amount of information on the different processes involved and factors affecting tuber development has been generated over the past 100 years (reviewed by Ewing and Struik, 1992; O'Brien et al., 1998; Jackson, 1999). From these studies it can be concluded that genotype, photoperiod (length of the dark period), temperature, water supply, nitrogen supply and physiological age of the mother tuber are the most important factors influencing plant development and tuber formation. However, there is still considerable debate on how determinant these factors are on tuber formation and plant development. For instance, physiological ageing of seed tubers is known to have a determinant influence on tuber initiation and the duration of the plant cycle (Struik and Wiersema, 1999). However, some studies (O'Brien et al., 1998) indicated that this is not usually the case, particularly, under standard agricultural practices.

This disagreement is probably due in great proportion to the fact that results are based on the evaluation of only one or a very limited number of genotypes (varieties). Furthermore, only a limited proportion of this research has been performed under field conditions, imposing a limitation to the extent to which these results can be related to a more global view on crop physiology. A great deal of attention has been paid to the distribution of number and size of tubers during the plant cycle, in order to identify some of the factors influencing these very complex traits (Struik et al., 1989). However, in these studies limited attention has been paid to the duration of stolon formation, the onset and degree of branching and the duration of the tuber initiation period, factors all having a determinant influence on the final number of tubers and their size distribution at the end of the growing season. Studies with very consistent

Figure 1. Schematic representation of morphological events related to plant development and tuber formation taking place during the life cycle of the potato plant.



results, on the other hand, have been obtained on the relationship between increase of tuber dry matter and the duration of the leaf canopy or the amount of light intercepted by the canopy (Collins, 1977; Allen and Scott, 1980).

The simultaneous occurrence of events related to tuber formation

It is widely accepted that conditions favourable for stolon formation are long days, warm temperatures and high levels of gibberellins. For tuber induction, on the other hand, short days, cool nights and low levels of gibberellins are the most favourable (Ewing and Struik, 1992). Surprisingly, in every potato plant and during a large part of the life cycle, it is possible to observe simultaneously, newly formed stolons, swelling stolon tips, tuber incipients and growing tubers of different sizes, while above ground level, the plant is growing, branching, flowering and even senescing (Ewing and Struik, 1992). This phenomenon makes it very difficult to explain from a physiological point of view, how it is possible that after the plant has been induced to tuberize, still new stolons are formed and that not all the stolon tips respond similarly and begin to swell and form tuber incipients within a short period of time. Surprisingly, this phenomenon has not been well characterised yet and there is very little information on the duration of the overlap between these events. Similarly, there is also overlap between the occurrence of later events, associated with tuber formation. Only when these events are monitored during the span of the plant cycle and compared among different genotypes, a more comprehensive picture of the physiological interrelations between these and other events taking place during the life cycle will emerge.

Tuber formation from a genetics perspective

Genetic features of the potato

Solanum tuberosum L., the most widely grown potato species world-wide, is a highly heterozygous and self-compatible tetraploid species with 2n=4x=48 chromosomes. Diploid species, on the other hand, are obligatory outbreeders due to self-incompatibility of the gametophytic type (Hawkes, 1990). Although self-compatible, *S. tuberosum* exhibits inbreeding depression after a few generations of selfing, which makes this species highly outcrossing and heterozygous with several alleles at each locus. Tetrasomic inheritance is complex as up to 8 different alleles descending from both parents could segregate independently in the progeny, resulting in 36 phenotypic classes. Therefore, most genetic studies in potato are done at the diploid level (Bradshaw and Mackay, 1994).

Genetic mapping of complex traits

Genetic mapping is based on the assignment of markers to linkage groups on the basis of the recombination values from all these pairwise combinations. In each linkage group the markers are placed in order, indicating the relative genetic distances between them.

Several molecular marker technologies are being used to generate genetic maps. RFLP markers (Botstein et al., 1980) are codominant and therefore very informative. However, generating RFLP marker data requires a relatively large amount of DNA, it is labour intensive and time consuming. In potato, several RFLP maps are available (Gebhard et al., 1991; Schäfer-Pregl et al., 1998). AFLP is a PCR-based DNA fingerprint technique (Vos et al., 1995). The principal advantage of AFLP is its capacity to analyse a large number of polymorphic loci simultaneously, throughout the genome with a single primer pair, without previous sequence knowledge. However, one major limitation of AFLP is its dominant nature and the difficulty in identifying allelic variants at a specific locus. In potato, Van Eck et al. (1995) using the CxE population with 67 genotypes, constructed an AFLP map, consisting of 264 markers generated from 6 primer combinations.

In a segregating population of potato, complex traits of significance in crop physiology, such as yield, tuber size distribution, senescence and tuber initiation, exhibit a quantitative inheritance. Quantitative traits show continuous phenotypic variation as a result of the combining allelic effect of several genes and their interaction with environmental conditions. The genetic loci controlling quantitative traits are known as quantitative trait loci (QTL).

QTL mapping is based on the association between phenotypic differences of the trait of interest and molecular markers located at specific positions on the linkage groups, using statistical tools. The QTL analysis approach leads to the identification of QTLs in terms of number, position, effects and interactions between them.

For a long time, QTL analysis of physiologically complex traits, such as yield and plant size, were done by evaluating a single component representing the trait of interest, once during the ontogeny of the trait. Although with this approach, genetic factors underlying these traits were detected, its major limitation was that traits were considered as isolated events taking place during the plant cycle, unrelated to genetic factors controlling other developmental components of the observed phenotype.

In recent years, however, an increasing amount of information has been generated in several crops, on the genetic dissection of developmental components of physiologically complex traits, such as plant growth (Bradshaw and Steller, 1995;

In Chapter 6, after having dissected the physiological parameters which are relevant for both above and below ground phenomena, the genetic dimension of these processes were incorporated; for this purpose the senescence syndrome was selected. The senescence process is characterised through the parameters: onset, duration, mid point, rate and end (duration of the plant cycle). QTL analysis was performed to identify genetic factors associated with these parameters. QTL analysis of the progress of senescence at each one of several evaluation dates is also presented.

In Chapter 7, the overall results are discussed with regard to the feasibility of using large populations for the genetic dissection and physiological characterisation of very complex traits in potato, in order to get a more complete understanding of the physiological and genetic basis underlying traits of economical relevance.

2

Sprouting of seed tubers during cold storage and its influence on the processes related to tuber formation in a crossing population of potato (*Solanum tuberosum* L.)

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growing season (6- 8 months later) to avoid or minimise pre-mature sprouting during storage (Van Loon, 1987). However, it has been widely observed that seed tubers stored at 4 °C will start to sprout spontaneously and that the time at which sprout growth begins is varietal dependent (Lindblom, 1970; Susnoschi, 1981a,b; O'Brien et al., 1983). The genetic make-up of the cultivars also plays a very important role in the effect of chronological age, temperature and other factors on PA (Ewing and Struik, 1992; Struik and Wiersema, 1999).

PA has been reported to have a major effect on time to emergence, number of stems, time to tuber initiation, duration of the life cycle (earliness), number of tubers, tuber size distribution, tuber quality and yield (Van der Zaag and Van Loon 1987; Ewing and Struik, 1992; Struik and Wiersema, 1999). However, there are contradictory results about the effect of PA on several of the above mentioned characteristics. These conflicting results could be related to differences in the genetic make up of the cultivars used, chronological age of the tubers, conditions during seed production, time and methods of haulm destruction, prevailing temperature during the period between haulm destruction and harvest, ways to express sprout growth, storage and sprouting temperatures, desprouting, apical dominance, light during storage, presence of "little potato", field versus greenhouse studies and planting date (Wurr, 1978a,b; Ewing and Struik, 1992; O'Brien et al., 1983; Struik and Wiersema, 1999).

Since we are studying in detail different aspects related to plant development and tuber formation in a population of potato (CxE), which, in general, has a very low or no dormancy and sprouts during storage, we decided to investigate a) the relationship between sprouting of seed tubers during storage at 4 °C and the duration of the dormant stage under conditions favourable for sprouting and, b) the influence of sprouting of seed tubers during storage at 4 °C on the initiation of processes related to tuber formation, duration of the plant cycle and on other plant and tuber related characteristics evaluated in the CxE population.

Materials and methods

Genetic background of plant material

The diploid backcross population (CxE) consisting of 250 individuals was obtained from the cross between C (USW5337.3; Hanneman and Peloquin, 1967) and E (77.2102.37; Jacobsen, 1980). Clone C is a hybrid between *S. phureja* PI225696.1 and *S. tuberosum* dihaploid USW42. Clone E is the result of a cross between clone C and the *S. vernei-S. tuberosum* backcross clone $VH^{3}4211$ (Jacobsen, 1978). The tetraploid varieties Astarte, Bintje, Gloria, Granola, Karnico, Première and Saturna were included as controls.

Agronomic details and design of the field experiments

The CxE population was multiplied under field conditions in 1998 (**Exp. 1**) in Wageningen (52° N lat.). Eight tubers from every genotype were planted on May 12th and plant maturity type was evaluated, allowing the identification of very early and very late genotypes. On September 21st (133 days after planting or DAP), one week after chemical vine killing and for every genotype separately, tubers were harvested and collected in two nylon bags containing about 50 tubers each. To allow wound healing, the bags were placed in chambers with forced warm air circulation for two weeks and then stored at 4 °C until the next growing season. On April 14th 1999, after 191 days at 4 °C, tubers were removed from storage and every bag of tubers was placed in individual trays to assess sprout growth. After this, tubers of similar size (30 - 40 mm Ø) were selected for all CE genotypes and the parental clones and placed for reconditioning at room temperature (18 °C average) and indirect natural light, to favour the development of sturdy sprouts and uniform shoot emergence. Tubers from the seven varieties were received from different sources, the day before the evaluation of sprout length in the CxE population. They were harvested in August 1998 and kept at 4°C until shipment. After the evaluation of the sprout length, the varieties were placed for reconditioning as done for the CxE population.

Also on May 12, 1998, 3 replications with 2 plants each of the CxE population, the parental clones and the varieties (**Exp. 2**) were planted next to Exp.1, and on September 21, 10 days after haulm killing, the tubers were harvested.

On April 29, 1999, again 3 replications with 2 plants each of the CxE population, the parental clones and the varieties (**Exp. 3**) were planted at the same location as Experiments 1 and 2, and on September 25, 10 days after haulm killing the tubers were harvested.

Also in 1999, a fourth experiment (**Exp. 4**) was planted on May 12th, in the vicinity of Wageningen, where 2 plants from the CxE population and parental clones were randomised within each of 11 blocks, corresponding each to a harvesting date: 29, 36, 42, 57, 64, 75, 89, 96, 110, 125 and 140 days after planting (DAP). Based on the preliminary information on plant maturity type and tuber size distribution, it was clear that several genotypes would have a life span beyond 140 DAP, therefore 47 of the latest genotypes were planted at random in 3 additional blocks, which were harvested at 155, 170 and 185 DAP. The seven varieties were randomised within 14 blocks. They were harvested at the same dates as the CE blocks. Experiment 4 was planted in a light sandy soil in order to facilitate the harvesting of the individual plants without damaging underground structures. Also, overhead irrigation was applied to Exp. 4 whenever needed in order to avoid undesired variation in stolon and tuber formation processes due to drought stress, as this stress may occur at different phenological

phases for the different genotypes. Plants were protected against *P. infestans* by weekly applications of fungicides. In order to determine the first date of evaluation, 28 plants from each one of the 7 earliest genotypes were planted next to the field experiment and three times per week two plants per genotype were harvested. When 5 out of the 7 genotypes showed stolon formation (29 DAP), the first block was harvested. In all the experiments the plant arrangement was 75 cm between rows and 40 cm between plants, within the row.

Characteristics under evaluation

From all characteristics evaluated on each plant and harvesting date, the following traits were analyzed after the classification of the CxE population according to the average length of sprouts in the seed tubers:

A) Above ground level:

- emergence;
- plant size: scale 1 = ≤ 40 cm, 2 = 41-60 cm, 3 = 61-80 cm, 4 = 81-100 cm and 5 = > 100 cm height;
- number of main stems (stems emerging directly from the seed tuber);
- number of secondary stems (stems with leaves that started as stolons followed by a switch to orthotropic growth);
- flowering stage: scale 0 (= no flowering yet) to 7 (= end of flowering). The onset of flowering was defined as the evaluation date at which the first open flower was observed;
- duration of the plant cycle: number of days from planting to death of the plant due to the process of senescence.

B) Stolon level:

- onset of stolon formation (first evaluation date at which at least one stolon was observed);
- number of stolons at onset;
- number of newly formed stolons;
- stolon length: proportion of short (1 to 10 cm), medium (11 to 25 cm), long (26 to 40 cm) and very long (> 40 cm) stolons;
- degree of stolon branching: scale 1 (= no branching) to 5 (= very intensive branching);
- onset of stolon tip swelling (first evaluation date at which at least one stolon tip was showing incipient swelling);
- number of swelling stolon tips.

C) Tuber level:

- number of tuber incipients (tuber initiation): number of stolon tip swellings with a diameter between 5 and 12 mm (approximately twice the diameter of the stolons, which have a variable thickness);
- tuber size distribution: all tubers produced by the individual plants at each evaluation date were harvested and separated into size categories, with steps of 5 mm, from 12 to 125 mm in diameter;
- tuber weight: from the 8th harvesting date, the weight of each tuber size class was recorded;
- secondary growth: observation of tubers on stolons emerging from the "eyes" of a developing tuber. Scale 1= no second growth to 5= all tubers have
- stolons emerging from the "eyes".

Evaluation of sprouting of seed tubers during storage at 4 °C

The two individual samples harvested in Exp. 1 and stored during 191 days at 4 °C were placed on plastic trays and the range in the length of the longest sprout (LS), independently of its position on the tuber, was evaluated using the following scale: LS-1= no visible sprouts; LS-2= sprouts between 1 and 4 mm; LS-3= sprouts between 5 and 9 mm; LS-4= sprouts between 10 and 19 mm; LS-5= sprouts between 20 and 29 mm and; LS-6= sprouts longer than 30 mm.

Evaluation of sprouting of seed tubers at 18 – 22 °C

After harvesting, the tubers from the Experiments 2 and 3 were kept at 18 – 22 °C for 32 (Exp. 2) or 38 days (Exp. 3). The tubers from every genotype and replication were placed in individuals plastic trays and the range of the length of the longest sprout was evaluated using the same scale described previously.

Calculated variables and statistical analysis

Analysis of Variance (ANOVA) and Tukey's mean separation test (P=0.05) were performed to calculate differences among sprout length (LS) categories in the number of days after planting (DAP) to the onset and end of the following processes: stolon formation, stolon tip swelling, stolon branching, tuber initiation, flowering and senescence. Also ANOVA was used to calculate differences among LS categories in plant size, incidence of second growth, number of main and secondary stems and total number of tubers at the end of the plant cycle, and number of newly formed stolons, and swelling stolon tips at each evaluation date. Correlations between all variables were calculated using the two-tailed Pearson Correlation Coefficient with $P<0.05$ and $P<0.01$. Thirteen CE clones were excluded from the analysis due to the low number of

observations available.

Environmental conditions during the field experiments

Data on the average daily air temperature (between 10 and 150 cm), soil temperatures (10 cm depth) and rainfall during 1998 and 1999 were obtained from the weather station "de Haarweg" of Wageningen University. In 1998 (May 5 to September 21) the average daily air temperature was 16.1 ± 2.9 °C, with a maximum of 20.8 ± 4.4 °C and a minimum of 9.5 ± 3.2 °C. The average daily soil temperature was 17.5 ± 2.2 °C, with a maximum of 19.5 ± 3.2 °C and a minimum of 15.7 ± 2.1 °C. In 1999 (April 29 to September 28), the average daily air temperature was 16.7 ± 3.0 °C, with a maximum of 33.9 ± 3.8 °C and a minimum of 9.6 ± 3.6 °C. The average daily soil temperature was 16.5 ± 3.0 °C, with a maximum of 17.1 ± 3.1 °C and a minimum of 16.0 ± 2.9 °C. In 1988 and 1999, the average daily rainfall was 3.8 ± 5.4 l/m^2 and 3.7 ± 4.6 l/m^2, respectively.

Results

Distribution of the CxE population, parental clones and varieties into sprout length categories

After 191 days in storage at 4 °C, 92% of the CxE population had ended dormancy (sprouts ≥ 1 mm) and 8% of the population did not show signs of sprout elongation (LS-1) (Table 1). The largest percentage (36%) had sprouts between 20 and 29 mm length (LS-5) and 14% had sprouts longer than 30 mm (LS-6). Both parents had sprouts (Table 2), but parent C had sprouts of 2 mm length (LS-2) whereas parent E had much longer sprouts (25 mm, LS-5). Sprouting is illustrated in Picture 1.

Differences in sprout length after long storage at 4 °C and after short storage at 18-22 °C

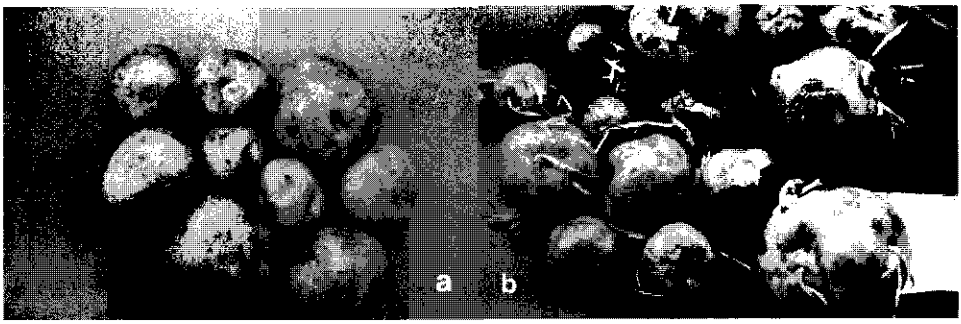
The same genotypes (8%) did not show signs of sprout growth either after 191 days in storage at 4 °C (Exp. 1) or after freshly harvested tubers were placed at 18 – 22 °C during 32 days (Exp. 2) or 38 days (Exp. 3) (Table 3). At harvest of all experiments, no visible sprout growth was observed in C, E, the progeny or the varieties. Sprout growth was not observed in tubers from the varieties after 32 or 38 days at 18 – 22 °C, contrasting the variable sprouting observed in the CxE population. A very high correlation coefficient was found for the comparison between results from Exp. 1 and Exp. 2 ($r = 0.91$), Exp. 1 and Exp. 3 ($r = 0.93$) and Exp. 3 and Exp.4 ($r = 0.98$). Temperature exerted a major influence on the rate of sprout growth.

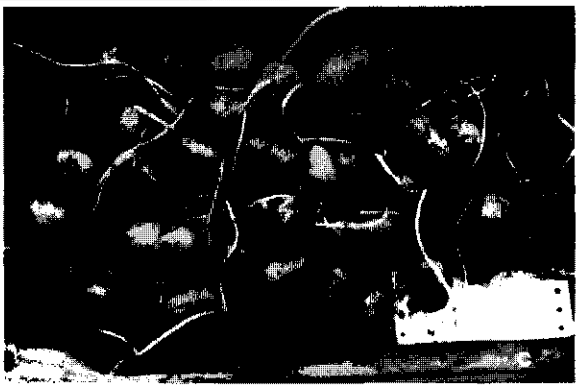
Table 1. Comparison of plant and tuber related traits between sprout length (LS) categories in the CxE population. Experiment 4.

	Sprout length categories (length of the longest sprout in mm)							
	1	2	3	4	5	6	MSE	p-value
	0	1 to 4	5 to 9	10 to 19	20 to 29	>30		
Percentage population	8	6	10	26	36	14		
Onset stolon formation[1]	30.6a	29.2b	29.3b	29.4b	28.7b	29.0b	3.8	<0.01
Onset stolon swelling[1]	47.3a	48.6a	44.2ab	42.7ab	39.6b	38.2b	149.2	<0.01
Onset stolon branching[1]	51.3a	52.1a	49.7a	48.9a	49.2a	47.5a	168.4	0.59
Onset flowering[1]	38.9a	39.7a	38.6a	39.0a	39.4a	39.0a	9.2	0.47
Duration plant cycle	130.9a	130.8a	120.0a	131.1a	123.8a	123.0a	883.5	0.15
Plant height scale[2]	3.2a	3.2a	3.1a	3.2a	3.1a	3.0a	0.12	0.13
Number main stems	3.5a	3.6ab	3.7abc	4.3cd	4.2bcd	4.8d	1.6	<0.01
Number secondary stems	0.9a	0.6a	1.1a	0.8a	1.0a	0.6a	4.0	0.06
Tubers/plant at 42 DAP	2.3a	6.0ab	10.4ab	6.8ab	10.0ab	12.8b	103.0	<0.01
Stolons/plant at 42 DAP	12.0a	15.3ab	17.1ab	17.5ab	17.9ab	21.2b	82.4	0.05
Tubers/plant at 57AP	8.9a	9.0a	16.5ab	16.4ab	22.8b	25.9b	219.2	<0.01
Tubers/plant[3]	70.1a	87.0b	56.1a	56.1a	66.6ab	65.0ab	2500.5	0.05
Tuber size (mm Ø)[3]	2.5a	2.4a	2.7a	2.5a	2.4a	2.4a	0.36	0.06
Total tuber weight (g)[3]	1442a	1459a	1350a	1319a	1422a	1249a	636829.4	0.67
Secondary tuber growth	0.08a	0.1a	0.07a	0.09a	0.07a	0.07a	0.07	0.06
Tubers/stem[3]	12.8a	16.1a	15.0a	14.2a	13.8a	13.0a	59.3	0.48

*Tukey's mean separation test, numbers in a row with common letters were not significantly different at p = 0.05; MSE= standard error of the mean [1]= in days after planting (DAP); [2]= scale: 1 = ≤ 40 cm, 2 = 41-60 cm, 3 = 61-80 cm, 4 = 81-100 cm and 5 = > 100 cm height; [3] = at the end of the plant cycle.

Picture 1. Tuber samples from the CxE population after 38 days at 18 – 22 °C (a, b and c) and after 191 days in storage at 4 °C (d). a: no visible sprouts (LS-1); b: sprouts between 20 and 29 mm length (LS-5); c: sprouts longer than 30 mm length; d: LS-1= no visible sprouts; LS-2= sprouts between 1 and 4 mm; LS-3= sprouts between 5 and 9 mm; LS-4= sprouts between 10 and 19 mm; LS-5= sprouts between 20 and 29 mm and; LS-6= sprouts longer than 30 mm.





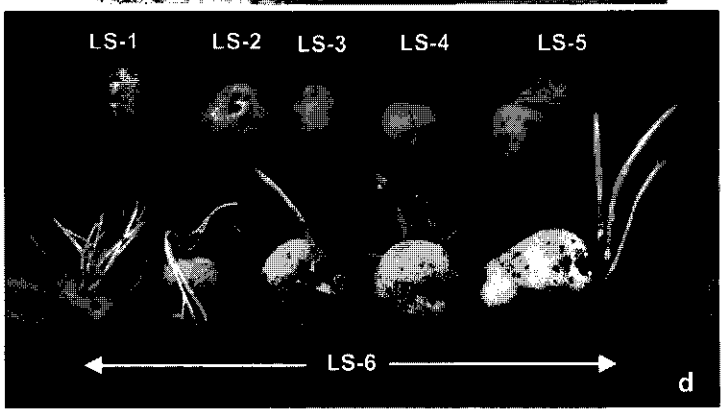


Table 2. Comparison between the CxE population, the parental clones (C and E) and the seven standard varieties in the average length of the longest sprout, number of stems per seed tuber, days after planting (DAP) to the onset of tuberization and duration of the plant cycle. Experiment 4.

Plant material	Sprout length (mm)	Stems/tuber	Onset stolon tip swelling*	Duration of the plant cycle*
C	2	3.8	57	140
E	25	5.0	36	110
C x E population	0- >30	1 – 10	29 – 89	75 - >185
Astarte	2	3.3	36	170
Bintje	2	6.0	36	140
Gloria	2	5.3	29	96
Granola	2	3.8	36	125
Karnico	2	6.0	36	185
Première	2	6.0	29	96
Saturna	2	4.5	36	140

* in days after planting

Influence of sprouting of seed tubers during storage on shoot emergence

Differences in shoot emergence were not directly related to differences in sprout length. The first plants began to emerge 14 days after planting and 7 days later (21 DAP), 62% of the population had emerged. At 29 DAP, 95.6% of the population (including the clones from the non-sprouting LS-1 category), the parental clones and all the varieties had emerged and had a very similar plant height. At 36 DAP only 3 clones (one out of two plants each) had some delay in emergence.

Sprouting during storage at 4 °C and the number of main stems, secondary stems, new stolons and stolon swelling tips

Highly significant differences were found among LS categories in the number of main stems per plant (Table 1). Clones without sprout elongation (LS-1) had on average 3.5 stems/plant, while clones with sprouts longer than 30 mm (LS-6) had 4.8 stems/plant. Similarly, C (LS-2) had 3.8 stems/tuber while E (LS-5) had on average 5 stems/tuber (Table 2). In contrast, the varieties had a variable number of stems/tuber, ranging from

Table 3. Distribution of the CxE population into length of sprout categories after 191 days at 4 °C (Exp.1) and after 32 (Exp. 2) and 38 (Exp. 3) days at 18 - 22 °C following harvesting.

	Sprout length categories (percentage CE population)					
	1	2	3	4	5	6
Exp. 1	8	6	10	26	36	14
Exp. 2	7	7	10	22	31	23
Exp. 3	7	6	9	20	39	19

3.3 stems for Astarte to 6.0 stems for Bintje, Karnico and Première, although all had 2 mm sprouts (LS-2). Since the same range in stem number was observed in the CE population within the categories LS-1 and LS-2, it is possible that genotypes within these categories were differing in the degree of apical dominance still present after reconditioning at 18°C. In the CxE population, the highly significant correlation ($p=0.01$) but with a relatively low correlation coefficient ($r = 0.35$) observed between LS categories and number of stems/plant is illustrated in Figure 1. The lower LS categories (1 to 3) had between 1 and 6 stems/plant, while categories 4, 5 and 6 had between 2 and 10 stems/plant, reflecting the wide variation in number of stems/tuber within LS categories. In the CxE population, no significant differences were found between LS categories in the number of secondary stems, number of new stolons and number of swelling tips per plant or per stem, on average across Exp. 2 or at each evaluation date, with exception of number of stolons per plant at 42 DAP (Table 1). CE genotypes in the LS-1 category produced 12 new stolons per plant in contrast to 21 new stolons per plant for genotypes in the LS-6 category.

Sprouting during storage at 4°C and the onset of the processes related to tuber formation

Small but significant differences were found between LS categories in the onset of stolon formation (Table 1). Genotypes without sprouting (LS-1) began to form stolons 31 days after planting instead of 29 days like in the other five LS categories. Significant differences were found also between LS categories in the number of days after planting to the observation of the first swelling stolon tip(s), the event considered as the first visual evidence of tuber induction. Genotypes with sprouts between 1 and 4 mm length (LS-2) began stolon tip swelling 10 days later than genotypes with sprouts longer than 30 mm (LS-6). However, there was a large variation in the onset of stolon tip swelling within LS categories (Figure 2). For instance, although 19 clones (8%) did

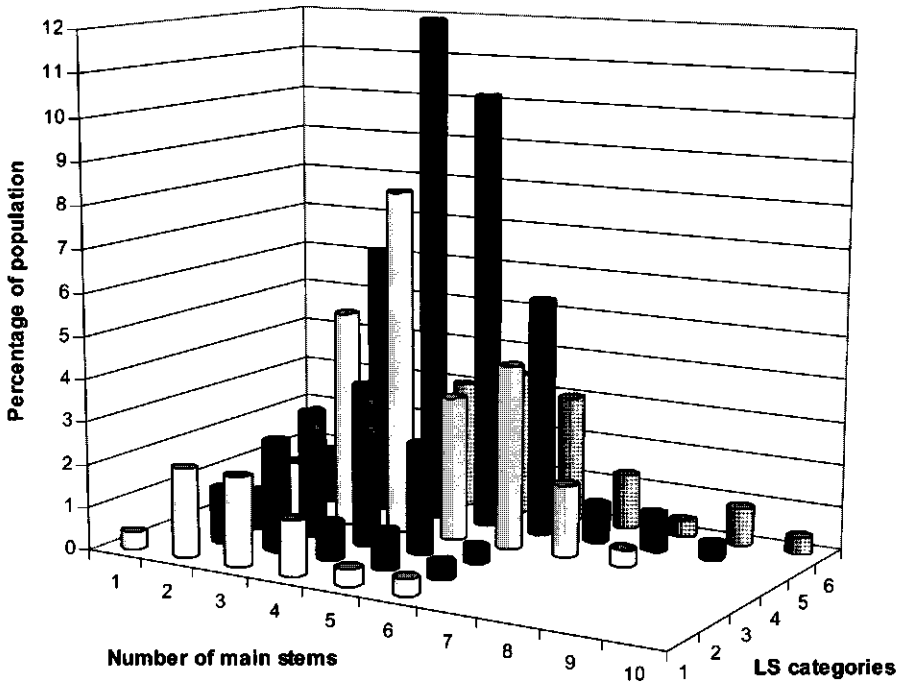


Figure 1. Distribution of the CxE population according to the sprout length (LS) categories and average number of main stems. Experiment 4.

not show sprout elongation (LS-1) during storage at 4 °C, they had the first swelling tips between 29 and 75 DAP like the other five LS categories. The highest percentage of the CxE population in the categories LS-4, LS-5 and LS-6, had the first swelling tips at 36 DAP just like the E (LS-5) parent. In contrast, only 1% of the genotypes in the category LS-2 showed the first swelling tips at 57 DAP, like the C parent (LS-2). On the other hand, the earliest varieties Gloria and Première began stolon tip swelling at 29 DAP in contrast to 36 DAP for the other 5 varieties (Table 2). Significant differences were not found between LS categories in the onset of stolon branching (Table 1).

Sprouting during storage at 4 °C and the number, size and weight of tubers

For the number of tubers per plant at each evaluation date, significant differences among LS categories were found only at 42 and 57 DAP (Table 1). The difference in number of tubers per plant between LS-1 and LS-6 categories at 42 and 57 DAP was

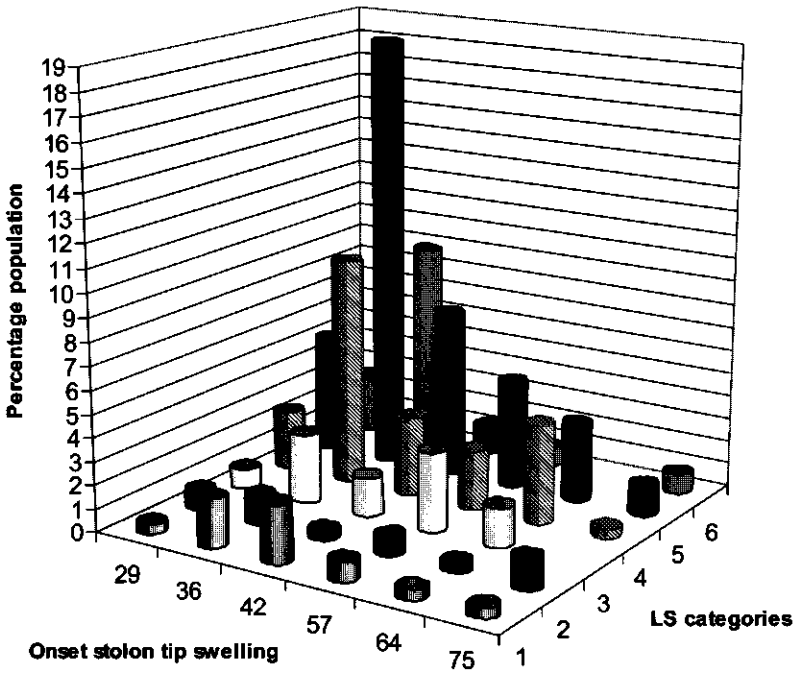


Figure 2. Distribution of the CxE population according to the sprout length (LS) categories and the onset of stolon tip swelling. Experiment 4.

11 and 17 tubers, respectively. Significant differences were found between LS categories in the total number of tubers at the end of the plant cycle (Table 1). Genotypes in the LS-3 and LS-4 categories had the lowest number of tubers per plant (56), in contrast to 87 in the LS-2 category. Genotypes without sprouts (LS-1) or sprouts longer than 30 mm (LS-6) had a similar number of tubers, 70 and 65 tubers, respectively. These results suggest that factors other than sprouting were influencing tuber number. No significant differences were found between LS categories in the average total tuber weight per plant, tuber size or the number of tubers per stem at the end of the plant cycle (Table 1).

Sprouting during storage at 4 ℃ and plant size, flowering, duration of the plant cycle and incidence of secondary growth

Although highly significant differences were found in the CxE population for plant height and the duration of the plant cycle, no significant differences were found between LS categories for these characteristics (Table 1). In general, in each LS

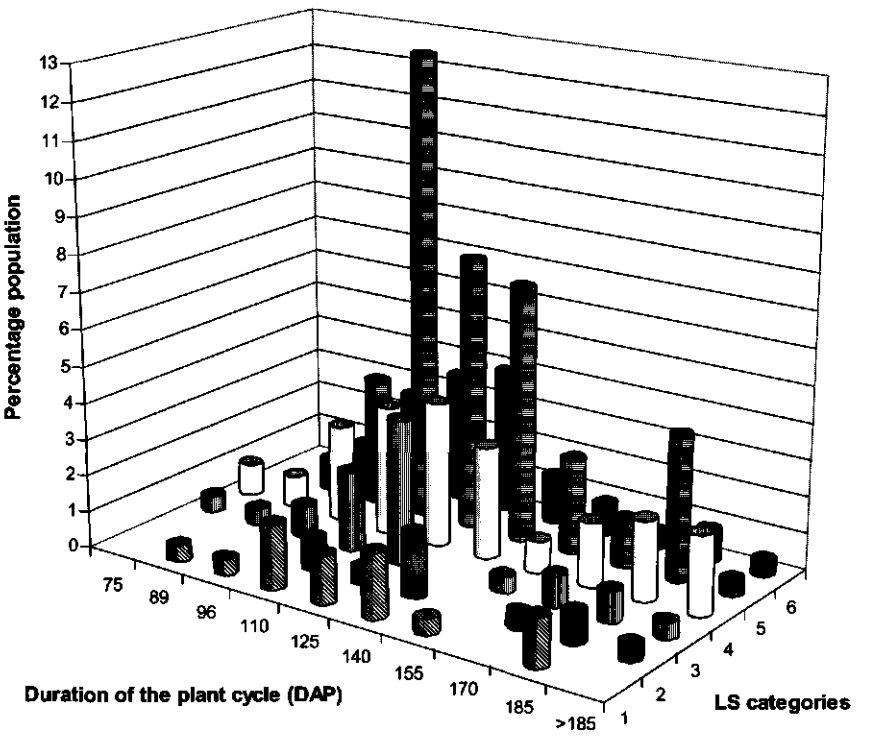


Figure 3. Distribution of the CxE population according to the sprout length (LS) categories and the duration of the plant cycle. Experiment 4.

category, the highest percentage of the population had a life span clustered within the range of the life span of the parental clones C (140 DAP) and E (110 DAP) (Figure 3). The varieties exhibited, as expected, a wide range in the duration of the plant cycle, from 96 DAP for Gloria and Première to 185 DAP for Karnico, although all had the same sprout length (LS-2). A similar range was observed in the CE clones from the LS-2 category. These results indicate a lack of strong correspondence between the ease to sprout at low temperatures (short or no dormancy) and the duration of the plant cycle. No significant differences were found between LS categories in the number of DAP to the onset of flowering (Table 1). All, the CxE population, parental clones and varieties, began to flower within a very narrow interval of time (36 to 42 DAP). The incidence of secondary growth was significantly related to both genotype and date of evaluation (data no shown), but not to the length of sprout growth at 4°C.

Discussion

The seed tubers from the CxE population were kept under the same conditions prior and during storage, however, there was a significant variation in the length of the longest sprout, which has been widely used to assess differences in physiological ageing of the seed tuber (Wurr, 1978a; Hartmans and Van Loon,1987; De Maine et al., 1998; Caldiz et al., 2001).

Significant differences in sprout length were found in the CxE population after long storage at 4 °C and after short storage at 18-22 °C, reflecting the influence of the *S. phureja* and *S. tuberosum* ancestry of this population. The main characteristics of *S. phureja* are the lack of dormancy, early maturity and short-day tuberization induction (Hawkes, 1990). Progenies involving long-day adapted *S. phureja* are known to have a more extensive sprouting and a greater sprout growth during storage at low temperatures than cultivars (Dodds and Paxman, 1961; De Maine et al., 1998).
In contrast, *S. tuberosum* varieties grown in temperate regions have been bred to have a long dormancy, variable maturity (early or main crop varieties) and long-day tuberization induction (Hawkes, 1990; Struik and Ewing, 1992). Thompson et al. (1980) found that the length of the dormancy period of *S. phureja* progenies placed at 21 – 27 °C was 20 days. In this study, tubers from the CxE population were kept at 18 – 22 °C for 32 or 38 days, after which more than 90% of the population was sprouting. Although both parents were sprouting, a very small percentage of the population did not show visible sprout growth at both 4 °C and 18 – 22 °C, indicating that these genotypes have a very strong dormancy. There was a high correlation between the average length of the longest sprout after long storage at 4 °C and short storage at 18 – 22 °C. In general, for genotypes with short or no dormancy, storage at 4 °C drastically reduced the rate of sprout growth compared to 18 – 22 °C.

It has been widely observed that tubers placed at temperatures suitable for sprout growth at the end of dormancy, usually exhibit a clear apical dominance (Krijthe, 1962; Struik and Wiersema, 1999) and that the longer the storage at 4 °C, the higher the number of stems per plant (Krijthe, 1962; Bodlaender and Marinus, 1987; Struik and Wiersema, 1999). Toosey (1964) observed that if seed tubers are sprouted at 12 – 15 °C immediately after the end of the dormancy period, a single sprout was produced, commonly at the apical end, which suppressed the growth of other sprouts. However, with storage at 2 – 4 °C, the number of sprouts per seed tuber increased as sprouting was delayed and all eyes could sprout similarly when the temperature was raised. In this study, significant differences were found between LS categories in the number of main stems per plant, those clones with the longest sprouts during storage at 4 °C, having the highest number of stems in the field. However, the correlation between

length of the longest sprout and the number of main stems per tuber, although highly significant, had a small r value. Besides differences in tuber size and/or number of "eyes" per seed tuber, this low correlation could be due to the fact that after long storage at 4 °C and reconditioning at 18 °C during several days, not in all CE clones the apical dominance had decreased in the same degree. This observation is further supported by the results from the seven varieties, which exhibited a variable number of stems/tuber even when all had a very similar sprout length and tuber size. Thus, differences in the length of the longest sprout could be directly related to the length of the dormancy period and to the rate of sprout growth at 4 °C (Wurr, 1978a,b; Thompson et al., 1980; De Maine et al., 1998) and partly related to the number of sprouts and hence the number of stems per tuber.

The higher number of stems associated with longer sprouts after long storage at 4 °C was reflected in a higher number of stolons and tubers per plant produced during early evaluation dates. However, at the end of the plant cycle, genotypes with an intermediate sprout length had the lowest number of tubers per plant, whereas genotypes with no sprouting or very long sprouts had a similar number of tubers. These results suggest that other genotype dependent factors such as the duration of the stolon formation period, the timing to the onset of stolon branching and the degree of stolon branching overrode the initial influence of sprout length on tuber number.

During storage at 4 °C, physiological ageing of the tubers still occurs (Struik and Wiersema, 1999), but the rate of this process would be relatively slow when tubers are kept sprout-free due to low temperature (Toosey, 1964) or faster when tubers are in not so deep dormancy (Reust, 1978). In addition, Van der Zaag and Van Loon (1987) indicated that in general, physiologically young seed tubers do not have sprouts at all, while physiologically old seed tubers have long sprouts. Similarly, O'Brien and Allen (1984) associated differences in physiological age among seed tubers exclusively to sprout length, and they considered that healthy seed tubers without sprouts, due to low storage temperatures or desprouting, have not aged.

Several authors (Wurr, 1978a,b; Madec and Perennec, 1962; Hartmans and Van Loon, 1987; and Ewing and Struik, 1992) had indicated that plants from physiologically old tubers began to form tubers and senesce earlier than plants from physiologically young tubers (very short or no sprouts). However, the very large variation observed in the onset of stolon tip swelling and duration of the plant cycle within groups of CE genotypes having a similar sprout length after long storage at 4 °C, suggests that if physiological ageing of the seed tubers took place during cold storage, this did not have a determinant influence on these events, among other tuber and plant related characteristics. This conclusion is supported by the results of Ali (1979) and Firman et al. (1991), who found that for most cultivars tested, the time

interval from emergence to tuber initiation was not affected by a wide array of physiological ageing, ranging from 0 to 1200 days-degree higher than 4 °C. O'Brien et al., (1998) also indicated that in general, the physiological age of seed tubers have little influence on the timing of tuber initiation, unless extreme ages are used, which in practice is quite uncommon.

In relation to tuber dormancy, Rojas-Beltran et al. (2000) stated that this is a stage in the ontogeny of the potato tuber that begins with its formation and ends with the sprouting of the tuber. Struik and Wiersema (1999) further indicated that this process can be divided into 3 phases: induction (at tuber initiation), maintenance (usually throughout the growing season and storage) and end or release (sprout growth of seed tubers after several weeks in storage or during reconditioning prior to planting).

The duration of the dormant period is highly dependent on genotype and environmental conditions, especially temperature, during tuber growth, from haulm destruction to lifting and during storage (Reust, 1978; Ewing and Struik, 1992; Struik and Wiersema, 1999). The environmental conditions during 1998 and 1999 were in the range considered highly favourable for plant development, tuber formation and maintenance of the dormancy stage (Claver, 1973; Van Heemst, 1986; Van Ittersum and Scholte, 1992a; Struik and Wiersema, 1999). However, most of the CxE population had a very short dormancy, as observed from the sprouting of newly harvested tubers. Although short dormancy has been associated with earlier onset of tuber initiation and senescence (Ewing and Struik, 1992), the CxE population began tuberization and senesced within a very wide period of time after planting. These results strongly suggest that the duration of the dormancy period and the physiological ageing during storage at 4 °C did not have a determinant influence on plant development and tuber formation in the CxE population. Furthermore, the wide array of combinations found between the duration of dormancy, onset of tuberization and the duration of the plant cycle suggest that these traits are controlled by different genetic factors, implying that these traits can be combined independently from each other by breeding and selection.

3

Temporal dynamics of tuber formation and plant related processes in a crossing population of potato (*Solanum tuberosum* L.)

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Abstract

The chronological relationships between stolon formation, stolon tip swelling, tuber initiation, flowering, senescence, growth and resorption of tubers were studied under field conditions in a diploid population of potato with 250 genotypes, the parental clones and 7 tetraploid varieties. Tuber initiation did not have a determinant influence on the timing of stolon formation, stolon tip swelling, flowering and duration of the plant cycle and very often proceeded stolon branching. The number and size distribution of tubers were largely influenced by the degree of stolon branching and the length of the stolon swelling period, whereas tuber resorption apparently had little influence on tuber number and size distribution. The peak production of stolons and swelling stolon tips took place largely within the flowering period, although in most cases, stolon tip swelling took place until the end of the plant cycle. More information on the general temporal relationships between events related to tuber formation and plant development will contribute to a better understanding of the physiological and genetic basis of the processes leading to the production of harvestable tubers.

Key words: flowering, phenology, plant cycle, potato, *Solanum phureja, Solanum tuberosum,* tuber formation

Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important crop in the world after maize, rice and wheat based on yield. Due to the crop's agronomic plasticity, it is grown and consumed in more countries than any other crop (Scott et al., 2000).
The growth and development of the potato plant can be divided into two main sets of events: (i) above ground level: the growth and senescence of the leaf canopy and the production of flowers and berries with their true seeds. (ii) below ground level: the formation of tubers. The later process can be subdivided into: a) the appearance and growth of stolons, diagravitropic stems arising from below ground nodes of the phototropic plant stems; b) the swelling of the sub-apical region of the stolon after halting longitudinal growth and, c) the formation of tuber incipients (swelling tips reaching 5 – 12 mm in diameter). The newly formed tubers will increase in size and finally they will mature, becoming dormant and the skin will set. Perhaps the most interesting and puzzling features of tuber formation are the apparent lack of synchrony between these developmental events and the simultaneous occurrence of different stages within every single plant. During a large part of the plant cycle, it is possible to observe newly formed stolons, swelling stolon tips, tuber incipients and growing tubers of different sizes, while above ground level, the plant is growing, flowering and even senescing. Tuber formation is the result of complex and dynamic relationships among several processes, all of them interacting in a plant, constantly exposed to a wide range of environmental changes.

A very large amount of information on the different processes involved and factors affecting plant development and tuber formation has been generated. This has been reviewed, among others, by Ivins and Bremner, 1965; Ewing and Struik, 1992; Almekinders and Struik, 1996; Kolbe and Stephan-Beckmann, 1997a,b; O'Brien et al., 1998; Jackson, 1999; Claassens and Vreugdenhil, 2000, and references therein. From these reviews it can be concluded that, among the abiotic and biotic factors affecting tuber formation, photoperiod (length of the dark period), temperature, water supply, nitrogen supply and physiological age of the mother tuber are the most important. However, there is still debate about how determinant these factors are on plant development and tuber related processes, particularly on tuber initiation. Also, there is disagreement in the literature on the strength of the interrelations between the above and below ground processes taking place in the potato plant. This lack of consensus is mainly due to the direct comparison of results generated under very different settings, such as field, greenhouse and growth chamber conditions, and the use of varieties differing in the duration of the plant cycle and physiological status of the seed tubers.

All this variability makes it difficult to generalise on the patterns of development followed during the life cycle of potato plants, grown under standard field conditions. Thus, in order to get more information on the general temporal relationships underlying the dynamics of tuber formation and other developmental processes during the potato life cycle, field data quantifying morphological events from a large and highly diverse population of potato grown under field conditions was analysed as a whole.

Materials and methods

Genetic background of plant material, design of field experiment and agronomic details

The genetic background of the plant material, the experimental design and agronomic details were given previously (Celis-Gamboa et al., 2002a). In brief, the diploid population CxE, consisting of 250 genotypes, is a backcross between *S. phureja* and *S. tuberosum*. The CxE population was planted on May 12, 1999, in the vicinity of Wageningen, in a light sandy soil in order to facilitate the harvesting of the plants and the observation and collection of most of the underground structures without damaging them. Two plants from the parental clones C and E and the progeny were planted at random within 11 blocks, each one harvested at 29, 36, 42, 57, 64, 75, 89, 96, 110, 125 or 140 days after planting (DAP). Three additional blocks were harvested at 155, 170 and 185 DAP, containing 47 of the latest CE genotypes. The varieties Astarte, Bintje, Granola, Gloria, Karnico, Première and Saturna, included as standards, were randomized within 14 blocks and harvested as the CxE population. Only data from 230 CE clones were used for analysis, 20 clones had to be excluded due to low number of observations available or to lack of information on the duration of the plant cycle.

Characteristics under evaluation

From all the characteristics evaluated on each plant and harvesting date, the following traits were analysed in order to obtain information on the phenology of the crop:

Above ground level:

- flowering stage: scale 0 (= no flowering yet) to 7 (= end of flowering);
- plant senescence: scale 1 (= whole plant green) to 7 (= no green tissue).

Below ground level:

Stolon level:

- number of newly formed stolons;

- degree of stolon branching: scale 1 (= no branching) to 5 (= very high degree of branching);
- number of stolon tips showing incipient swelling.

Tuber level:
- number of tuber incipients: number of stolon tip swellings with a diameter between 5 and 12 mm (approximately twice the diameter of the stolon, which has a variable thickness);
- number of growing tubers: all tubers larger than 12 mm produced by the individual plants at each evaluation date were harvested and counted;
- number of resorbed tubers: "empty" tubers or tubers with a translucent appearance in most cases still attached to the stolon.

Calculated variables and statistical analysis

The onset of stolon formation and stolon tip swelling were defined as the first of several consecutive evaluation dates at which stolons and swelling tips were observed, respectively. The end of these processes was defined as the first of several consecutive evaluations at which no more stolons or swelling stolon tips were observed.

Since swelling of the stolon tips is considered the first visual evidence that the process of tuberization has begun, we will consider the onset of stolon tip swelling as the onset of tuberization (tuber initiation). Another reason to consider the onset of stolon tip swelling as equivalent to tuber initiation is that the transition from swelling stolon tip to tuber incipient could not be determined with precision, since this would require more frequent evaluations.

The onset of stolon branching was defined as the first evaluation date at which branching of stolons was observed. The onset of the flowering process was defined as the evaluation date at which the first open flower was observed. The end of flowering was considered to be the evaluation date after which no more flower buds or open flowers were present. The onset of senescence was defined as the last evaluation date at which no signs of senescence were observed, since for many genotypes, the transition from a green plant to a more or less advanced stage of senescence began in between two consecutive evaluations. In this way the onset of senescence was standardised and it was independent of the speed of the process. The end of the process of senescence (duration of the plant cycle) was defined as the first of several consecutive evaluations at which no more green leaf or stem tissue was observed.

Analysis of Variance (ANOVA) was performed to evaluate differences among genotypes in the number of days after planting (DAP) to the onset and end of the following processes: stolon formation, stolon tip swelling, stolon branching, tuber

initiation, flowering and senescence. The duration of the flowering and senescence processes were also analysed. ANOVA was also used to evaluate differences among genotypes and evaluation dates in the number of new stolons, swelling stolon tips, tubers in each size category and tubers resorbed. Correlation among all variables was calculated using the two-tailed Pearson Correlation Coefficient and testing with $p < 0.05$ and $p \leq 0.01$.

Environmental conditions during field experiments

Data on the average daily air temperature (between 10 and 150 cm), soil temperatures (10 cm depth) and rainfall from 1999 were obtained from the weather station "de Haarweg" from Wageningen University. From April 29 to September 28 (0 to 140 DAP), the average daily air temperature was 16.7 ± 3.0 °C, with a maximum of 33.9 ± 3.8 °C and minimum of 9.6 ± 3.6 °C. The average daily soil temperature was 16.5 ± 3.0 °C, with a maximum of 17.1 ± 3.1 °C and a minimum of 16.0 ± 2.9 °C. The average daily rainfall was 3.7 ± 4.6 l/m^2. From September 29 to November 12, the period during which three additional evaluations were done on 47 late to very late genotypes, the average daily air temperature was 9.8 ± 3.0 °C, with an average daily maximum of 14.5 ± 2.9 °C and a minimum of 5.2 ± 4.0 °C. The average daily soil temperature was 12.3 ± 1.8 °C, with a maximum of 12.7 ± 1.9 °C and a minimum of 11.9 ± 1.8 °C. The average daily rainfall was 1.4 ± 2.5 l/m^2.

Results

Temporal differences in the onset of stolon formation, stolon tip swelling, flowering and senescence

Highly significant differences were found between CE genotypes for all the traits evaluated (Table 1). The CxE population began the process of stolon formation (Table 2) and flowering (Table 4) within a very short interval of time, 29 to 36 DAP and 36 to 42 DAP, respectively. All the varieties began to form stolons at 29 DAP and to flower at 42 DAP. In contrast, the onset of stolon tip swelling (Table 3) and senescence (Table 5) took place across a much wider interval of time. The CxE population began the process of stolon tip swelling within a period of 46 days (29 to 75 DAP), while the varieties had swelling stolon tips within 7 days (29 to 36 DAP). In the CxE population, the first tuber incipients were observed within a period of 46 days (29 to 75 DAP), whereas all varieties showed the first tuber incipients similarly and very early in the growing season (36 DAP). On the other hand, the CxE population began the process of senescence between 64 and 170 DAP. Likewise, the varieties, showed a wide range in the onset of senescence, from 89 DAP (Gloria and Première) to 140 DAP (Astarte and

Table 1. Minimum, maximum, mean and standard error values of all the variables considered and found significantly different between genotypes at $P < 0.01$.

Variable	Minimum	Maximum	Mean	MSE
Onset stolon formation	29	36	31.2	2.13
End stolon formation	57	>185	88.9	13.69
Duration stolon formation	21	>185	60.9	14.47
Onset stolon tip swelling	29	75	41.9	14.75
End stolon tip swelling	64	>185	110.3	36.67
Duration stolon tip swelling	6	161	67.2	36.01
Onset stolon branching	no[2]	93	49.2	25.36
Degree stolon branching	0	5	3.2	0.37
Onset tuber resorption	no[3]	140	79.4	196.01
Number new stolons*	0	90	6.2	2.30
Number swelling tips*	0	45	6.4	3.51
Number tubers < 12 mm Ø *	0	78	15.4	4.31
Number tubers > 12 mm Ø*	0	47.8	10.4	3.90
Number resorbed tubers *	0	29	2.8	0.92
Onset flowering	36	42	39.2	6.62
End flowering	64	155	86.0	20.03
Duration flowering	22	119	43.8	25.3
Onset senescence	64	170	91.7	12.7
End of senescence[1]	75	>185	125.8	29.8
Duration senescence	11	89	39.4	20.09

= per evaluation date; MSE= standard error of the mean; 1 = equivalent to the duration of the plant cycle; 2 = no stolon branching; 3 = no tuber resorption

Karnico). The parental clones C and E began stolon tip swelling, flowering and senescence within the range of the CxE population (Tables 2, 3, 4, 5). The most contrasting difference between the parental clones was found for the onset of stolon tip swelling. In C, the first swelling stolon tips were observed 21 days later than in E.

Table 2. Distribution of the CxE population (%), parental clones and varieties for the onset and end of stolon formation.

	Stolon formation													
	End (DAP)													
Onset (DAP)	**42**	**57**	**64**	**75**	**89**	**96**	**110**	**125**	**140**	**155**	**170**	**185**	**>185**	**MDO**
29	P	1.3	20.0^{Gl}	28.6^{C,E,S}	11.8	9.4^{Gr}	5.1^{A,B}	6.4	1.3 ^{K}		2.1	2.6	2.1	88.9
36			3.0	3.0	1.3	0.9	0.4		0.9	0.9	0.4		0.4	100
MDE	0	1.3	24.3	54	67.2	77.4	83	89.4	91.5	92.3	94.9	97.4	100	

A = Astarte; B = Bintje; Gl = Gloria; Gr = Granola; K = Karnico; P = Première; S = Saturna; C = female parent; E = male parent; MDO and MDE = marginal distribution of onset and end, respectively.

Table 3. Distribution of the CxE population (%), parental clones and varieties for the onset and end of stolon tip swelling.

	Stolon tip swelling												
	End (DAP)												
Onset (DAP)	**57**	**64**	**75**	**89**	**96**	**110**	**125**	**140**	**155**	**170**	**185**	**>185**	**MDO**
29	P	2.2	2.2^{Gl}	0.9	1.7	0.9		0.4					8.3
36		8.1	6.5 ^{S}	8.2 ^{E}	5.6	6.0 $^{Gr, B}$	3.9	2.6 ^{A}	0.9	0.9 ^{K}			51
42		0.8	2.6	3.4	2.2	2.6	2.6	3.9	0.4		0.9		70.2
57					0.9	3.0 ^{C}	3.4	3.0	0.9	1.7	0.9	0.9	84.9
64						0.4	1.3	0.4	0.4	0.4	2.6	0.9	91.3
75							1.3		1.7	1.3	2.2	2.2	100
MDE		11.1	22.4	34.9	45.3	58.1	70.5	80.8	85.1	89.4	96	100	

A = Astarte; B = Bintje; Gl = Gloria; Gr = Granola; K = Karnico; P = Première; S = Saturna; C = female parent; E = male parent; MDO and MDE = marginal distribution of onset and end, respectively.

Table 4. Distribution of the CxE population (%), parental clones and varieties for the onset and end of flowering.

Flowering									
	End (DAP)								
Onset (DAP)	64	75	89	96	110	125	140	155	MDO
36	7.9	13.8 ^{E}	6.3	2.9	2.1	2.1		0.4	35.4
42	3.8	24.6 $^{Gl, S, B, P, C}$	16.7 ^{Gr}	4.2 $^{K, A}$	2.1	10.4	2.1	0.8	100
MDE	11.7	38.4	72.9	80.0	84.2	96.7	98.8	100	

A = Astarte; B = Bintje; Gl = Gloria; Gr = Granola; K = Karnico; P = Première; S = Saturna; C = female parent; E = male parent; MDO and MDE = marginal distribution of onset and end, respectively.

Table 5. Distribution of the CxE population (%), parental clones and varieties for the onset and end of senescence (duration of the plant cycle).

Senescence											
	End (DAP)										
Onset (DAP)	75	89	96	110	125	140	155	170	185	>185	MDO
64	1.3	1.7	4.3	6.1	1.7	0.4					15.7
75		1.7	6.1	16.1 ^{E}	10.9	1.7	0.4				52.6
89			0.4 $^{Gl, P}$	2.2	2.6	2.2	0.4				60.4
96				0.4	3.5 ^{Gr}	5.2 $^{S, B, C}$	0.4	1.7	0.4		72.2
110					2.2	4.8	1.3	1.3	2.6		84.3
125						0.4	2.2	0.9	3.5		91.3
140							0.4	0.9 ^{A}	1.3 ^{K}	1.7	95.7
155									2.2	1.7	99.6
170										0.4	100
MDE	1.3	4.8	15.7	40.4	61.3	76.1	81.3	86.1	96.1	100	

A = Astarte; B = Bintje; Gl = Gloria; Gr = Granola; K = Karnico; P = Première; S = Saturna; C = female parent; E = male parent; MDO and MDE = marginal distribution of onset and end, respectively

Temporal differences in the end of stolon formation, stolon tip swelling, flowering and senescence

In the CxE population, the end of both stolon formation (Table 2) and stolon tip swelling (Table 3) took place within a very similar interval of time, 57 to > 185 DAP and 64 to >185 DAP, respectively. For the varieties, the end of stolon formation ranged from 42 DAP (Première) to 140 DAP (Karnico), whereas the end of stolon tip swelling ranged from 57 DAP (Première) to 170 DAP (Karnico). With regard to flowering (Table 4), the CxE population and the varieties ended this process within a period of 91 (64 to 155 DAP) and 21 days (75 to 96 DAP), respectively. For the end of senescence (Table 5), in the CxE population there were genotypes with shorter (75 DAP) and longer plant cycle (first signs of senescence at 185 DAP) than the varieties. Gloria and Première (the earliest varieties) completed the plant cycle at 96 DAP, whereas Karnico (the latest variety) senesced at 185 DAP. The parental clones, C and E, ended stolon formation, stolon tip swelling, flowering and senescence within the range of the CxE population (Tables 2, 3, 4, 5). The most contrasting difference between the parental clones was found in the duration of the plant cycle (end of senescence), E had a life span 30 days shorter (110 DAP) than C (140 DAP).

Duration of the processes of stolon formation, stolon tip swelling, flowering and senescence

A wide variation in the duration of the processes of stolon formation, stolon tip swelling, flowering and senescence was found not only in the CxE population and parental clones but also in the set of varieties (Tables 2, 3, 4, 5). In the CxE population, the formation of stolons was the longest process taking place during the plant cycle (28 to 156 days), followed by the production of stolon tip swellings (22 to 143 days), the production of flowers (22 to 119 days) and the senescence of the leaf canopy (11 to 89 days) (Tables 2, 3, 4, 5). For the varieties, in contrast, stolon tip swelling was the longest process, ranging from 28 days (Première) to 134 days (Karnico), followed by stolon formation, from 33 (Première) to 111 days (Karnico and Astarte), flowering production, from 33 (Gloria and Première) to 54 days (Karnico), and canopy senescence, from 7 (Gloria and Première) to 45 days (Karnico). In the parental clones, the longest process was stolon tip swelling (53 days), followed by stolon formation (46 days), flowering, 33 (C) and 39 days (E), and senescence, 44 (C) and 35 days (E).

Based on the first evaluation date at which at least 75% of the population reached the onset and end of these four processes, it was found that at 29 DAP, 89% of the population, the parental clones and all the varieties had begun stolon formation (Table 2), and 13 days later (42 DAP) all of them were flowering (Table 4). After another 15

days (57 DAP), 85% of the population, both parents and all the varieties had swelling stolon tips (Table 3) and 7 days later (64 DAP), 91% of the population, both parents and all the varieties had tuber incipients. Finally, after another 46 days (110 DAP), 84% of the population, both parental clones and 5 out of 7 varieties were senescing (Table 5). On the other hand, for the end of these processes, 77% of the population, both parents and 4 out of 7 varieties had ended stolon formation at 96 DAP. Also at 96 DAP, 80% of the population, both parents and all the varieties had ended flowering. Fifty four days later (140 DAP), 81% of the population, both parents and all the varieties, except Karnico, had ended stolon tip swelling. And, also at 140 DAP, 76% of the population, both parents and 5 out of 7 varieties had completed the life cycle.

Temporal relationships between plant development and tuber formation processes

Considering the number of days required by at least 75% of the population to reach the peak (highest number recorded during the experiment) production of new stolons, swelling stolon tips and tubers (size not considered) (Figure 1), in only 14 days (75 to 89 DAP), 80% of the population reached the peak production of tubers, while reaching the peak production of new stolons and swelling stolon tips required a longer period of time, 28 days (29 to 57 DAP) and 46 days (29 to 75 DAP), respectively. In Figure 1, the times at which the peak production of new stolons, swelling stolon tips and tubers took place with the progression in time of flowering and senescence are also compared. It was found that during the period between 42 and 75 DAP, 97% and 83% of the population had reached the peak production of stolons and swelling tips respectively, while during the same period the whole population had reached full bloom and already 50% of the population had ended the flowering period. Thus, the peak production of both stolons and swelling stolon tips took place within the range of the flowering period. On the other hand, between 75 and 89 DAP, 80% of the population had reached the peak production of tubers and also during this period, 54% of the population was either showing signs of plant senescence (49%) or had already completed the life cycle (5%). These results suggest that during this period, for a large proportion of the population, tuber initiation had reached its maximum and now a large portion of the tubers were in the phase of fast growth, while the plants were senescing.

Fluctuations over time in the number of new stolons, stolon tip swellings, tuber incipients and growing tubers

Initially, the number of new stolons was higher than that of new swelling tips or tubers (Figure 2). However, after 42 DAP, a continuous decrease in the production of new stolons took place, reaching almost zero at 140 DAP. On the other hand, the production of new swelling stolon tips was relatively constant and higher than that of

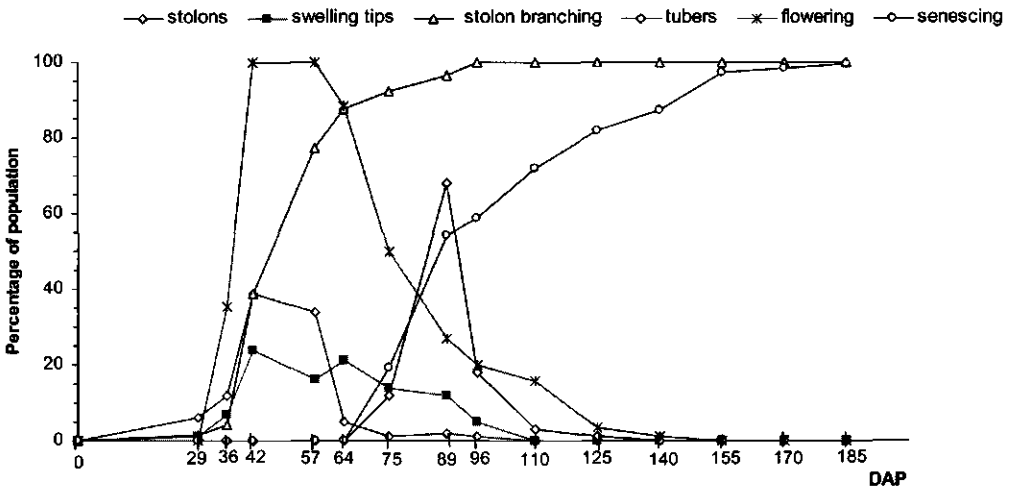

Figure 1: Percentage of population reaching the peak production of stolons, swelling stolon tips and tubers. The percentage of genotypes showing stolon branching, flowering and senescence at each evaluation date are included.

stolons at the end of the experiment, suggesting that branching stolons were providing new tuber sites. In fact, during the period from 42 to 64 DAP, the total number of new stolons decreased from 8429 to 3429 and the percentage of the population showing stolon branching increased from 39 to 88. Meanwhile, the total number of tubers increased drastically from 4105 to 20248, reflecting the increase in tuber sites due to stolon branching.

The fact that the total number of swelling stolon tips was much lower than the total number of tubers at each evaluation date, strongly suggests that not only a vast majority of swelling stolon tips became growing tubers, but also that the transition from swelling tip to growing tuber took place within a very short period. The total number of tubers produced by the population at each evaluation date showed a dramatic increase over time. Within a period of 32 days (57 to 89 DAP), the number of tubers increased from 8913 to 33380 tubers per harvested block (date). However, during the next seven days (89 to 96 DAP) some interesting changes in the number of tubers were found. The total number of tubers decreased from 33380 to 31368 and the number of resorbed tubers increased from 603 to 1920. In addition, for the first time, the number of tuber incipients (< 12 mm Ø) decreased from 19904 to 15280 and the number of growing tubers (>20 mm Ø) surpassed the number of tuber incipients (from 13476 to 16088). After 96 DAP, the number of growing tubers increased steadily while the number of tuber incipients decreased more markedly, indicating that from

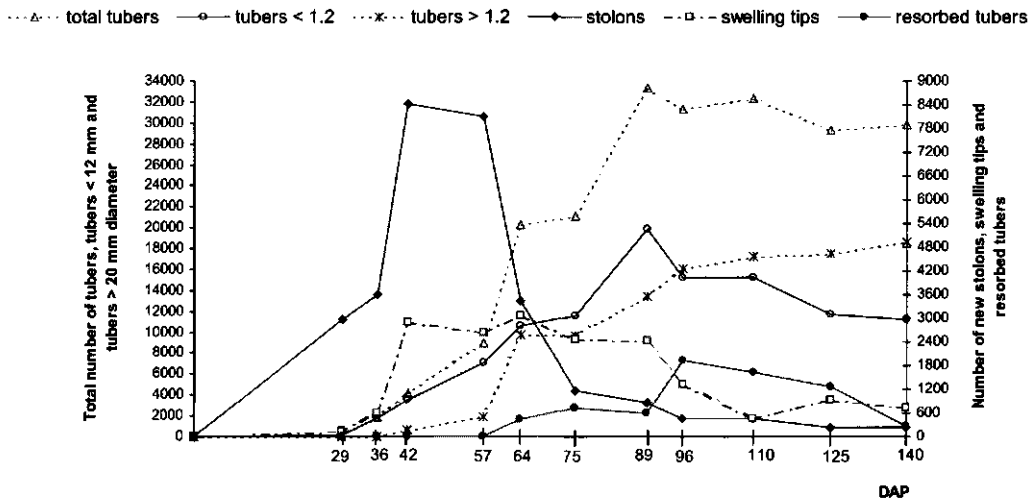

Figure 2. Total number of new stolons, swelling stolon tips, tubers < 12 mm Ø (tuber incipien tubers > 20 mm Ø (growing tubers), total tubers and resorbed tubers at each evaluation date.

this date onwards, plants were in a period of fast tuber growth, an observation further supported by the fact that 59% of the population was either showing signs of plant senescence (43%) or had completed the life cycle (16%).

Correlations between the onset and end of plant development and tuber formation processes

Highly significant correlations were found between the onset and end of the different processes and other plant and tuber characteristics (Table 6). The end of stolon formation was closely correlated with both the onset and end of stolon tip swelling. The end of flowering was highly correlated with the end of stolon formation and the onset and end of stolon tip swelling. Both, the onset and end of senescence were highly correlated with the end of stolon formation, the onset and end of stolon tip swelling and the end of flowering. Interestingly, plant size was highly correlated with the end of flowering and both the beginning and end of senescence. These results suggest that many of these events occurred at a similar relative developmental time. The fact that the number of tubers was significantly correlated with the end of stolon tip swelling, the onset of stolon branching, the degree of branching and with both the onset and end of senescence, but not with the number of new stolons or the end of stolon formation is noteworthy.

Table 6. Correlations between the onset and end of the processes of stolon formation, stolon tip swelling, tuber setting, flowering, senescence and other tuber and plant related characteristics

	O- senescence	E- senescence	E- stolon formation	O- stolon tip swelling	E- stolon tip swelling	E-flowering	O- stolon branching	Degree branching
E- senescence	0.88**							
E- stolon formation	0.76**	0.77**						
O- swelling	0.77**	0.75**	0.66**					
E- swelling	0.85**	0.89**	0.75**	0.69**				
E- flowering	0.83**	0.80**	0.70**	0.74**	0.74**			
O-stolon branching	0.07	0.04	0.04	0.10	0.08	0.04		
Degree of stolon branching	0.46**	0.49**	0.56**	0.41**	0.52**	0.45**	0.32**	
Number of tubers[1]	0.30**	0.25**	0.26**	0.16*	0.33**	0.16*	0.18**	0.23**
Number of tubers resorbed[1]	0.21**	0.22**	0.12**	0.13*	0.11*	0.25**	0.02	0.03
Plant size	0.54**	0.66**	0.59**	0.50**	0.55**	0.56**	0.03	0.45**
Number of new stolons[1]	0.14*	0.02	0.20**	0.01	0.07	0.03	0.19**	-0.45**
Number of swelling stolon tips[1]	0.16*	0.05	0.20**	0.07	0.22**	0.02	0.24**	0.56**

Pearson coefficient of correlation, 2-tailed curve, *= $p < 0.005$; **= $p < 0.001$; numbers without a symbol = no significant correlations; O = onset, E = end; [1] = average over the plant cycle.

Association between stolon formation and stolon tip swelling

The onset of stolon formation preceded the swelling of the stolon tips but the interval of time between both events was variable, 7 to 46 days (Figure 3). In contrast, the varieties began both processes within a very short interval of time (7 days) (Tables 2 and 3). From the distribution of the CxE population according to number of days between the onset of tuber initiation and the end of stolon formation (Figure 4), there was a large variation in the end of stolon formation between genotypes that began stolon tip swelling similarly. For instance, between genotypes that began stolon tip swelling at 29 DAP, there were genotypes that ended stolon formation between 57 and 110 DAP. However, between genotypes that began stolon tip swelling at 36 DAP, the end of stolon formation ranged from 57 to > 185 DAP. On the other hand, between genotypes that began stolon tip swelling much later (64 DAP), stolon formation ended between 64 and > 185 DAP. Thus, the onset of stolon tip swelling did not have a determinant influence on the duration of the stolon formation period.

For the end of these two processes (Figure 5), it was observed that 70% of the CxE population ended stolon tip swelling, 7 to 113 days after the last newly formed stolon was observed, 25% ended both processes simultaneously, and only 5% of the CxE population ended stolon tip swelling before the last newly formed stolon was observed. In contrast, the parental clones ended stolon tip swelling 35 and 14 days after the end of stolon formation (Tables 2 and 3). On the other hand, 5 out of 7 varieties ended stolon tip swelling 11 to 30 days after the end of stolon formation, while in Saturna and Bintje both events took place at a similar time. These results indicate that in a large majority of the genotypes, stolon tip swelling was taking place in the branches of previously formed stolons, since more swelling stolon tips were observed during a variable period of time following the observation of the last newly formed stolon(s).

Association between stolon formation and senescence

Between CE genotypes that began stolon formation at the same time (29 or 36 DAP), a large variation in the duration of the plant cycle was found (75 to > 185 DAP) (Figure 6). The number of days between the onset of stolon formation and the end of senescence ranged from 39 to more than 156. On the other hand, 40% of the CxE population ceased stolon formation, 7 to 65 days before the first signs of senescence were observed, 33% of the population showed both events at the same time, whereas 27% of the population ended stolon formation, 11 to 57 days after the process of senescence had begun (Figure 7). Likewise, the varieties ended stolon formation before (Gloria, Première and Saturna), simultaneously (Granola, Astarte and Karnico) or after the onset of senescence had begun (Bintje) (Tables 2 and 5).

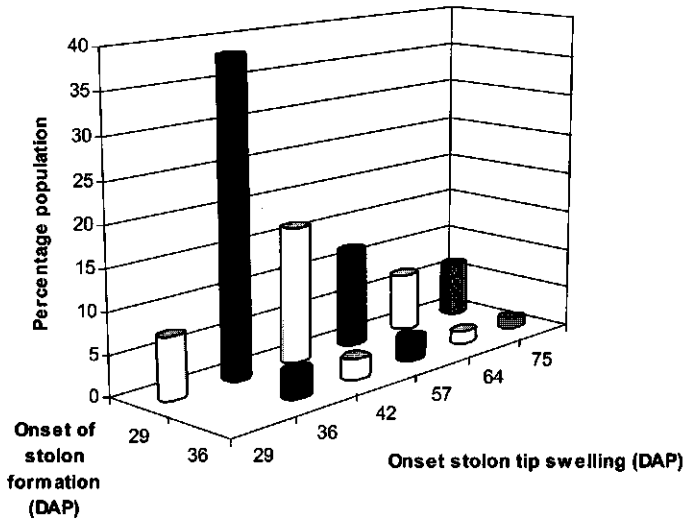

Figure 3. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the onset of both stolon formation and stolon tip swelling.

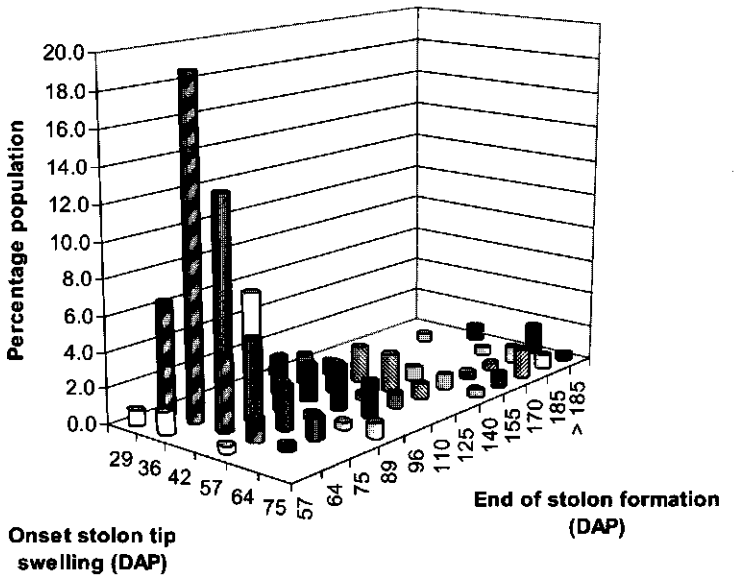

Figure 4. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the onset stolon tip swelling and the end of stolon formation.

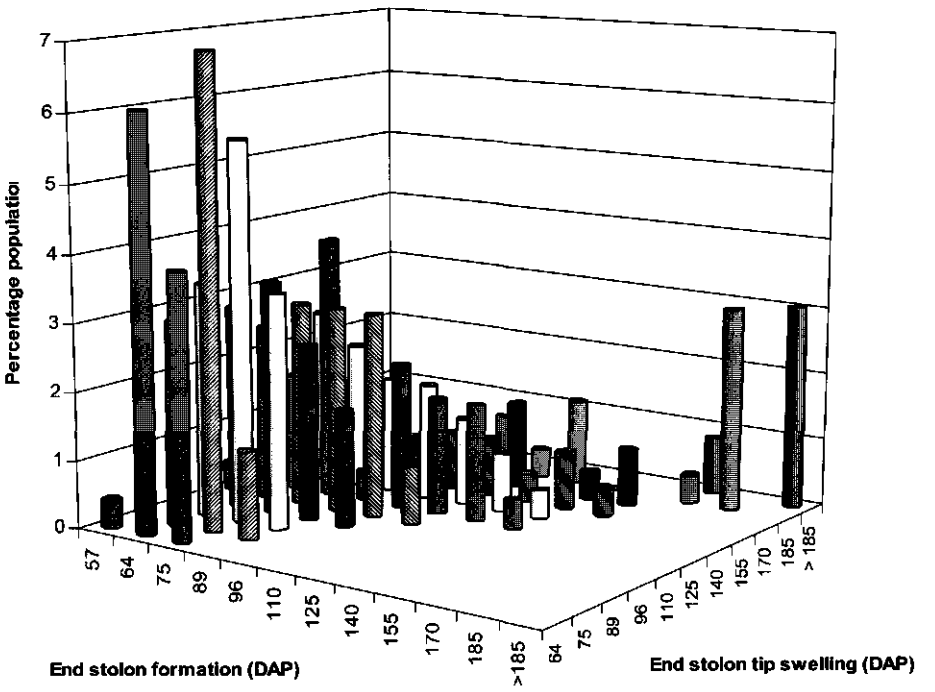

Figure 5. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the end of both stolon formation and stolon tip swelling.

These results show that for a large majority of the genotypes under evaluation, the end of stolon formation preceded senescence. On the other hand, a variable number of days was found between the observation of the last newly formed stolon and the end of the life cycle (end of senescence) (Figure 8). In the CxE population this period ranged from 11 to 106 days, in the parental clones, from 76 (C) to 21 days (E) and, in the varieties, this interval ranged from 29 days (Granola) to 65 days (Saturna) (Tables 2 and 5).

Association between stolon tip swelling and senescence

In both, the CxE population (Figure 9) and the varieties (Tables 3 and 5), large differences in the duration of the plant cycle were observed between genotypes that had begun stolon tip swelling simultaneously. Indicating that the onset of stolon tip swelling, as the first visual manifestation of tuber induction did not have a determinant influence on the duration of the plant cycle. On the other hand, the largest percentage of the population (73%) ended stolon tip swelling after the process of senescence had begun (11 to 75 days), whereas 14 and 13% of the CxE population ended stolon tip

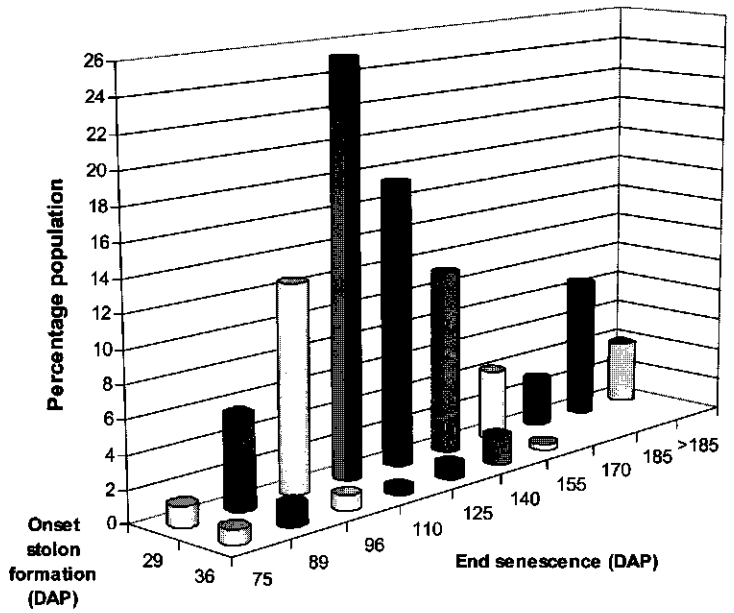

Figure 6. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the onset of stolon formation and the end of senescence.

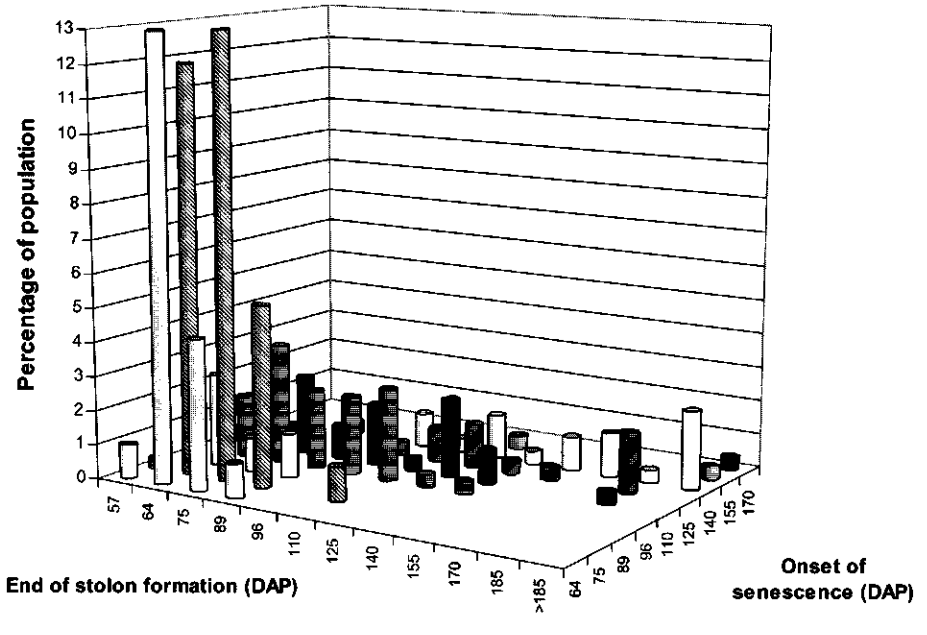

Figure 7. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the end of stolon formation and the onset of senescence.

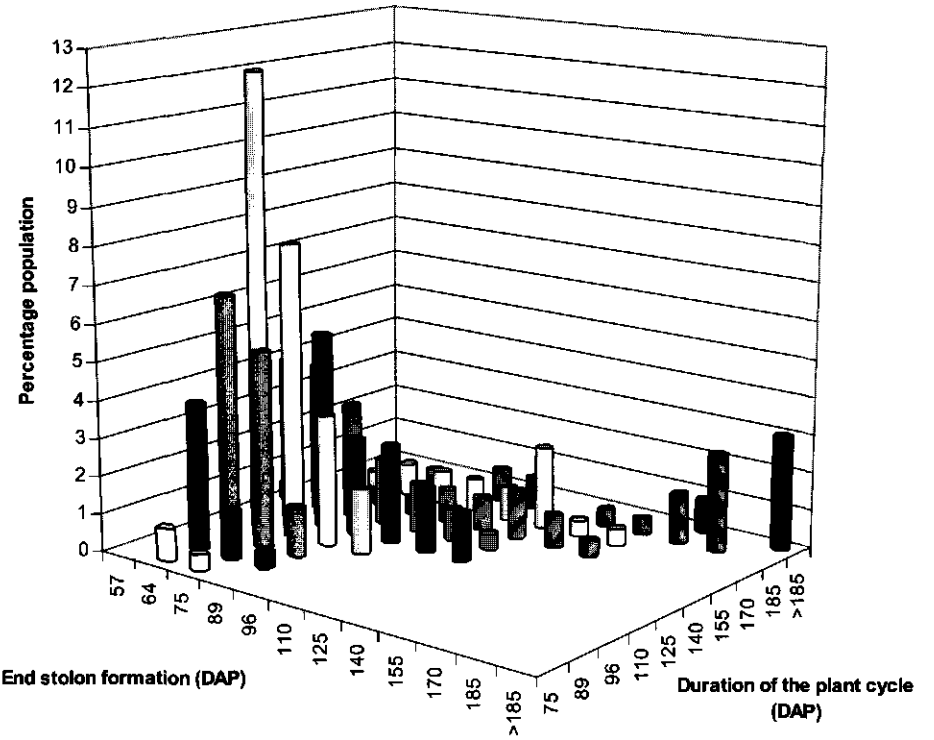

Figure 8. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to both the end of stolon formation and the duration of the plant cycle.

swelling before (7 to 44 days) or at the same time as the onset of senescence, respectively (Figure 10). In contrast, 5 varieties (Gloria, Première, Astarte, Karnico, and Saturna) ended stolon tip swelling before the onset of senescence, while Granola and Bintje ended this process 14 days after senescence had begun (Tables 3 and 5). These results suggest that for a large majority of the genotypes evaluated, the external stimulus inducing tuberization was present during most of the plant cycle, inducing the tips of the stolon branches to swell, giving rise to new tuber incipients, although the plants were progressing in senescence.

Furthermore, a large variation was found in the interval of time between the end of stolon tip swelling and the end of the plant cycle (Figure 11). In the CxE population, this gap ranged from 11 to 80 days, in the parental clones, from 21 (E) to 30 days (E), whereas for the varieties this time interval ranged from 15 (Granola and Karnico) to 65 days (Saturna). These results suggest that the duration of the period during the plant cycle with conditions conducive to stolon tip swelling was different among genotypes.

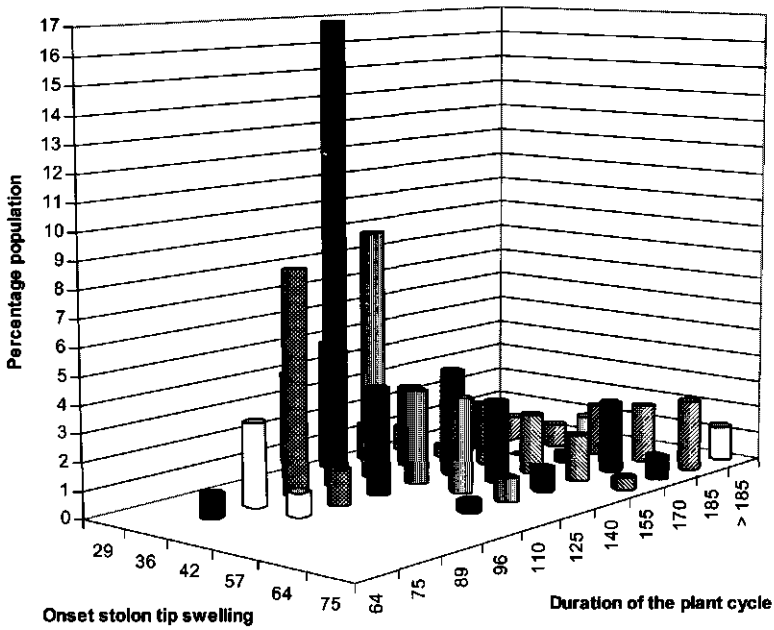

Figure 9. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the onset of stolon tip swelling and the duration of the plant cycle.

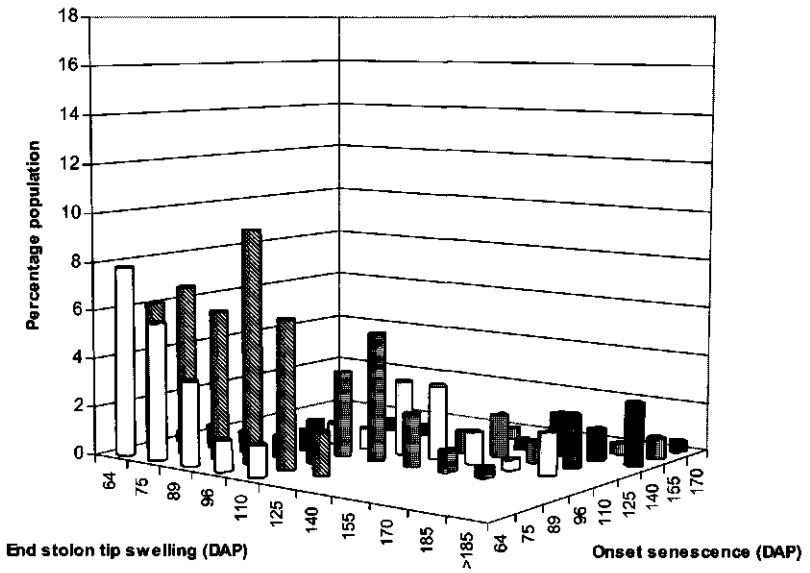

Figure 10. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the end of stolon tip swelling and the onset of senescence.

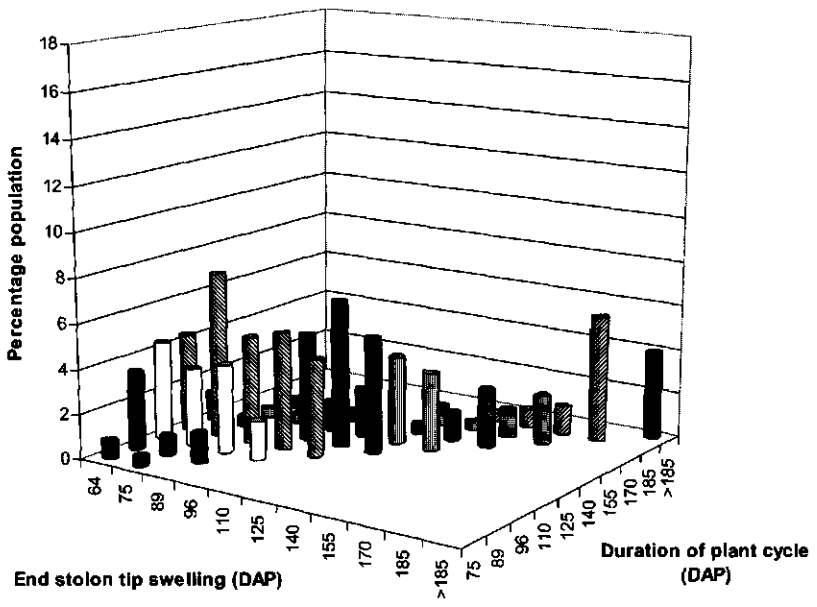

Figure 11. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the end of both stolon tip swelling and the duration of the plant cycle.

Associations between stolon tip swelling and flowering

Based on the number of DAP to the onset of both flowering and stolon tip swelling (Figure 12), 30%, 34% and 36% of the CxE population had the first stolon tip swellings before (6 to 13 days), simultaneously with, or after (6 to 39 days) the first open flower was observed, respectively. In contrast, E began to form tuber incipients 15 days after the first open flower was observed, whereas C began tuber initiation and flowering simultaneously (36 DAP) (Table 4). On the other hand, Gloria and Première had the first swelling tips at 29 DAP, in contrast to 36 DAP for Bintje, Saturna, Granola, Astarte and Karnico (Table 4).

In relation to the period between the onset of stolon tip swelling and the end of flowering (Figure 13), for those genotypes that began tip swelling after a similar number of days, the flowering period ended 7 to 80 days later. On the other hand, Gloria and Première began stolon tip swelling and ended flowering at 36 and 75 DAP, respectively. However, although the other five varieties began stolon tip swelling at the same time (42 DAP), Bintje and Saturna ended flowering at 75 DAP, Granola at 89 DAP while Astarte and Karnico ended flowering 54 days after (96 DAP). These results lead to the conclusion that the onset of tuberization did not have a determinant influence on the process of flowering.

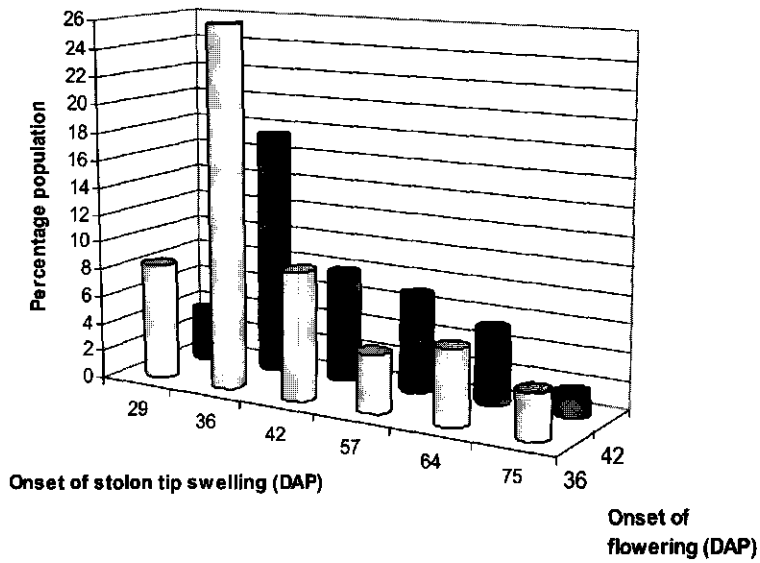

Figure 12. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the onset of both stolon tip swelling and flowering.

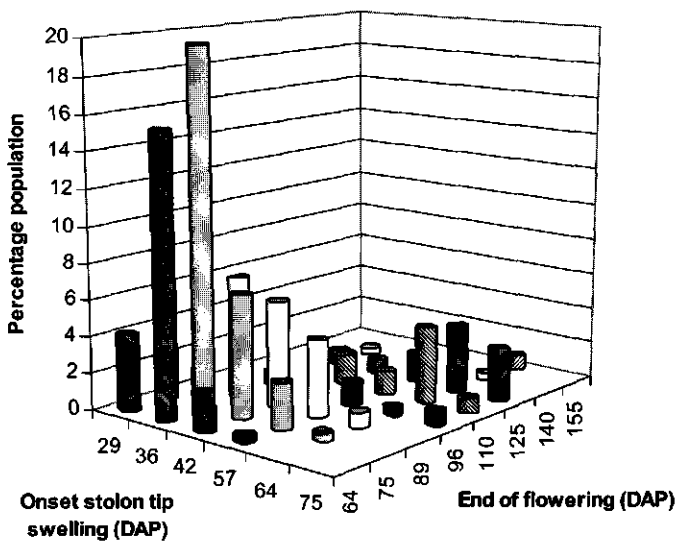

Figure 13. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the onset of stolon tip swelling and the end of flowering.

Association between the end of flowering and the onset of senescence

The comparison of the duration of the period between the end of flowering and the onset of senescence (Figure 14) shows that there was a wide variation in the number of days between these two events. For 44% of the population, the end of flowering preceded the onset of senescence, but the gap between these two events was variable (7 to 60 days). In contrast, 18% of the population ended flowering after the process of senescence had begun but the interval was narrower (11 to 29 days). On the other hand, for 40% of the population these two events took place after a similar number of days.

All varieties, in contrast, ended flowering before the onset of senescence, but there were large differences in the duration of the period between these two events, from 7 days (Granola) to 51 days (Astarte) (Tables 4 and 5).

These results show that from the end of shoot growth, which is considered to finish near the end of the flowering period, a variable number of days elapsed before the plant began the process of senescence. The duration of this period could be a determinant factor influencing final tuber size and weight.

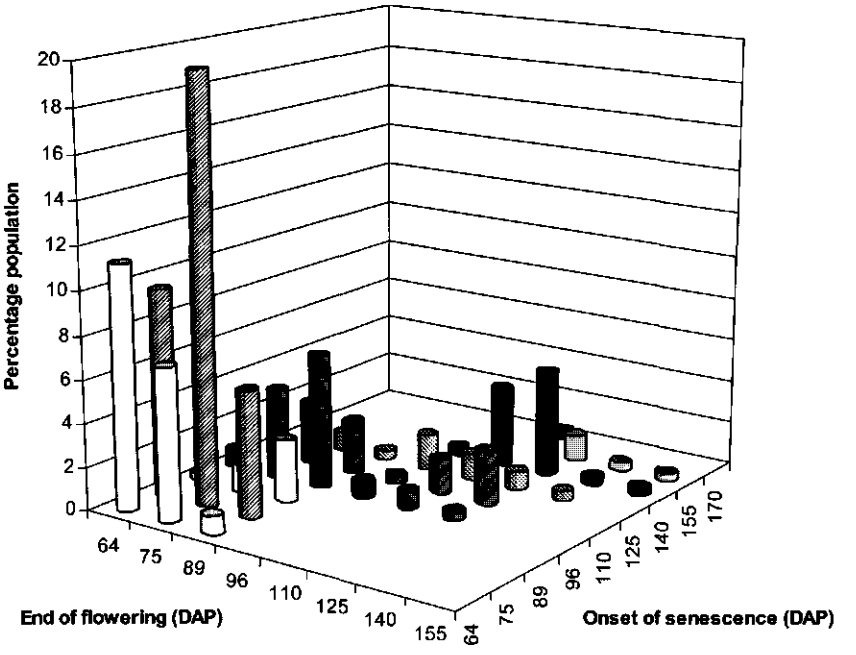

Figure 14. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the end of flowering and the onset of senescence.

Association between the end of flowering and the end of stolon formation and stolon tip swelling

From the distribution of the CxE population according to the number of DAP to the end of both stolon formation and flowering (Figure 15) and to the end of both stolon tip swelling and flowering (Figure 16), it was found that while the largest percentage of the population (62%) ended stolon formation either before (7 to 89 days) or after a similar number of days, 76% of the population ended stolon tip swelling after the end of flowering (11 to 106 days). On the other hand, both parental clones and 4 out of 7 varieties (Granola, Bintje, Astarte and Karnico) ended both stolon formation and stolon tip swelling after the end of flowering (Tables 1, 2 and 3).

Discussion

Chronology of tuber formation events

Highly significant differences were found among CE genotypes for all the tuber formation and plant related variables evaluated. Furthermore, when the dynamics of stolon formation, stolon tip swelling, stolon branching, flowering and senescence were compared using the population as a unit, some generalizations about the chronological relationships between these processes can be drawn. As expected, the onset of stolon formation, swelling of the stolon tips and formation of tuber incipients took place sequentially.

Surprisingly, although the CxE population, parental clones and varieties had a very wide range in the duration of the plant cycle, all began stolon formation and flowering within a very narrow period. This strongly supports the observation about the possible lack of (the need for) a specific signal inducing stolon initiation, produced in the leaves and triggered by an environmental stimulus, at least for plants originated from seed tubers (Vreugdenhil and Struik 1989). In contrast, the onset of stolon tip swelling took place within a broad number of days, suggesting that genetic differences in the length of the critical photoperiod, associated with the *S. tuberosum* and *S. phureja* backgrounds (Hawkes, 1990), could be influencing tuber induction.

Several authors (Krijthe, 1955; Ivins and Bremner, 1965; Jeffe; Kolbe and Stephan-Beckmann, 1997a; O'Brien et al., 1998) concluded, based on the observation of a very limited number of varieties, that the majority of tubers are initiated within a very short interval of time, 1 to 3 weeks. Furthermore, O'Brien et al. (1998), indicated that under conditions highly conducive to rapid growth, tuber initiation could be completed in a very short interval of time (2 – 3 days). However,

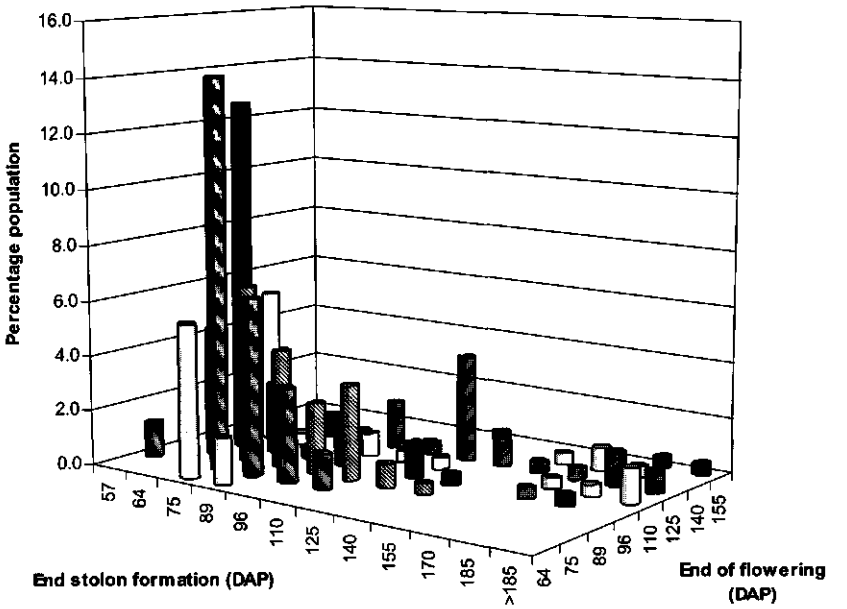

Figure 15. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to both the end of stolon formation and flowering.

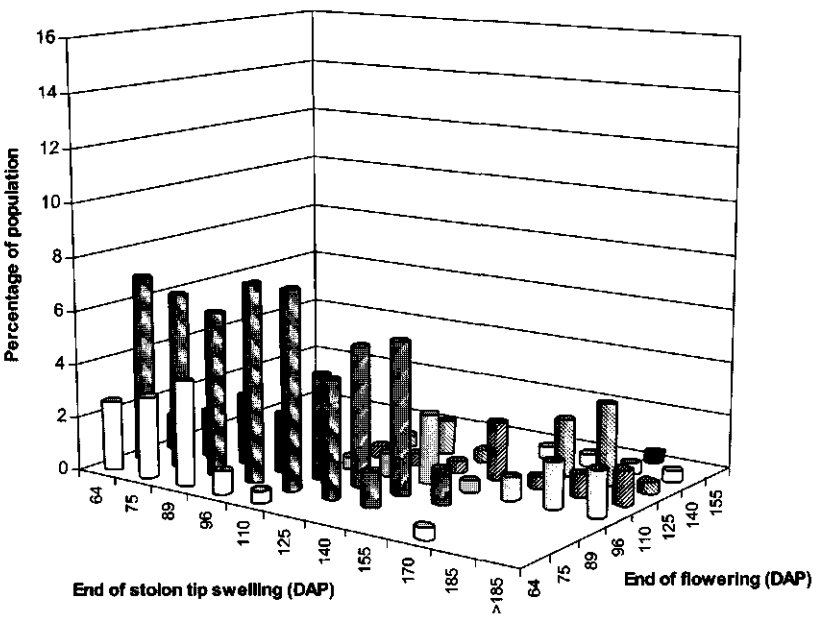

Figure 16. Frequency distribution (%) of the CxE population according to the number of days after planting (DAP) to the end of both stolon tip swelling and flowering.

from the counting of all the stolon swelling tips and tubers of different sizes during 14 evaluation dates, it is clear that genotypic differences were more determinant for the duration of tuber initiation than the prevailing environmental conditions. The weather conditions and management of the experimental plots were very favourable for potato. However, very distinct differences in the duration of stolon tip swelling were observed, not only within the CxE population but also between the varieties used as controls. In most of the cases, stolon tip swelling and formation of tuber incipients took place during most of the plant cycle, as was also observed by Clark (1921) and Sale (1976).

Among CE genotypes that began stolon tip swelling after a similar number of days, there were very large differences in the period between the onset of stolon tip swelling and the end of stolon formation, stolon tip swelling, flowering and senescence. The same was observed for the varieties. These results contrast with the observations made by Milthorphe (1963), Ivins and Bremner (1965), Bremmer and Radley (1966) and Ewing and Struik (1992), based on the observation of a limited number of varieties, on the determinant influence of tuber initiation on the development of the potato plant. Ivins and Bremner (1965) and Slater (1963) indicated that the rate of tuber growth and the time to foliage senescence are related to the amount of leaf growth present at tuber initiation. However, the seven varieties evaluated in this study began stolon tip swelling shortly after shoot emergence, when the plants were small and yet they showed very distinct differences in the number of days to the end of stolon formation, stolon tip swelling, flowering, duration of the plant cycle and the final plant size (data not shown). Similar observations could be drawn from the evaluation of the 250 CE genotypes. Furthermore, after the onset of stolon tip swelling, both the CE clones and the varieties kept flowering during a variable period of time, which is an indicator of growth of the leaf canopy (Almekinders and Struik, 1996). In this regard, Firman et al. (1995) concluded after evaluating several contrasting cultivars under field conditions, that the majority of the leaf surface in many cultivars is produced after the onset of tuber initiation. Thus, tuber initiation (stolon tip swelling) should be regarded only as one of the several developmental stages taking place during the potato life cycle, although playing a very crucial role in the establishment of the population of growing tubers.

Under field conditions, the assessment of the underground processes leading to the production of harvestable tubers rely on the frequent and destructive sampling of plants. In order to facilitate this assessment, the flowering period often has been regarded as the above ground process that could be readily linked to the period of tuber formation. Several growth stage keys have been proposed to monitor the phenology of the potato crop. Most of these growth stage keys give particular

importance to the flowering process during the potato life cycle (Sparks and Woodbury, 1959; Anon. 1987; Grieß, 1987). However, Jefferies and Lawson (1991) indicated that there was no evidence to link flowering and tuber development, besides the fact that some cultivars do not flower or abort buds during early stages of development. Nevertheless, the flowering period has been widely associated with tuber initiation (Krijthe, 1955; Pallais, 1987; Almekinders and Wiersema, 1991). Clark (1921) indicated that in general, tuber formation begins at about the end of the period of flower bud development and for late varieties, when the buds have started to open or even later. Similarly, Firman et al. (1991) concluded that under field conditions, the initiation of flower primordia always precedes tuber initiation, with a variable interval of time between the two stages due to genetic differences. Indeed, this was the case for most of the CxE population and only in a relatively small proportion of the population, the first swelling stolon tips were observed, few days before the first open flower was observed. The varieties, in contrast, began stolon tip swelling shortly before or at the onset of flowering. Since in this study, the time to the first open flower was considered as the onset of flowering, but in fact, flower initiation had begun well before this event, it seems that indeed, flower initiation begins before or at the same time as tuber initiation in plants from seed tubers. In addition, for a very large proportion of the CxE population, the peak production of stolons and swelling stolon tips took place within the flowering period, although, for many genotypes, including the varieties, the processes of stolon tip swelling and formation of tuber incipients proceeded beyond the end of flowering. From these results it can be concluded that in general, under favourable conditions for plant growth, the flowering period could be considered as an indicator of tuber formation. In addition, the extent of the flowering period could be also considered as an indicator of the rate of leaf production and plant growth, as was suggested by Firman et al. (1995). This is further supported by the high correlations that we found between the end of flowering and plant size.

Tuber number

An aspect that has received limited attention under field conditions, for practical reasons, has been the determination of the temporal relationships between stolon formation, stolon tip swelling, stolon branching, tuber resorption and tuber number. Lovell and Booth (1969) and Ewing and Struik (1992) indicated that factors, which favour vigorous haulm growth and delay tuber initiation, also favour stolon development and branching. Ewing and Struik (1992) indicated that long days, high temperatures and high levels of gibberellins, factors unfavourable for tuber initiation, stimulate stolon branching. However, for a large proportion of the CxE population and all the varieties, after the onset of stolon tip swelling, the production of new stolons

decreased rapidly but stolon branching began, which was reflected in a drastic increase in the total number of tuber incipients and growing tubers within a short period of time. Contrary to the observation by Lovell and Booth (1969), in many cases, the onset of stolon tip swelling preceded stolon branching and the degree of stolon branching increased over time but it was highly genotype dependent. These results suggest that tuber initiation and stolon branching can take place simultaneously and that the different control mechanisms can be triggered by the same environmental conditions.

On the other hand, Ewing and Struik (1992) indicated that one of the reasons that makes it difficult to predict the number of tubers reaching marketable size is the resorption or decay of a large number of small tubers. They also indicated that the proportion of resorbed tubers depends on the number of tuber incipients, since there is a limit to the number of tuber incipients reaching large sizes. Based on the results of Cho and Iritani (1983), Ewing and Struik (1992) estimated that between 23 and 44% of the tubers initiated were resorbed. In our study, since the field experiment was planted in a light sandy soil, we were able to dig out the individual plants with a minimum of disturbance and to retrieve tubers remaining in the soil. Contrary to the observation related to the study of Cho and Iritani, the total percentage of resorbed tubers ranged from 2 to 6 %, while the reduction over time in the total number of tubers ranged from 3 to 12%. Our results suggest that tuber resorption does not play a crucial role in defining the final marketable yield and size distribution; instead, the degree of stolon branching, the duration of the stolon tip swelling period and the ability of the small growing tubers to reach a large size, all factors highly genotype dependent, might be more important.

Some final remarks

In relation to the process of senescence, we found very high correlations between the onset/end of senescence and most of the processes evaluated. These results strongly suggest that senescence could be the process having the most determinant influence on the temporal dynamics of all the other processes taking place in the potato plant.

The very wide diversity found in both the CxE population and the varieties for the temporal relationships between plant development and tuber related processes illustrates why evaluation of a very limited number of genotypes has complicated reaching a consensus on the general pattern of development in the potato plant. This diversity also leads to the conclusion that the generation of a "universal" model of plant development in potato is unrealistic. A much better understanding of the physiological and genetic factors controlling the temporal relationships between tuber formation and plant development is needed in order to take advantage more effectively of the developmental plasticity of the potato.

4

Dissecting the complexity of tuber formation in potato (*Solanum tuberosum* L.)

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Abstract

A large and diverse population of diploid potato was classified according to the duration of the plant cycle into five categories, in order to identify features related to tuber formation and plant related processes distinguishing these plant types. In general, genotypes with a very short life cycle (< 90 days after planting) could be described as plants of small size with a short period of stolon formation, stolon tip swelling and flowering, early stolon tip swelling, very little or no stolon branching and fast progress of canopy senescence. In contrast, genotypes with a very long life cycle (≥ 170 days after planting), could be described as having a large plant size, profuse and long flowering period, large production of stolons during most of the plant cycle, profuse stolon branching, late but long period of stolon tip swelling and subsequent formation of tuber incipients and slow progress of canopy senescence. Furthermore, at the end of the plant cycle, genotypes with a long life cycle had a much higher tuber yield (in general) than genotypes with a short life cycle. Between groups of genotypes with different duration of the life cycle, differences were also found for the duration and chronological order of events related to plant development and tuber formation. The evaluation of a large and highly diverse population of potato was very useful to detect such differences. Results illustrate the complexity of the relationships between all the events taking place during the life cycle of the potato plants.

Keywords: flowering, plant cycle, potato, tuber formation, *Solanum phureja*, *Solanum tuberosum*, senescence, stolon formation

Introduction

Tuber-bearing *Solanum* species are considered to have a short-day type of reaction, since they will be induced to tuberize only, or more rapidly, if they have been exposed to daylight phases shorter than a critical daylength (Vreugdenhil and Struik, 1989). The tuber inducing factor(s) about which still little is known, is (are) synthesised in the leaves and transported basipetally to the stolon tips, where tubers are formed (Ewing and Struik, 1992). However, not in all available stolon tips, tubers will be formed at the same time. Thus, the complexity of tuber formation lies partly in the fact that within every potato plant and during a variable period of time, all developmental stages of tuber formation: stolon initiation, stolon elongation and halting of longitudinal growth, tuber induction and swelling of the sub-apical region of the subtending stolon, take place simultaneously (Vreugdenhil and Struik, 1989; Celis-Gamboa et al., 2002b). This phenomenon brings as a consequence the presence of growing tubers of different sizes, which will cause a variable tuber size distribution at harvest.

A large amount of research has focused on the detailed comparison of the morphological and physiological features of plants with contrasting duration of the plant cycle. However, there is still limited information on the temporal dynamics of plant and tuber related processes taking place in plants differing in the duration of the plant cycle. This information would be useful for a better understanding of the genetic and environmental factors influencing the synchronicity of tuber formation. In a previous paper (Celis-Gamboa et al., 2001b), we analysed the changes over time of several processes related to plant development and tuber formation, considering a large and highly diverse population of diploid potato as a whole. From this analysis, we were able to obtain a more general and integrated view of the dynamics of the different processes taking place during the potato life cycle. Now, after the classification of this population into five categories, according to the duration of the plant cycle, ranging from very short to very long life cycle genotypes, the temporal dynamics of major processes and several other related characteristics were compared to find the general features characterising these plant types.

Materials and methods

Plant material and experimental design

The genetic background of the plant material, the experimental design and agronomical details were given previously (Celis-Gamboa et al., 2002a). In brief, the diploid population CE, consisting of 250 genotypes is a backcross between *S. phureja* and *S. tuberosum*. The CxE population was planted on May 12, 1999, in the vicinity of Wageningen (52° N), in a light sandy soil in order to facilitate the harvesting of the plants and recover all the underground structures without damage. Two plants from the parental clones C and E and the progeny were planted at random within 11 blocks, one block was harvested at 29, 36, 42, 57, 64, 75, 89, 96, 110, 125 and 140 days after planting DAP. Three additional blocks were harvested at 155, 170 and 185 DAP, containing 47 of the latest CE genotypes. The varieties: Astarte, Bintje, Granola, Gloria, Karnico, Première and Saturna, included as standards, were randomised within 14 blocks and harvested as the CxE population.

Characteristics under evaluation

1- Onset and end of tuber formation and above ground processes

In order to determine the number of days after planting (DAP) to the onset and end of the processes of stolon formation, stolon tip swelling, stolon branching, tuber initiation, flowering and senescence, the following traits were monitored at each evaluation date:

- number of newly formed stolons;
- number of stolon tips with incipient swelling;
- number of tuber incipients: swelling of a stolon tip with a diameter between 5 and 12 mm (approximately twice the diameter of the stolon);
- flowering stage: scale 0 (= not flowering yet) to 7 (= end of flowering);
- plant senescence: scale 1 (= whole plant green) to 7 (= no green tissue).

2- Other characteristics under evaluation

Above ground level:

- plant size: scale: 1 = ≤ 40 cm, 2 = 41-60 cm, 3 = 61-80 cm, 4 = 81-100 c and 5 = > 100 cm height;
- number of main stems per plant (stems originated from the seed tuber);
- number of secondary stems (stems with leaves that started as stolons followed by a switch to orthotropic growth).

Stolon level:

- stolon length: proportion of short (1 to 10 cm), medium (11 to 25 cm), long (26 to 40 cm) and very long (> 40 cm) stolons;
- degree of stolon branching: scale 1 (= no branching) to 5 (= very high degree of branching).

Tuber level:

- tuber size distribution: all tubers produced by the individual plants at each evaluation date were harvested, separated into size categories from 5 to 125 mm in diameter, using a set of sieves with 5 mm difference, and counted;
- tuber weight: from 96 DAP, the weight of every tuber size category was recorded;
- number of resorbed tubers.

All these variables were described in detail in a previous paper (Celis-Gamboa et al., 2002b).

3- Duration of tuber formation and plant related processes

The duration of stolon formation, stolon tip swelling, flowering and senescence were estimated by calculating the number of days after planting (DAP) at which a given process ended minus the DAP at which it began. The rates of progress (speed) of the flowering and senescence processes were estimated by calculating the average slope of the curves constructed with the data collected on the progress of these processes during the period between the onset and end.

4- Meteorological data

Data on the average daily air temperature (between 10 and 150 cm above ground level), soil temperature (10 cm depth) and rainfall from 1999 were obtained from the weather station "de Haarweg" of Wageningen University. A summary of the prevailing weather conditions during the experiment were given previously (Celis-Gamboa et al, 2001b). In addition, the following variables were determined:

Daylength (Dl): the time from morning to evening civil twilight;

effective daily air temperature (T): calculated according to the formulae proposed by Van Heemst (1986):

$T = Ta - 7$, if $7 < Ta \leq 18$

$T = 29 - Ta$, if $18 < Ta \leq 29$

$T = 0$, if $Ta \leq 7$ or $Ta > 29$

where: T is the effective daily air temperature (°C)

Ta is the average daily (24-h) air temperature (°C)

Table 1. Average number of days after planting (DAP) to the onset and end of stolon formation, stolon tip swelling, stolon branching, tuber setting, flowering and senescence for genotypes differing in length of the plant cycle categories in the CxE population.

Trait	Duration of the plant cycle categories					
	Very Early	Early	Intermediate	Late	Very late	MSE
Number of genotypes	15	92	70	22	41	
Onset stolon formation	29.0a	29.2a	29.3a	30.5b	29.3a	2.34
End stolon formation	62.3a	67.2ab	79.3b	99.0c	134.4d	392.17
Onset stolon tip swelling	35.1a	35.3a	42.8b	50.3c	62.3d	79.75
End stolon tip swelling	67.3a	89.2b	114.7c	134.6d	172.8e	394.08
Onset tuber incipients (< 12mm Ø)	35.9a	37.1b	45.9b	55.2c	66.3d	86.14
Onset tubers > 20 mm Ø	46.4a	48.3a	58.7b	67.1c	79.6d	102.83
Onset stolon tip swelling to onset tubers > 20 mm Ø	11.3a	13.0a	15.8a	16.8a	17.2a	89.13
Onset tubers > 20 mm Ø to end of plant cycle	46.7a	65.3b	81.2c	102.6d	118.4e	115.46
Onset stolon branching	48.8a	50.4a	46.9a	50.2a	50.8a	167.67
Onset flowering	37.5a	39.0ab	39.7b	39.1ab	40.3b	6.91
End flowering	72.3a	75.5a	85.0b	105.5c	119.8d	145.38
Onset tuber resorption	78.9ab	84.8b	87.6b	73.8a	79.1ab	708.40
Onset of senescence	67.4a	72.6a	89.7b	111.2c	136.1d	144.13
Duration plant cycle	84.6a	105.1b	130.7c	160.0d	185.0e	49.51

Tukey's mean comparison, values with common letter are not significantly different at p = 0.05; MSE = Standard error of the mean

Table 2. Average number of days after planting (DAP) to the onset, end and duration of stolon formation, stolon tip swelling, flowering and senescence for the control varieties. Also the onset of stolon branching, onset of tuber initiation, and rate of flowering and senescence are presented.

Trait	Days after planting (DAP)						
	Première	Gloria	Granola	Saturna	Bintje	Astarte	Karnico
Senescence category	VE	VE	E	I	I	L	VL
O- stolon formation	29	29	29	29	29	29	29
E - stolon formation	42	64	96	75	110	110	140
D- stolon formation	13	35	67	46	81	81	111
O - stolon tip swelling	29	29	36	36	36	36	36
E - stolon tip swelling	57	75	110	75	110	140	170
D- stolon tip swelling	28	46	77	39	74	107	134
O - stolon branching	42	42	42	42	42	42	42
O - flowering	42	42	42	42	42	42	42
E - flowering	75	75	89	75	75	96	96
D- flowering	33	33	47	33	33	54	54
O - senescence	89	89	96	96	96	140	140
E - senescence	96	96	125	140	140	170	185
D- senescence	7	7	29	44	44	30	45

VE = very early; E = early; I = intermediate; L = late; VL = very late;O = onset; E = end; D = duration

Table 3. Differences in the duration of the processes (in days) of stolon formation, stolon tip swelling production, flowering and senescence, rates of flowering and senescence (days^{-1}) between genotypes from different duration of the plant cycle categories.

Process	Duration of the plant cycle categories (DPC)					
	Very early	Early	Intermediate	Late	Very late	MSE
Stolon formation	33.3a	38.1ab	49.5b	66.7c	91.4d	314.5
Stolon tip swelling	32.2a	53.4b	71.5c	79.4c	108.8d	439.0
Flowering	34.8a	36.5a	45.4b	66.3c	79.5d	142.9
Rate flowering	2.86a	3.57b	4.76c	5.26cd	7.69d	0.008
Senescence	17.2a	32.3b	40.9c	48.8cd	52.7d	135.5
Rate senescence	2.56a	4.54b	5.88bc	6.66c	8.33c	0.007

Tukey's mean comparison. Values with common letter are not significantly different at p = 0.05; MSE = Standard error of the mean.

Chronological differences in the onset and end of individual processes between duration of life cycle categories

The range of variation in the number of DAP to the onset and end of the different processes among CE genotypes belonging to the same DPC category and between DPC categories is depicted in Figure 1.

Between genotypes from the same DPC category, a wide variation was observed in the timing of all the processes, except for the onset of stolon formation and flowering, which for the whole population occurred within a very short interval of time.

Also distinct differences were found between DPC categories in the degree of overlapping between the different developmental stages related to both tuber and plant processes. For instance, for the process of stolon tip swelling, in the VE, E and I categories there was an overlap between the last genotype beginning stolon tip swelling and the first one to end this processes, while in the L and VL categories, particularly for the former, during 50 days all the L genotypes had swelling stolon tips before the first genotypes ended this
process.

In all DPC categories, some genotypes began the formation of tuber incipients very early in the plant cycle. For instance, in all DPC categories, there were genotypes that had the first tuber incipients at 42 DAP and in all categories, except the VL, there were

genotypes with tuber incipients already at 36 DAP. These results suggest that the onset of tuber initiation and the duration of the life cycle were not directly related. Between CE genotypes from the same DPC categories, the end of senescence took place within a very narrow interval of time, in comparison with the onset of this process, reflecting differences in the duration and rate of senescence. In contrast, all varieties (except Karnico) began stolon formation, flowering, tuber initiation and stolon branching after a similar number of days, although they had large differences in the duration of the plant cycle (Figure 2). On the other hand, between varieties with the same number of DAP to the end of senescence, differences in the duration of stolon formation and stolon tip swelling were found.

Differences between DPC categories in other tuber and plant related characteristics

CE genotypes with longer plant cycle had significantly larger plant size and had a higher proportion of long stolons, which were more branched than in earlier genotypes (Table 4). For instance, the VE genotypes had an estimated plant height of 48 cm, while plants from the VL genotypes were taller than 100 cm and very often they were close to 150 cm in height. In the VE genotypes, all stolons were shorter than 25 cm length with very low or no branching, while in the VL genotypes, the stolons were highly branched and some of them were close to 1 m length. Except for the number of secondary stems and the degree of stolon branching, a similar trend was observed for the varieties (Table 5).

For instance, Karnico was 33 cm taller than Première, although both had very similar number of main stems per plant. Furthermore, 80% of the stolons in Première were about 10 cm long, whereas in Karnico, 70% of the stolons were between 20 and 30 cm long.

Some significant but small differences were found among categories for the production of new stolons and number of swelling tips at each evaluation date. On average, for the VE category were observed 5 new stolons and 1 swelling tip at each evaluation date, while for the VL genotypes 8 new stolons and 4 swelling tips were observed (data not shown). In contrast, small differences were found between varieties in the number of new stolons or swelling tips (data not shown).

Changes in the total number of tubers per plant during the life cycle

At 42 DAP, the VE and E genotypes had a very similar tuber number and much higher, 15 and 19 tubers respectively, than in the I (6), L (1) and VL (0) genotypes (Figure 3). However, 24 days later (64 DAP), the E genotypes had the highest number of tubers (55), although followed very closely by the I genotypes (51 tubers per plant). Also at this date, the L genotypes had slightly higher number of tubers (35) than the

VE genotypes (33). Nevertheless, 25 days later (89 DAP), by the end of the life cycle of the VE genotypes, this category had the lowest (37) and the I and L categories had the highest number of tubers per plant (80), followed by the E (63) and VL categories (57). However, seven days later, the I and VL genotypes had the highest tuber number (80), followed by the L (73 tubers) and the E genotypes (57 tubers). Finally, by the end of the plant cycle of most of the VL genotypes (185 DAP), the VL genotypes had the highest number of tubers per plant (90) followed by the L (74), I (65), E (57) and VE (37) categories.

Also there were differences between DPC categories in the duration of the period during which there was a continuous increase in tuber number (Figure 3). It was found that the later the category, the longer this period was and it took place progressively later during the growing season. For instance, in the VE genotypes, this period included most of the plant's cycle, from 36 to 64 DAP and there was a rapid increase in tuber number (from 8 to 33). In contrast, in the VL category, a very drastic increase in tuber number took place between 64 and 155 DAP (from 6 to 108 tubers). It is quite possible that the number of tubers could increase even further in the VL genotypes, but because of the cold temperatures during November the plants died.

In general, after the peak production of tubers was reached, there was a variable decrease in tuber number. The I and VL genotypes showed the most drastic decrease in tuber number, from 82 to 65 and from 108 to 90 tubers per plant, respectively.

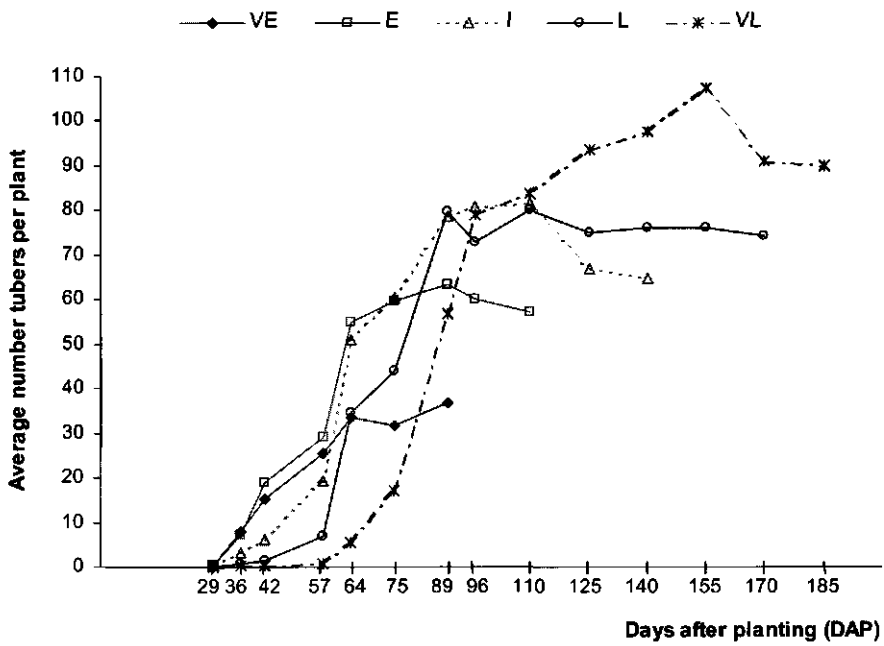


Figure 3. Differences between DPC categories in the average number of tubers per plant, from planting to the end of the life cycle. VE = very early, E = early, I = intermediate, L= late, VL = very late.

Changes in the average number of tuber incipients and growing tubers per plant during the life cycle

Differences between DPC categories were found in relation to changes over time in the number of tuber incipients and growing tubers per plant. In every DPC category, tuber incipients reached a maximum number and then the number began to decrease during the last part of the plant cycle (Figure 4).

In the VE category, the number of tuber incipients increased from 2 to 20 in a period of 28 days (29 to 57 DAP), after which a 50% reduction was observed. From this date, the number was very similar until the end of the plant cycle. On the other hand, the E, I and L categories reached the peak production of tuber incipients at a similar time (89 DAP), but the number of tubers was very different: 28, 45 and 56 tubers, respectively. From this date, there was a continuous decrease in the number of tuber incipients to 18, 21 and 24 tuber incipients respectively. In contrast, during a period of 32 days (64 to 96 DAP), the VL genotypes had an increase in tuber number from 4 to 55 tubers per

Figure 1: Range of variation in days after planting to the onset and end of stolon formation, stolon tip swelling, flowering, senescence, onset of formation of tuber incipients and onset of stolon branching of CE genotypes grouped according to the duration of the plant cycle. DAP = days after planting; DL= daylength in hours and minutes; aDD = accumulative degree days; O = onset; E = end; Z = more than 185 days.

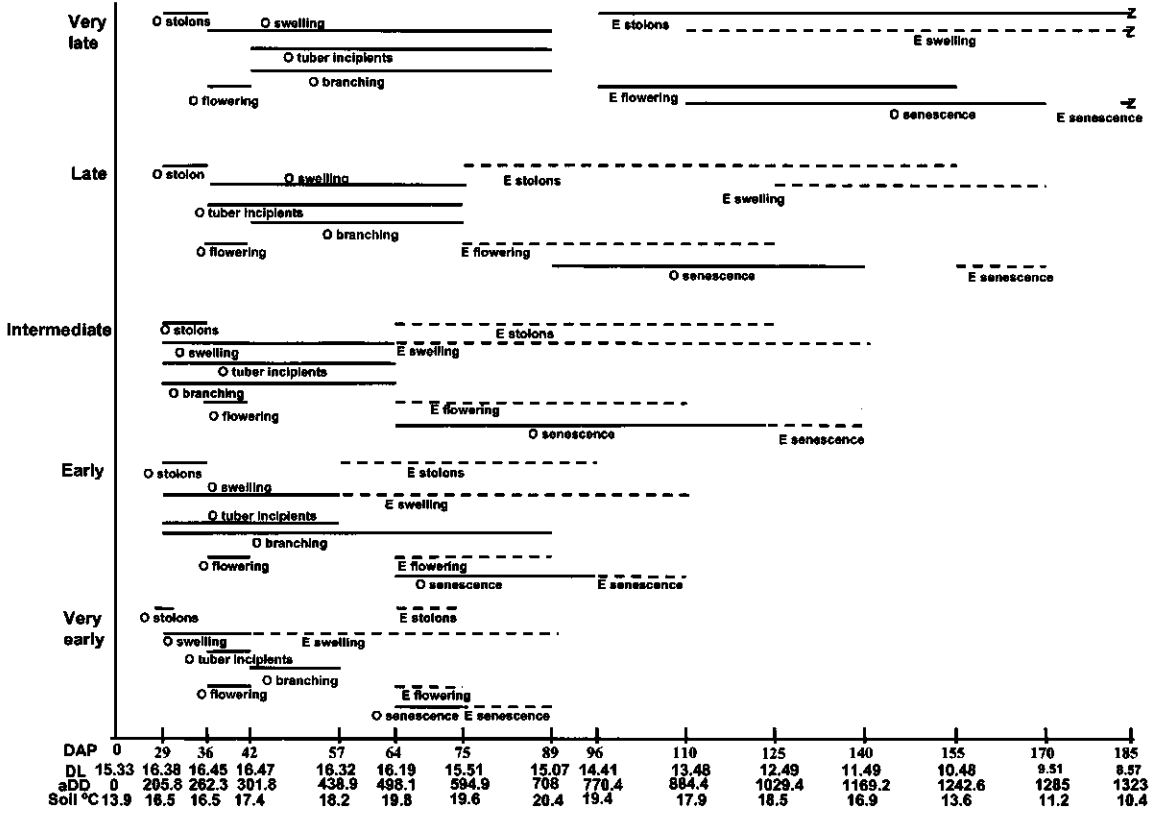


Figure 2. Differences between varieties in the duration of stolon formation, stolon tip swelling, flowering, senescence, onset of formation of tuber incipients and onset of stolon branching of CE genotypes grouped according to the duration of the plant cycle. DAP = days after planting; DL= daylength in hours and minutes; aDD = accumulative degree days; O = onset; E = end; Z = more than 185 days.

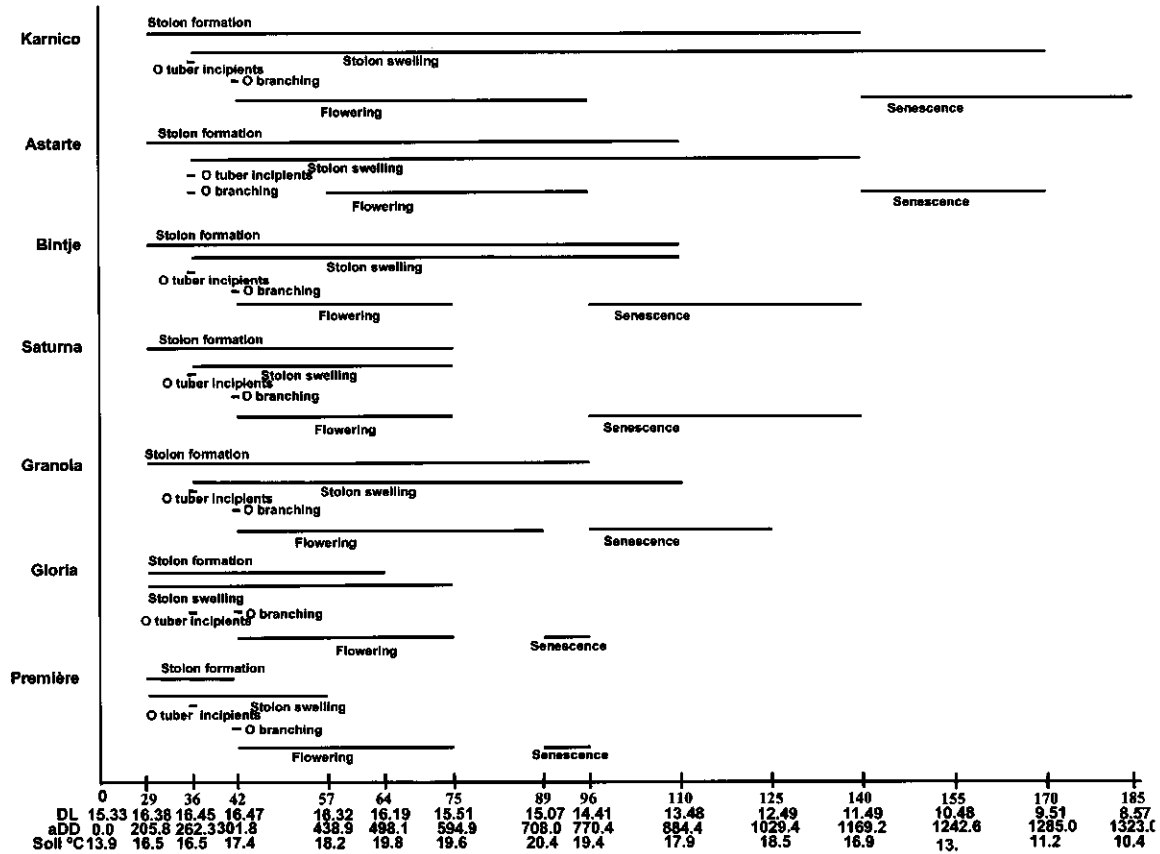


Table 4. Differences between DPC categories for some stolon and plant related characteristics.

	Duration plant cycle categories (DPC)					
	Very early	**Early**	**Intermediate**	**Late**	**Very late**	**MSE**
Plant height (cm)	2.7a (48[1])	2.9b (61)	3.1c (78)	4.3d (88)	5.0e (125)	0.05
Number of main stems	4.6a	4.5a	4.2a	4.2a	4.3a	1.90
Number of secondary stems	0.1a	0.4ab	0.7bc	0.8cd	1.0d	0.46
Stolon length %[2]	57:43:0:0	49:34:13:4	40:36:21:3	48:26:18:8	39:34:19:8	
Degree of branching	2.8a	3.1ab	3.4ab	3.2ab	3.6b	1.55
Number of new stolons[3]	5.8a	6.3a	5.5a	5.3a	6.3a	7.90
Number of swelling tips[3]	2.3a	3.7b	3.7b	3.1ab	4.0b	6.71
Number of tubers resorbed[4]	5.4a	6.2a	6.7a	4.9a	5.4a	43.4
Number of tubers per stem	5.9a	10.5ab	15.0bc	18.4cd	21.6d	40.48
Length of the longest sprout[5]	4.2a	4.4a	4.0a	4.0a	4.2a	1.77

Tukey's mean comparison. Values with common letter are not significantly different at p = 0.05; [1]= approximated plant size in cm; [2] = percentage of short: medium: long: very long stolons; [3] = average number observed at each evaluation date; [4] = value at 140 DAP; [5] = after 191 days in storage at 4 °C, scale 1 (no visible sprouts; 2 (1 to 4 mm), 3 (5 to 9 mm), 4 (10 to 19 mm), 5 (20 to 20 mm) and 6 (> 30 mm).

Table 5. Differences between varieties in the number of days after planting (DAP) for some stolon and plant related characteristics.

	Première	Gloria	Granola	Saturna	Bintje	Astarte	Karnico
DPC category[1]	E	E	I	I	I	L	VL
Plant maturity type[2]	9	8	5	6	6	3	2.4
Plant height (cm)	60	51	67	68	77	98	93
Number of main stems	4	10	6	4	7	4	3
Number secondary stems	0	0	0	0	0	0	0
Stolon length distribution[3]	80:20:0:0	30:50:20:0	70:30:0:0	50:50:0:0	40:60:0:0	40:40:20:0	30:30:40:0
Degree of branching	3	2	3	3	3	2	3
Number of new stolons	3	4	3	3	5	3	3
Onset of tubers > 20 mm Ø[4]	36	36	42	42	42	57	42
Onset of stolon tip swelling to onset tubers > 20 mm Ø[4]	7	7	6	6	6	21	6
Onset of tubers > 20 mm Ø to end of plant cycle[4]	60	60	83	93	93	113	143

[1] = DPC = duration of the plant cycle categories: E = early, I = intermediate, L = late, VL = very late; [2]According to Joosten, 1991; [3] = proportion of short : medium : large : very large; [4] = in days.

plant, but is was not immediately followed by a reduction in number like in the VE, E, I and L genotypes. Instead, it remained rather constant during 29 days, after which the number decreased until the end of the plant cycle to 38 tuber incipients per plant. In relation to the number of growing tubers (Figure 5), in general, the number of tubers kept increasing until the end of the life cycle, to 27, 39, 44, 50 and 52 tubers per plant for the VE, E, I, L and VL categories, respectively.

Changes in the average size of growing tubers during the life cycle

Significant differences were found between DPC categories in the number of days from planting to the first evaluation at which growing tubers were observed (Table 1). This period ranged from 46 to 80 DAP, for the VE and VL categories respectively. On the other hand, there were no significant differences between DPC categories in the number of days that elapsed from the onset of stolon tip swelling to the date at which the first tubers larger than 20 mm Ø were observed (Table 1). However, it was progressively longer from the VE to the VL categories, 11 to 17 days, respectively. In contrast, for the varieties, a very short and similar period of time (7 days or less) elapsed between the onset of stolon tip swelling and the onset of tuber growth, except for Astarte (21 days) (Table 5). In relation to the increase in size of growing tubers (Figure 6), at the end of the plant cycle, the VE genotypes had the largest tubers (40 mm), followed by the I (36 mm), E (35 mm), L (34 mm) and VL (30 mm). Interestingly, all categories had a reduction in average tuber size from 57 to 64 DAP, except the VE genotypes, which had such reduction during the period from 36 to 42 DAP. After this event, growing tubers from the VE and E genotypes continued to increase in size until the end of the plant cycle, whereas the I, L and VL genotypes had some fluctuations in the average tuber size, quite possibly due to the incorporation of a variable number of new growing tubers into the existing "pool", causing a reduction in the average tuber size.

Changes in average tuber weight during the last part of the growing season

At the first evaluation date at which the fresh tuber weight was evaluated (96 DAP), the E genotypes had the highest tuber weight per plant (1266 g), followed by the I (1259 g), L (709 g), VE (799 g) and VL (394 g) genotypes (Table 6). However, 29 days later (125 DAP), the VL genotypes surpassed in tuber weight the VE genotypes. At 140 DAP, the I genotypes still had the highest tuber weight per plant, although the L and VL genotypes were consistently increasing in tuber weight. Finally, by the end of the plant cycle,

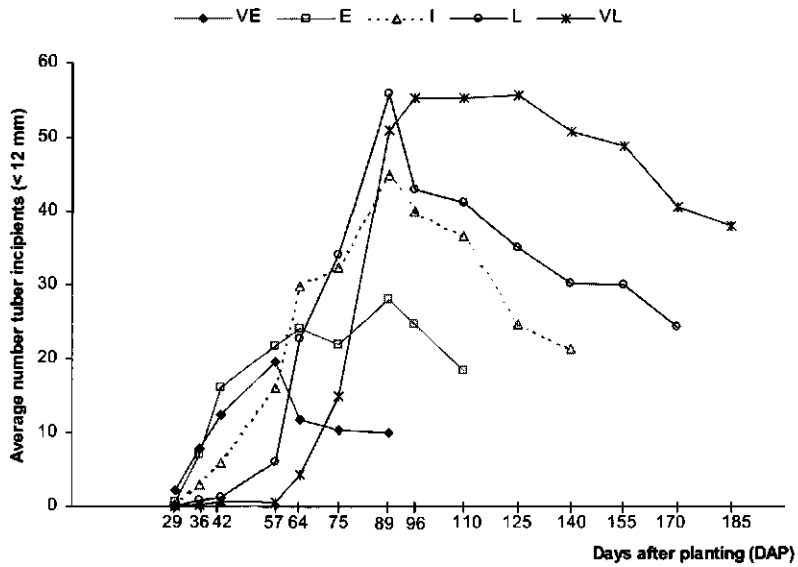


Figure 4. Differences between DPC categories in the average number of tuber incipients (<12 mm Ø), from planting to the end of the plant cycle. VE = very early, E= early, I = intermediate, L = late and VL = very late.

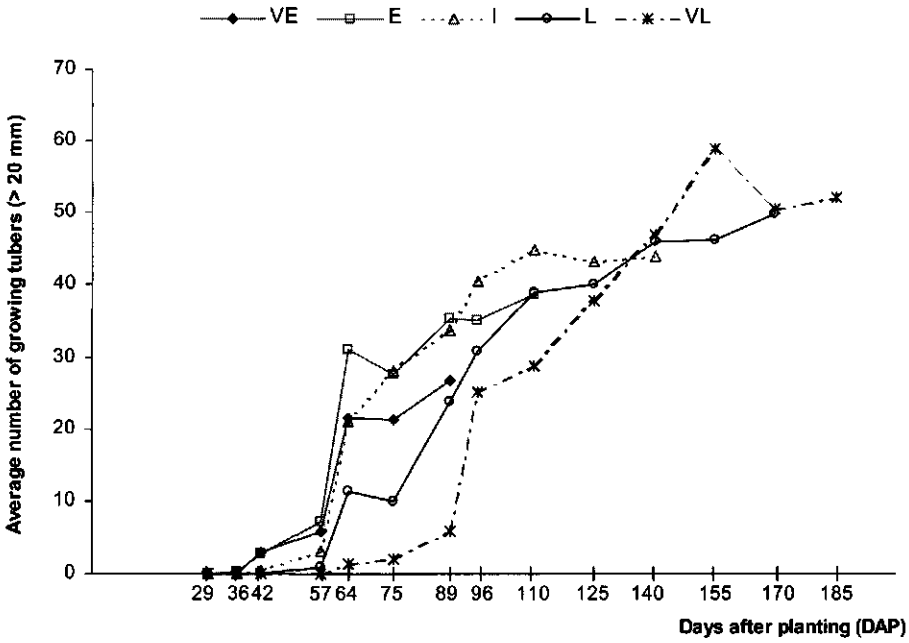


Figure 5. Differences between DPC categories in the average number of growing tubers (> 20 mm Ø), from planting to the end of the life cycle. VE = very early, E = early, I = intermediate, L= late, VL = very late.

within each of these five categories based on the duration of the plant cycle. This approach allowed us to assess the plasticity in the order of events leading to tuber formation and its association with tuber yield and plant development.

Materials and methods

Plant material and experimental design

The genetic background of the plant material, the experimental design and agronomical details were given previously (Celis-Gamboa et al., 2001a). In brief, the diploid population CE, consisting of 250 genotypes is a backcross between *S. phureja* and *S. tuberosum*. The CxE population was planted on May 12, 1999, in the vicinity of Wageningen (52 °N), in a light sandy soil in order to facilitate the harvesting of the plants and to recover all the underground structures without damage. Two plants from the parental clones C and E and the progeny were planted at random within 11 blocks, each one harvested at 29, 36, 42, 57, 64, 75, 89, 96, 110, 125 and 140 days after planting DAP. Three additional blocks were harvested at 155, 170 and 185 DAP, containing 47 of the latest CE genotypes. The varieties: Astarte, Bintje, Granola, Gloria, Karnico, Première and Saturna were included as standards and were randomised within 14 blocks and harvested as the CxE population.

Identification of highly contrasting genotypes in the CxE population

Giving the wide variation observed in the CxE population in the timing (days after planting) of the different plant and tuber related processes monitored, the following variables were used to identify genotypes with highly contrasting characteristics:

- duration of the plant cycle;
- duration of stolon formation;
- average number of new stolons observed at each evaluation date;
- onset and degree of stolon branching;
- onset of stolon tip swelling;
- average number of swelling tips at each evaluation date;
- duration of the stolon tip swelling process;
- number of days from planting to the observation of the first tuber(s) larger than 20 mm Ø (onset of tuber growth);
- number of days from the onset of stolon tip swelling to the onset of tuber growth;
- number of days from the onset of tuber growth to the end of the plant cycle (period of tuber growth);
- number of tubers smaller than 12 mm Ø (tuber initials) at the end of the plant cycle;
- number of tubers larger than 20 mm Ø (growing tubers) at the end of the plant

cycle;
- total number of tubers per plant at the end of the plant cycle;
- number of tubers per stem;
- tuber size at the end of the plant cycle;
- tuber weight at the end of the plant cycle;
- specific gravity: (5000/total fresh tuber weight) * under water weight of tubers;
- length of the longest sprout after storage of seed tubers at 4 °C during 191 days: 1= no visible sprouts; 2= sprouts between 1 and 4 mm; 3= sprouts between 5 and 9 mm; 4= sprouts between 10 and 19 mm; 5= sprouts between 20 and 29 mm and; 6= sprouts longer than 30 mm;
- duration of the flowering period;
- duration of the process of senescence.

These variables and the methodology used to assess them have been described in detail previously (Celis-Gamboa et al., 2002 b,c).

Initially, the CE genotypes were classified according to the duration of the plant cycle into 5 categories: very early, early, intermediate, late and very late. For every group a matrix table containing the data for all the variables considered was constructed. For each variable, the genotypes with the lowest and highest values were selected. At the end, the genotypes that were most frequently chosen for having extreme values for the individual variable were selected. From this first round of classification, 53 genotypes were identified. Using the same set of variables, a new data matrix was constructed for the 53 genotypes and a new round of classification was carried out, resulting in the selection of 26 genotypes. A new data matrix was constructed resulting in the selection of the two most contrasting genotypes per category.

To facilitate the identification of the genotypes throughout the text, the genotype number is preceded by the codes VE (very early), E (early), I (intermediate), L (late) or VL (very late), to indicate the duration of the plant cycle category to which it belongs, as previously described (Celis-Gamboa et al., 2001c).

Evaluation of the progress of senescence

The process of senescence was defined as the period between the last observation at which the plant did not have signs of senescence (this date was considered the time of the onset of the process), to the first date at which the plant was dead (end of the process). The progress of senescence was monitored using the following scale: 1= green plant; 2= upper leaves with the first signs of yellowing (light green); 3= yellow leaves; 4= 25% of tissue brown; 5= 50% of tissue brown; 6= more than 75% of tissue

brown; 7= dead plant.

Evaluation of the flowering process

The progress of flowering was monitored using the following scale: 0= no open flowers yet; 1= onset of flowering: evaluation date at which the first open flower was observed; 2= about 50% of the flower buds open on the primary stems; 3= full bloom: most of the flowers on primary inflorescences open and fully expanded; 4= past full bloom: most flowers from primary inflorescences already dropped and second- or third- order stems with flower buds and open flowers; 5= about 75% of all open flowers already dropped from second-, third- or fourth- order stems; 6= last open flowers observed in second-, third- or fourth- order stems; 7= end of flowering: no more developing buds or open flowers observed.

Chronological order of events related to stolon formation, stolon branching, stolon tip swelling, flowering, senescence and tuber growth

In order to identify differences in the chronological sequence of the onset and end of stolon formation, stolon tip swelling, flowering and senescence, as well as the onset of stolon branching and onset of tuber growth, independently of the duration of the plant cycle, the number of days after planting to the different events was expressed as percentage of the duration of the plant cycle. To facilitate graphical representation and comparison with other traits, the average fresh tuber weight per plant at the end of the plant cycle was expressed as percentage of 5000 g (rTweight).

Results

Several plant and tuber related characteristics describing the selected CE genotypes are presented in Table 1 and illustrated in Pictures 1, 2 and 3.

Differences in the process of senescence between selected genotypes

The progress of senescence in the selected genotypes and parental clones is shown in Figure 1. VE-196 and VE-743 completed the process of senescence in the shortest period (11 days), whereas in VL-690 and VL-703, the process of senescence lasted 30 days. Highly contrasting differences were found between the late genotypes. In L-732, the process of senescence took place in 15 days, in contrast to the remarkably slow process of senescence in L-758 (80 days). In the parental clones C and E, the process of senescence lasted 44 and 35 days, respectively, and it was within the range observed in the selected progeny.

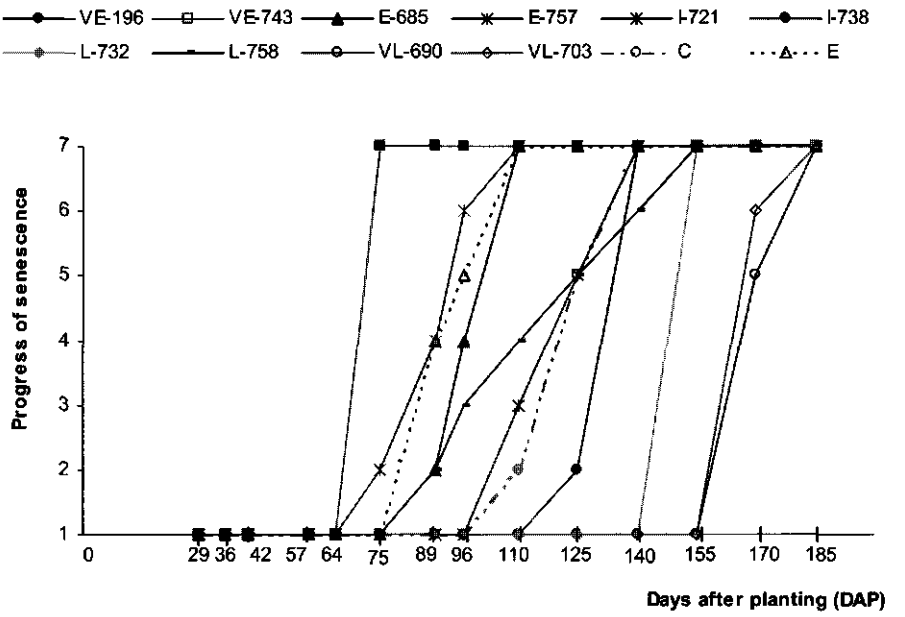


Figure 1. Progress of senescence in the selected genotypes and parental clones. VE = very early, E = early, I = intermediate, L = late, VL = very late, C and E = parental clones.

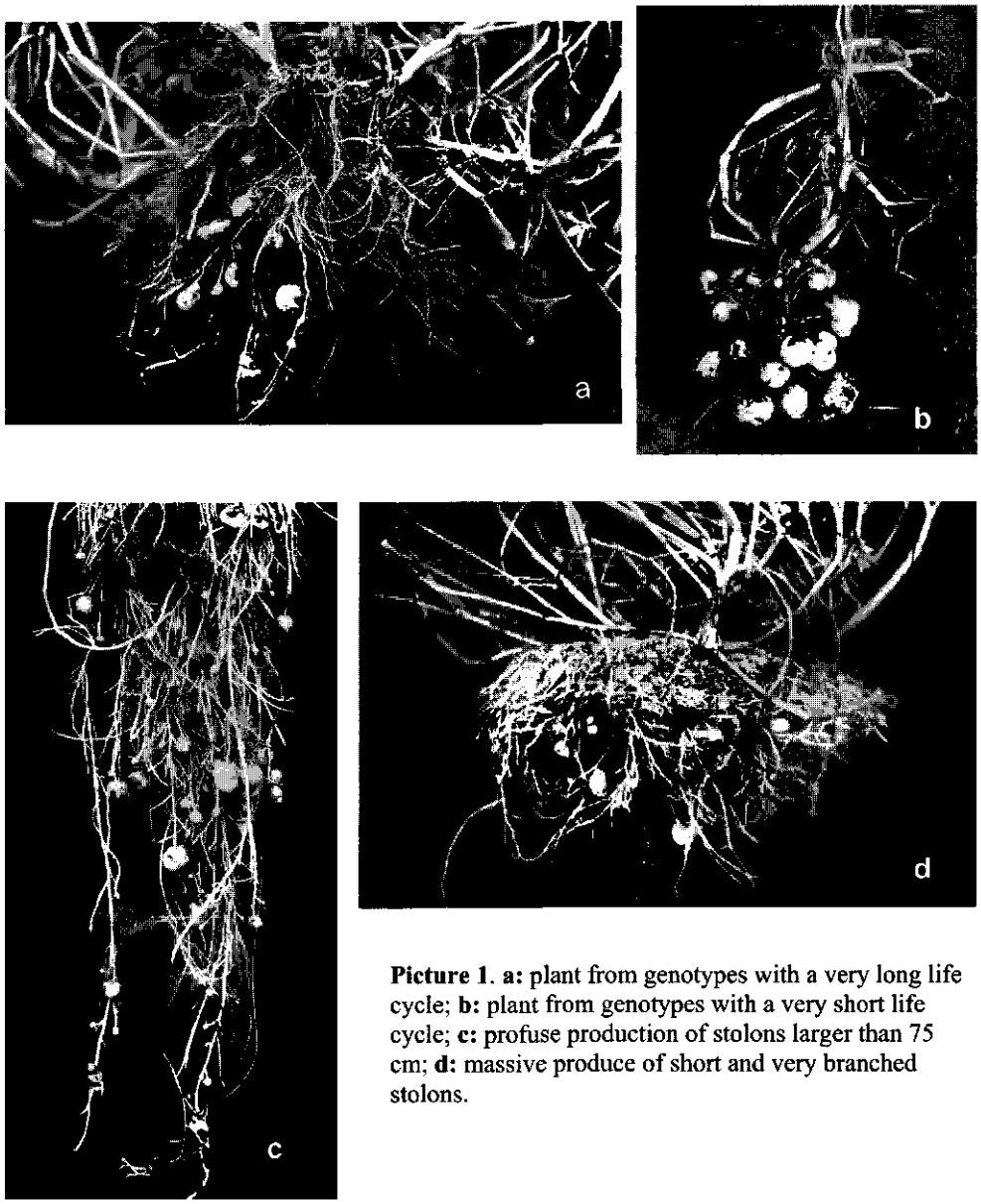
Differences in the process of flowering between selected genotypes

E-757 had the shortest flowering period (28 days) followed by VE-196 and E-685 (33 days). VL-693 and VL-703 had the longest flowering period, 113 and 98 days respectively (Figure 2). The most contrasting difference in the duration of the flowering period was found between the intermediate (I) genotypes. I-738 was flowering 36 days longer than I-721. Five genotypes began flowering at 36 DAP (VE-743, E-757, E-721, I-738, I-732) including the parental clones and another 5 began flowering 42 DAP (VE-196, E-685, L-758, VL-690 and VL-703). At 57 DAP, all genotypes had about 50% of the flower buds on the primary inflorescences open (flowering scale = 2). From 64 DAP, two clearly distinct groups could be distinguished, those with a short flowering period (VE-196, VE-743, E-685, E-757, I-721, L-758, C and E) and those with a prolonged flowering period (I-738, L-732, VL-690 and VL-703). The duration of the flowering period was directly related to plant size (Table 1). In every category, the genotype with the longest flowering period had the tallest plant.

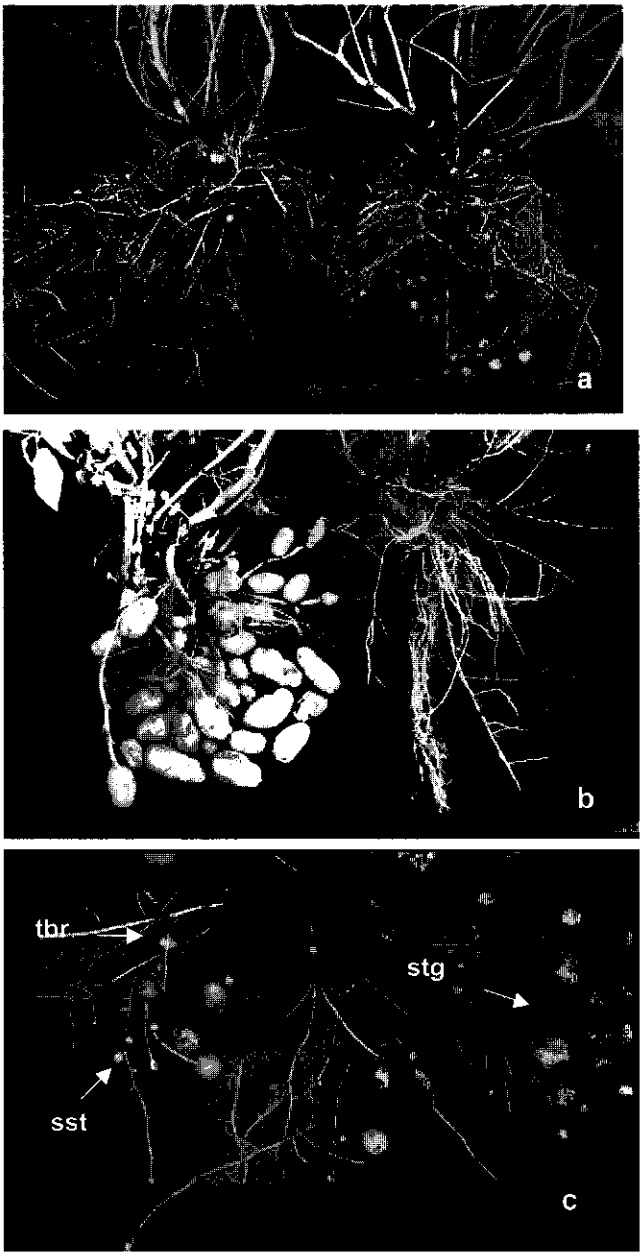
Table 1. Comparison of several plant and tuber related characteristics for selected CE genotypes and parental clones

	Selected CE Genotypes										Parents	
	VE-196	**VE-743**	**E-685**	**E-757**	**I-721**	**I-738**	**L-732**	**L-758**	**VL-690**	**VL-703**	**C**	**E**
Duration plant cycle (days)	75	75	110	110	140	140	155	155	185	185	140	110
Number of stolons[1]	2.8	4.7	3.9	7.6	6.6	5.1	5.8	3.0	8.1	4.2	4.9	5.8
Degree stolon branching[2]	0	4	2	3	2	3	3	5	2	2	2	2
Stolon length[3]	100^{s}	$60^{s}40^{m}$	$50^{s}50^{m}$	$80^{s}20^{m}$	$20^{s}40^{m}40^{l}$	$30^{s}40^{m}30^{l}$	$40^{s}60^{m}$	$50^{s}50^{m}$	$50^{s}50^{m}$	$40^{s}60^{m}$	100^{s}	$50^{s}50^{m}$
Number of swelling tips[1]	1	1	2	4	5	1	2	2	3	1	2	3
O-STSw to O-tuber growth[4]	27	7	6	12	15	8	9	10	3	11	5	25
O-tuber growth to E-SE[4]	44	61	48	67	70	54	52	73	69	48	59	48
Number of tubers < 12 mm	8	13	10	21	18	10	51	8	44	5	6	13
Number of tubers > 20 mm	4	5	7	26	27	10	22	10	36	7	7	15
Number of tubers/plant	12	18	18	48	46	20	73	17	80	12	13	27
Tuber pattern[5]	1,4	1,2,3,4	1,2	1,2,3,4,5	1,2	1,2,3,5	1,2,3,4,5	1,2	1,2,3,5	1,2	1,2,4	1,2,4
Tuber size (mm)	33	22	27	27	26	23	22	21	24	37	13	15
Tuber weight/plant (g)	400	1650	73	2400	2400	1026	1455	569	1750	398	586	2112
Specific gravity	253	432	62	405	326	369	425	79	319	383	344	431
Number of berries per plant	0	8	5	0	0	0	20	0	0	15	15	20
Dormancy[6]	4	4	5	6	4	4.5	5.5	1	4	1	2	5
Plant size (scale)[7]	3.0	2.9	3.0	2.9	3.2	3.6	3.7	3.0	3.8	3.8	3.0	3.1
Number of stems/plant	3.5	3.6	4.4	4.5	5.6	3.1	3.7	1.9	3.4	2.5	3.8	4.8
Number of tubers/stem	4.6	5.9	6.8	11.7	11.3	10.6	41.2	14.4	31.2	8.6	9.3	6.1

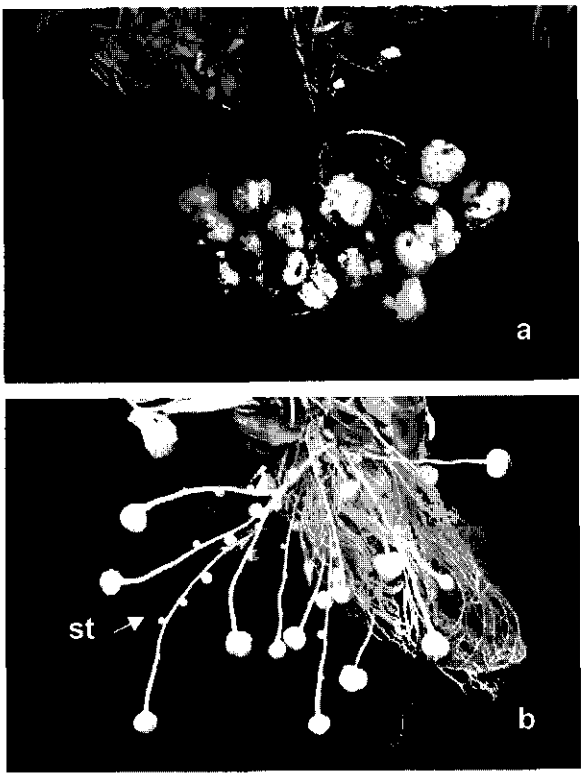
O-STSw = onset of stolon tip swelling; E-SE = end of the plant cycle; **1**: average number at each evaluation date; **2**: degree of stolon branching: 0 = no stolon branching to 5 profuse stolon branching; **3**: proportion of short (s), 1 to 10 cm; medium (m), 1 to 25 cm and long stolons (l) 26 to 40 cm length; **4** : as proportion of the plant cycle; **5**: position of tubers on stolons: 1 = at stolon tip of main stolon; 2 = on branches; 3= sessile, along main stolons; 4= tubers on very short main stolons surrounding the mother tuber; **5** = secondary tubers emerging from a growing tuber; **6**: sprouting of seed tubers during storage, LS-1= no visible sprouts; LS-2= sprouts between 1 and 4 mm; LS-3= sprouts between 5 and 9 mm; LS-4= sprouts between 10 and 19 mm; LS-5= sprouts between 20 and 29 mm and; LS-6= sprouts longer than 30 mm. **7** = plant size: scale 1 = ≤ 40 cm, 2 = 41-60 cm, 3 = 61-80 cm, 4 = 81-100 cm and 5 = > 100 cm height.

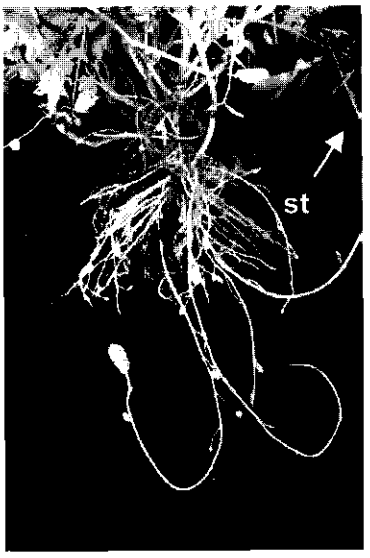


Picture 1. **a:** plant from genotypes with a very long life cycle; **b:** plant from genotypes with a very short life cycle; **c:** profuse production of stolons larger than 75 cm; **d:** massive produce of short and very branched stolons.



Picture 2. a: plants from genotype with a very long life cycle with a massive production of branched stolons and tubers; **b:** genotype with very early tuber formation (left) and genotype very late in tuber initiation at 75 days after planting; **c:** plant with secondary tuber growth (stg), sessile tubers (sst) and tubers on stolon branches (tbr).





Picture 3. a: tubers of uniform size on very short stolons; **b:** tubers on stolon tips and sessile tubers (st), no stolon branching; **c:** tubers on very long stolons and shift to secondary stem formation (st).

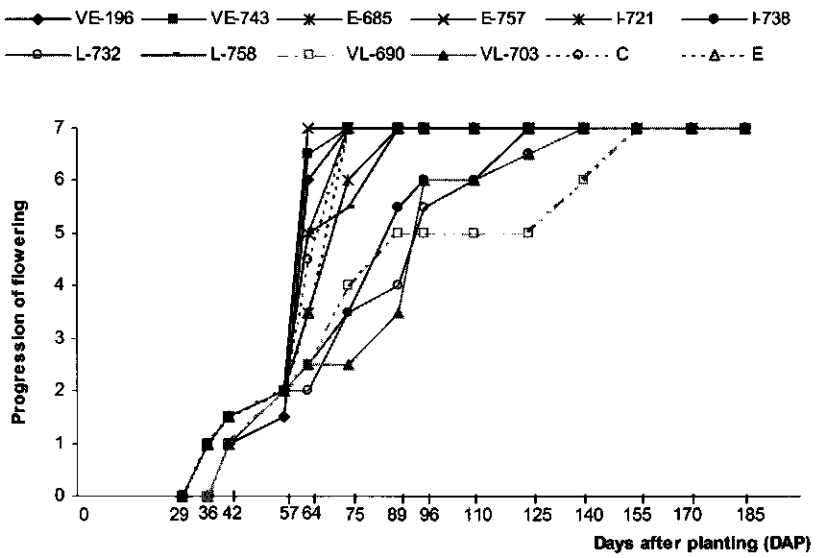


Figure 2. Progress of flowering in selected genotypes and parental clones. VE = very early, E = early, I = intermediate, L = late, VL = very late, C and E = parental clones.

Differences between selected genotypes in the relative occurrence of events related to flowering, senescence and tuber formation during the plant cycle

Large differences were found between the selected CE genotypes in the relative time at which the onset and end of stolon formation, stolon tip swelling, flowering and senescence, tuber growth and tuber resorption took place (Table 2). As expected, stolon formation was the first event that took place. The VL genotypes began stolon formation when just 16% of the plant cycle had elapsed, in contrast to 40% in the VE genotypes. Similarly, VL-690 was the first to start stolon branching (23% of the plant cycle) in contrast to VE-743 which began stolon branching after 75% of the plant cycle had elapsed, or it did not take place like in VE-196. On the other hand, surprisingly, E-757, I-721 and L-758 were the first to begin stolon tip swelling (25% of the plant cycle) and E-685 was the last to begin this process (52%), followed very closely by VE-196, VE-743, I-738, L-732 and VL-703, which all began stolon tip swelling after 48% of the plant cycle had elapsed.

There was not a direct association between the duration of the plant cycle (in days after planting) and the proportion of the life cycle, at which the first swelling tips (the first visual evidence of tuber initiation) were observed. In contrast, flowering began progressively later from the VL genotypes, 23% of the plant cycle, to the VE ones, 55

– 56% of the plant cycle.

The first growing tubers (larger than 20 mm Ø) were observed at a similar proportion of the plant cycle in VL-690 (35%), L-758 (37%) and E-757 (38%), whereas VE-196 had the first growing tubers relatively much later in the life cycle (75%). On the other hand, VL-690 and VL-703 began tuber resorption after 48% of the plant cycle had passed, whereas E-757 , VE-196 and VE-743 began tuber resorption relatively much later in the plant cycle (80 – 85%). In I-738 and L-732, tuber resorption was not observed.

The selected VL genotypes had the most contrasting differences for the end of stolon formation. While VL-703 ended stolon formation after just 40% of the plant cycle had elapsed, VL-690 ended this process almost at the end of the plant cycle (92%). VL-703 and I-721 were the first genotypes to end stolon formation (40%) whereas VL-690, L-732 and E-685 were the last genotypes to end this process, after 92, 90 and 87% of the plant cycle had elapsed, respectively. On the other hand, L-758 ended stolon tip swelling at 57% of the plant cycle. In contrast to I-721, which had new swelling stolon tips until the end of the plant cycle. About the duration of the flowering period, VE-196, VE-743, I-738 and L-732 were producing flowers until 90% of the plant cycle had elapsed, whereas in E-757 and L-758, the end of flowering took place much earlier (57%). L-732 and L-758 had the most contrasting differences in the proportion of the plant cycle after which the process of senescence began, 90 and 48%, respectively. Interestingly, the VE and VL genotypes began this process at similar relative time, 85% of the plant cycle.

Differences between varieties in the relative occurrence of events related to plant development and tuber formation during the plant cycle

Despite the large differences between varieties in the duration of the plant cycle, much less variation was observed in the proportion of the plant cycle at which the onset and end of plant development and tuber formation events took place (Table 3).

The onset of stolon formation, stolon branching, stolon tip swelling, tuber growth and flowering took place relatively earlier from the VE to the VL varieties. For instance, in Gloria and Première, the first stolons were observed after 30% of the plant cycle had elapsed, in contrast to 22% for Granola, Saturna and Bintje, and 17% for Astarte and Karnico. A similar trend in the grouping of varieties was observed for the onset of stolon branching, stolon tip swelling and flowering. On the other hand, for the end of these processes, Saturna, Bintje, Astarte and Karnico ended flowering at similar proportion of the plant cycle (52 to 56%), in contrast to Gloria and Première that had open flowers during a larger proportion of the plant cycle. In contrast, the intermediate varieties, Saturna and Bintje began the process of senescence after 70% of the plant

cycle had elapsed, whereas Gloria, Première, Granola and Karnico began this process at similar relative time (76 to 78% of the plant cycle). Karnico was the last variety to began senescence (82%).

Sequential order of events taking place during the life cycle of selected genotypes
Between selected genotypes, a wide variation was found in the order at which plant development and tuber formation events were taking place in the period between the onset of stolon formation and the end of the plant cycle (Table 4). Large changes in sequence of events took place in relation to the end of stolon formation, end of stolon tip swelling and end of flowering.
For instance, in VE-196, the end of stolon formation took place at a similar proportion of the plant cycle as the onset of tuber growth, while in VE-743, the onset of stolon branching was the event coinciding with the end of stolon formation. Furthermore, in VE-196, the end of stolon formation took place after both the onset of flowering and the end of stolon tip swelling, whereas in VE-743, the end of stolon formation took place before the end of stolon tip swelling. On the other hand, in E-685 and VL-690, the end of stolon formation took place after the end of flowering and the onset of senescence, and at the same time as the end of stolon tip swelling. In E-757 the end of stolon formation preceded these three events but in VL-703, stolon formation took place very early in the plant cycle and at similar time as the onset of stolon branching. Interestingly, when each event was considered individually and its order of occurrence compared between the selected CE genotypes, much less differences were found. For instance, the onset of flowering was the 2nd event taking place in the life cycle of 8 out of the 10 CE genotypes. For the onset of stolon tip swelling, in 4 CE genotypes (VE-196, VE-743, I-721 and L-758) this was the 2nd event during the life cycle, while in 4 other genotypes (E-685, I-738, L-732 and VL-690) this was the 4th event in the plant cycle.

More variability was found in the moment during the life cycle at which the onset of stolon branching, onset of tuber growth, onset of tuber resorption and the end of stolon formation took place. The onset of stolon branching was the 2nd (E-685 and VL-690) and 3rd (I-738, L-732 and VL-703) event during the plant cycle, while for the remaining 7 genotypes, either it did not take place (VE-196) or ranged between the 4th (L-758) and the 8th (E-757) event in the plant cycle. On the other hand, the onset of tuber growth was the 2nd (VE-743), 3rd (E-757), 4th (VE-196, I-721, VL-690), 5th (E-685, I-738, L-732, L-758) or 7th (VL-703) event taking place during the plant cycle. In contrast, while I-738 and L-732 did not have tuber resorption, this was the 5th (VL-703), 6th (VE-196, E-685, VL-690), 7th (VE-743, I-721) or 10th (E-757, L-758) event during the plant cycle. The end of stolon formation, on the other hand, was the 3rd

Table 2. Differences between selected CE genotypes and parental clones in the onset and end of plant and tuber related processes expressed as proportion (in %) of the plant cycle.

	Selected CE genotypes										Parents	
	VE-196	VE-743	E-685	E-757	I-721	I-738	L-732	L-758	VL-690	VL-703	C	E
Duration plant cycle*	75	75	110	110	140	140	155	155	185	185	140	110
Events												
O- Stolon formation	39	39	26	26	21	21	19	23	16	16	21	26
O- Stolon branching	n.s.b	76	38	68	46	33	30	30	23	41	42	38
O- Stolon tip swelling	48	48	52	26	26	46	48	27	35	48	41	33
O- Tuber growth	76	48	58	38	41	54	57	37	35	59	46	58
O- Tuber resorption	85	85	68	81	54	n.r.	n.r.	71	48	48	54	81
O- Flowering	56	52	38	33	28	28	25	27	23	23	26	33
E- Stolon formation	76	76	87	52	41	64	90	57	92	41	54	68
E- Stolon tip swelling	56	85	87	68	100	79	90	57	92	84	79	87
E- Flowering	90	90	68	58	64	89	90	57	84	76	54	68
O- Senescence	85	85	68	58	69	79	90	48	84	84	69	68

* = In days; n.s.b = no stolon branching; n.r.= no resorption

Table 4. Differences between selected CE genotypes and parental clones in the chronological order in the onset and end of plant and tuber formation events taking place during the potato life cycle.

	Selected CE genotypes										Parents	
	VE-196	VE-743	E-685	E-757	I-721	I-738	L-732	L-758	VL-690	VL-703	C	E
Onset stolon formation	1	1	1	1	1	1	1	1	1	1	1	1
Onset stolon branching	n.s.b	5	2	8	6	3	3	4	2	3	3	4
Onset stolon tip swelling	2	2	4	3	2	4	4	2	4	5	3	2
Onset tuber growth	4	2	5	3	4	5	5	5	4	7	6	5
Onset tuber resorption	6	7	6	10	7	n.r	n.r.	10	6	5	3	9
Onset flowering	3	4	2	2	2	2	2	2	2	2	2	2
End stolon formation	4	5	9	5	4	6	6	7	9	3	7	6
End stolon tip swelling	6	7	9	8	10	7	6	7	9	9	10	10
End flowering	9	10	6	6	8	9	6	7	7	8	7	6
Onset senescence	6	7	6	6	9	7	6	6	7	9	9	6
End plant cycle	10	11	11	11	11	10	10	11	11	11	11	11

n.s.b = no stolon branching; n.r.= no resorption.

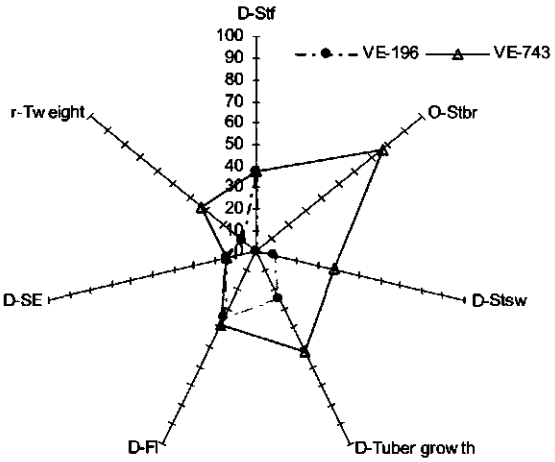
Table 5. Differences between control varieties in the chronological order in the onset and end of plant development and tuber formation events taking place during the potato life cycle.

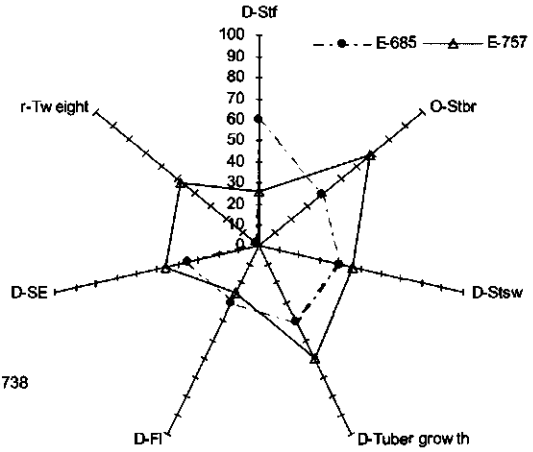
	Varieties						
	Gloria	**Première**	**Granola**	**Saturna**	**Bintje**	**Astarte**	**Karnico**
Duration plant cycle	96	96	125	140	140	170	185
	Early*		Intermediate			Late	Very late
Onset stolon formation	1	1	1	1	1	1	1
Onset stolon branching	4	4	3	3	4	3	3
Onset stolon tip swelling	1	1	2	2	2	2	2
Onset tuber growth	3	3	3	5	2	5	3
Onset flowering	4	4	3	3	4	3	3
End stolon formation	6	4	7	6	8	7	7
End stolon tip swelling	7	7	9	6	8	8	9
End flowering	7	8	6	6	6	6	6
Onset senescence	9	9	7	9	7	8	7
End plant cycle	10	10	10	10	10	10	10

*** according to the classification of the CxE population**

flowering period had the largest relative tuber weight, in the L genotypes, the opposite was observed. L-732 had a larger proportion of the plant cycle devoted to flowering (65%) and a shorter tuber growth period (43%) than L-758 (30 and 63%, respectively). However, L-732, had a higher relative tuber weight (30%) than L-758 (10%). Interestingly, L-732 spent 42% of the plant cycle on stolon tip swelling and just 10% in the process of senescence, while L-758 spent 30 and 52% of the plant cycle, respectively. A different situation was observed in the very late genotypes. VL-690 spent a higher proportion of the plant cycle in stolon formation (76%), stolon tip

Figure 3. Differences between selected CE genotypes and parental clones in the proportion of the plant cycle devoted to stolon formation (Stf), stolon tip swelling (Stsw), tuber growth, flowering (Fl) and senescence (SE). The onset of stolon branching (Stbr) and the relative tuber weight (r-Tweight) are also included. O = onset; D = duration.





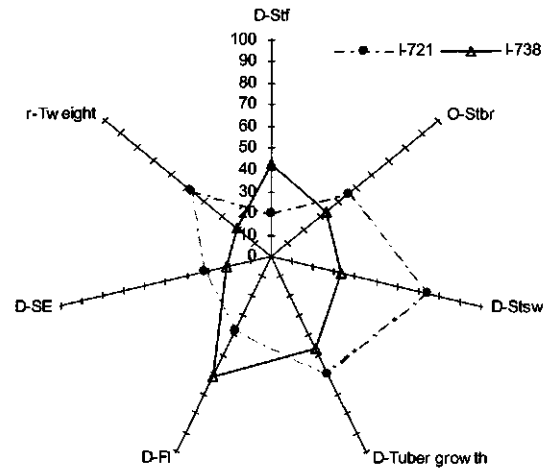
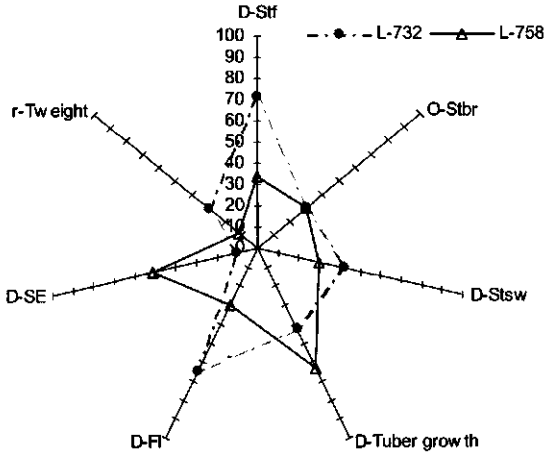
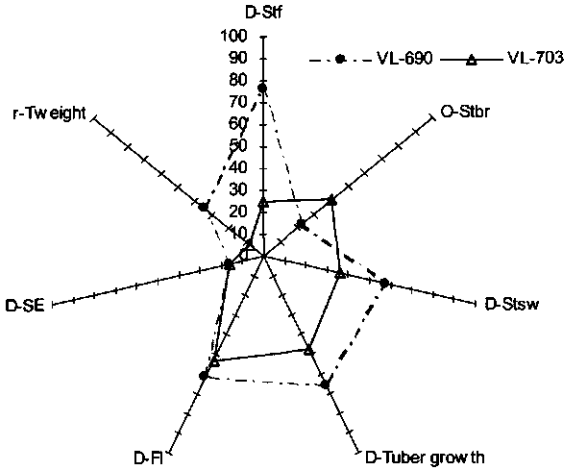


Figure 3. Continued





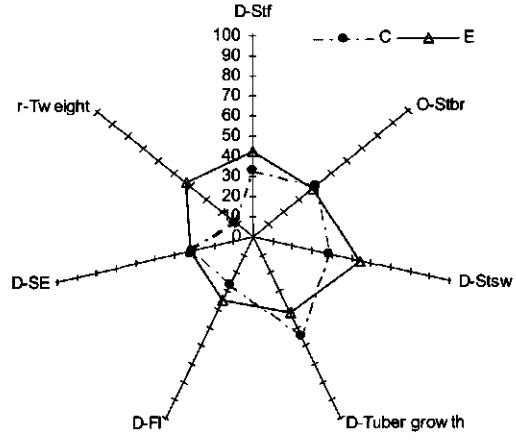
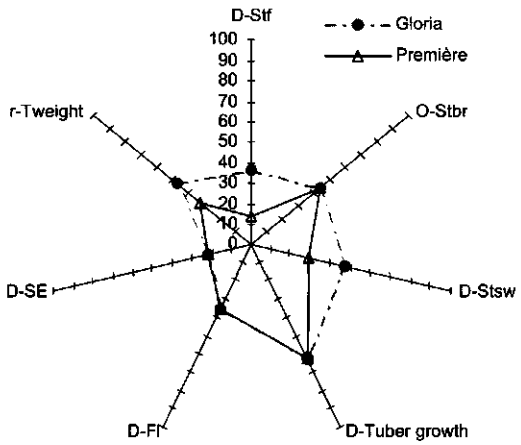
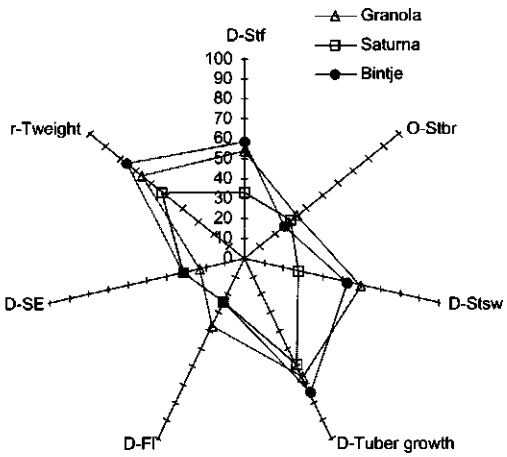
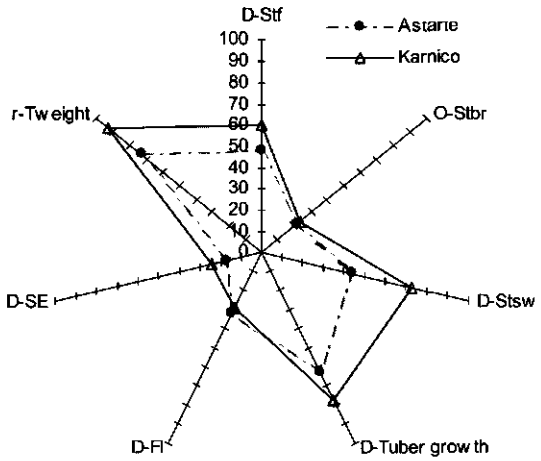


Figure 4. Differences between control varieties in the proportion of the plant cycle devoted to stolon formation (Stf), stolon tip swelling (Stsw), tuber growth, flowering (Fl) and senescence (SE). The onset of stolon branching (Stbr) and the relative tuber weight (r-Tweight) are also included. O = onset; D = duration.







swelling (57%) and flowering (61%) than VL-703 (25, 36 and 53% respectively) and it also had a higher relative tuber weight (35%) than VL-703.

In contrast to the large differences found between the selected CE genotypes in the proportion of the plant cycle spent in the processes considered, relatively fewer striking differences were found in the parental clones C and E (Figure 3f). The largest difference was in the relative yield, which was very low in C (10%) compared to 40% in E, even though E had a shorter tuber growth period than C. In addition, C also had shorter stolon formation, stolon tip swelling and flowering periods than E. In summary, among the 5 CE genotypes with the highest relative tuber weight, 3 genotypes had a longer period of stolon formation, 3 had a later onset of stolon branching, all had a longer period of stolon swelling, 4 had a longer tuber growth period, 3 had a shorter flowering period and 2 had a longer senescence period than the less yielding counterparts. These results suggest that the duration of the stolon tip swelling and tuber growth periods were directly related to the final tuber yield. However, high yield could be observed even when there was large variation in the duration of other processes such as stolon formation, flowering and senescence, making it difficult to identify the most favourable duration for these events.

Differences in the duration of stolon formation, stolon tip swelling, flowering and senescence between control varieties

Differences in the proportion of the plant cycle devoted to stolon formation, stolon tip swelling, flowering, senescence and tuber growth were found also between varieties (Figures 4a, 4b and 4c). Karnico spent 60 and 70% of the plant cycle in stolon formation and stolon tip swelling respectively, in contrast to 15 and 30% in Première, respectively. Furthermore, in Karnico, tuber growth took place during 80% of the plant cycle in contrast to 60% for Saturna.

For all varieties, the flowering period was shorter than in the selected CE genotypes, in Saturna, flowering took place during 40% of the plant cycle, in comparison to 25% in Bintje and Astarte. Also the process of senescence was shorter in the varieties than in the CE genotypes, with Astarte having the shortest senescence period (20%) and Bintje and Saturna the longest of all varieties (30%). On the other hand, Astarte and Karnico began stolon branching very early in the plant cycle (20%), in contrast to Gloria and Première (45%). In relation to the relative yield, in general, the varieties had a higher relative tuber yield than the CE clones, with Karnico having the highest (93%) and Première the lowest (33%) relative yield of all varieties.

If we compare the duration of the processes between varieties, using Karnico as reference, all had a shorter stolon formation period, 5 out of 6 varieties had a later onset of stolon branching and all had shorter stolon tip swelling and tuber growth

periods than Karnico. In addition, 4 varieties had shorter flowering and senescence periods, while 2 varieties had a shorter flowering period and a longer senescence process than Karnico. Thus, it seems that for the varieties, a long stolon formation period, early stolon branching, long stolon tip swelling and tuber growth periods, with a relatively short flowering period and a long senescence were the most favourable conditions for very high yield.

Discussion

Using several plant and tuber formation characteristics, 10 highly contrasting genotypes were selected from the widely diverse CE population. Large differences were found between the selected genotypes in the proportion of the plant cycle after which the onset and end of stolon formation, stolon tip swelling and flowering took place. In general, these processes began earlier in the plant cycle for the late genotypes than for the early ones. The same trend was observed for the varieties. Similar results were obtained by Grieß (1987), who comparing a main crop and two early varieties found that flowering began after 25 and 32% of the plant cycle had elapsed, respectively. In relation to the duration of the flowering period, large differences were also found between the selected genotypes. However, interestingly, the VE genotypes were flowering until almost the end of the plant cycle (only 1 or 2 flowers still present), which is not expected for very early and determined genotypes (O'Brien et al., 1998; Almekinders and Struik, 1996). Plant height was directly related to the extent of the flowering period and the duration of the plant cycle, both indicators of the degree of determinacy of the growth habit (Firman et al., 1995).

In relation to the process of senescence, the very early and very late CE genotypes had a very similar and short process of senescence, in comparison with almost half of the plant cycle devoted to senescence by one of the early and one of the late CE genotypes. Grieß (1987) also found that in two early and one main crop varieties, 50% of the plant cycle was devoted to the process of senescence. In addition, between the selected CE genotypes, we found differences in the rate of senescence. In the very early genotypes, there was a faster senescence of the leaf canopy than in the other CE genotypes. Furthermore, between CE genotypes with similar duration of the plant cycle, there were differences in the rate of senescence, suggesting that a persistent canopy could result from a) a long period of green leaves followed by a very fast senescence process or from b) a shorter green leaf period followed by a gradual senescence of the plant. Firman et al. (1995) indicated that this last pattern of senescence could be potentially the most efficient for tuber yield, because less photosynthate would be used in stem and leaf production. However, we did not find a

strong relationship between the duration of the senescence process and the relative tuber yield between the selected CE genotypes, suggesting that other factors were also influencing final tuber yield. Among these factors, we found that in the selected CE genotypes, the duration of stolon tip swelling and tuber growth were directly related to yield, reflecting their determinant influence on tuber number and size, respectively. The same was observed for the varieties. Radley et al. (1961) indicated that although the duration of tuber bulking (growth) is of great importance, the extension of this period by inducing early tuberization by means of an early planting could be ineffective, due to a reduction in leaf area and early senescence. However, between the selected genotypes and in the CxE population, in general, there was a large variation in the duration of the tuber growth period between genotypes with similar duration of the plant cycle, suggesting that these two characteristics could be combined by breeding and selection.

A large amount of research has been focused on the comparison of characteristics related to tuber initiation and yield between varieties contrasting in earliness (Collins, 1977; Meyling and Bodlaender, 1981; Almekinders and Struik, 1996; Firman et al.,1995; O'Brien et al., 1998). However, very little information is available on the differences in the duration of stolon formation, stolon tip swelling, flowering and senescence between genotypes differing in the duration of the plant cycle. In the selected CE genotypes, a wide array of possibilities in the duration of stolon formation, stolon tip swelling (as the first physical evidence of tuber induction), flowering and senescence was observed. There were genotypes that combined, for instance, a very short stolon formation period with very long flowering, early stolon tip swelling, late stolon branching and a short senescence process. A narrower array of alternatives in the duration of these events was observed in the varieties.

Between CE genotypes with high tuber yield it was not possible to identify a clear general pattern of duration for the different processes considered, due to the large array of possibilities observed in these highly contrasting genotypes. For the varieties, on the other hand, using Karnico (the highest yielding variety in this study) as reference, it was possible to identify a general pattern for the duration of these processes: long stolon formation, stolon tip swelling and tuber growth periods, a relatively short flowering, a slightly long senescence process and early stolon branching seem to be favourable for high yield. However, it is important to keep in mind that Karnico is a starch variety and consequently, high total yield and starch content were major targets during its breeding process. In the other 6 varieties, yield
as well as tuber quality attributes, such as uniform tuber size were determining breeding and selection processes. Thus, during the process of selection to obtain the variety with the desired characteristics, indirectly, the onset and duration of processes

such as stolon formation, stolon tip swelling, tuber growth, flowering and senescence were being combined and selected. This can explain why in the varieties much less differences were observed in the onset and duration of these processes, regardless of highly contrasting differences in the number of calendar days to the end of the plant cycle. In contrast, the CE genotypes used in this study were chosen from a highly diverse and unselected population of potato, originated from the cross between two contrasting species (*S. phureja* and *S. tuberosum*). This plant material allowed us to assess some of the large and perhaps unexpected plasticity present in potato for the occurrence of events related to tuber formation and plant development.

Our results make more evident the complexity of the chronological relationships between all the processes taking place during the potato life cycle, adding more difficulties to the attempt to explain the role of environmental factors and gibberellin levels in the modulation of tuber formation and plant development, particularly under field conditions.

A very large amount of information on the processes controlling and the factors affecting stolon formation, tuber formation and flowering has been generated and reviewed among others by Ivins and Bremner (1965), Vreudenhil and Struik (1989), Ewing and Struik (1992), Almekinders and Struik (1996), Almekinders (1993), O'Brien et al. (1998), Jackson (1999) and references therein. However, a large proportion of this research, largely done under controlled conditions, has been focused on the factors influencing the occurrence, particularly the onset, of these processes. Nevertheless, still limited and sometimes contradictory information is available on the factors influencing and mechanisms controlling the onset and duration of tuber related processes. A potato plant is able to produce tubers only if it has been induced to tuberize. The consensus in the literature is that short days (long nights with low temperatures) are tuber-inducing conditions in potato (Driver and Hawkes, 1943; Ewing, 1978; Ewing and Struik, 1992). A tuber-inducing component(s) formed in the leaves is (are) transported basipetally to the stolon tips, where it will cause the swelling of the subapical region of the stolon. It is common to say at this stage that the plant has been induced to tuberize. When the stolon tip swelling reaches twice the diameter of the stolon, it is said that tuber initiation has begun (Ewing and Struik, 1992; O'Brien et al., 1998). In general long days with warm temperatures promote vegetative growth: shoot elongation and the formation of numerous, long branched stolons. In addition flowering is also favoured by long days with moderate temperature. These environmental conditions increase levels of gibberellins in the plant. After the plant is exposed to short days, there is a well described decrease in the level of giberellins, which is associated with a reduction of stolon elongation, favouring tuberization (Vreugdenhil and Struik, 1987).

The induction and growth of tubers have been related to profound changes in the overall morphology of the potato plant, due to the preferential diversion of assimilates to the tubers: cessation of stem growth and axillary branching, end of flowering shortly after tuber initiation and hastening of the senescence process (Ivins and Bremner, 1965; Ewing and Struik, 1992; Martinez-Garcia et al., 2002). Thus, it seems that early tuber initiation necessarily results in small plants with limited leaf area and low yield, whereas late tuber initiation leads to larger plants with higher yield. However, among CE genotypes and control varieties that began tuber formation at similar relative times, there were marked differences in the duration of stolon formation, flowering and the senescence process. There were several genotypes that began tuber initiation very early in the plant cycle, and yet produced long branched stolons and flowered profusely during a large proportion of the plant cycle, had a very large canopy and a very long plant cycle (155 days or more). These characteristics are usually associated with plants poorly induced to tuberize, having high levels of gibberellins (Vreugdenhil and Struik, 1987). Our observations contradict the known association between high levels of gibberellins and the inhibition of tuberization. Results inconsistent with this generalization have also been obtained from the study of anti-phyB (phytochrome B) potato plants (Jackson and Prat, 1996; Jackson et al., 2000), as well as from the comparison under field conditions of large numbers of varieties with contrasting earliness (O'Brien et al., 1998).

The very wide array of combinations in the duration of plant development and tuber formation events observed in the CxE population and control varieties, as well as the very well known lack of synchronicity of events leading to harvestable tubers, suggest the possibility of the existence of several genetic factors controlling tuberization and plant development in potato. Gene expression is induced or repressed by environmental stimuli and regulatory elements such as gibberellins.

The remarkable plasticity in the timing and intensity of the morphogenetic events taking place during the life cycle of the potato plant is evident, not only after placing a large population of genotypes, segregating for the duration of the plant cycle under the same field conditions, but also after exposing single genotypes to different photoperiod and temperature regimes. In this regard, Steward et al. (1981), placed a single genotype under four combinations of photoperiod and temperature regimes and obtained such dramatic changes in the morphology of the potato plants, that brought the question of how under the influence of these two factors, plants so remarkably different could emerge within the limits of a single genome. On the other hand, we placed under the same field conditions, a large population of potato originated from a cross between two potato species differing in daylength requirements for tuberization. We found also a vast array of possibilities in plant morphology, resembling those

observed by Steward and co-workers. Both studies show evidence of the remarkable plasticity present in potato. These results imply that several genes controlling developmental events are affected differently by the same environmental conditions or alternatively, large blocks of genes are triggered by these external stimuli producing as a consequence particular patterns of development. A much better understanding of the physiological and genetic factors underlying tuber formation and plant development will bring us closer to the dreamed ability to manipulate tuber formation to satisfy specific requirements set by the seed industry, processing companies and consumers.

6

Genetic analysis of senescence in potato (*Solanum tuberosum* L.)

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Abstract

Major events taking place during the process of senescence were evaluated in a large mapping population of potato, comprising 250 genotypes, segregating for the duration of the plant cycle. Three Quantitative Trait Loci (QTLs) on chromosome 5 were found to be associated with the duration of the plant cycle and the onset, duration, mid point and rate of the senescence process. Other QTLs associated with these traits were found on chromosomes 6, 9 and 12. During the ontogeny of senescence, the QTLs on chromosome 5 were detected during most of the senescence process, whereas QTLs on chromosomes 1, 4, 7, 8 and 12 were detected only at the beginning or at the end of this process. QTL analysis of components of a process in addition to time-related QTL analysis during the ontogeny of the process is an effective strategy to identify genetic factors underlying complex developmental processes like senescence.

The fact that QTLs, associated with senescence, duration of the plant cycle, tuber formation and carbohydrate metabolism and transport have been detected on chromosome 5 lead to the hypothesis that chromosome 5 harbors a major fraction of the genetic machinery required during senescence. Our results show that the evaluation of large populations segregating for major features of complex processes like senescence, is a powerful tool to identify genetic factors associated with major developmental components. We also show that this is a very effective strategy to identify contrasting genotypes, which could be used to identify and characterize senescence-associated genes.

Key words: earliness, life cycle, plant maturity type, potato, QTL, senescence, senescence-associated genes, tuber formation

Introduction

Plant senescence is an important stage in the life cycle of plant species of monocarpic habit (coupling whole-plant senescence to reproduction) (Leopold, 1961). Functionally, plant senescence is not just a degeneration process, but is also a developmental and recycling process. During plant senescence, assimilates and nutrients accumulated during the growing stage are translocated from the senescing organs to developing seeds and storage organs (Smart 1994; Gan and Amasino, 1997; Lohman et al., 1994).

Leaf yellowing is the most obvious marker of plant senescence. Although the regulatory mechanisms controlling the timing of leaf senescence remain elusive, the actual process of leaf senescence has been extensively characterized in several species (reviewed by Leopold, 1965; Smart, 1994; Buchanan-Wollaston, 1997). Common features observed across several species have led to the concept of the senescence syndrome, defined as "an orderly sequence of events involving the turnover of macromolecules and lipids and the transport of mobilizing nutrients out of the senescing tissue" (Hensel et al.,1993). Thus, leaf senescence occurs according to a predictable and synchronous developmental program and it involves the co-ordinated expression of many nuclear genes (Smart, 1994). At the cellular level, organelles are disassembled in a precise hierarchy. Chloroplasts are the first organelles to be affected during the senescence program, while nucleus and mitochondria are last (Smart, 1994). The most widely used marker to monitor the senescence syndrome is the loss of chlorophyll (yellowing) associated with the degeneration of the chloroplast internal structure (Weaver et al. 1998).

It has been known for many years that the main diversity in crop yield is due to differences in the duration of photosynthetic activity (Watson,1952). There has been considerable interest in breeding crop varieties with delayed senescence in the hope of increasing crop productivity. For example, delayed senescence with concomitant preservation of the photosynthetic apparatus of the *Gd1d2* mutant of soybean increases yield by 44% (Guiamet et al., 1990). Stay green varieties of maize and sorghum have been used in breeding programs to enhance the yield of these crops (Thomas and Smart, 1993).

A major goal of studies on the genetic and physiological basis of senescence is to identify major regulatory genes that are responsible for the overall control of the process. At least 10 different classes of senescence-associated genes (SAGs) have been distinguished, based on temporal patterns of expression during leaf development (Smart, 1994; Buchanan-Wollaston, 1997). Interestingly, the majority of the senescence-associated genes isolated up to date, have been identified using methods

aimed at the isolation of any cDNA clone that represents an mRNA species that occurs specifically in senescing leaves (and not in green leaves), or, that has a significantly increased level of expression in senescing leaves relative to green leaves (Smart, 1994). Given the relatively limited success of this strategy, it is believed that the identification and characterization of mutants with an altered senescence process would be a more powerful strategy to identify these elusive regulatory genes (Buchanan-Wollaston, 1997; Chandlee, 2001). Because this type of mutants is not common, progress toward a more complete understanding of the regulation of the senescence syndrome is expected to be slow until some of these regulatory genes are cloned and studied (Chandlee, 2001).

It is surprising that a more straightforward strategy involving the evaluation of large populations segregating for senescence-related features, to identify interesting genotypes, has yet not been well documented.

From the analysis of extensive data collected on a highly polymorphic and well documented mapping population of potato (CE), we found very large differences between genotypes in the duration of the plant cycle, in the timing of occurrence of morphological events related to tuber formation and flowering (both reproductive processes coupled to senescence), and in senescence (Celis-Gamboa et al., 2002b,c).

Since the most obvious sign of senescence is yellowing of the leaves, the progress of senescence could be divided into stages, on the basis of the amount of yellowing that has occurred. We found large differences between genotypes in the onset, duration and rate of the senescence process. These results rendered this population suitable for the genetic analysis of major components of the process of senescence in potato. Such an analysis could be a major step towards a better understanding of physiological and genetic factors underlying tuber formation and yield potential in potato.

Materials and methods

Plant material, design of field experiment and agronomical details

The genetic background of the plant material, the experimental design and agronomical details were given previously (Celis-Gamboa et al., 2002a). In brief, the diploid population CxE, consisting of 250 individuals is a backcross between *S. phureja* and *S. tuberosum*. The CxE population was planted on May 12, 1999, in the vicinity of Wageningen (52° N), in a light sandy soil in order to facilitate the harvesting of the plants and to recover all the underground structures without damage. Two plants from the parental clones C and E and the progeny were planted at random within 11 blocks, each one harvested at 29, 36, 42, 57, 64, 75, 89, 96, 110, 125 and

140 days after planting DAP. Three additional blocks were harvested at 155, 170 and 185 DAP, containing 47 of the latest CE genotypes.

Evaluation of variables related to haulm senescence

The process of plant senescence was described by 5 components: onset, end, duration, mid point and rate (Figure 1). The onset of senescence (OSE, in days after planting), was defined as the last observation date at which foliage of the plant still did not have signs of senescence (leaf yellowing). The end of senescence was considered to be the first evaluation date at which the haulm did not have any green tissues left (leaves or stems). The number of days from planting to the end of senescence was defined as the duration of the plant cycle (DPC, in days after planting). The duration of the senescence process (DSE, in days) was defined as the number of days between the onset and the end of senescence. The mid point of senescence (MpSE, in days after planting) was defined as the number of days from planting until 50% of the senescence process had elapsed.

The progress of haulm senescence was assessed for each genotype from planting to the end of the plant cycle, using the following scale: 1= green plant; 2= upper leaves with the first signs of yellowing (light green); 3= yellow leaves; 4= 25% of haulm tissue brown; 5= 50% of haulm tissue brown; 6= more than 75% of haulm tissue brown; 7= dead plant. With this information curves of the progress of senescence were constructed and the slopes between consecutive data points and the mean slope were calculated. The mean slope was considered the rate (speed) of the senescence process (RSE; $days^{-1}$), defined as the number of days elapsed between consecutive stages of senescence.

Evaluation of plant maturity type

On May 12, 1998 and April 29, 1999, 3 replications with two plants each of the 250 CE genotypes, the parental clones and 7 standard varieties (Astarte, Bintje, Granola, Gloria, Karnico, Première and Saturna) were planted in a clay soil in Wageningen. Plant maturity type was evaluated using an ordinal scale ranging from 2 (green and vigorous; late to very late) to 10 (dead; very early plant types). The evaluation was performed between 110 and 120 days after planting (DAP), when the mid- early variety Bintje was showing the first signs of senescence ("bending knees").

Evaluation of tuber formation and plant development related characteristics

In order to study the influence of the timing of occurrence of tuber formation and other plant developmental events on the process of senescence, the following variables were

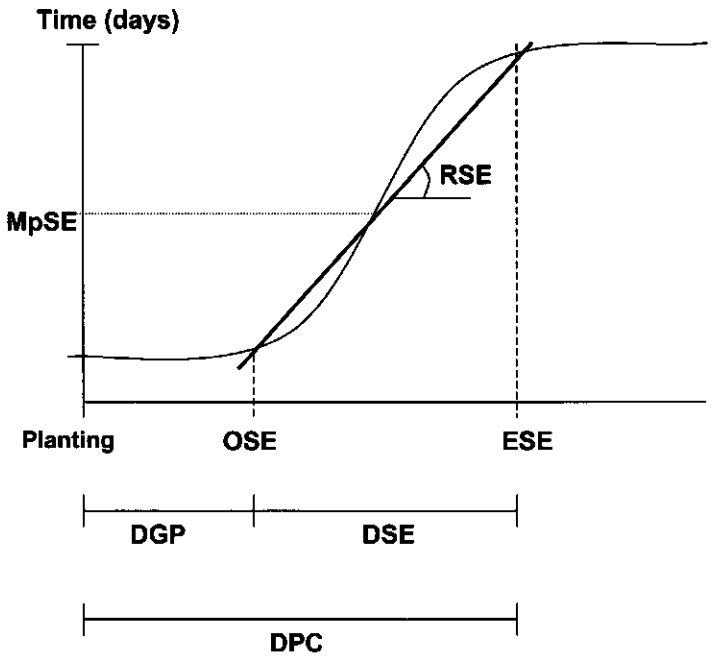


Figure 1. Schematic representation of variables describing the senescence process: onset (OSE), end (ESE), duration (DSE), mid point (MpSE) and rate (RSE) of senescence; duration of the green foliage period (DGP): from planting until the onset of senescence; duration of the plant cycle (DPC): from planting until the end of senescence.

assessed for every genotype:

- onset of stolon formation: first evaluation at which at least one stolon was observed (days after planting);
- end of stolon formation: first of several consecutive evaluations after which no more newly formed stolons were observed (in days after planting);
- duration of stolon formation period: number of days elapsed between the first and last date at which newly formed stolons were observed (in days);
- mid point of the stolon formation period: number of days from planting to 50% of the stolon formation period (in days after planting);
- onset of stolon tip swelling: first evaluation date at which at least one stolon tip was showing incipient swelling (in days after planting);
- end of stolon tip swelling: first of several consecutive evaluations after which no more new swelling stolon tips were observed (in days after planting);
- duration of stolon tip swelling period: number of days elapsed between the first and last date at which newly formed stolon tip swellings were observed (in days);

- mid point of the stolon tip swelling period: number of days from planting to 50% of the stolon tip swelling period (in days after planting);
- mid point of the tuber initiation period: number of days from planting to 50% of the tuber initiation period. Tuber incipients were described as swelling stolon tips between 5 and 12 mm Ø;
- rate of increase in the number of tuber incipients: with the number of tuber incipients recorded at each evaluation date, an accumulative curve was constructed. After calculating the slopes between consecutive evaluation dates, the mean slope was calculated and considered as the rate of increase in number of tuber incipients (in $days^{-1}$);
- onset of the tuber growth period: evaluation date at which the first tuber(s) reaching at least 20 mm Ø were observed (in days after planting);
- duration of the tuber growth period: number of days from the onset of tuber growth to the end of the plant cycle (in days);
- onset of flowering: evaluation date at which the first open flower was observed (in days after planting);
- end of flowering: evaluation date after which no more flower buds or open flowers were observed (in days after planting);
- duration of the flowering period: number of days elapsed between the onset of flowering (first evaluation date at which at least one open flower was observed) to the end of this process (in days);
- mid point of the flowering period: number of days from planting to 50% of the flowering period (in days after planting);
- plant size: scale: 1 = ≤ 40 cm, 2 = 41-60 cm, 3 = 61-80 cm, 4 = 81-100 cm and 5 = > 100 cm height.

Statistical analysis

Analysis of variance was carried out for all traits considered and the broad sense heritability (h^2_b) was calculated. Pearson correlations between variables were calculated on the basis of the means over replicates. Twenty four CE clones were excluded from the analysis due to low numbers of observations or lack of information on the duration of the plant cycle (beyond 140 or 185 days).

To assess the influence of plant related and tuber formation events on DPC, OSE, DSE, MpSE and RSE, a multiple regression model was constructed using the RSELECT procedure of GenStat 5. The Mallows Cp value was used as criterion for goodness of fit. This procedure evaluates all possible subsets of predictor variables and selects a small number of best regression models, among which the final selection can be chosen using additional criteria (e.g. relevance of physiological relationships). The

selected set of predictor variables was used to fit the final model with a restriction on the constant term to be zero.

Generation of AFLP markers and map construction

Genomic DNA was isolated from very young leaves and shoots ground in liquid N_2, according to the CTAB protocol as described by Bernatzky and Tanksley (1995). The AFLP procedure was performed as described by Vos et al. (1995), using the following 15 *Eco*RI/*Mse*I based primer combinations: E+AAC/M+CAA, E+AAC/M+CAC, E+AAC/M+CAG, E+AAC/M+CAT, E+AAC/M+CCA; E+AAC/M+CCT, E+AAC/M+CTA, E+AAC/M+CTG, E+ACA/M+CAA, E+ACA/M+CAC, E+ACA/M+CAT, E+ACA/M+CTA, E+AGA/M+CTC, E+ATG/M+CTA and E+ATG/M+CTC. AFLP markers were designated with the name of the primer combination used followed by the mobility of the amplification products relative to the Sigma 10 base ladder (Research Genetics, Huntsville, AL, USA). AFLP markers were visually scored as dominant markers, i.e. as presence (ab) or absence (aa) of the band.

The markers were divided into three groups according to the genetic models: <abxaa> markers segregating 1:1 in the mother; <aaxab> markers segregating 1:1 in the father and <abxab> markers segregating 3:1 in both parents or 'bridge markers' (Ritter, 1990). All markers were scored twice visually for checking scoring errors. Linkage analysis was performed according to the double pseudo-testcross approach for cross pollinating populations (Grattapaglia & Sederoff 1994) and using the software JoinMap 2.0 (Stam, 1993; Stam and Van Ooijen, 1995). Markers were preliminary assigned to linkage groups using a LOD threshold value of 4.0 and a preliminar marker order was determined. To check for singletons, this data set was displayed in Excel, where a graphical genotyping could be assessed after conditional formatting of the cells, where recombinant events were displayed as a change of colour of cells in a column. Since singletons are likely to be scoring errors and they can seriously distort marker order of a linkage map (Van Os et al., 2000), singletons were re-evaluated on the original films. Subsequently, markers were assigned to linkage groups using a LOD threshold of 3.0. The grouping of the markers was done with the separate <abxaa> and <aaxab> markers and resulted in a linkage map for each parent separately. The calculations of the linkage maps were done using all pairwise recombination estimates smaller than 0.499, threshold value for LOD higher than 0.01, no fixed orders and the Kosambi's mapping function. Using the 3:1 AFLP markers as "allelic bridges" markers, an integrated map was also constructed after combining the aligned maternal and paternal linkage groups. Special consideration was given to the fact that the order of the 1:1 segregating markers in the integrated map was the same as in the maternal and paternal maps. When changes in this order were observed, the

option of "fixed-order-files" was used.

QTL mapping

Quantitative traits were tested for normality and when neccesary transformed (square root (y+3/8) or 10log (y=1)). Using the software package MapQTL® version 4.0 (Van Ooijen, 2000), normally distributed quantitative traits were analyzed by interval mapping (Lander and Botstein, 1989), multiple QTL mapping (Jansen and Stam, 1994). If quantitative traits were not normally distributed after transformation, QTLs were identified by the Kruskal-Wallis test (Kruglyak and Lander, 1995). The criteria for detecting a QTL was set by a significance level of $p \leq 0.005$ in the Kruskal-Wallis test (Van Ooijen, 2000). For interval mapping and multiple QTL mapping methods, a threshold LOD value of 3.0 was chosen after performing a permutation test, as implemented in MapQTL® version 4.0. QTLs found in common to more than one trait were named based on the parental map and linkage group number. When more than one QTL was found in the same linkage group, the code was followed by a consecutive number, e.g. E5-1, E5-2, etc. All QTLs detected by the Kruskal-Wallis test or by interval mapping below the threshold value were tested for two and three-way interactions by regression analysis. After identification of QTLs related to the duration of the plant cycle and the onset, duration, mid point and rate of senescence, QTL additive effects and the effects of interactions were estimated by regression coefficients of a linear model fitted with the markers having the highest LOD value in the QTL region as regressors. After testing all possible interactions between the QTLs found by interval mapping and multiple QTL mapping (MQM), only those that were significant were allowed into the model.

In those cases where after interval mapping, the LOD profile showed the presence of more than one peak well above the LOD threshold value, cofactors were introduced at either of the peak positions, at all peak positions simultaneously and at the peak positions plus some additional makers (Qi et al., 2000). With all these cofactor configurations, the presence of one, two or three QTLs positioned on a chromosome was determined.

Given the high correlation between the duration of the plant cycle and all the variables considered, three sets of phenotypic data were used for QTL analysis, the raw phenotypic data and the non-standardised residuals after correcting the raw data for the duration of the plant cycle and plant maturity type (PMT).

Results

Phenotypic data on plant development and tuber formation

Data on the timing of occurrence and duration of events related to stolon formation, stolon tip swelling, tuber initiation, tuber growth, flowering and senescence were presented and analysed elsewhere (Celis-Gamboa et al., 2002b,c).

Phenotypic data of senescence related variables

A very large variation in DPC, OSE, DSE, MpSE, RSE and PMT was observed in the CxE population. The frequency distributions for these variables are given in Figure 2. DPC ranged from 75 to more than 185 days after planting, OSE ranged from 64 to 170 days, DSE ranged from 7 to 80 days, MpSE ranged from 70 to 178 days, RSE ranged from 1 to 13 $days^{-1}$ and PMT ranged from 3 to 10. Transgressive segregation was observed in each of these variables. The parental clones C and E had on average 125 DAP, 86 DAP, 40 days, 106 DAP, 7 $days^{-1}$ and 7.5, for DPC, OSE, DPC, MpSE, RSE and PMT, respectively. The broad sense heritability was very high for all the traits, DPC (0.95), OSE (0.98), DSE (0.85), RSE (0.96) and PMT (0.86).

Differences in the duration of senescence between CE genotypes with similar duration of the plant cycle

Between CE genotypes with similar duration of the plant cycle, there were large differences in the duration of the senescence process (Table 1). For instance, among genotypes with a life cycle of 96 days, DSE ranged from 7 to 32 days, whereas between genotypes with a life cycle of 155 days, DSE ranged from 15 to 80 days. In contrast, the parents C and E had a life cycle of 140 and 110 days, respectively, and the process of senescence lasted 44 and 35 days, respectively.

Progress of senescence

The distribution of the CxE population at different stages of senescence during the experiment is depicted in Figure 3. At 75 DAP, 80% of the population still had a green canopy whereas 20% of the population was at different stages of senescence, including 3% of CE clones that had ended their life cycle. The percentage of CE clones ending the plant cycle increased progressively and at 185 days, only 5% of the population was still in the process of senescence. Differences in the rate of progress of senescence were also observed between CE genotypes with similar DPC (Figure 4). Except for genotypes with a very short plant cycle (75 to 89 days), where plant senescence was very fast, genotypes varied in rate of senescence, despite very similar DPC (Figures 4b, 4c, 4d, and 4e). Differences in the overall rate of progress of senescence after

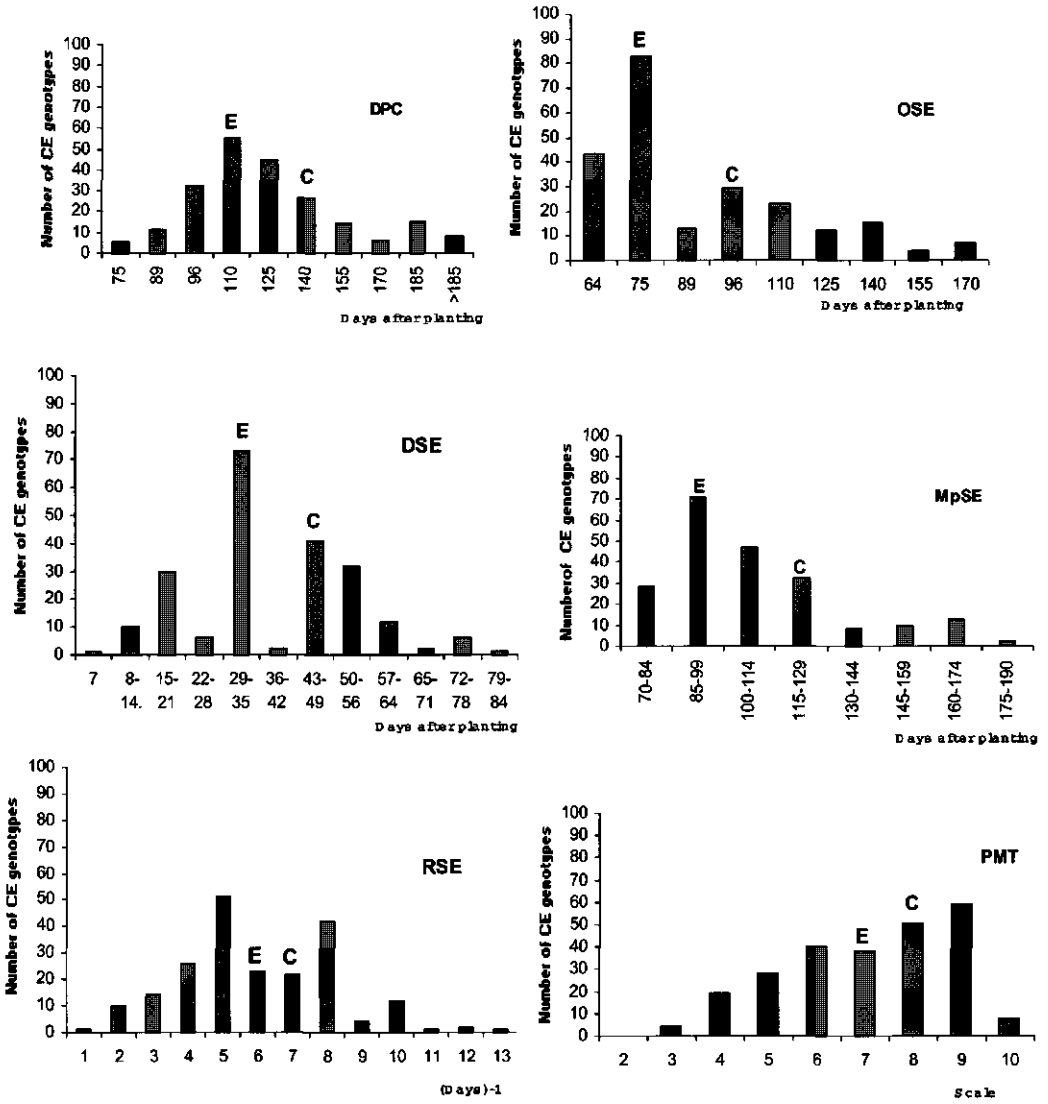


Figure 2. Frequency distribution of the CxE population for the duration of the plant *after planting) and the onset (OSE; days after planting), duration* (DSE; days), *mid p* after planting) and rate (RSE; days $^{-1}$) of senescence; plant maturity type (PMT; scal maturing to 10= very early maturing). C (female parent), E (male parent) of CE popı

Table 1. Distribution of the CxE population, according to the duration of the senescence period (DSE) and the duration of the plant cycle (DPC). C and E = parental clones.

	Duration of the plant cycle (DPC) in days after planting									
DSE	**75**	**89**	**96**	**110**	**125**	**140**	**155**	**170**	**185**	**>185**
7			1							
8-14	5	5								
15-21			19	5	4	1	1			
22-28		6								
29-35			12	33 [E]	13	9	3	2	2	
36-42					2					
43-49				18		11 [C]	4	2	6	
> 45										8
50-56					24	4				
57-63					1		5	1	4	
64-70						1	1			
71-77						1		1	1	
78-84							1			

grouping the CxE population according to the duration of the plant cycle, resulting in genotypes with a very short, short, intermediate, long and very long life cycle are shown in Figure 4f. On average, the rate of senescence decreased from the very early to the very late genotypes. C and E had a rate of senescence, intermediate between these two extreme categories.

Associations between the duration of the plant cycle, the onset, duration, rate and mid point of senescence

In general, very high correlations were found between DPC, OSE, DSE, MpSE and RSE (Table 2). DPC was (by definition) highly correlated with MpSE (r =0.98) and to a lesser extent to OSE (r =0.88). Much lower correlations were found between DPC and DSE (r =0.59) and RSE (r =-0.52), reflecting the large variation in RSE between genotypes with similar duration of the plant cycle (Figure 4). Correlation between

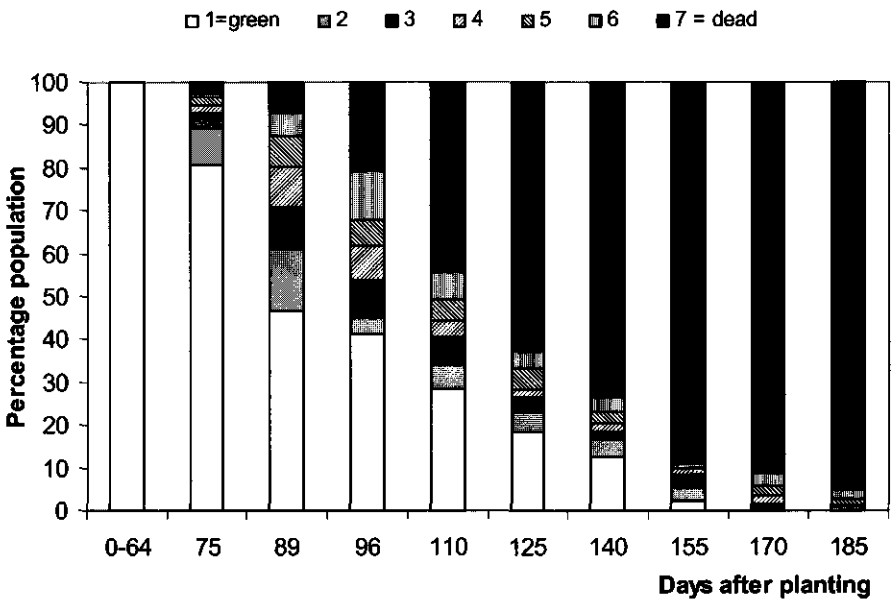


Figure 3. Distribution of the CxE population over different stages of senescence at each evaluation date.

OSE and DSE and OSE and RSE were relatively poor, reflecting the large variation in DSE and RSE found among genotypes with similar OSE.

Association between DPC, OSE, DSE, MpSE, RSE and morphological events related to stolon formation, stolon tip swelling, tuber initiation, tuber growth and flowering

High and significant correlations were found between DPC, OSE, DSE and MpSE and morphological events related to reproductive development, i.e. stolon formation, stolon tip swelling, tuber initiation, tuber growth and flowering (Table 2).

The end of stolon formation was slightly more correlated to DPC, OSE, DSE and MpSE (0.27 to 0.72) than the duration (-0.24 to 0.68) or mid point (-0.22 to 0.69) of the stolon formation period, whereas RSE was negatively but similarly correlated to the end, duration and mid point of the stolon formation period (-0.22 to –0.24).

The mid point of the stolon tip swelling period was best correlated to DPC (r =0.87), OSE (r =0.84), DSE (r =0.41), MpSE (r =0.87) and RSE (r = -0.35), whereas the onset of this process was (overall) the least correlated to DPC (r =0.70), OSE (r =0.74), DSE (r =0.20) and MpSE (r =0.73). On the other hand, the mid point of the tuber initiation period was more correlated to DPC (r =0.97), OSE (r =0.89) and MpSE (r =0.97), whereas the rate of increase of tuber incipients was less correlated , r= -0.77, -0.55, and 0.77, respectively.

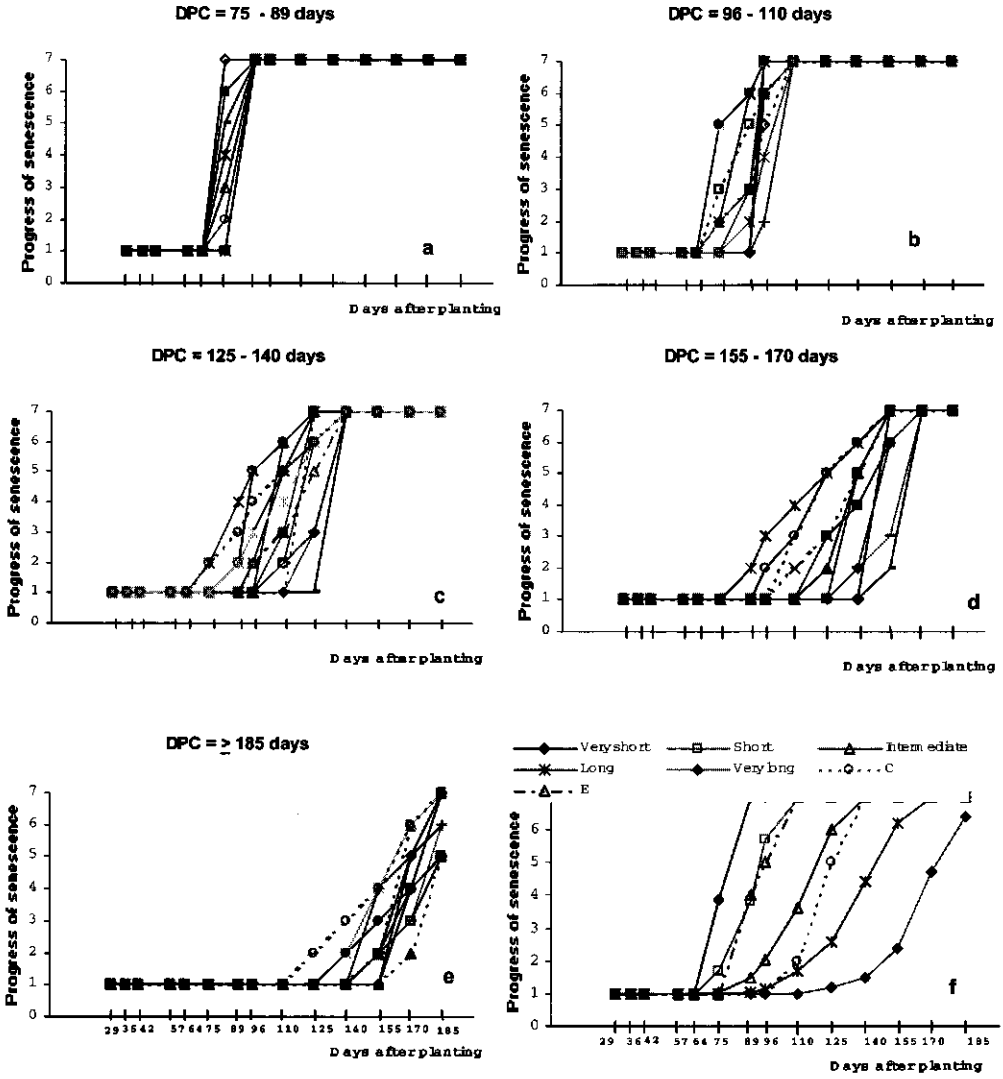


Figure 4. Differences in the duration and progress of senescence between CE genotypes differing in the duration of the plant cycle (DPC). Each curve corresponds to an individual CE clone.

Table 2. Correlations between stolon formation, stolon tip swelling, flowering, tuber initiation, tuber growth, duration of the plant cycle and the process of senescence. DPC = duration plant cycle, OSE = onset senescence; DSE: duration of senescence; MpSE = mid point senescence; RSE = rate of senescence.

	DPC	**OSE**	**DSE**	**MpSE**	**RSE**
DPC	1.0				
OSE	0.88**	1.0			
DSE	0.59**	0.16*	1.0		
MpSE	0.98**	0.96**	0.40**	1.0	
RSE	-0.52**	-0.15^{ns}	-0.86**	-0.37**	1.0
Onset stolon formation	-0.03^{ns}	0.06^{ns}	0.22*	0.09^{ns}	-0.10^{ns}
End stolon formation	0.71**	0.72**	0.27*	0.72**	-0.23*
Duration stolon formation	0.66**	0.67**	0.23*	0.68**	-0.24*
Mid point stolon formation period	0.67**	0.67**	0.26*	0.69**	-0.22*
Onset of stolon tip swelling	0.70**	0.74**	0.20*	0.73**	-0.16^{ns}
End of stolon tip swelling	0.83**	0.78**	0.42**	0.82**	-0.37**
Duration of stolon tip swelling	0.61**	0.66**	0.40**	0.68**	-0.35**
Mid point stolon tip swelling	0.87**	0.84**	0.41**	0.87**	-0.35**
Rate increase of tuber incipients (< 12 *mm* Ø)	-0.77**	-0.55**	0.56**	-0.77**	0.53**
Mid point tuber initiation period	0.97**	0.89**	0.53**	0.97**	-0.43**

Table 2. Continued

	DPC	OSE	DSE	MpSE	RSE
Onset tuber growth period	0.71**	0.72**	0.27**	0.73**	-0.19**
Duration tuber growth period	0.90**	0.73**	0.66**	0.85**	-0.58**
Rate increase of growing tubers	-0.76**	-056**	0.59**	-0.70**	0.62**
Mid point tuber growth period	0.97**	0.89**	0.53**	0.97**	-0.44
Onset of flowering	0.19 ns	0.19 ns	0.11 ns	0.19 ns	-0.08 ns
End of flowering	0.78**	0.82**	0.25**	0.81**	-0.20**
Duration flowering period	0.77**	0.81**	0.24**	0.80**	-0.20**
Mid point flowering period	0.78**	0.82**	0.25**	0.81**	-0.20**
Plant size	0.78**	0.76**	0.30**	0.80**	-0.26**

Pearson correlation coefficient, ns = no significant; * p = 0.05; ** p = 0.01; DPC = duration plant cycle, OSE = onset senescence; DSE: duration of senescence; MpSE = mid point senescence; RSE = rate of senescence.

In relation to the process of tuber growth, the mid point of the tuber growth period was the variable most related to DPC (r =0.97), OSE (r =0.89) and MpSE (r =0.97) and the rate of increase of growing tubers was the least related to OSE (r =-0.56), while the onset of the tuber growth period was the least correlated to DSE (r= 0.27). For the process of flowering, in contrast, the end, duration and mid point of the flowering period were similarly correlated to DPC (0.77 – 0.78), OSE (0.81 – 0.88) and MpSE. (0.80 – 0.81). The size of the plants, on the other hand, was slightly more correlated to MpSE (r =0.80), followed by DPC (r =0.78), OSE (r =0.76), DSE (r =0.30) and RSE (r= -0.26).

In general, plant and tuber related variables were more correlated to DPC than to OSE, MpSE or DSE, except for the onset of stolon tip swelling and the process of flowering, due to the large variation in timing of these events between plants with similar DPC. On the other hand, the mid point of tuber initiation and tuber growth periods had the highest correlation to both DPC and MpSE (r =0.97). These extreme correlations were not unexpected, since for a large proportion of the CxE population, during most of the plant cycle new tuber incipients were formed and they were

continuously added to the existing pool of growing tubers. RSE was the senescence variable most poorly correlated with stolon formation, stolon tip swelling and flowering ($r < 0.40$). However, slightly higher correlations were found between RSE and the rate of tuber incipients ($r = 0.53$) and the rate of growing tubers ($r = 0.62$) and to a smaller extent to the mid point of tuber initiation and growing tubers ($r = -0.45$).

Regression analysis of tuber formation and plant related events on the process of senescence

Due to the high correlations between tuber formation, plant related characteristics, and DPC, OSE, DSE, MpSE and RSE, only 3 or 4 variables were enough to account for more than 80% of the variation in the senescence parameters.

For instance, due to the very high correlation found between the mid point of the tuber growth period and DPC, a model including this variable alone accounted for 95% of the variance in DPC:

$$DPC = 0.97\ MpTG \qquad R^2 = 0.948$$

However, when MpTG was excluded, a model including the onset (OTG) and duration (DTG) of the tuber growth period still accounted for 95% of the variance associated to DPC:

$$DPC = 0.73\ DTG + 0.40\ OTG \qquad R^2 = 0.951$$

On the other hand, when tuber-related variables were excluded from the model, the mid point of the stolon tip swelling period (MpStsw) and plant size accounted for 80% of the variation in DPC:

$$DPC = 0.63\ MpStsw + 0.32\ plant\ size \qquad R^2 = 0.796$$

For OSE, a model including the mid point of tuber growth (MpTG), the mid point of the flowering period (MpFl) and plant size (PS) accounted for 83% of the phenotypic variation in this variable:

$$OSE = 0.53\ MpTG + 0.24\ MpFl + 0.21\ PS \qquad R^2 = 0.829$$

For the duration of the senescence process (DSE), the rate in number of growing tubers (RTG), the mid point of the tuber growth period (MpTG), plant size (PS) and the mid point of the flowering period (MpFl) accounted for 49% of the variation for DSE:

$$DSE = -0.46\ RTG + 0.70\ MpTG - 0.3\ PS - 0.36\ MpFl \quad R^2 = 0.487$$

For the mid point of the senescence period, a model including the mid point (MpTG) and the duration (DTG) of the tuber growth period, the mid point of the flowering period (MpFl) and plant size (PS) accounted for 96% of the variation in MpSE:

$$MpSE = 0.66\ MpTG + 0.24\ DTG + 0.10\ MpFl + 0.08\ PS \quad R^2 = 0.961$$

For the rate of senescence, a model including the rate in number of growing tubers (RTG), the mid point of the tuber growth period (MpTG), plant size (PS) and the mid point of the flowering period (MpFl) accounted for 49% of the variation for RSE:

$$RSE = 0.62\ RTG - 0.43\ MpTG + 0.32\ MpFl + 0.20\ PS \quad R^2 = 0.487$$

Thus, as previously observed, DSE and RSE were the senescence variables less associated with plant development and tuber formation variables.

Map construction

From the AFLP analysis, 403 markers were obtained. These markers were used to construct a maternal (C) and a paternal map (E) with 12 linkage groups each. The C map consisted of 140 markers spanning 919 cM and the E map consisted of 178 markers spanning 784 cM of the potato genome. The length of the 12 linkage groups in the C map ranged from 11.7 cM to 135.9 cM and those of the E map ranged from 5.6 cM to 120.3 cM. Since the maternal (C) and paternal (E) maps could not be integrated unambiguously, due to the low informativeness of the dominantly scored 3:1 AFLP "bridge" markers, QTL mapping was performed on the separated parental maps. Assignment of the chromosome number to the linkage groups was done using as reference the AFLP map previously constructed using 67 CE genotypes (Van Eck et al, 1995). To identify the origin of the map, C or E parent and the linkage group, each linkage group is preceded by the letter C or E, followed by the linkage group number, e.g. E5.

QTLs associated with the progress of senescence

The QTLs found associated with the progress of senescence (scale 1 = green foliage to 7 = death), from 75 days (evaluation date at which the genotypes with the shortest

Table 3. Map position of markers with the highest K-value of Quantitative Trait Loci detected for the progress of senescence during the period between 75 and 185 days using the Kruskal-Wallis test.

DAP	Chrom. number* (QTL name)	Marker	Position (cM)	Progress of senescence (scale 1 to 7)		
				Presence of marker		
				+	0	Additive effect
75	C7 (C7)	E+ATG/M+CTA-172	3.2	1.25	1.69	-0.44
	E5 (E5-1)	E-AAC/M+CAG-425	63.4	1.63	1.07	0.56
	E5 (E5-2)	E+AAC/M+CTC-27	34.5	1.71	1.30	0.41
	E5 (E5-3)	E+ACA/M+CAA-349	0.0	1.17	1.76	-0.59
	E9 (E9)	E+AAC/M+CAT-560	15.8	1.90	1.18	0.72
89	E5 (E5-1)	E-AAC/M+CAG-425	63.4	3.45	1.51	1.94
	E5 (E5-2)	E+AAC/M+CTC-27	34.5	3.38	1.86	1.52
	E5 (E5-3)	E+ACA/M+CAA-349	0.0	2.08	3.45	-1.37
	E9 (E9)	E+AAC/M+CAT-560	15.8	3.18	2.18	1.00
96	C5 (C5)	E+ACC/M48-456	65.8	3.14	4.00	-0.86
	E5 (E5-1)	E-AAC/M+CAG-425	63.4	4.91	1.96	2.95
	E5 (E5-2)	E+AAC/M+CTC-27	34.5	4.65	2.42	2.23
	E5 (E5-3)	E+ACA/M+CAA-349	0.0	2.88	4.47	-1.59
110	C5 (C5)	E+ACC/M48-456	65.8	4.02	5.06	-1.04
	E5 (E5-1)	E-AAC/M+CAG-425	63.4	5.97	2.83	3.14
	E5 (E5-2)	E+AAC/M+CTC-27	34.5	5.80	3.24	2.56
	E5 (E5-3)	E+ACA/M+CAA-349	0.0	3.87	5.41	-1.54
125	C5 (C5)	E+ACC/M48-456	65.8	4.60	6.04	-1.44
	E5 (E5-1)	E-AAC/M+CAG-425	63.4	6.32	4.01	2.31
	E5 (E5-2)	E+AAC/M+CTC-27	34.5	6.59	4.24	2.35
	E5 (E5-3)	E+ACA/M+CAA-349	0.0	4.67	6.34	-1.67

DAP = days after planting; * C = female map, E = male map

Table 3. continued.

DAP	Chromosme number* (QTL name)	Marker	Position (cM)	Progress of senescence (scale 1 to 7)		
				Presence of marker		
				+	0	Additive effect
140	C5 (C5)	E+ACC/M48-456	65.8	5.23	6.45	-1.22
	E5 (E5-1)	E-AAC/M+CAG-425	63.4	6.93	4.84	2.09
	E5 (E5-2)	E+AAC/M+CTC-27	34.5	6.80	5.08	1.72
	E5 (E5-3)	E+ACA/M+CAA-349	0.0	5.33	6.66	-1.33
155	C5 (C5)	E+ACC/M48-456	65.8	6.09	6.80	-0.70
	E5 (E5-1)	E-AAC/M+CAG-425	63.4	6.90	5.82	1.05
	E5 (E5-2)	E+AAC/M+CTC-27	34.5	6.83	6.11	0.72
	E5 (E5-3)	E+ACA/M+CAA-349	0.0	6.15	6.93	-0.78
	E12 (E12)	E+AAC/M+CAC-106.6	34.8	6.14	6.82	-0.68
170	C5 (C5)	E+ACC/M48-456	65.8	6.57	6.42	-0.35
	C1 (C1)	E+AAC/M+CCT-190	31.1	6.59	6.94	-0.35
	E4 (E4)	E+ACA/M+CAA-279	46.8	6.88	6.61	0.27
	E5 (E5-1)	E-AAC/M+CAG-425	63.4	6.94	6.50	0.44
	E5 (E5-2)	E+AAC/M+CTC-27	34.5	6.94	6.61	0.33
	E5 (E5-3)	E+ACA/M+CAA-349	0.0	6.62	6.97	-0.35
	E12 (E12)	E+AAC/M+CAC-106.6	34.8	6.58	6.95	-0.37
185	E8 (E8)	E+ACA/M+CAC-208.4	12.3	7.00	6.76	0.24

DAP = days after planting; * C = female map, E = male map

plant cycle were already senescing) to 185 days (end of the experiment) are summarised in Table 3. Due to lack of normality of the data for several of the evaluation dates, QTLs were detected using the Kruskal-Wallis test ($p \leq 0.005$). From 75 to 170 days, three QTLs in chromosome E5 were identified: E5-1, E5-2 and E5-3. Interestingly, at each evaluation date, the same marker position associated to each one of these QTLs had consistently the highest K-value. The presence of marker loci on E5-1 and E5-2 was always associated with a more advanced stage of senescence, whereas the presence of the marker locus on E5-3 always had the opposite effect. For instance, at 75 days, plants having the marker locus on E5-1 were starting the process of senescence (1.6), whereas plants without this marker locus were mostly green (scale 1.1). In contrast, plants with marker locus E5-3 were still rather green (scale 1.2), whereas plants lacking this marker locus had a more advanced stage of senescence (scale 1.8). On the other hand, plants with or without the marker on E5-2 were already starting the process of senescence, but plants with this marker QTL had a more advanced stage of senescence (scale 1.7) than plants without it (1.3). A similar trend was observed until 170 days. At 96 and 110 DAP, the most contrasting differences in the average stage of senescence associated with the presence/absence of the markers for these three QTLs loci were found. For instance, at 96 days, plants with the marker associated with E5-1 were in a much more advanced stage of senescence (scale 4.9) than plants without this marker (scale 1.9). At 110 days, plants with E5-1 had a senescence value of 6.0, whereas plants without it had a value of 2.8.

At 75 days, besides the three QTLs in E5, two more QTLs (C7 and E9) with opposite effects on the stage of senescence were detected. The presence of C7 was associated with an earlier stage of senescence (scale 1.3) than in absence of the marker (scale 1.7). In contrast, plants with the marker on E9 (also present at 84 days) had a more advanced stage of senescence (scale 1.9) than those without it (scale 1.2). Thus, at 75 days, plants with E5-1, E5-2 or E9 had an earlier onset of senescence than plants with C7 or E5-3.

At 155 days, a new QTL (E12) was identified, which had a similar effect to that associated with E5-3. Plants with E12 where in a less advanced stage of senescence (scale 6.1) than those in which the marker was absent (scale 6.8).

At 170 days, two new QTLs (C1 and E4) were identified but with opposite effects on the progress of senescence. The presence of the marker on C1 was associated with a less advanced stage of senescence (6.6), whereas the presence of E4 marker was associated with a more advanced stage of senescence (6.9).

At 185 days only one and new QTL was detected (E8). All plants with this marker were dead (scale 7), whereas the absence of it was associated with a less advanced senescence (scale 6.7).

There was a dynamic interaction between the significance of the K-value of the markers associated to these QTLs and the progress of senescence, during the period between 75 and 185 days (Figure 5). The onset of the senescence process was particularly related to the QTLs C7 and E9, whereas the end of this process was particularly related to C1, E4 and E8. The QTLs E5-1, E5-2 and E5-3, on the other hand, were present during most of the senescence period.

QTLs related to the duration of the plant cycle and the onset, duration, mid point and rate of senescence

Using interval mapping and the raw data, only QTLs on chromosome 5 were detected for DPC and the components of the senescence process. However, after using the markers associated to these QTLs as cofactors in MQM, QTLs on chromosmes 6 and 12 were identified (Table 4). Interestingly, after performing interval mapping on the phenotypic data upon correction for the duration of the plant cycle, nothing was found. However, in addition to QTLs on chromosomes 6 and 12, a new QTL on chromosome 9 was found associated to OSE and MpSE after correcting the raw data for PMT (Table 4).

The QTLs E5-1, E5-2 and E5-3 were found associated with DPC and accounted for 41% of the phenotypic variation. For OSE, three QTLs were found: E5-1, E9 and E12. These three QTLs and the interaction between E5-1 and E12 accounted for 39% of the phenotypic variation for OSE (Table 4).

For DSE three QTLs were identified: E5-2, E5-3 and E6, which accounted for 23% of the phenotypic variation. For MpSE, besides E5-1 and E5-3, the QTL E12 and the interaction between E5-1 and E12 accounted for 39% of the phenotypic variation. In contrast, for RSE, QTLs E5-3 and E6 and their interaction accounted for 20% of the phenotypic variation.

Interestingly, only after correcting the data for PMT, the QTL 9 was detected in association with DPC, OSE and MpSE, accounting for 7, 4, and 4% of the phenotypic variation, respectively (Table 4). Thus, E5-1 was found associated with DPC, OSE and MpSe; E5-2 to DPC and DSE, while E5-3 was associated to DPC, DSE, MpSE and RSE. The QTL E6, on the other hand, was associated with DPC, OSE and MpSE, whereas E12 was related to OSE and MpSE (Figure 6).

Furthermore, plant size and all the variables considered in relation to stolon formation, stolon tip swelling, tuber initiation, tuber growth and flowering, as well as several other morphological events recorded during the experiment, were found associated with one, two or the three QTLs on chromosome E5. New QTLs associated with these traits were found only after markers on E5-1, E5-2 and E5-3 were used as co-factors in MQM or after correcting the raw data for plant maturity type (data not

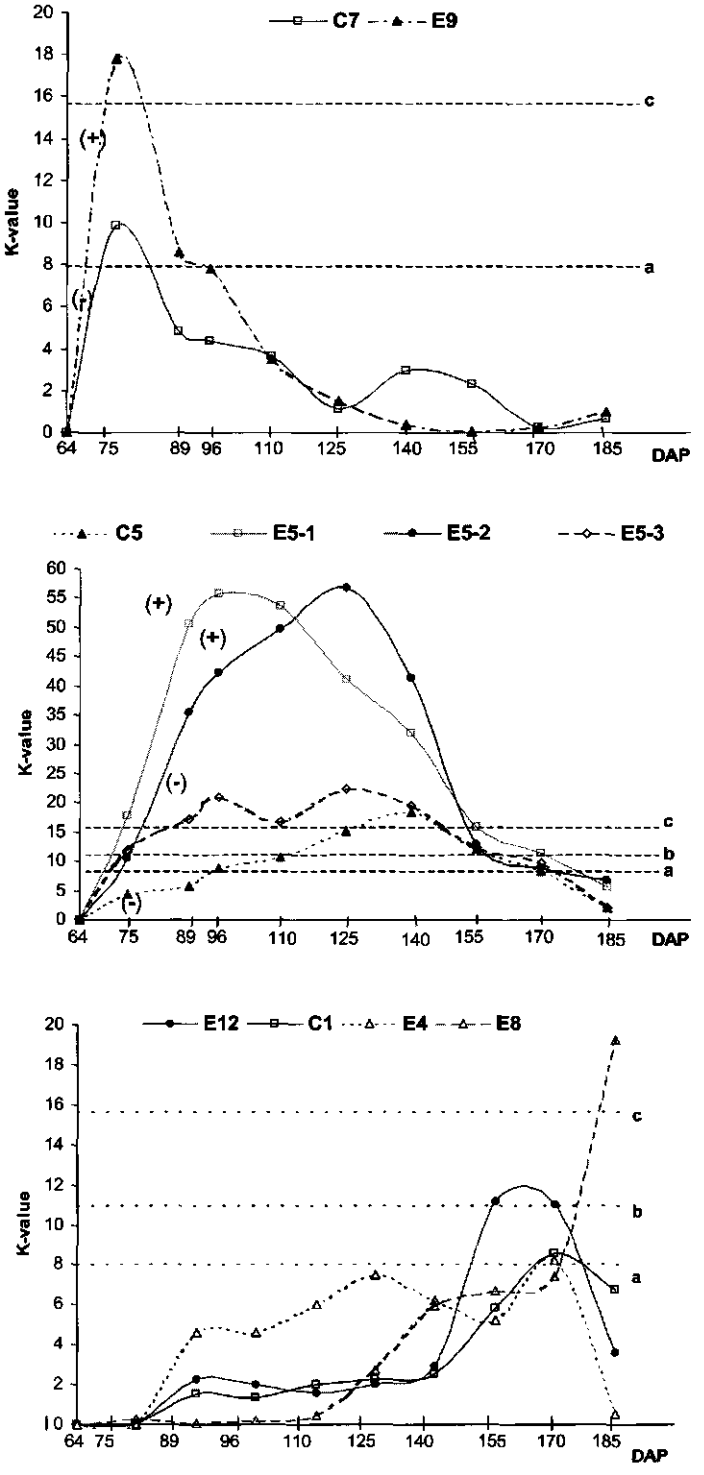


Figure 5. K-value profiles of QTLs detected during the progress of senescence in the period from 75 to 185 days after planting (DAP), using the Kruskal-Wallis test (p-value: a = 0.005, b = 0.001, c =0.0001). A "-"sign indicates a delaying effect and a "+" sign indicates an advancing effect in the progress of senescence.

Table 4. Quantitative trait loci associated with the duration of the plant cycle (DPC), onset (OSE), duration (DSE), mid point (MpSE), rate (RSE) of senescence and plant maturity type (PMT).

Trait	QTL information					
	Name	Chrom.	Marker	Position	LOD	% Exp. variation
DPC	E5-1	E5	E-AAC/M+CAG-425	63.4	12.61	40.9[1]
	E5-2	E5	E+AAC/M+CTC-27	34.5	9.95	
	E5-3	E5	E+ACA/M+CAA-349	0.0	6.63	
	E9[3]	E9	E+AAC/M+CAT-560	26.4	3.12	6.9[2]
OSE	C5	C5	E+ACC/M48-456	65.8	3.29	39.3[1]
	E5-1	E5	E-AAC/M+CAG-425	63.4	11.70	
	E12	E12	E+AGA/M+CTC-30	11.4	3.82	
		E5-1*E12				
	E9[3]	E9	E+AAC/M+CAT-560	26.4	3.10	4.1[2]
DSE	E5-2	E5	E+AAC/M+CTC-27	34.5	5.58	22.8[1]
	E5-3	E5	E+ACA/M+CAA-349	0.0	5.22	
	E6	E6	E+AAC/M+CCT-502	0.0	3.48	
MpSE	E5-1	E5	E-AAC/M+CAG-425	63.4	11.30	38.9[1]
	E5-3	E5	E+ACA/M+CAA-349	0.0	4.77	
	E12	E12	E+AGA/M+CTC-30	11.4	3.18	
		E5-1*E12				
	E9[3]	E9	E+AAC/M+CAT-560	26.4	4.55	4.1[2]
RSE	E5-3	E5	E+ACA/M+CAA-349	0.0	5.69	19.0[1]
	E6	E6	E+AAC/M+CCT-502	0.0	3.59	
		E5-3* E6				
PMT	E5-1	E5	E32/m49-425	63.4	14.25	40.3[2]

[1] = calculated by multiple regression analysis; [2] = by interval mapping; [3] = detected after correcting data for PMT

QTLs related to plant maturity type

Since significant differences ($p = 0.05$) were found between the PMT values recorded in 1998 and 1999, both data sets as well as the two-year average score were used for QTL identification. Only one QTL (E5-1) was found associated to PMT. Since the LOD value and the percentage of explained variation was very similar for the three PMT data sets (data not shown), only the mean PMT is presented. E5-1 had a LOD score of 14.25 and accounted for 40% of the phenotypic variation.

Additive and epistatic effects of QTLs related to DPC, OSE, DSE, MpSE, RSE and PMT

A summary of the main effects and epistatic interactions of the QTLs found associated with DPC, OSE, DSE, MpSE, RSE and PMT is presented in Table 5.

For DPC, presence of the markers on E5-1, E5-2 and E9 shortened the duration of the plant cycle 23, 16 and 7 days, respectively, whereas with E5-3, plants had a life cycle 11 days longer than average. E5-1 and E9 had an accelerating effect on OSE (14 and 8 days before average), E12 delayed this event 22 days. Interestingly, when markers on E5-1 and E5-12 were present, OSE was 13 days earlier than on average, the same effect as when E5-1 (was present alone).

For DSE, the presence of marker E5-2 shortened the process of senescence by 9 days, while the presence of E5-3 or E6 extended the senescence process by 6 and 7 days, respectively. For MpSE, presence of E5-1 or E9 advanced senescence by 15 and 8 days, respectively, whereas presence of E5-3 or E12 delayed this event by 10 and 20 days, respectively. However, when both E5-1 and E12 were present, MpSE took place considerably earlier than on average (13 days), an effect similar as with E5-1 was present alone.

For RSE, the presence of marker on E5-3 or E6 decreased the rate of senescence in 10 and 12 days, respectively, whereas the interaction between these 2 QTLs slowed down the rate of senescence by 15 days.

For PMT, plants with the marker on E5-1 had a much earlier maturity type (2.7 points).

The differences found between QTLs in the magnitude, their effect (positive or negative), as well as in the number of QTLs associated with the duration of the plant cycle and the four variables related to the process of senescence, offer an explanation for the large diversity found in the CxE population for the combination of these 5 variables, which is depicted in Figure 7.

Figure 6. Chromosomes and location of QTLs controlling the duration of the plant cycle (DPC) and the onset (OSE), duration (DSE), mid point (MpSE) and rate (RSE) of senescence, and plant maturity type. C and E refer to chromosomes belonging to the female (C) or male (E) parental maps. *** = QTLs identified during the progress of senescence by the Kruskal-Wallis test. Markers in bold had the highest LOD value or K value.

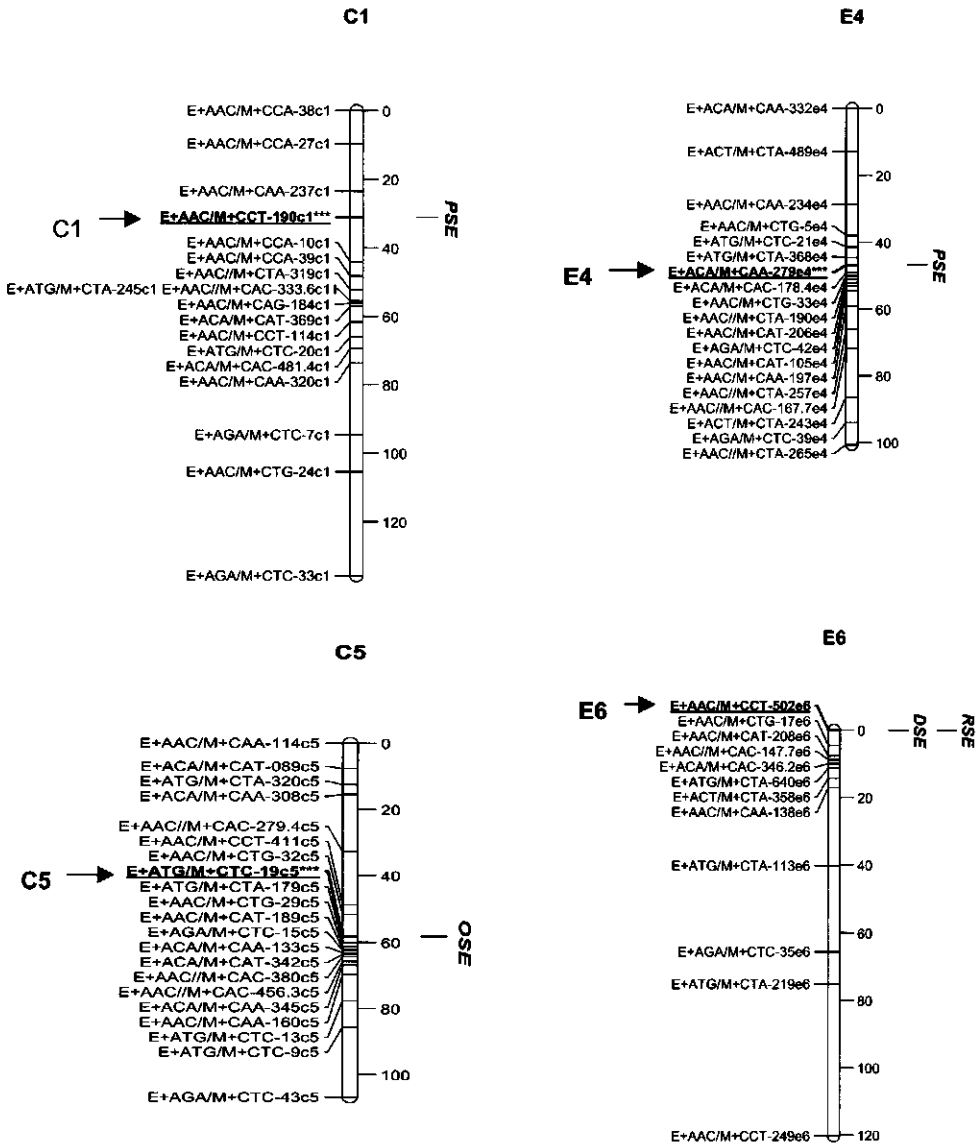


Figure 6. Continued

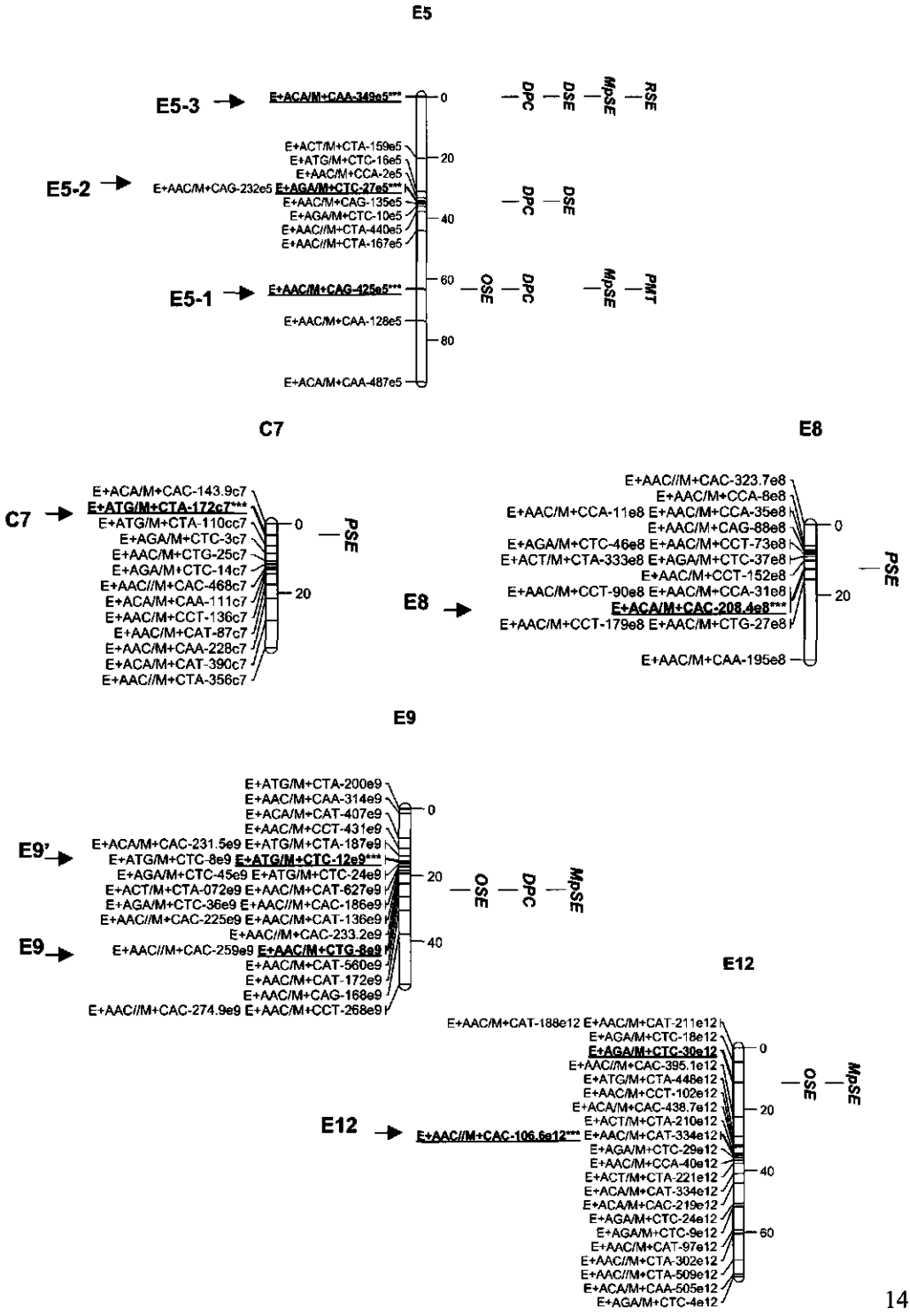


Table 5. Additive and epistatic effects of QTLs for the duration of the plant cycle (DPC), the onset (OSE), duration (DSE), mid point (MpSE), rate (RSE) of senescence and plant maturity type (PMT), estimated by linear regression models.

QTLs		Traits (population's mean)					
Name	Position cM	DPC[1]	OSE[1]	DSE[1]	MpSE[1]	RSE[2]	PMT[3]
		125.5	89.1	37.5	106.1	5.0	7.5
E5-1	30.5	- 23.17	- 13.80		- 14.54		2.7
E5-2	59.4	- 16.01		- 8.61			
E5-3	93.9	11.18		5.65	10.44	-10.20	
E6	0.0			7.15		-11.9	
E9	24.4	- 7.16	- 8.00		- 7.60		
E12	11.4		22.33		20.27		
E5-3 * E6						+14.9	
E5-1 * E12			- 13.2		-13.27		

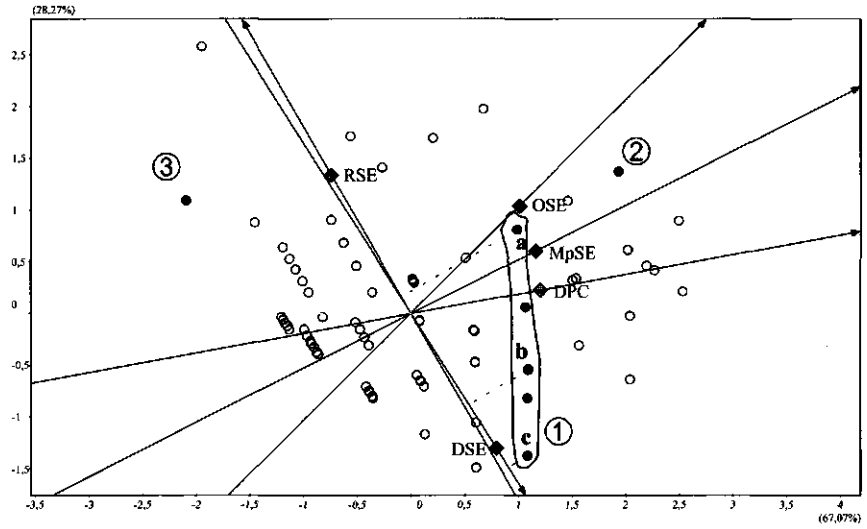
1 = in days; 2 = in $days^{-1}$; 3 = scale 2 to 10

For instance, in example 1, there are 5 clusters of genotypes, with a life cycle of 155 days. However, these clusters differed markedly in OSE, DSE, MpSE and RSE. For instance, the clusters a, b and c, had a DSE of 30, 59 and 80 days respectively. On the other hand, the cluster of genotypes in example 2, had a long life cycle, late MpSE, late OSE but shorter DSE and faster RSE than average. In contrast, the cluster of genotypes in example 3, had a short life cycle, an early OSE, and MpSE, a very short DSE and a very fast RSE.

Combined effects of E5-1, E5-2 and E5-3 on the duration of the plant cycle and the variables related to senescence

The three QTLs found on chromosome 5 were controlling DPC, OSE, DSE, MpSE

Figure 7. Biplot of the first two principal axes for the variables: duration of the plant cycle (DPC) and the onset (OSE), duration (DSE), mid point (MpSE), rate (RSE) of senescence. The intersection is equal to the mean for each one variable. To read off the values on the trait axes, perpendicular lines are drawn from the cluster of genotypes (circles) to the axes. The arrow indicates an increasing value above the mean. Each one of the numbers 1, 2 and 3 refers to examples for the variation found in the CxE population for these variables. In example 1, five clusters of CE genotypes (black dots) have a DPC of 155 days after planting, in the cluster a, b and c, a perpendicular to the DSE and RSE were drawn. Cluster c had the longest DSE and the slowest RSE, whereas cluster a had the shortest DSE and the fastest RSE among these 5 clusters.



and RSE. However, interestingly, the combined presence and/or absence of these three QTLs had a different effect on these variables (Table 6) than when each one was considered independently (Table 5).

For instance, when marker E5-3 was absent, plants with E5-1, E5-2 and E5-1+E5-2 had a life cycle of 140, 123 and 106 days, respectively (Table 6). On the other hand, when only marker E5-3 was present, DPC was the longest (155 days). However, with the combined presence of E5-2+E5-3, E5-1+E5-3 and all three markers present, DPC was reduced to 137, 127 and 111 days, respectively. Something similar was observed for DSE and MpSE (Table 7). Interestingly, when these three QTLs on chromosome E5 were considered together, the presence of marker on E5-2 caused a larger reduction in DPC than the presence of marker on E5-1, which was the opposite to what was

found when they were analyzed individually (Table 5). **Table 6**. Combined effect of QTLs E5-1, E5-2 and E5-3 on the average duration of the plant cycle (DPC) and the onset (OSE), duration (DSE), mid point (MpSE) and rate (RSE) of senescence and plant maturity type (PMT).

			DPC	OSE	DSE	MpSE	RSE	PMT
Marker alleles present		***E5-3***	**0**	**0**	**0**	**0**	**0**	**0**
	E5-1	***E5-2***						
	0	**0**	121.5	89.0	32.5	105.2	5.1	6.5
E5-2		**1**	123.1	88.5	34.63	104.9	4.9	6.9
E5-1	**1**	**0**	140.0	96.0	44.0	118.0	7.3	7.2
E5-1 + E5-2		**1**	105.6	74.3	31.29	89.0	4.2	9.6
			DPC	**OSE**	**DSE**	**MpSE**	**RSE**	**PMT**
		E5-3	**1**	**1**	**1**	**1**	**1**	
	E5-1	***E5-2***						
E5-3	**0**	**0**	154.7	109.0	46.7	129.8	6.3	5.7
E5-2 + E5-3		**1**	136.5	98.8	37.8	117.6	5.5	7.1
E5-1 + E5-3	**1**	**0**	126.9	83.5	43.4	105.2	6.4	7.5
E5-1 +E5-2 + E5-3		**1**	110.8	76.7	34.2	93.7	4.9	9.2

Nevertheless, when both markers on E5-1 and E5-2 were present, they were associated with the most drastic reduction in DPC (106 days), although followed very closely by the effect of the three QTLs on E5 together (111 days), meaning that in the presence of E5-1 and E5-2, the effect of E5-3 was almost null.

For PMT, when the marker on E5-1 was present, plants had a rather early maturity type (7.2), but when E5-2 was also present, plants had a much earlier maturity type (9.6). In contrast, when E5-3 was the only one marker QTL present, plants had a late maturity type (5.7), supporting the effect of these three QTLs on the duration of the plant cycle.

Influence of QTLs E5-1, E5-2, E5-3 and E12 on the duration of the plant cycle

OSE and MpSE have QTLs E5-1, E12 and their interaction in common, whereas DPC has in common with OSE and MpSE the three QTLs on chromosome E5 (Table 5, Figure 6). Since OSE and MpSE were highly correlated to DPC, it was interesting to

assess how these four QTLs were influencing DPC Table 7. Contrary to the estimated

Table 7. Combined effect of QTLs E5-1, E5-2, E5-3 and E12 on the average duration of the plant cycle (DPC) and the onset (OSE), duration (DSE), mid point (MpSE) and rate (RSE) of senescence.

				DPC	OSE	DSE	MpSE	RSE
			E12	0	0	0	0	0
QTLs present	***E5-1***	***E5-2***	***E5-3***					
	0	0	0	115.3	82.0	33.3	98.7	5.2
E5-3			1	148.9	96.0	54.4	116.8	7.2
E5-2		1	0	120.0	86.0	31.5	101.7	4.7
E5-2 +E5-3			1	125.5	92.5	33.0	109.0	4.4
E5-1	1	0	0	no plants with this marker combination				
E5-1+ E5-3			1	127.1	76.9	50.3	102.0	7.3
E5-1+ E5-2		1	0	105.7	75.11	30.6	90.4	4.4
E5-1+ E5-2 +E5-3			1	112.8	80.83	32.0	96.8	4.5
				DPC	**OSE**	**DSE**	**MpSE**	**RSE**
			E12	1	1	1	1	1
	E5-1	***E5-2***	***E5-3***					
E12	0	0	0	140.0	110.0	30.0	125.0	5.0
E5-3 + E12			1	158.6	118.2	42.8	137.4	5.8
E5-2 + E12		1	0	132.5	96.0	36.5	114.2	5.9
E5-2+ E5-3 + E12			1	140.2	100.8	39.3	120.5	5.9
E5-1+ E12	1	0	0	140.0	96.0	44.0	118.0	7.2
E5-1+ E5-3 +E12			1	126.7	88.7	38.0	107.7	5.9
E5-1+ E5-2 +E12		1	0	105.5	73.7	31.8	89.6	4.0
E5-1+ E5-2 + E5-3+E12			1	110.2	75.3	34.9	92.7	5.0

reducing effect of marker on E5-1 on DPC (23 days shorter), when the three QTLs on

chromosome E5 were analysed together with QTL E12, surprisingly, plants with marker E5-1 only had the longest DPC (140 days). However, from Table 7, we can see that in the CxE population, there were no plants having the marker on E5-1 without having the marker on E12. Furthermore, having E12 alone also gave an average DPC of 140 days, the same average DPC obtained with marker on E5-1 or markers on E5-1+ E5-12. Thus, this rather contradictory effect of E5-1 was in fact due to the presence of marker on E12. On the other hand, when E5-1, E5-3 and E12 were present together, the average DPC decreased to 127 days, although independently E5-3 and E12 had an extending effect on DSE. When markers on E5-1, E5-2 and E12 were present, a further reduction in DPC was observed (106 days). Interestingly, this reduction was the same as the one obtained when only E5-1 and E5-2 were present. Thus, it seems that there was no effect of E12 in this QTL combination. However, when all four QTLs were present, DPC was further reduced, but just 3 days shorter than when only E5-1, E5-2 and E5-3 were present (113 days). Thus, also in combination with E5-1, E5-2 and E5-3, the QTL E12 did not have an influence on DPC. These results indicate that indeed QTL E12 is not directly related to DPC and only when E5-1 is present alone, DPC is influenced by this QTL. These results further indicate that chromosome 5 harbors major genetic factors controlling the duration of the plant cycle.

Discussion

Map construction

We were able to obtain 12 highly stable linkage groups for each parent. The validity of the generated maps was confirmed upon mapping of tuber shape and flower color in the same chromosomes and similar locations as previously reported using 67 CE genotypes (Van Eck et al., 1994; Van Eck et al., 1993). However, we were not able to integrate the parental maps unambiguously given the low informativeness of dominantly scored 3:1 AFLP "bridge" markers.

Plant maturity type

Plant maturity type is not yet a very well described trait, which gives an overall "impression" of the advancement of the life cycle of the potato. Plant maturity type considers aspects such as yellowing of the foliage, flowering stage and the overall plant architecture ("bending knees"). Regardless of the ambiguity in the description and differences in the scale used to evaluate this trait, plant maturity type is rutinarely used and highly regarded for the evaluation of breeding material (Tai and Young, 1984; Lynch and Kosub, 1988; Joosten, 1991). The broad sense heritability for plant maturity type have been reported ranging from 0.17 to 0.65 for segregating

populations (Van Dam et al., 1999; Chen et al., 2001), whereas h^2 was ranging from 0.58 to 0.62 using a limited number of clones (Tai and Young, 1984; Lynch and Kosub, 1988). However, from the evaluation of plant maturity type in the CxE population, either using 67 genotypes (Jacobs et al., 1995) or using 250 CE genotypes (this study), h^2 was 0.87 and only one major QTL on chromosome 5 was found, confirming the results from Collins et al., 1999). The presence of the marker linked to this QTL was associated with a substantial increase on plant maturity type (earliness). Plant maturity type is evaluate once during the growing season, usually at the time when the mid-early variety Bintje is showing the first signs of senescence ("bending kness"). This evaluation usually takes place between 110 to 120 days after planting and under long day conditions. From the evaluation of the progress of senescence in the CxE population, we found that 110 days was the best date to discriminate between early and late maturing genotypes, confirming the criterion on the timing to assess this trait.

Variation in senescence pattern

Large differences in the timing of occurrence of the onset (duration of the "green period") and rate of progress of the senescence process were found between CE clones with very similar duration of the plant cycle. A delayed senescence is considered very important in increasing yield in annual crops, if a long plant cycle can be completed within the growing season. This has been shown in the studies related to "stay-green" variants in several species such as maize and sorghum (Smart et al. 1995).

Thomas and Smart (1993) identified two types of functionally "stay-green" plants. Type A is associated with alterations in genes involved in the onset of senescence, while Type B is associated with the regulation of the rate of senescence. In the CxE population, among genotypes with the same duration of the plant cycle, there were genotypes that began senescence earlier or later and genotypes that progressed in senescence faster or slower than on average. On the other hand, between genotypes that began senescence at the same time, there were large differences in the duration of the senescence process and in consequence, in the duration of the plant cycle. These results show that using a large population, segregating for the duration of the plant cycle, it is possible to identify genotypes with very particular features, including genotypes that could be considered functionally "stay-green" type of plants. The use of this strategy for the identification of contrasting genotypes could be very useful for the identification and characterization of senescence-associated genes, contributing towards a better understanding and possibly manipulation of the senescence syndrome for economical advantage.

Relation between senescence and other developmental processes

A major goal of senescence studies is the identification of major regulatory genes responsible for the overall control of the process (Chandlee, 2001). Al least 10 classes of senescence-associated genes (SAGs) have been distinguished, based on the temporal pattern of expression during leaf development. However, interestingly, 9 of these 10 SAG classes are also expressed during other plant developmental stages (Smart, 1994; Buchanan-Wollaston, 1997).

In the CxE population, a very large number of variables related to plant development and tuber formation were evaluated. Surprisingly, all of them were mapped on chromosome 5 and only after using the markers associated to QTLs E5-1, E5-2 and E5-3 as covariables, new QTLs related to these traits were found.

A diverse set of traits related to plant maturity and vigour (Jacobs et al., 1995; Collins et al., 1999), potato tuber yield and starch content (Schäfer-Pregl et al.,1998), carbohydrate metabolism and transport (Chen et al., 2001) and tuberization (Van der Berg et al., 1996; Simko et al., 1999) have been mapped on different regions of chromosome 5. Chen et al. (2001) mapped the Sut2 locus, a sucrose transporter-like gene, which was closely linked to QTLs for tuber starch content, tuber yield, plant maturity and vigour. They hypothesized that a sucrose sensor or sucrose transporter type of allele could be affecting the efficiency of translocation of sucrose from the leaves to the tubers, affecting in consequence all these traits.

It is tantalising to speculate that chromosome 5 harbors a major fraction of the genetic machinery responsible for the senescence syndrome in potato, having a pleiotropic effect on many other traits, given the surprising coincidental location on chromosome 5 of trait loci related to plant development, tuber formation, metabolism and transport, duration of the plant cycle and major components of the senescence process. These results also support the hypothesis on the evolutionary origin of the senescence syndrome, i.e. senescence is a developmental program that was likely pieced together from other developmental programs containing activities and functions necessary for senescence (Bleecker, 1998; Chandlee, 2001).

Complexity of senescence

Evidence on the complexity of the senescence process is given by the large variation found in the CxE population for the duration of the plant cycle and the onset, duration, mid point and rate of senescence. These results can be explained by the large array of possible alternatives that emerge from the six QTLs found associated with these variables on chromosomes 5, 6, 9 and 12.

The congruence of QTLs related to the duration of the plant cycle and components of the senescence process suggest a pleiotropism of genes. In quantitative genetics it is

assumed that trait correlation can be attributed to the effect of pleiotropy or to the tight linkage of genes. If pleiotropism was the major reason, the coincidence of both, the location of QTLs for related traits and the directions of their genetic effects can be expected. If the close linkage of genes was the major reason, the direction of the genetic effect of QTLs for different traits may be different, although the coincidence of the locations of QTL can still be expected . In our study, a general coincidence of traits related to senescence was observed. The marker associated to a QTL for DPC was also associated with one or more senescence components. Also, the "direction" of the effects of these QTLs was the same, regardless of the senescence component. These results suggest that pleiotropism rather than close linkage of different QTLs might be the major reason for the correlation found between related traits. However more detailed genetic analysis are required to confirm this observation.

The presence of 3 QTLs on chromosome 5 of potato is not unexpected. QTLs identified in the upper, middle and lower part of chromosome 5 have been associated to late blight (Sandbrink et al., 2000; Gebhard and Valkonen, 2001) and starch metabolism (Chen et al., 2001). Furthermore QTLs related to tuberization have been mapped on the upper (Simko et al., 1999) and middle (Van der Berg et al., 1996) region on chromosome 5.

The congruence of QTLs related to the components of the senescence process, supports the observations of Gan and Amasino (1997) on the likely existence of several genetic pathways that are interconnected to form a senescence regulatory network. This implies that elimination of the activity of one gene in the regulatory network, would have very limited effect on the overall progression of senescence.

Perhaps the most commonly used strategy to identify senescence-associated genes is through the comparison of transcript products isolated from senescing and green tissues (Lohman et al., 1994). However, one of the major limitations of this strategy is that it is not representing the natural process of senescence at the whole plant level (Buchanan-Wollaston, 1997). In the CxE population, we monitored the progress of senescence under field conditions. From the QTL analysis of the progress of senescence at each evaluation date, we were able to identify QTLs which were detected either at the beginning, at the end or during the course of life cycle of the CxE population. Interestingly, at the first evaluation dates (when only the short life cycle genotypes were already senescing), we were able to identify a QTL located on chromosome 7, whereas during the last evaluation dates, (when only very late genotypes were still senescing), we were able to identify three QTLs, located on chromosomes 1, 4 and 8. As expected, the three QTLs on chromosome E5 were the only ones detected in all, except the last evaluation date. The smooth changes in the profile of all the QTLs detected during the experiment, progressing from very low to a

maximum value and then decreasing or vice-versa is noteworthy.

Our results show that a strategy involving the evaluation of several components of a given process as well as the progress of the process in time, can be used to monitor complex processes like senescence on a massive scale, providing that there is an easy and highly related scorable marker available. Yellowing of the senescence leaves or whole plants is considered a good marker to monitor senescence and the degree of yellowing has been associated with changes in the number and intensity of SAGs' expression (Weaver et al., 1998). In this study, monitoring the progress of senescence based on the proportion of yellowing tissue present in the plant was a simple but effective way to detect QTLs and to monitor the temporal dynamics of their profiles. Our results show the potential use of this strategy to identify marker loci, as the first lead into chromosome regions involved in the progress of the process, speeding up the search for related genes.

To our knowledge, this is the first report where a highly diverse population, instead of extreme genotypes or mutants has been used to identify genetic factors related to major components of the senescence syndrome, as well as genetic factors which are detectable only at specific stages during the ontogeny of the process.

7

General discussion

The CxE population

In the Laboratory of Plant Breeding, Wageningen University, the CxE population has been widely used for the genetic study of different characteristics of the potato. Using a subset of 67 CE clones, the genetic basis of disynapsis and tuber flesh colour were elucidated (Jongedijk and Ramana, 1988). Furthermore, Jacobs (1995) constructed an RFLP map on which genes for anthocyanin pigmentation and tuber skin colour (Van Eck et al., 1994a), tuber shape (Van Eck et al., 1994b), flower colour (Van Eck et al., 1993) and plant maturity (Van Eck and Jacobsen, 1995) were mapped. Also, an AFLP map was constructed, consisting of 264 markers (Van Eck et al.,1995). In order to study more complex traits, with quantitative inheritance, the CxE population was enlarged to 250 genotypes. In addition, an improved AFLP map including the 250 genotypes and 403 AFLP markers was constructed (Celis-Gamboa et al., 2002e). Currently, besides the genetic analysis of tuber formation and plant development, the extended CE population is being used for the genetic analysis of cooking quality traits and physical properties of tuber starch. In addition, using the CxE population, the first transcript map of potato was constructed (Brugmans et al., 2002) and a metabolite map is being developed. All these efforts and strategies are focused towards a more comprehensive understanding of the potato genome, to make more effective use of the genetic diversity present in this crop.

We have now contributed to a more broad understanding of tuber formation and plant development in potato, merging crop physiological and genetic aspects.

Physiological ageing

From the vast number of studies on the physiological and agronomical aspects related to tuber formation in potato, it is widely accepted that photoperiod (length of the dark period), temperature, water supply, nitrogen supply and physiological age of the mother tuber are the most important factors influencing plant development and tuber formation (Ewing and Struik, 1992; O'Brien et al., 1998). For this study, the CxE population was planted under field conditions and to ensure that plants were growing under very favourable conditions, the experimental plot was fertilized properly and overhead irrigation was applied when needed, to avoid water stress. However, a large majority of the CE genotypes had very short or no dormancy and consequently, during storage of seed tubers until planting time, tubers sprouted. According to Krijthe (1962) and O'Brien et al. (1983), physiological ageing (physiological condition) of seed tubers is reflected by the degree of sprouting. Ewing and Struik (1992) and Struik and Wiersema (1999) have indicated that physiological ageing has a major effect on tuber

initiation, plant size, duration of the plant cycle, number of stems and tubers per plant. However, Firman et al. (1991) and O'Brien et al. (1998) showed evidence contradicting this conclusion. Since seed tubers from most of the CE genotypes had sprouts of variable length, it was important to assess the relationship between the degree of sprouting and the onset of tuberization, the duration of the plant cycle and several other plant and tuber-related characteristics. This study would give us an indication of how much of the variation observed in these traits was due to variation in sprouting (physiological ageing) of the seed tubers.

As expected, we found a strong correlation between the degree of dormancy and the length of the sprouts after storage, although it was clear that the low storage temperature drastically reduced the rate of sprout growth (Chapter 2). However, the length of the sprouts was not significantly related to the onset of stolon tip swelling, the first visual manifestation indicating that tuber initiation has begun. The same lack of strong correlation was found for the duration of the plant cycle, plant size and for many other variables considered.

Our results indicate that dormancy and physiological ageing do not have a determinant influence on the onset of tuberization, duration of the plant cycle and several other events taking place during the life cycle. This implies that the large variation in the timing of occurrence of events such as stolon formation, stolon branching, tuber initiation, tuber growth, flowering and senescence observed in the CxE population was due to other factors, likely related to genetic differences. Furthermore, the large array of alternatives found in the CxE population in the onset of tuberization, duration of the plant cycle and degree of dormancy, led us to conclude that these traits are probably controlled by different genetic factors.

Effect of tuber initiation on plant development

Since the reports by Ivins (1963), Ivins and Bremner (1965) and Bremner and Radley (1966), on the determinant influence of tuber initiation on plant development, this association has been widely accepted in potato research. This apparently profound influence of tuber initiation on plant development has been reviewed by Vreugdenhil and Struik (1989) and Ewing and Struik (1992). Also, O'Brien et al. (1998) indicated that the acceptance of tuber initiation as a key event in the potato life cycle has greatly influenced research on growth and plant development. However, they indicated that in several studies supporting this view, tuber initiation was not recorded or was estimated rather than monitored directly. There were also differences in the criteria used to establish the onset of tuber initiation. Furthermore, in many cases this conclusion was reached from growth chamber experiments.

We evaluated several components of plant development such as plant size, duration

of flowering and senescence periods and the duration of the plant cycle. In addition, we monitored several events related to tuber formation, such as onset of stolon tip swelling and number of tuber incipients (<12 mm Ø). However, we could not find the strong correlation between tuber initiation and plant development, either at the whole population level (Chapter 3) or between groups of genotypes with the same duration of the plant cycle (Chapter 4). For instance, at the population level, there were genotypes that had a very long life cycle and yet they had the first tuber incipients very early in the plant cycle. Furthermore, within groups of genotypes with very similar duration of the plant cycle, onset of tuber formation was very variable among genotypes. In addition, the control varieties began tuber initiation at the same time and yet, very large differences in the duration of the plant cycle were observed. We concluded that tuber initiation should be regarded as one of several developmental steps taking place during the potato life cycle, playing a crucial role in the establishment of the population of growing tubers, but not the only one doing so.

Duration of tuber initiation

The duration of tuber initiation is another aspect on which there are differences of opinion in literature. Jefferies and Lawson (1991) and O'Brien et al. (1998) concluded that tuber initiation takes place during a very limited period of time (2 - 3 weeks). O'Brien et al. (1998) further indicated that in field experiments, the duration of this event is much shorter than in commercial fields because plant emergence is variable in practice. In Chapters 3 and 4, we present the changes in the number of swelling stolon tips, tuber incipients and growing tubers during the progress of the experiment and it is clear that tuber initiation took place during a longer period than expected. Although it can be argued that these results are a reflection of the unselected nature of the CxE population, also in the control varieties, a variable and relatively long period during with tuber incipients were formed, was observed. These results support the observations by Clark (1921) and Sale (1976), who found that tuber incipients were formed during most of the plant cycle. If synchronicity of plant emergence would have such an influence on the duration of tuber initiation, regardless of genotype (or varietal) differences, controlling tuber size distribution and tuber number would be easier.

Tuber number and size distribution

It is not surprising that an impressive amount of attention has been given to the factors influencing the number and size distribution of tubers at harvest. Tuber size distribution is a key factor in potato production, since tubers of specific size are required by the seed industry, fresh market and processing factories. Several studies on

the influence of water stress, air and soil temperature, pathogen infection, number of primary stolons, tuber resorption, seed size, spacing and other agronomical practices have been reported (e.g., Burton, 1989). However, in relation to the dynamics of the number of stolons, tuber incipients and growing tubers, as well as on the influence of stolon branching on tuber number and size distribution, during the life cycle of the potato plant, available information is focused on one or a very few of these variables. There were no studies yet where all these variables were monitored together, on a large number of genotypes and, even less, under field conditions.

In Chapter 3 we found that the number of tuber incipients increased drastically after stolon branching began. We also found that tuber resorption had a very limited influence on final tuber number. We concluded that final tuber number and size distribution are greatly influenced by the degree of stolon branching and the duration of the stolon tip swelling period. Also, the duration of the plant cycle had a determinant influence on tuber number and size distribution (Chapter 4).

Overlapping of developmental events

Vreugdenhil and Struik (1989) suggested that variability in tuber size distribution could be due to the lack of synchronicity of tuberization. A universal phenomenon in the potato is the simultaneous presence of newly formed stolons, swelling stolon tips, tuber incipients and growing tubers of diverse sizes, simultaneously on each plant. A question that arises from this phenomenon is for how long this overlapping takes place and if there are differences between genotypes in the duration of this overlap. This information could prove useful for the understanding of the physiological and genetic basis of tuber formation.

We found very distinct differences in the time to the onset, end and duration of stolon formation and stolon tip swelling. Differences were also found in the time to the onset of stolon branching and tuber resorption (Chapter 3). Differences in the timing of these events were reflected in the duration of the overlap between events, like between the formation of new stolons, stolon branching and formation of tuber incipients, overlap clearly shown from the comparison between groups of genotypes differing in the duration of the plant cycle (Chapter 4, Figures 1 and 2).

Validity of the data

At this point, it is relevant to address the question whether the data collected to determine the onset and end points of major morphological events such as stolon formation, stolon tip swelling, tuber initiation, flowering and senescence were valid. Since we were interested in recording as many variables as possible, the evaluations were destructive. At each evaluation date we were evaluating a new set of plants.

Interestingly, when a morphological event such as stolon formation was observed for the first time, consistently we kept observing new stolons until a date when for the first time, newly formed stolons were not present on the set of plants being evaluated. From this date, in most of the cases, no more new stolons were observed. A very consistent pattern was also observed for other events such as the senescence process (Chapter 6), giving us the confidence that it was possible to reliably assess about the onset and end of morphological events taking place during the life cycle of individual genotypes. Furthermore, in the year 2000, a small set of CE genotypes with the shortest and longest life cycle were planted under very similar conditions like in 1999, and the onset of stolon tip swelling was evaluated at intervals of 2 - 3 days. Without exception, the onset of stolon tip swelling took place very similarly like in 1999, indicating that under very similar conditions, the timing of this event is quite reproducible.

Chronological order of developmental events

We found very distinct differences in the chronological order of events related to stolon formation, stolon tip swelling, stolon branching, flowering and senescence, between groups of genotypes differing in the duration of the plant cycle (Chapter 4) or between individual genotypes (Chapter 5). Differences found between the CxE population and control varieties in the onset, duration and chronological order of events leading to harvestable tubers, clearly reflect how during the selection process, the temporal occurrence of these events is being tailored (indirectly) to meet specific needs (Chapters 3, 4 and 5). For instance, in the CxE population, there was a large variation in the order at which events related to plant development and tuber formation began and ended (Figure 1, this Chapter). For the varieties, on the other hand, the onset of events related to plant development and tuber formation was very similar, despite large differences in the duration of the plant cycle. However more differences were observed between varieties for the end of these events. These results suggest that for the varieties an early onset and a certain order of these events is more related to yield and quality attributes than the ending time and order of these events, which during the selection process was being indirectly tailored by the breeder.

The observed variation in the timing of occurrence to the onset, end and duration of plant developmental and tuber formation events, is likely to be associated with the well known range of adaptation of the potato. Potato is grown in altitudes ranging from 0 to more than 4000 meters above see level, making potato the most widely grown crop in the world (Scott, 2000). The remarkable plasticity of potato plants to respond to a wide range of environmental conditions, has been well documented through the monitoring

of the dramatic changes in plant morphology, following exposure of single genotypes to different light and temperature regimens (Steward et al., 1981). This plasticity was also reflected in our study, by the large variation found in the CxE population in the temporal occurrence of morphological events taking place during the plant cycle. This plasticity reflects the complexity of physiological and genetic aspects, underlying tuber formation and the life cycle of the potato plant. It can be speculated that several genes controlling developmental events are affected differently by the same environmental conditions, or alternatively, that blocks of genes are triggered by these external stimuli producing in consequence particular patterns of development.

In the past, physiologists and molecular biologists were trying independently to understand the nature of complex processes such as flowering and senescence, but these efforts had relatively limited success. More recently, an increasing number of studies, in which the physiological events and the genetic factors controlling them are analyzed together, have been reported (Koornneef et al., 1991; Koornneef et al., 1997). In most cases, these studies involve the analysis of one individual genotype, either a mutant, a transgenic plant or a variety. However, frequently, the limited availability of plant material imposes an important limitation to the rate of progress on the understanding of complex traits such as senescence (Chandlee, 2001). Recently, more studies in which populations of plant species were used for physiological and genetic analysis of complex traits, have been presented (Jamaux et al., 1997; Ellis et al., 1997). Interestingly, studies of this type, under field conditions, are not yet available in potato. We studied in the CxE population, the onset, duration and rate of the senescence syndrome (Chapter 6). Within groups of genotypes with similar duration of the plant cycle, we found a large variation in the duration and rate of this process. Some of these genotypes could be considered as functionally "stay-green" (Thomas and Smart, 1993). Furthermore, we were able to identify QTLs associated with the different components of the senescence process, as well as QTLs that were detected only at specific points in time during the progress of senescence of the CxE population. These results show that large and highly diverse populations can be used to dissect major processes taking place during the life cycle of the plants. This strategy can give an indication of the number of QTLs and time at which these genetic factors are detectable, which could be very useful later on, in expression studies. We will use this strategy to detect genetic factors associated to stolon formation, stolon tip swelling and flowering. The amount of data available on so many aspects about the potato plant is now available to explore the physiological and genetic aspects of each one of them. We hope to be able to find a relationship between correlated traits and the genetic factors controlling them.

A joint venture of plant physiologists and molecular biologists to study and unravel the complexity of major processes in plants will be the most efficient and fruitful strategy to understand and manipulate these processes for agricultural advantage.

Figure 1. Differences in the chronological order and the proportion of the plant cycle at which, events related of plant developmental and tuber formation took place between CE genotypes grouped according to the duration of the plant cycle.

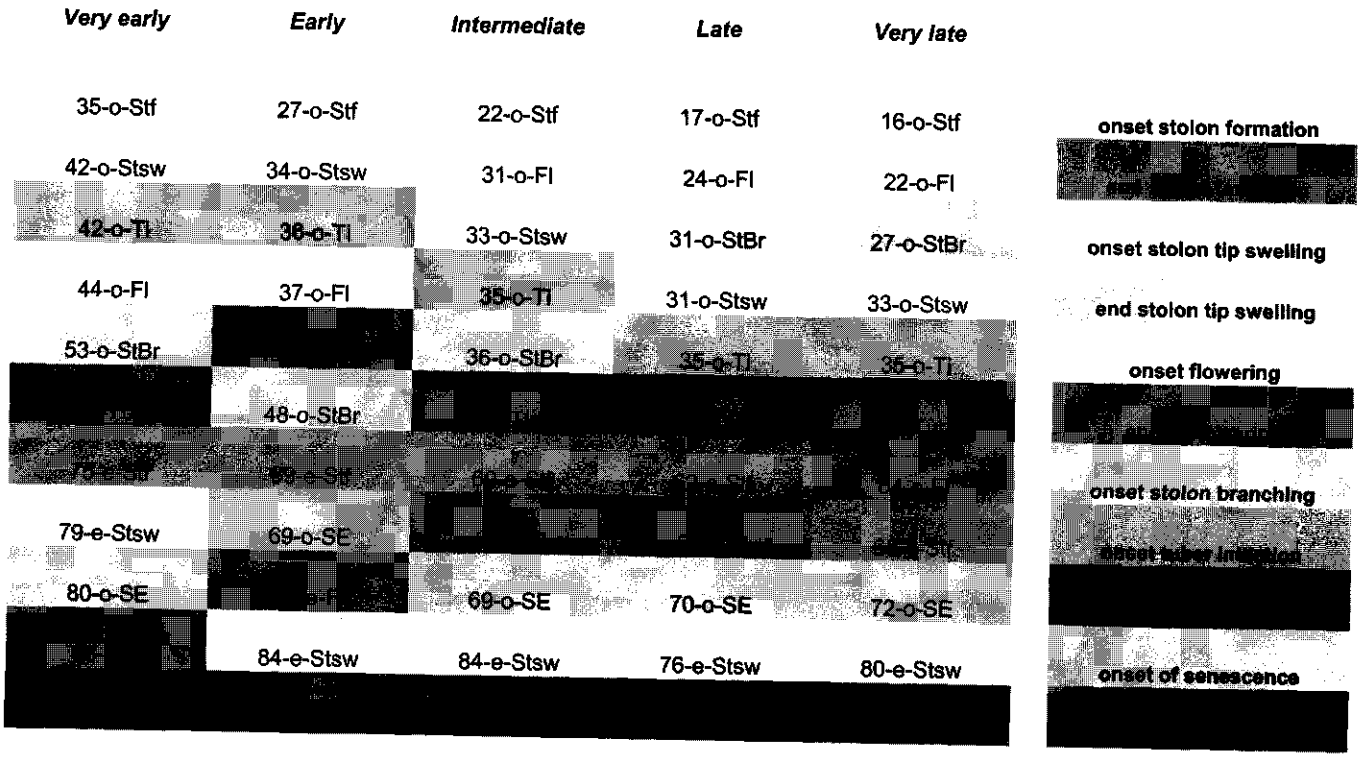
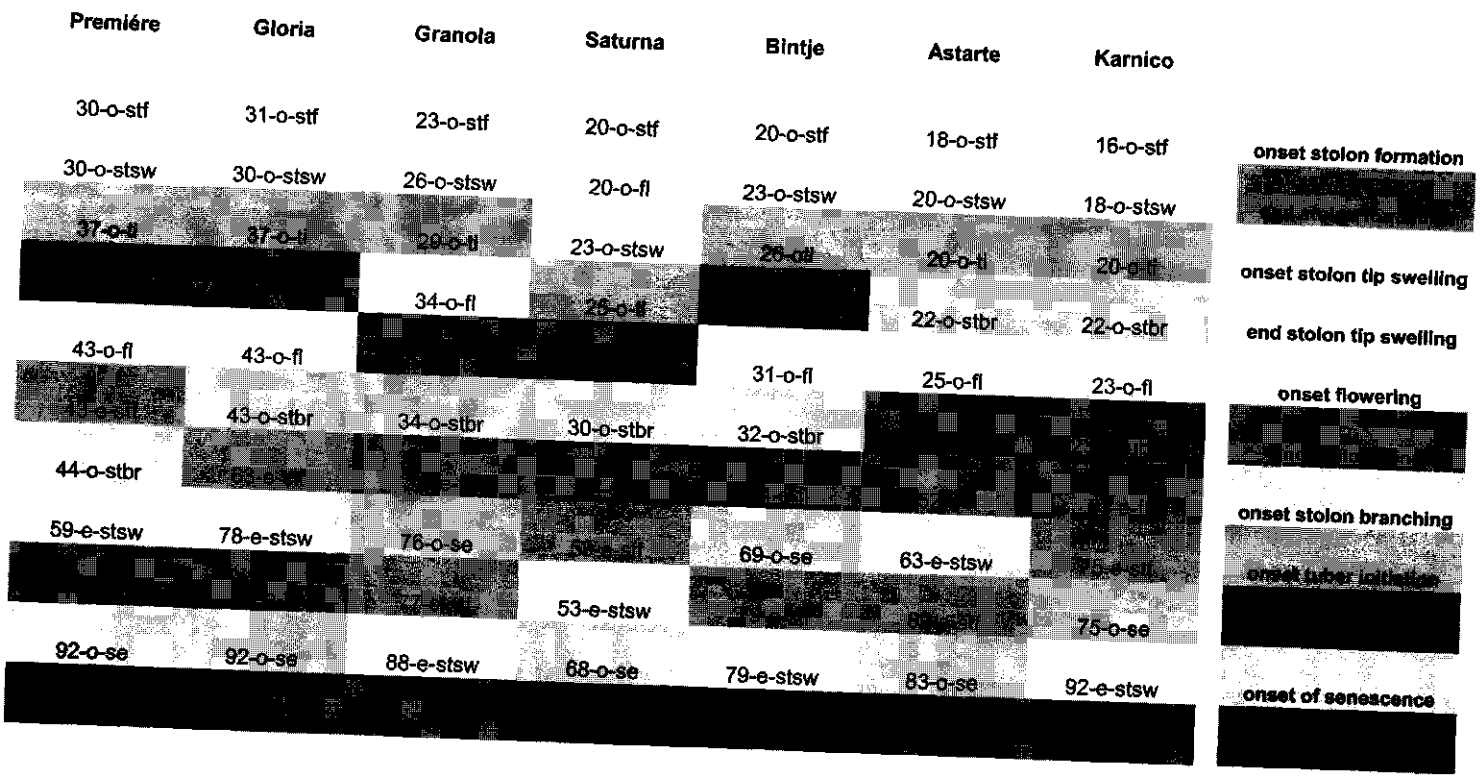


Figure 2. Differences in the chronological order and the proportion of the plant cycle at which events related of plant development and tuber formation took place between control varieties.



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Summary

Summary

This thesis describes the results of an analysis of the major developmental events taking place during the life cycle of potato, based on records from 250 genotypes, from the highly diverse CE population. The aim was to generate a massive amount of data to built a framework of crop physiological relationships, and use the data set to begin with the identification of genetic factors controlling major developmental processes like senescence.

Physiological ageing of seed tubers during storage influences tuber initiation and plant development. Since the majority of the CxE population had short or no tuber dormancy, seed tubers sprouted during storage. Because physiological ageing is associated with the intensity of sprouting during storage and with tuber formation processes after planting, it was necessary to investigate the association between sprout length, as an indicator of physiological ageing, and the timing of occurrence of events related to tuber formation and the duration of the plant cycle. Among genotypes of the CxE population, sprout length at the end of the storage period, was not significantly related to tuber initiation or other events related to tuber formation. Associations were also not found between sprout length and the duration of the plant cycle, plant size and several other variables recorded. We conclude that the variation in sprouting of seed tubers was not the main factor responsible for the large variation observed in the CxE population in the timing of occurrence of tuber formation and plant developmental events.

In order to identify general temporal relationships between stolon formation, stolon branching, stolon tip swelling, tuber initiation, tuber growth, flowering and senescence, the extensive data on plant development were analysed at the population level. As expected, the onset of stolon formation, swelling of the stolon tips and tuber initiation took place sequentially. Despite the large variation found in the CxE population and control varieties in the duration of the plant cycle, all began to form stolons and to flower within a remarkably short period of time. Results strongly suggest that the onset of stolon formation and flowering is not necessarily triggered by an environmental stimulus. The peak production of stolons and swelling stolon tips took place during flowering. However, while in most genotypes, stolon formation was confined to the flowering period, stolon tip swelling proceeded beyond the end of flowering and ended during the senescence period. The number and size distribution of tubers were largely influenced by the degree of stolon branching and the length of the stolon swelling period, and apparently to a smaller extent by the resorption of tubers. Tuber initiation did not have a determinant influence on the timing of stolon formation, stolon tip swelling, flowering and duration of the plant cycle, contradicting extensive literature supporting the determinant influence of tuber initiation on plant development.

Differences in the timing of events related to plant development and tuber formation were further analysed after grouping the CxE population according to the duration of the plant cycle. Genotypes with life cycles shorter than 90 days had a small plant size, a short period of stolon formation, stolon tip swelling and flowering, early tuber initiation, very little or no stolon branching and fast progress of canopy senescence. In contrast, genotypes with life cycles longer than 170 days, had a large plant size, profuse and long flowering period, large production of stolons during most of the plant cycle, profuse stolon branching, late but long period of stolon tip swelling and subsequent tuber initiation and slow progress of canopy senescence. Differences were also found between groups of genotypes in the chronological order at which tuber formation and plant developmental events took place during the plant cycle. Our results show that although in every potato plant there is a period when there is a simultaneous presence of newly formed stolons, swelling stolon tips, tuber incipients and growing tubers, there was no chaos, but a well-orchestrated order influenced by the duration of the plant cycle.

In order to get more information on the complexity and plasticity in the timing of occurrence of events taking place during the life cycle of a potato plant, the two most contrasting genotypes found in each of the five groups of genotypes (clustered according to the duration of the plant cycle) were compared in great detail. Large differences were found between these selected genotypes in the proportion of the plant cycle after which events related to stolon formation, stolon branching, stolon tip swelling, tuber growth, flowering and senescence took place. It was not possible to find a clear association between the relative timing of occurrence of these events and tuber yield at the end of the plant cycle. However, for the varieties, a long period of stolon formation, stolon tip swelling and tuber growth, a relatively short flowering, a long senescence process and early stolon branching appeared to be favourable for high tuber yield. The large variation found in the CE genotypes and varieties in the order of occurrence of morphological events related to plant development and tuber formation demonstrates the remarkable plasticity present in potato and the influence of the selection process during the developing of varieties on the timing of events leading to high yields.

Very high correlations were found between tuber formation and plant developmental events and the duration of the plant cycle (end of the senescence process). In addition, very large differences were found in the CxE population in the onset, duration, mid point and rate of the senescence process. We decided to investigate the genetic factors underlying these variables, describing the process of senescence in potato. We found between two to four QTLs associated with each one of these senescence variables,

located on chromosomes 5, 6, 9 and 12 of the male (E) map. Each one of these QTLs was found in common to at least two of these senescence variables. The effect of these QTLs on different senescence variables was in the same direction, suggesting pleiotropic rather than close linkage interactions between genetic factors. From the QTL analysis of the progress of senescence (amount of yellowing of the plant canopy) during the life cycle of the CxE population, new QTLs were identified, which were present either at the beginning, at the end or during most of the senescence process.
Our results show that the evaluation of variables describing complex traits during the phenology of a large mapping population, can be a powerful strategy to describe a process at both physiological and genetic levels.

Samenvatting

De belangrijkste stappen in de ontwikkeling tijdens de levenscyclus van de aardappel zijn onderzocht en beschreven in dit proefschrift. Het doel van dit onderzoek was het in kaart brengen van de gewasfysiologische variatie en van de genetische factoren die de fysiologische eigenschappen bepalen. Daarvoor is een uitgebreide set van gewasfysiologische data gebruikt, die werd verkregen door middel van gedetailleerde analyse van 250 genotypen van de zeer diverse C x E populatie. De eigenschap veroudering werd als model uitgekozen voor de koppeling tussen gewasfysiologische en genetische data.

Fysiologische veroudering van aardappelpootgoed tijdens bewaring beïnvloedt de latere plantontwikkeling en knolaanleg. Aangezien de meeste genotypen van de CxE populatie geen of slechts een zeer korte kiemrust kenden, waren de meeste knollen van de CxE populatie reeds begonnen te spruiten tijdens de bewaring. Fysiologische veroudering wordt geassocieerd met de intensiteit van spruitvorming tijdens bewaring en met knolvormingsprocessen na planten. Daarom was het noodzakelijk de associatie tussen lengte van de spruit en het tijdstip waarop de processen die gerelateerd zijn aan knolvorming vast te stellen, alsmede de lengte van de levensduur van de plant te bepalen. Voor de genotypen van de CxE populatie was geen significante relatie te vinden tussen lengte van de spruit aan het eind van de bewaarperiode en knolinitiatie of andere processen die verband houden met knolvorming. Er was ook geen sprake van associatie tussen lengte van de spruit en de lengte van de levenscyclus van de plant of hoogte van de plant. De conclusie lijkt daarom gerechtvaardigd dat de variatie in de het spruiten van pootgoed van de CxE populatie niet de hoofdreden was voor de enorme variatie in tijdstip en mate van knolvorming en andere ontwikkelingsgerelateerde eigenschappen in de CxE populatie.

Teneinde algemene, tijdgerelateerde relaties tussen stoloonvorming, stoloonvertakking, stoloonzwelling, knolaanleg, knolgroei, bloei en plantveroudering te kunnen vast stellen werd de omvangrijke dataset betreffende plantontwikkeling op populatieniveau geanalyseerd. Zoals verwacht kon worden, vonden begin van stoloonvorming, zwelling van de stolonen en knolaanleg na elkaar plaats. Ondanks de grote variatie in de lengte van de levenscyclus die niet alleen werd vastgesteld in de CxE populatie maar ook in zeven controlerassen, begonnen alle genotypen stolonen te vormen en te bloeien binnen een zeer korte periode. De resultaten wekken sterk de indruk dat stoloonvorming en bloei niet door milieustimuli geïnduceerd worden. De piekproductie van stolonen en van zwellingen aan de stolonen vond plaats tijdens de bloei. Terwijl in de meeste genotypen de stoloonvorming alleen plaats vond tijdens de bloeiperiode ging stoloonzwelling door, ook nadat de bloei was geëindigd, tot ver in

de verouderingsfase van de planten. Het aantal knollen en de knolgrootteverdeling werden sterk beïnvloed door de de mate van stoloon- vertakking en de duur van de stoloonzwellingsperiode en in veel mindere mate door de resorptie van knollen. In tegenstelling tot wat in de zeer uitgebreide literatuur beweerd wordt, bleek knolinitiatie geen bepalende invloed te hebben op plantontwikkeling.

Verschillen in de tijdstippen waarop de processen plaats vinden die van belang zijn voor plant-ontwikkeling en knolvorming werden verder geanalyseerd door de CxE populatie te verdelen in vijf groepen al naar gelang de duur van de levenscyclus van de planten. Planten (genotypen) met een levenscyclus korter dan 90 dagen waren over het algemeen klein, hadden een korte periode waarin stolonen werden gevormd en zwelden en vertoonden weinig of geen stoloonvertakking, vroege knolinductie, een korte bloeiperiode en een snelle veroudering van het bladerdek. Daarentegen waren planten met een levenscyclus van meer dan 170 dagen over het algemeen groot, met een grote productie van stolonen gedurende het grootste deel van de levenscyclus en uitbundige stoloonvertakking. Verder werden deze planten gekenmerkt door een late maar wel langdurige periode van stoloonzwelling en dus knolinductie, uitbundige bloei en een lange bloeiperiode en een langzame veroudering van het bladerdek. Er werden ook opmerkelijke verschillen vastgesteld tussen groepen van planten voor de chronologische volgorde waarin zich knolvorming en andere plant gerelateerde ontwikkelingsprocessen voltrokken tijdens de levenscyclus. De resultaten laten zien dat alhoewel er in elke aardappelplant een periode bestaat waarin tegelijkertijd nieuw gevormde stolonen, zwellende stolonen, beginnende knollen en groeiende knollen aanwezig zijn, dit niet het resultaat is van een chaotisch proces, maar van een nauw georkestreerde volgorde die bepaald wordt door de lengte van de levenscyclus van de plant.

Om meer informatie te verkrijgen over de complexiteit en plasticiteit van de verschillende gebeurtenissen tijdens de levenscyclus van de plant, werden de twee meest contrasterende genotypen in elk van de vijf groepen nader met elkaar vergeleken. Grote verschillen werden gevonden tussen de geselecteerde genotypen in het percentage van de levenscyclus waarop gebeurtenissen plaats vonden die gerelateerd zijn aan stoloonvorming, stoloonvertakking, stoloonzwelling, knolgroei, bloei en veroudering. Het bleek niet mogelijk om een duidelijke associatie te vinden tussen deze gebeurtenissen en knolopbrengst aan het einde van de levenscyclus. Voor de zeven rassen gold dat een lange periode van stoloonvorming, stoloonzwelling en knolgroei, vroege stoloonvertakking, een relatief korte bloei periode en een lang verouderingsproces optimaal waren voor een hoge knolopbrengst. De grote variatie die

gevonden werd in de CxE populatie en de rassen in de volgorde waarin zich de verschillende processen, gerelateerd aan plant-ontwikkeling en knolvorming, voltrokken, toont de grote plasticiteit van het gewas aardappel. Het laat ook zien wat de invloed van het selectieproces is op de tijdstippen van de verschillende gebeurtenissen die van belang zijn in de plantontwikkeling om te komen tot de hoog opbrengende rassen.

Teneinde de gewasfysiologische resultaten in een genetisch kader te kunnen plaatsen werd de levenscyclus van de plant genomen als modeleigenschap. Er werden zeer sterke correlaties gevonden tussen knolvorming en plantontwikkelingsgerelateerde processen en de duur van de levenscyclus (ofwel het einde van het verouderingsproces). Grote verschillen werden ook gevonden in de CxE populatie voor het begin, de duur, het midden en de snelheid van het verouderingsproces. De genetische factoren die ten grondslag liggen aan deze eigenschappen werden derhalve bepaald. Er werden tussen de twee en vier QTLs gevonden voor elk van deze verouderingsvariabelen op respectievelijk koppelingsgroepen 5, 6, 9 en 12 van de mannelijke (E) kaart. Elk van deze QTLs was geco-lokaliseerd met één of meerdere van de verouderingsvariabelen. Het effect van de QTLs van de verschillende variabelen was in dezelfde richting. Of de veroudering werd versneld of verliep juist langzamer. Door de tijdgerelateerde QTL-analyse konden verschillende QTLs geïdentificeerd worden die of aan het begin, of aan het einde of gedurende het grootste deel van het verouderingsproces aanwezig waren. Dit proefschrift laat zien dat het uiteenrafelen en evalueren van de verschillende componenten van complexe fenotypische eigenschappen in een grote splitsende populatie een zeer krachtige strategie is om processen op zowel fysiologisch als genetisch gebied beter te leren begrijpen.

Resumen

Resumen

Esta tesis describe los resultados obtenidos luego del análisis de los eventos principales que tuvieron lugar durante el ciclo de vida de 250 genotipos de la altamente diversa población de papa CxE. El objetivo fue la generación de una masiva cantidad de datos para la contrucción de un marco de relaciones fisiológicas para iniciar la identificación de los factores genéticos controlando procesos principales tales como la senescencia.

El envejecimiento fisiológico de los tubérculos-semilla durante el almacenamiento ejerce una influencia sobre la iniciación de la tuberización y el desarrollo de la planta de papa. Puesto que la mayoría de los genotipos de la población CxE tienen muy poca o ningúna dormáncia, los tubérculos-semilla brotaron durante el almacenamiento. Debido a la asociación entre envejecimiento fisiológico, la intensidad del brotamiento durante el almacenamiento y el proceso de formación de los tubérculos luego de la siembra, fue necesario investigar ésta asociación.

En la población CxE, la longitud de los brotes no fué significativamente relacionada con la iniciación de la tuberización ú otros eventos relacionados con este proceso. No se encontró una fuerte asociación entre la longitud de los brotes y la duración del ciclo de vida, el tamaño de las plantas o cualquiera otra de las muchas otras variables estudiadas. Estos resultados llevan a concluir que la variación en longitud de los brotes de los tubérculos-semilla no fué el factor principal, responsable de la gran diversidad observada en la población CxE en el momento de ocurrencia de eventos relacionados con la formación de los tubérculos y el desarrollo de las plantas.

A fin de identificar relaciones temporales entre la formación, ramificación y engrosamiento del ápice de los estolones, la iniciación de la tuberización, el crecimiento de los tubérculos, la floración y la senescencia, la masiva cantidad de datos disponibles sobre el desarrollo de las plantas fue analizada a nivel de población. Como se esperaba, el inicio de la formación de estolones, el engrosamiento del ápice de los estolones y la iniciación de la tuberización tuvo lugar secuencialmente. A pesar de la gran variación observada en la duración del ciclo de vida en la población CxE y variedades de referencia, todas comenzaron a formar estolones y a florecer dentro un sorprendentemente corto período de tiempo. Estos resultados fuertemente sugieren que la iniciación de la estolonización y floración no son necesariamente inducidos por un estímulo ambiental. El pico de producción de estolones y ápices engrosados tuvo lugar durante el período de floración. Sin embargo, mientras que en la mayoría de los genotipos CE, la formación de estolones estuvo confinada al proceso de floración, el engrosamiento de los ápices de los estolones (la primera evidencia visual del proceso de tuberización) continuó mucho más allá de la floración, finalizando durante la senescencia de la planta. El número y distribución del tamaño de los tubérculos de

papa estuvo largamente influenciado por el grado de ramificación de los estolones y la duración del período de engrosamiento de los ápices y, aparentemente en mucha menor medida por la reabsorción de tubérculos. La iniciación de la tuberización no ejerció una influencia determinante sobre el momento durante el cual la iniciación de la formación de estolones, engrosamiento de los ápices de los estolones y la foración tuvieron lugar, así como sobre la duración del ciclo de vida, hechos que contradicen la extensiva literatura científica apoyando la existencia de una influencia determinante por parte de la iniciación de la tuberización sobre el desarrollo de la planta.

Diferencias en el momento de ocurréncia de eventos relacionados con el desarrollo de la planta de papa y la formación de tubérculos fuéron analizados detalladamente luego de la clasificación de la población CxE en cinco grupos, de acuerdo a la duración del ciclo de vida. Genotipos con un ciclo de vida menor de 90 días, tuvieron un tamaño mucho menor, un período más corto de formación de estolones, engrosamiento de ápices y floración; una tuberización mas temprana, muy poca o ningúna ramificación de los estolones y una más rápida senescencia del follaje, que genotipos con un ciclo de vida mayor de 170 días. Contrastando las características de los genotipos de ciclo corto, los genotipos de ciclo largo, fuéron plantas de gran tamaño, con un período de profusa producción de flores, una gran producción de estolones durante la mayor parte del ciclo de vida, profusa ramificación de los estolones, tardía pero prolongada producción de ápices engrosados y consecuentemente un prolongado início de la tuberización y, finalmente un lento proceso de senescencia. Entre éstos grupos de genotipos, también fueron encontradas diferencias en el orden cronológico en cual eventos relacionados con la formación de los tubérculos y desarrollo de la planta tuvieron lugar durante el ciclo de vida.

Nuestros resultados demuestran que aunque en cada planta de papa, hay un período donde la producción de nuevos estolones, el engrosamiento del ápice de los mismos, y la iniciación y crecimiento de tubérculos tienen lugar simultáneamente, ésta coincidencia no es caótica, sino al contrario, donde existe un order muy bien orquestrado, influenciado por la duración del ciclo de vida.

A fin de obtener mas información sobre la complejidad y plasticidad en el momento de ocurréncia de los eventos que tienen lugar durante el ciclo de vida de la papa, los dos genotipos mas contrastantes, identificados en cada uno de los 5 grupos obtenidos, fueron comparados mas detalladamente. Grandes diferencias fueron encontradas, entre los genotipos seleccionados, en la proporción del ciclo de vida durante cual eventos relacionados con la formación y ramificación de los estolones, engrosamiento de los ápices, crecimiento de los tubérculos, floración y senescencia tuvieron lugar.

No pudimos encontrar una clara asociación entre la ocurrencia relativa de todos estos eventos y el rendimiento al final del ciclo de vida. Sin embargo, para las

variedades evaluadas parece ser que las condiciones mas favorables para un alto rendimiento estan relacionadas con un período prolongado de formación de estolones, engrosamiento de los ápices y crecimiento de los tubérculos, un período relativamente corto de floración, un prolongado proceso de senescencia y una ramificación temprana de los estolones.

La gran variación encontrada enthre los genotipos CE y las variedades en el orden de ocurrencia de eventos morfológicos relacionados con el desarrollo de la planta y la formación de tubérculos, demuestra la sorprendente plasticidad presente en el cultivo y el efecto que ejerce el proceso de mejoramiento, durante el desarrollo de nuevas variedades, sobre el momento durante el cual eventos que conllevan a un alto rendimiento tienen lugar.

Muy altas correlaciones fueron encontradas entre todos los eventos relacionados con los tubérculos y desarrollo de la planta y la duración del ciclo de vida (fin del proceso de senescencia). Ademas, grandes diferencias fueron encontradas en la población CxE en el inicio, duración, punto medio y velocidad del proceso de senescencia. Por esta razon decidimos investigar los factores genéticos controlando estas variables.

Encontramos entre 2 y 4 QTLs (genes de carácter quantitativo) asociados con cada una de estas variables. Localizados en los cromosomas 5, 6, 9 y 12 del mapa genético del clon paterno (E). Cada uno de éstos QTLs fué encontrado en común con al menos 2 de estas variables. Puesto que el efecto de éstos QTLs sobre las diferentes variables de senescencia fueron en en mismo sentido (positivos o negativos), suguiere la existencia de una relación pleiotrópica y no de linkage entre estos factors genéticos. Como resultado del análisis genético quantitatívo del progreso de la senescencia (grado de amarillamiento del follage) durante el ciclo de vida de la población CxE, nuevos QTLs fueron identificados, los cuales fueron detectados sólo al comienzo, al final o durante la mayor parte del proceso de senecencia.

Nuestros resultados demuestran que la evaluación de variables, describiendo procesos complejos que tienen lugar durante la fenología de una población grande, desarrollada para el mapeo genético, es una estrategia poderosa para la descripción de processos a nivel fisiológico y genético.

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