Breeding is more than genetics:

a non-genetic toolkit to improve hybrid potato breeding

Julia Stockem

Propositions

1. For a hybrid breeding programme to be successful, agronomy is as important as genetics.
   (this thesis)

2. In precision agriculture, the current seed tuber size classification system must be changed into one based on seed tuber weight.
   (this thesis)

3. The outcome of a study is determined by the traits that we choose to measure.

4. The absence of certain values can be as meaningful as the actually measured values.

5. To reach one's goals it is essential to timely decide when something is finished.

6. Positive discrimination is wrong.

Propositions belonging to the thesis, entitled
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Abstract

For potato, diploid hybrid breeding is a novel technology that has recently been developed. The implementation of this technology in an efficient breeding programme raised new questions. Besides the genetic variation that is present in a breeding programme, breeders have to deal with environmental variation in all stages of the breeding process. Partly, this environmental variation can be used to assess genotypic variation under specific conditions. For another part, variation is a nuisance that needs to be dealt with, to limit its effect on the outcome of variety trials where the goal is to evaluate different genotypes and select for the best inherent performance.

To decrease unwanted variation in breeding trials two approaches were used. On the one hand, to assess to variation due to starting material differences. Therefore, we determined the effect of the seedling tuber quality traits tuber weight and number of eyes on the performance of plants grown from these seed tubers in the field as well as on their yield.

Large variation in single-tuber weight was found in a seedling tuber batch of size class 35-45 mm, with up to a factor 4.6 difference in weight between the highest and lowest sizes. A higher seedling tuber weight resulted in higher yield. With the variation in weight found in the seedling tuber batch, this trait can result in unwanted variation in breeding trials. The number of eyes of seedling tubers affected plant growth rather than yield. A higher number of eyes led to more stems per plant, however, this was compensated by fewer tubers per stem, resulting in no effect on yield.

On the other hand, to decrease unwanted variation in breeding trials we determined the effect of plot size and develop models to calculate variation when using different plot sizes and shapes. This was done for the following relevant traits: total tuber weight, number of tubers, tuber shape, standard deviation of tuber shape, tuber volume, and standard deviation of tuber volume. The developed models can be used to determine the plot size that is needed for the required degree of precision in a trial.

Environmental variation can also be applied to determine how genotypes respond in different environments in fewer cycles of selection and testing. In climate room trials we found that increasing air temperature decreases number of tubers and weight per tuber, while increasing the day/night difference in temperature led to an increase in weight per tuber. Increasing light intensity and increasing the percentage of far-red light in the spectrum led to increases in number of tubers. For these responses, we found variation among the used parent lines, and one relatively heat-tolerant line was identified. This line can be used to develop heat-tolerant hybrids.

Environmental variation was also applied by performing variety trials in different locations, in which genotype × environment interaction and yield stability of a large set of hybrid materials and standard commercial cultivars was determined. Experimental test-hybrids only slightly underperformed compared to commercial cultivars in terms of yield. Stability of yield components was comparable to the commercial cultivars.

In this thesis, we applied environmental variation in breeding trials to enhance the breeding process. Moreover, we determined ways to deal with unwanted variation. We have shown that limiting variation in seedling tuber weight can decrease the variation in yield in cultivar trials. We also developed models that can be used to determine the required plot size for the required degree of precision.

Keywords: hybrid potato, variation, plot size, seedling tuber quality, light, temperature, Genotype × Environment

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Chapter 1

General introduction

1.1 Hybrid potato breeding

Potato is one of the most important food crops in the world, with a global production of 376 million tonnes in 2021 (FAO). Worldwide, it is the third most consumed crop after wheat and rice (Devaux et al. 2014, 2021), and in comparison with other staple crops, it has a high nutritional value (Devaux et al. 2021). Moreover, potato can be grown in a wide range of environments (Devaux et al. 2014).

To obtain improved cultivars it is important to have an efficient breeding system. In potato, there are two breeding systems that are currently being used: traditional breeding with tetraploids, and hybrid breeding using diploid material.

In traditional potato breeding, two tetraploid cultivars are being crossed in the first year of the breeding cycle (Bradshaw 2021). The true seeds that are derived from the crosses between two cultivars are genetically diverse. They are sown, and from that moment on each genotype will be multiplied by making clones. These clones are being grown in increasing numbers of increasingly large fields for the next five to ten years, and selections will be made. A cultivar that is derived from this process will be multiplied for another 5−7 years to generate enough starting material for ware crop production (Figure 1.1). Due to the complex genetics of tetraploid material, it is hard to steer or predict the outcome of these crossings. This complexity, together with the large number of selection criteria in potato, results in a low genetic improvement of yield (also called “genetic gain”) in tetraploid cultivars in the past century (Douches et al.1996; Piepho et al. 2014; Agha et al. 2023).

Unlike for many major field crops, vegetables, and ornamental crops (ter Steeg et al., 2022), hybrid breeding has only recently been introduced in potato. In hybrid breeding, diploid instead of tetraploid material is being used because of simpler genetics. In tetraploids, more unfavourable alleles can be ‘hidden’ compared to diploids; this makes the inbreeding process easier in diploids (ter Steeg et al. 2022). Moreover, homozygosity is reached faster when inbreeding diploids instead of tetraploids (Lindhout et al. 2018). In the past, lack of self-compatibility and strong inbreeding depression hampered the success of hybrid breeding in potato (Lindhout et al. 2011). Self-compatibility through the S-locus inhibitor (*Sli*) gene enabled hybrid breeding in potato (Eggers et al. 2021; Ma et al. 2021). Parent lines are developed by performing self-pollinations over several generations. With each generation, the parent lines become more homozygous (Lindhout et al. 2011), and after five to seven generations of self-pollinations the lines can be phenotypically uniform (Bradshaw 2022). Between the generations of self-pollinations, selections for desired traits are made based on phenotype as well as making use of molecular markers. With this process, vigorous inbreeding lines are obtained, which is considered as a breakthrough in hybrid breeding as inbreeding depression was one of the bottlenecks (Lindhout et al. 2018). An issue of introgressing the *Sli* gene from *Solanum chacoense*, the donor of *Sli*, could be linkage drag of unfavorable traits (Clot et al. 2020). Eggers et al. (2021) however reported no problems with deleterious alleles coming from crossing with this wild species. In test-crosses, hybrid performance is being evaluated and used to select inbred parent lines. Similar to tetraploid breeding, this selection process requires five to ten years when starting with heterogeneous germplasm (Figure 1.1). When starting from an improved population of parent lines, in which disadvantageous alleles already were removed by selection, the selection process towards a hybrid is shorter than is the case in conventional breeding based on tetraploid genotypes. Another difference with traditional tetraploid breeding is that existing parent lines can be improved with new traits much more rapidly. For example, resistance genes can be introgressed into the parent lines of a hybrid, as demonstrated by Su et al. (2020) with resistance genes against *Phytophthora infestans*. Besides the speed, an advantage of hybrid breeding is heterosis, where the hybrid outperforms the parent lines (Lindhout et al. 2011; Adams et al. 2022; Bradshaw 2022; ter Steeg et al. 2022).

Starting material of a hybrid cultivar is the hybrid true potato seed (HTPS) that is derived from a cross between the homozygous parent lines. HTPS as starting material has several advantages compared to seed tubers. The multiplication of starting material is much faster, because one female plant can produce thousands of seeds in one growing season. Once a hybrid cultivar has been developed, it takes only two more seasons to produce enough starting material for ware crop production (Figure 1.1). These seeds need less space for transport and storage, and they can be stored for more than 40 years (Pallais 1987). In contrast, transport and storage of seed tubers is much more difficult and costly as they are bulky. Moreover, seed tubers become physiologically older during storage (Struik & Wiersema 1999; Blauer et al. 2013), causing loss of growth vigour. The period that seed tubers can be stored depends on the cultivar, the conditions during seed tuber production and the storage conditions (van der Zaag & van Loon 1987; Struik & Wiersema 1999), but is usually shorter than a year. Another advantage of HTPS over using seed tubers is that they are almost disease free while disease accumulation is a well-known problem in seed tuber production (so-called "seed degeneration") (Struik & Wiersema 1999; Almekinders et al. 2010). This high phytosanitary quality of hybrid cultivars increases yield potential especially in developing countries where availability of healthy starting material is low (de Vries et al. 2016) and disease pressure is high (Gildemacher et al. 2009; Damtew et al. 2018).

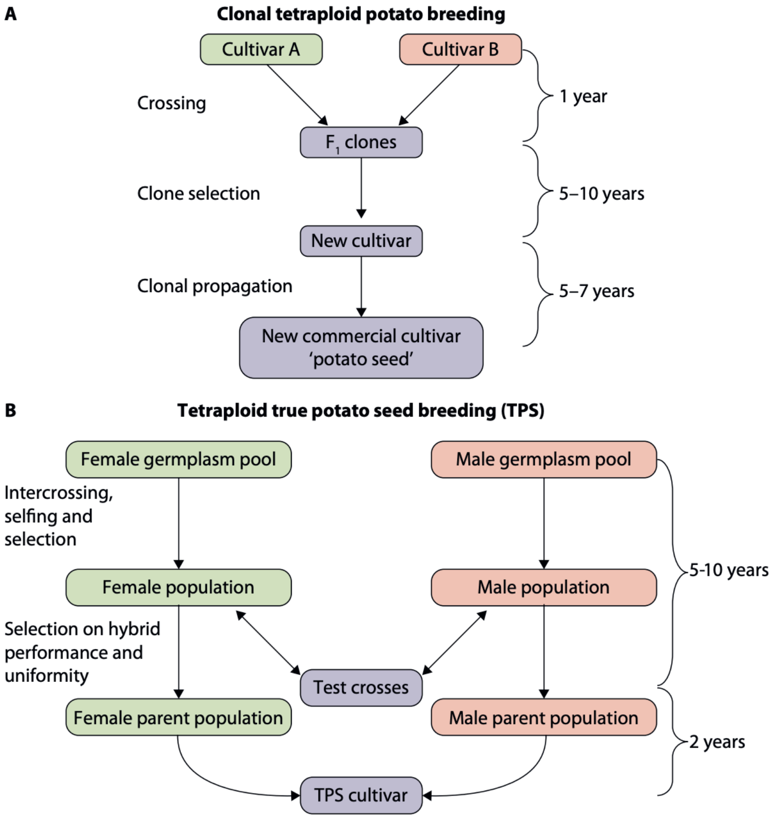

Figure 1.1: Scheme of a traditional tetraploid potato breeding system (A) and a diploid hybrid potato breeding system (B). Source: de Vries et al. (2023)

1.2 Requirements for a successful hybrid breeding programme

For a successful hybrid potato breeding programme, several genetic as well as non-genetic components are required (Figure 1.2). Below, the main components will be described.

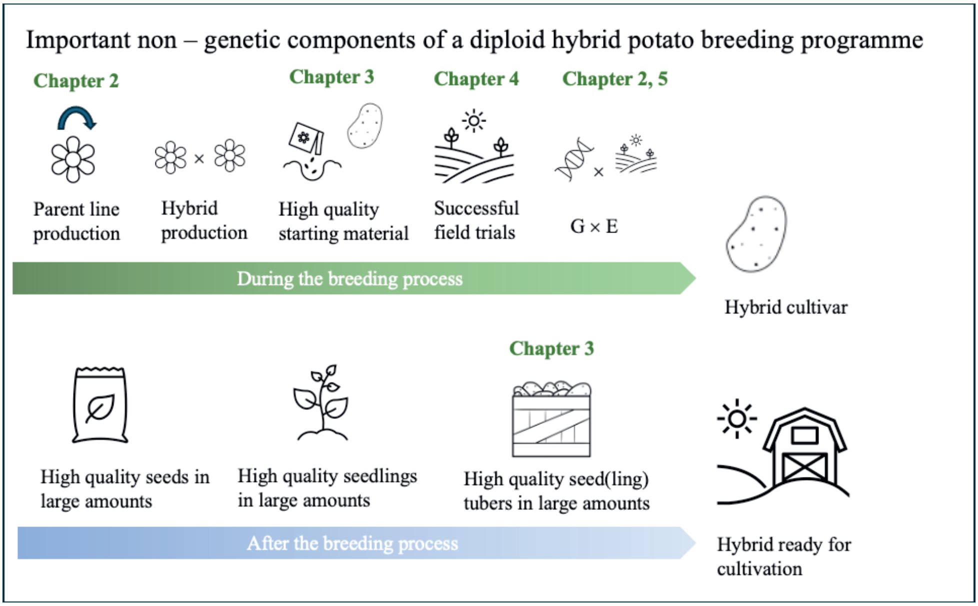

Figure 1.2: Important non-genetic components of a successful hybrid potato breeding programme.

1.2.1 Genetics

Possible routes for homozygous parent lines are the production of double haploids, or by performing self-pollinations. Although the process to homozygosity is shorter when producing double haploids, it is hard to use them for breeding due to low plant vigour (Bradshaw 2009; Jansky & Spooner 2018). To develop homozygous parent lines by performing self-pollinations, it is essential to create self-compatibility (Eggers et al. 2021), while diploid *Solanum tuberosum* is to a large extent self-incompatible. Moreover, more dedicated breeding can be reached by using molecular markers. When a molecular marker is available for certain traits such as resistance against late blight, this enables the breeder to select for these traits as soon as a seedling has produced enough leaf material for sampling, which takes approximately three weeks after sowing. This technique makes the breeding process more efficient because the selections can be made in an early stage without waiting for the phenotype to express, and it enables stacking of several traits (Jiang 2015). Moreover, molecular markers can be used during the inbreeding process to test the level of homozygosity, and the most homozygous plants can be selected to reach the required level of homozygosity faster. Another application is the use of genomic prediction models, as demonstrated by Adams et al. (2023) for the traits dry matter content, average tuber volume and total tuber yield. So, molecular markers can be used to speed up the process of producing desired parent lines for producing hybrids.

Molecular markers can also be used to improve existing elite parent lines. By marker assisted backcrossing new traits can be inserted into a hybrid. For example, resistance genes against *Phytophthora infestans* have been crossed into the parent lines of a hybrid. By backcrossing two times with the original parent and one selfing, the original parents could be recreated with the resistance gene (Su et al. 2019). So, obviously, genetics are essential for hybrid breeding. However, a hybrid breeding programme also consists of non-genetic components that are needed for a successful breeding programme. These will be described in the following section.

1.2.2 Non-genetic components of a hybrid breeding programme

Producing parent lines and HTPS

To develop parent lines, several generations of self-pollinations are required. These selfings can be produced in a greenhouse. Inbred lines generally have a lower plant vigour than hybrids, and often they suffer from inbreeding depression. This makes it hard to grow them in outside field conditions. Moreover, growing plants in a greenhouse and subsequent hand pollination decrease the chance for cross pollination by insects. Also hybrid seed production can be done in controlled environments using inbred parent lines. Because the parents are self-compatible, selfing should be avoided, for example through emasculation before the flowers reach the anthesis stage. Pollen is collected from the male parent and used to pollinate emasculated female flowers.

To stimulate flowering and seed set, precise climate management is needed in the greenhouse. The combination of light and temperature has been shown to be an important factor in flower production, where the rate of producing new flowers increases with increasing temperature up to a certain maximum (Almekinders & Struik 1996). Light intensity as well as the photoperiod and light spectrum can affect abundance of flowering in potato and the proportion of successful berry set (Almekinders & Struik 1996; Plantenga et al. 2019). For example, increasing the light intensity as well as imposing long days were shown to increase flower bud development and advance flowering (Almekinders & Struik 1996; Plantenga et al. 2019). The responses to temperature and light intensity differ among genotypes. Moreover, there are three-way interactions between genotype, temperature and light intensity. For a breeding programme with many different genotypes in one greenhouse this means that is impossible to optimize conditions for all individual plants. Nevertheless, knowledge on the effects of environment on flower and seed production is essential in hybrid breeding based on true potato seed, as seeds are required in the breeding process as well as for the end-product.

High-quality field trials

During the inbreeding process, test crosses are made to determine the genetic potential and the combining ability of the parent lines. Based on the performance of these test hybrids in the field, selections are made in the population of parent lines. Also more advanced hybrids are tested in the field to assess their performance in different locations. Traits that are important for the industry, such as total yield, tuber size and tuber shape or dry matter concentration of the tubers are measured and compared among hybrids and locations. The optimal values for these traits can be different, depending on the use of the harvestable product, the tubers. For example, the production of French fries requires oblong tubers with a low sugar content for a good frying colour, while for the production of starch the dry matter production and starch content should be as high as possible. As selections of the whole breeding programme are based on these field trials, it is essential to perform high quality and reliable field trials. The goal in these breeding trials is to select hybrids that perform best for the traits of interest because they combine the right alleles; however, in field trials there are other factors such as the environmental conditions and variation in the field that affect plant performance rather than only the genotype (see for more explanation below).

Type of starting material

The result of hybrid breeding in diploid potato is hybrid true potato seed (HTPS) as starting material when a new hybrid cultivar has been developed. This is different from traditional breeding, where tetraploid true potato seed (TPS) is only used once, right after crossing two cultivars. Subsequently, the clones are grown from seed tubers. Several pathways from HTPS towards a ware crop can be described (Figure 1.3) (van Dijk et al. 2021). One possibility is to produce seedlings in a nursery and transplant them to the field. In this system, the seeds are sown into trays that contain individual plugs, for example 104-plug trays filled with peat-based substrate. A good temperature for germination is between 16 and 21 °C (Lam 1986). Approximately six weeks after sowing, when the plantlets have five to eight leaves (Kacheyo et al. 2023), they can be transplanted into a field for either seedling tuber production or ware tuber production. When seedling tubers were produced, they can be grown in a subsequent year for ware tuber production. Another possibility could be direct sowing of HTPS to the field (Figure 1.3).

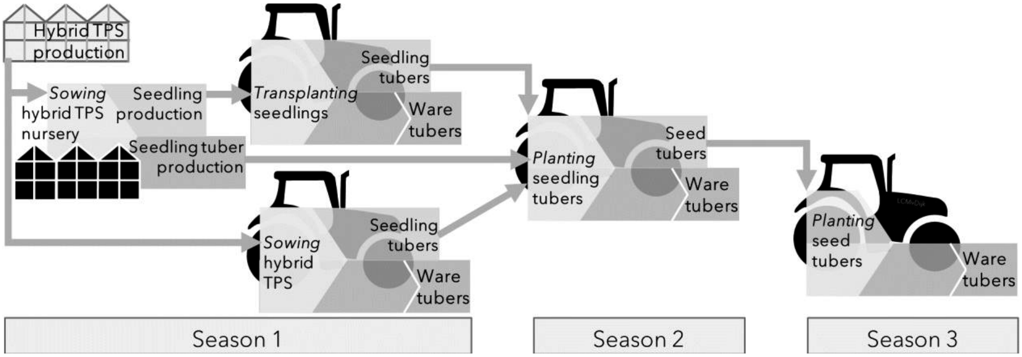

Figure 1.3: Potential pathways from hybrid true potato seed to a ware crop. Source: van Dijk et al. (2021)

Starting material

Starting material of high quality is important when starting a crop from seedlings as well as from seed(ling) tubers. In any potato crop, it is advantageous to have complete ground cover early in the season. One reason is lower weed pressure, because less light reaches the emerging weed seedling. Moreover, a higher leaf area index enables a higher light interception by the plant, leading to higher plant growth rates, and cooler soil temperatures which are conducive to tuberization and reduce evaporation. In potato, a higher light interception can therefore lead to increased tuber yield (Khurana & McLaren 1982). For both types of starting material that means that a fast initial growth in the beginning of the season is advantageous. However, usually initial growth and increase of ground cover are slower in a seedling-grown crop than in a seed tuber-grown crop (Struik & Wiersema 1999).

When starting a crop from seedlings there usually is a transplant shock after transplanting seedlings from a nursery to the open field. After transplanting, it takes 1−2 weeks until the plant starts growing. Using seedlings of high quality is expected to decrease the duration of this transplant shock, leading to faster growth in the beginning and earlier complete ground cover. Moreover, seedling survival after transplanting is expected to be better when using high quality seedling.

Also seed(ling) tuber quality can have large effects on plant development and yield. In tetraploid cultivars, seed tubers are used as starting material, and an important aspect of seed tuber quality is the physiological age, which is the developmental stage of a seed tuber (Struik 2007a). The physiological age of a seed tuber can affect sprouting of the tuber, development of the plant in the field and with that it can also affect yield. It is influenced by growing conditions of the mother plant during the season of seed tuber production, as well as the storage conditions. Moreover, there can be an effect of earlier field seasons on the physiological age of the seed tuber.

In tetraploid cultivars, seed tubers are multiplied over several field seasons before they are used as starting material for a ware crop. In seedling tubers, there is only effect of one growing season on the quality because they always start from clean HTPS. This means that there is less accumulation of diseases over seasons, leading to healthier starting material. For physiological age, there is only the effect of one growing season. It is unknown to what extent physiological age or other tuber quality traits such as tuber weight affect plant development and yield when starting from seedling tubers.

Variation

In this thesis, two types of non-genetic variation are distinguished, namely unwanted variation and wanted variation. The unwanted variation disturbs the outcomes of the breeding programme, while the wanted variation is applied by breeders to make the breeding process more efficient by creating variation that allows genetic variation in certain characteristics to express itself. Below, both types will be explained further.

Wanted variation

Environmental variation can be applied to make the breeding programme more efficient. One way is to steer the development of plants to a required phenotype. For example, in tetraploid cultivars low temperature stimulates tuber development while high temperature stimulates shoot growth (Struik et al. 1989a). When seedling tubers need to be made from parent lines in the greenhouse, this knowledge can be used to improve the tuber production and be more efficient.

Another possibility of applying environmental variation to improve selections is conducting stress tests. This can be done under controlled conditions in the greenhouse or in climate rooms. For example, parent lines can be tested for heat tolerance to produce hybrids that are tolerant for high temperatures as well. The tolerance in the hybrids should be confirmed later on under field conditions.

Besides these specific stress tests in hybrids, it is important to test the performance of hybrids in different environments. Plant performance is affected by environmental factors, and different genotypes can respond differently to environmental variation. (Bradshaw 2021) The extent of genotype × environment (G × E) interaction can be different between traits. During the breeding pipeline, the G × E interactions are tested by performing variety trials in several different locations. This way, cultivars can be selected that perform well in specific environments or for yield stability, where cultivars perform stable over different environments. In a tetraploid breeding programme, more time is needed to multiply seed tubers, therefore, it takes a couple of years before multilocation trials can be performed (Tiemens-Hulscher et al. 2013; Bradshaw 2021). In diploid breeding, the multiplication of starting material is faster due to the production of HTPS. Hundreds of seeds can be produced in one crop cycle in the greenhouse, and in the following field season seedling tubers can be produced. These seedling tubers could be used for multilocation trials in the subsequent year. Therefore, the decision for the moment of conducting G × E in hybrids is not seed(ling) tuber limited, as it is in tetraploid breeding. However, it is unknown to what extent G × E interaction can be measured in diploid seedling tubers, and whether the variation is similar to what is found in tetraploid clones grown from seed tubers.

Unwanted variation

The field trials that are performed in a breeding programme usually aim to measure differences between the genotypes that are planted. Based on the performance of the hybrids selection in parent lines can be made.

Within a trial field environmental variation is present that can lead to variation between plots. For example, there can be gradients of nutrients in the soil or differences in soil type and moisture. As a result, differences between hybrids that are tested can be the consequence of field variation rather than the genetics, making the results of the trial less precise. The trial design, number of replicates, plot size and plot shape are essential to collect meaningful data, which show the potential of a genotype rather than the field variation. Another type of variation that can disturb the outcomes of breeding trials that was explained above is the type and quality of starting material. In this thesis, spatial variation in field trials and variation originating from seedling tubers will be addressed. The possible effect on the outcome of a trials will be investigated, and we will determine methods on how to deal with this variation.

1.2.3 Logistics after the breeding process

In Section 1.2.2, I described agronomic components that are important for the success of a hybrid potato breeding programme. Parts of these are still relevant once a hybrid cultivar has been developed (Figure 1.2). When a new variety is released, a large amount of starting material needs to be produced in order to grow the hybrid on a large scale. In hybrid breeding, thousands of true seeds can be produced in one crop cycle in the greenhouse. In contrast to seed production in a breeding programme, many plants of the same male and female line are grown now, so greenhouse conditions can be optimized for the production of large numbers of high quality from these lines. High quality seeds are important to reach high percentages of germination, uniform germination, and to produce vigorous seedlings of high quality.

In medium or high-tech cropping systems it is most likely that seed(ling) tubers remain the most important starting material for ware tuber production, because the system is optimized for planting and growing tubers. That means that high quality seedling tuber production is an important step after the production of HTPS. As explained in Section 1.2.2, high quality seedlings are important for a good plant establishment in the field and high initial growth rates, so also for seedling and eventually ware tuber production we need the knowledge to grow high quality seedlings and seedling tubers.

1.3 Aim and outline of this thesis

In this thesis, I want to support breeders in dealing with non-genetic variation in their breeding programme. For unwanted variation, which disturbs the outcome of breeding trials and thus their selection and multiplication programme, I will develop tools to decrease the impact on results. Furthermore, we will apply environmental variation under controlled conditions as well as under field conditions. Eventually the goal is to make the breeding process more efficient.

The thesis consists of six chapters: the general introduction (this Chapter 1), four research chapters (Chapter 2−5) and a general discussion (Chapter 6).

In **Chapter 2**, I report on investigations into the effects of light intensity, the percentage of far-red light in the spectrum and temperature on tuber production under controlled conditions. The goal was to determine the effect of high temperature on tuber production of inbred lines, and to find environmental settings that improve seedling tuber production of parent lines under controlled conditions. Three separate climate room trials were conducted. In the first trial, four different temperature regimes were tested in combination with four different light intensities. In the second trial, the effect of high temperature was investigated without increasing the maximum temperature. This was done by increasing the duration of a period with high temperature during the day. In the last trial, I determined the effect of the day/night temperature differential in combination with three levels of far-red light in the spectrum.

Chapter 3 deals with the quality of diploid hybrid seedling tubers. For tetraploid cultivars, it is known that seed tuber quality affects the plant performance and yield of a crop. For diploid hybrids, this effect is unknown, and for making selections it is important to distinguish whether plant performance and yield is the result of seedling tuber quality or genetics. Therefore, the effect of seedling tuber quality on plant growth and yield was investigated. In this chapter, I selected seedling tuber weight and the number of eyes per seedling tuber as quality traits to investigate. The presence of variation in these characteristics in a seedling tuber lot was determined, and in two field trials, I investigated the effect of differences in seedling tuber weight and number of eyes per seedling tuber on plant performance and yield parameters.

In **Chapter 4**, I assessed the plot dimensions that are needed for field trials to test the performance of diploid hybrid potato. The goal was to provide a tool to determine the plot dimensions that are needed to reach the required degree of precision in a trial. As a measure of variation the least significant difference, expressed as a percentage of the trial mean (LSD%) was used. Two field trials were conducted in two subsequent years containing diploid hybrids as well as an existing tetraploid cultivar. Based on these trials, I derived relationships between LSD% and plot size and shape for the following the tuber traits: total tuber weight per plant, number of tubers, tuber volume, standard deviation of tuber volume, tuber shape and standard deviation of tuber shape. The outcome of this research will help breeders determine the needed plot dimensions, depending on the trait of interest and the required precision of the results.

In **Chapter 5**, I describe the results of field trials with diploid test hybrids and with tetraploid commercial cultivars that were performed in different, contrasting locations. In this chapter we compare the performance of diploid test hybrids and of tetraploid cultivars on the level of yield and yield components. Moreover, I quantified the G × E interaction of tuber yield and yield components in the tetraploid cultivars and diploid test hybrids. The goal was to benchmark diploid test-hybrids against commercial cultivars and to compare the extend of G × E interaction between the two.

Chapter 6 is the general discussion where the main findings of this thesis will be presented. Methods to apply environmental variation in a breeding programme will be discussed, and I will show results of applied variation under climate room conditions and under field conditions in a hybrid potato breeding programme from Chapter 2 and Chapter 5. Moreover, sources of non-genetic variation that breeders can encounter in their breeding programme will be discussed. Results of Chapter 3 and Chapter 4 will provide ways to decrease the effect of unwanted variation on the results of breeding trials. The findings will help to make breeding programmes more efficient.

Chapter 2

Shedding light on a hot topic: tuberisation in potato

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Abstract

Growing small seedling tubers from true seed, comparable with mini tubers, in controlled conditions could be a method to multiply healthy starting material of potato. In indoor farming systems, the conditions can be optimised for high production. In field trials, it is impossible to investigate the effects of environmental factors such as temperature and light separately. In this study, we performed three climate room experiments in which the effects of light intensity, temperature and percentage of far-red light in the light spectrum on tuber production were assessed. We found that increasing the average temperature reduced tuber number and tuber weight. Increasing the diurnal temperature variation while keeping the average temperature equal resulted in increased tuber size. The light treatments on the other hand only affected the number of tubers per plant: increasing light intensity and increasing the percentage of far-red light in the spectrum enhanced the number of tubers. Moreover, interaction in tuber production between inbred lines and temperature was significant, with some inbred lines being relatively tolerant to high temperature. These findings will help breed for heat tolerant varieties and optimise growing conditions for tuber production in indoor farming systems.

2.1 Introduction

In potato, hybrid breeding was recently developed as a new breeding system (Eggers et al. 2021; Jansky et al. 2016; Lindhout et al. 2011; Zhang et al. 2021). In hybrid breeding, homozygous diploid inbreeding lines are developed by performing self-pollination during several generations. By subsequently crossing two inbred lines, a hybrid true seed (HTPS) genotype is produced. Compared with traditional breeding, developing new cultivars by hybrid breeding has several advantages. For example, the breeding process is faster due to simpler genetics (Su et al. 2020), storage and transport of true seeds are easier, and with true seeds, the starting material is more disease-free compared with traditional breeding involving clonal multiplication (Lindhout et al. 2018; Stokstad 2019). Also for molecular genetic research, it is advantageous to work at the diploid level rather than at the tetraploid level (van Lieshout et al. 2020; Wang et al. 2020; Witek et al. 2016). For example, mapping studies are more efficient in diploid populations due to less allelic variation (Endelman and Jansky 2016; Meijer et al. 2018; Prinzenberg et al. 2018).

There are several ways to multiply starting material in potato. One example is the production of mini tubers from in vitro plantlets under controlled conditions (Struik and Wiersema, 1999). With hybrid breeding, small seedling tubers can be produced from true seeds. The advantage is that they can be produced under controlled conditions as well, keeping the plants and tubers free from diseases (Buckseth et al. 2016). For mini tuber production, it is possible to grow the plants in glasshouses in soil, as well as in completely controlled systems in soilless media (Bradshaw 2021; Tierno et al. 2014); there also are possibilities for the production of small seedling tubers from true seeds (Chujoy and Cabello 2007). Using a vertical farming system could provide a further advantage, since growing conditions can be optimised for tuber production and the cultivation can be independent of location, climate or growing season (van Delden et al. 2021).

Under well-watered conditions, temperature and light are the main environmental drivers for plant growth. Furthermore, potato yield is strongly affected by these environmental conditions (Bodlaender, 1963; Haverkort, 1990), in a cultivar-dependent way (Kooman et al.

1996a). Stockem et al. (2020) showed that in diploid hybrid potato genotype by environment (G × E) interaction is a prominent factor influencing phenotypic variation. In field trials, it is impossible to disentangle the effect of different environmental factors on tuber yield. Using climate rooms with controlled conditions provides the opportunity to investigate the effect of different environmental factors separately. In this way, we can determine the conditions that are needed for successful mini tuber production in vertical farms. Moreover, it is possible to generate fundamental knowledge about potato development that would not be possible in field trials.

Temperature affects shoot and tuber growth differently. For tetraploid cultivars, it was shown that a high temperature (up to 28°C) stimulates shoot growth, while it delays tuber formation (Struik 2007b; Trapero-Mozos et al. 2018). An average temperature of approximately 17°C is generally considered as optimal for tuber formation (Ewing, 1981), although this optimal temperature for tuber formation may differ among cultivars and depends on photoperiod (Struik 2007b). Tuberisation in potato is induced by the signal StSP6A, a homologue of FLOWERING LOCUS T (FT), which is involved in flowering in Arabidopsis (Abelenda et al. 2019). Stolon growth and tuber formation can be affected by high temperature due to altered hormone signalling and the reduction of StSP6A expression required for continued tuber growth (Hastilestari et al. 2018; Lehretz et al. 2019; Struik et al. 1989b). Moreover, tuber yield can be decreased by reduced leaf area or leaf:stem ratios, by reduced production of assimilates at supra-optimal temperatures for photosynthesis, by reduced partitioning of assimilates to tubers, by reduced activity of starch synthesising enzymes (Mohabir and John 1988) and by a decrease in sink strength of the tubers, which also impedes starch accumulation in the tubers (Dahal et al. 2019; Ewing 1981; Hastilestari et al. 2018).

Light is another important factor that differs among environments. Relevant aspects of light are photoperiod, light intensity and light spectrum (Plantenga et al. 2016). Photoperiod is known to have a major effect on plant development, and its effect can be different in different stages. For example, tuber formation of some cultivars can depend on short days to be able to produce tubers (Ewing and Struik 1992; Jackson et al. 1996). Light intensity is important for photo-morphogenetic effects such as branching, but also for CO2 assimilation, and increasing light intensity leads to increased assimilation rates (Stutte et al. 1996). Plantenga et al. (2016) showed that light spectrum also affects tuber initiation. Adding far-red light to the spectrum accelerated tuber initiation, while additional blue light delayed tuber initiation. This makes light one of the driving environmental cues that determine tuber yield.

Using completely controlled plant production facilities makes it possible to optimise conditions for mini tuber production. Moreover, yield potential as well as the sensitivity to environmental factors such as light and temperature differs among genotypes. Therefore, we performed a series of climate room trials in which we aimed to disentangle the effects of temperature and light on tuber production in several diploid potato genotypes. The potato lines used in our trials are day-neutral for tuber formation; therefore, we focused on the effects of light intensity and light spectrum. The results of this research will enhance our fundamental knowledge on the effect of light and temperature on tuber production in potato. Moreover, they will help optimise conditions for tuber production in controlled environments, such as mini tuber production facilities in seed producton schemes, and in vertical potato farms or life support systems in space (Wheeler 2006).

2.2 Materials and methods

Three different climate room trials (Trials 1–3) were conducted to investigate the effects of light intensity, light spectrum, elevated temperature and their interactions on tuber production in diploid genotypes, grown from true potato seeds. In Trial 1, we investigated the effect of average temperature and light intensity on tuber production. In this trial, average temperature was increased by increasing the day and night temperature while keeping the day/night difference constant, thus, besides average temperature also the maximum temperature was increased and this could be an important factor in itself. In Trial 2, we wanted to rule out the maximum temperature as a confounding factor. We investigated the effect of increased average temperature on tuber production by increasing the period of high temperature during the day and keeping minimum and maximum temperature constant. The goal of Trial 3 was to investigate the effect of day/night temperature difference and the amount of far-red light in the light spectrum on tuber production.

Plant material

In all trials, inbred lines (IL1–IL11) were used that were obtained after several generations of self-pollination by Solynta, as described by Lindhout et al. (2011, 2018). All lines were developed by manual self-pollinating for between 6 and 10 generations. These lines were selected based on their phenotypic uniformity in tuber and plant habit. IL5 is a selfing of Solyntus, a highly homozygous diploid potato line, which was released with its sequence information in 2020 (van Lieshout et al. 2020).

In Trial 1, the inbred lines IL1, IL2, IL3 and IL4 were used. Trial 2 was performed with IL5, IL6, IL7, IL8 and IL9, and in Trial 3, we used IL2, IL6, IL10 and IL11. We chose to use a variety of inbred lines to assess the phenotypic variation that can be observed under contrasting light and temperature treatments. Different inbred lines were used because the results of the trials were used as input information for Solynta's hybrid breeding programme. During the execution of the experiments, new improved inbred lines were developed. These improved lines were used for the follow-up trials.

Treatments

Trial 1: Average temperature and light intensity

In Trial 1, true seeds of four inbred lines (IL1, IL2, IL3 and IL4) were sown in 104-plug trays with plugs of peaty substrate (Obturo, Van der Knaap, Wateringen, Netherlands). The trays were placed in a climate room with a constant temperature of 18°C, a day length of 16 h, a relative humidity (RH) of 80%, a CO2 concentration of 400 ppm and a light intensity of 150 µmol m^{-2} s^{-1} at plant level. Light spectrum was the medium far-red (FR) spectrum as described in Table 2.1. No additional seed treatments were applied. Twelve days after sowing, the light and temperature treatments started. Four weeks after sowing, the seedlings were transplanted into 4-L pots containing peat-based substrate (Horticoop, Bleiswijk, Netherlands).

Table 2.1: Light spectra used in Trials 1, 2 and 3.

Light color	No FR (%)	Medium FR (%)	Extra FR (%)
Red (R) Wavelength: 660 nm	82	75	69
Blue (B) Wavelength: 460 nm	12	11	10
Green (G) Wavelength: 500 - 570 nm	6	5	4
Far-red (FR) Wavelength: 730 nm	0	9	17

Four different temperature regimes (20/16, 24/20, 28/24 and 32/28°C day/night) and three different light intensities (250, 400 and 550 µmol m^{-2} s^{-1} at plant level) were applied at a day length of 16 h. Light spectrum was not changed when the treatments started, RH was 75% during the night and 55% during the day and CO2 concentration was 1400 ppm. This high CO2 concentration was chosen to make sure that CO2 was not a limiting factor for plant growth. The plants were grown under these treatments until senescence.

Trial 2: Elevated temperature

Seeds of five different inbred lines (IL5, IL6, IL7, IL8 and IL9) were sown into 104-plug trays with plugs of peaty substrate (Obturo, Van der Knaap, Wateringen, Netherlands). They were placed in climate rooms set at a day/night temperature regime of 24/18°C, with a day length of 16 h, a RH of 80%, a CO2 concentration of 700 ppm and a light intensity of 400 µmol m^{-2} s^{-1} at plant level. In this trial, a lower CO2 concentration was chosen than we used in Trial 1, because pilot trials after Trial 1 revealed that elevating CO2 concentration beyond 700 ppm did not increase plant growth anymore (unpublished results). Light spectrum during germination was the medium FR spectrum in Table 2.1. No additional seed treatments that could enhance germination were applied. At 4 weeks after sowing, seedlings were transplanted to 3-L pots with peat-based potting soil (Horticoop, Bleiswijk, Netherlands), they continued to grow in these climate conditions until start of flowering (BBCH stage 613–615; Kacheyo et al. 2021). In this trial, 3-L pots were used because of space limitation.

In this trial, we were specifically interested in the effect of high temperature during the tuberisation period and not the whole plant cycle. Therefore, temperature treatments started when the plants started flowering (BBCH stage 613–615; Kacheyo et al. 2021), assuming that the start of tuber initiation coincides roughly with flowering (Celis-Gamboa et al. 2003). The plants were exposed to different temperature regimes for 5 weeks (Table 2.2). In these temperature treatments, the average temperature was increased by applying different durations of 30°C during the day, but without altering the minimum and maximum temperature.

Table 2.2: Temperature regimes of Trial 2

Regime	Hours of night temperature (18 °C)	Hours of basic day temperature (24 °C)	Hours of heat during the day (30 °C)	Average temperature (°C)
1	8	16	0	22.0
2	8	15	1	22.3
3	8	12	4	23.0
4	8	10	6	23.5
5	8	8	8	24.0
6	8	4	12	25.0
7	8	0	16	26.0

After 5 weeks of treatment, the plants were placed in the greenhouse until senescence. Greenhouse conditions were set to a day/night temperature regime of 24/18°C. Additional lighting was supplied with high pressure sodium (HPS) lamps. RH varied between 30% and 90%.

Trial 3: Temperature differential and light spectrum

Seeds of four different genotypes (IL2, IL6, IL10 and IL11) were sown into 104-plug trays with plugs of peaty substrate (Obturo, Van der Knaap, Wateringen, Netherlands). They were placed in climate rooms at a temperature of 18°C, with a day length of 16 h, a RH of 80%, a CO2 concentration of 700 ppm and a light intensity of 150 µmol m^{-2} s^{-1} at plant level. Light spectrum during germination was the medium FR spectrum (Table 2.1). No further seed treatments were applied. One week after sowing, when the seeds were germinated (BBCH scale 009 as described by Kacheyo et al. (2021)), the temperature and light spectrum treatments started. Four weeks after sowing, the seedlings were transplanted into 4-L pots with peat-based potting soil (Horticoop, Bleiswijk, Netherlands).

Three different light spectra that varied in percentage of far-red light (Table 2.1) and three different temperature treatments (Table 2.3) were imposed in all possible combinations. Light-emitting diodes (LEDs) in the climate rooms consisted of individually dimmable red, blue, green and far-red LEDs and were set to implement the light spectra described in Table 2.1. The different treatments were separated by white screens to prevent interference of the different light spectrum treatments. The light intensity was 400 µmol m^{-2} s^{-1} at plant level, with a daylength of 16 h. RH for all treatments was 60% during the day and 80% during the night with a constant CO2 concentration of 700 ppm. The plants were grown under these treatments until senescence.

Table 2.3: Temperature regimes that were applied in Trial 3. Day length was 16 hours.

Temperature settings	Average temperature (°C)	Day temperature (°C)	Night temperature (°C)	Temperature differential day/night (°C)
Treatment 1	23.0	23	23	0
Treatment 2	22.7	24	20	4
Treatment 3	23.0	26	17	9

In all experiments, tubers were harvested after plant senescence. For each plant, tuber number and total tuber weight per plant were recorded and average tuber weight (in g/tuber) was calculated.

The climate rooms were placed adjacent to each other, and similarly constructed (Green Simplicity). In Trials 1 and 3, a split-split-plot design was used with temperature as main-plot, light treatment as sub-plot (in Trial 1 light intensity, in Trial 3 light spectrum) and inbred line as sub-sub-plot. Trial 2 was a split-plot design with temperature as main-plot and inbred line as sub-plot. Trial 1 consisted of three replicated blocks with three plants per plot, Trial 2 had six blocks with four plants per plot and Trial 3 had four blocks with four plants per plot. For statistical analysis, average values of these three or four plants were used. To ensure strict and distinct temperature treatments, the main plots in all trials were not assigned randomly but were fixed in separate climate rooms.

Statistical analysis was performed using R (R Core Team 2021). Temperature treatments in all trials were in fact only pseudo replicated, as is very common in growth chamber research. To cope with that statistical problem, we performed an AMMI analysis from the Agricolae package (de Mendiburu 2020) with the sub-, and sub-sub-plots in one column. This analysis breaks down the interactions with the environments, in this case, the temperature treatments, into principal components. Here, we examined the AMMI output to detect any evident clusters in the residuals far from the origin, which would be indicative of interactive effects between our experimental treatments and the specific climate chamber used. In this case, no evident clusters were found. Furthermore, residuals were checked for normal distribution, using a Q–Q plot. In case residuals were not distributed normally, data were log or square root transformed. For Trials 1 and 3, the ANOVA for split-split plot designs was used from the Agricolae package (de Mendiburu 2020). For Trial 2, the ANOVA for split-plot designs from the same package was used. For all trials, Fisher's LSD was used as post hoc test.

2.3 Results

Temperature regime affects tuber weight and number

Increasing the temperature from 20/16 to 32/28°C day/night (Trial 1) led to a decrease in tuber weight and fewer tubers, resulting in a reduced total tuber fresh weight (Table 2.4, Figure 2.1). Although tuber production of all inbred lines was affected by the increase in temperature, the sensitivity to elevated temperature was different (Table 2.4). For example, inbred line IL3 did not produce tubers at the 28/24°C treatment, while the other inbred lines did. However, at 32/28°C, no tuber formation in any of the inbred lines was observed. The maximum tolerable temperature for tuber production must be between 28/24°C and 32/28°C for these inbred lines. In this experiment, not only the average temperature was increased, but also both the day and night temperatures.

Table 2.4: P-values of analysis of variance (ANOVA) of inbred lines grown under four different temperature regimes (20/16, 24/20, 28/24 and 32/28 °C day/night) and three different light intensities (250, 400, 550 µmol m^{-2} s^{-1}) (Trial 2). Significant effects are indicated by asterisks: p value <0.01: **, <0.001: *. Data of number of tubers were square root transformed before analysis.**

Factor	Total tuber fresh weight	Average tuber weight	Number of tubers
Replicate	0.51	0.26	0.64
Temperature	$1.16 \cdot 10^{-7}$ ***	0.004 **	$3.34 \cdot 10^{-7}$ ***
Light intensity	0.002 **	0.49	0.009**
Temperature × light intensity	0.008 **	0.19	0.32
Inbred line	$4.13 \cdot 10^{-13}$ ***	$1.55 \cdot 10^{-9}$ ***	$6.18 \cdot 10^{-12}$ ***
Inbred line × Temperature	$5.26 \cdot 10^{-14}$ ***	$2.27 \cdot 10^{-5}$ ***	$2.89 \cdot 10^{-4}$ ***
Inbred line × Light intensity	0.54	0.36	0.64
Inbred line × Temperature × Light intensity	0.74	0.81	0.89

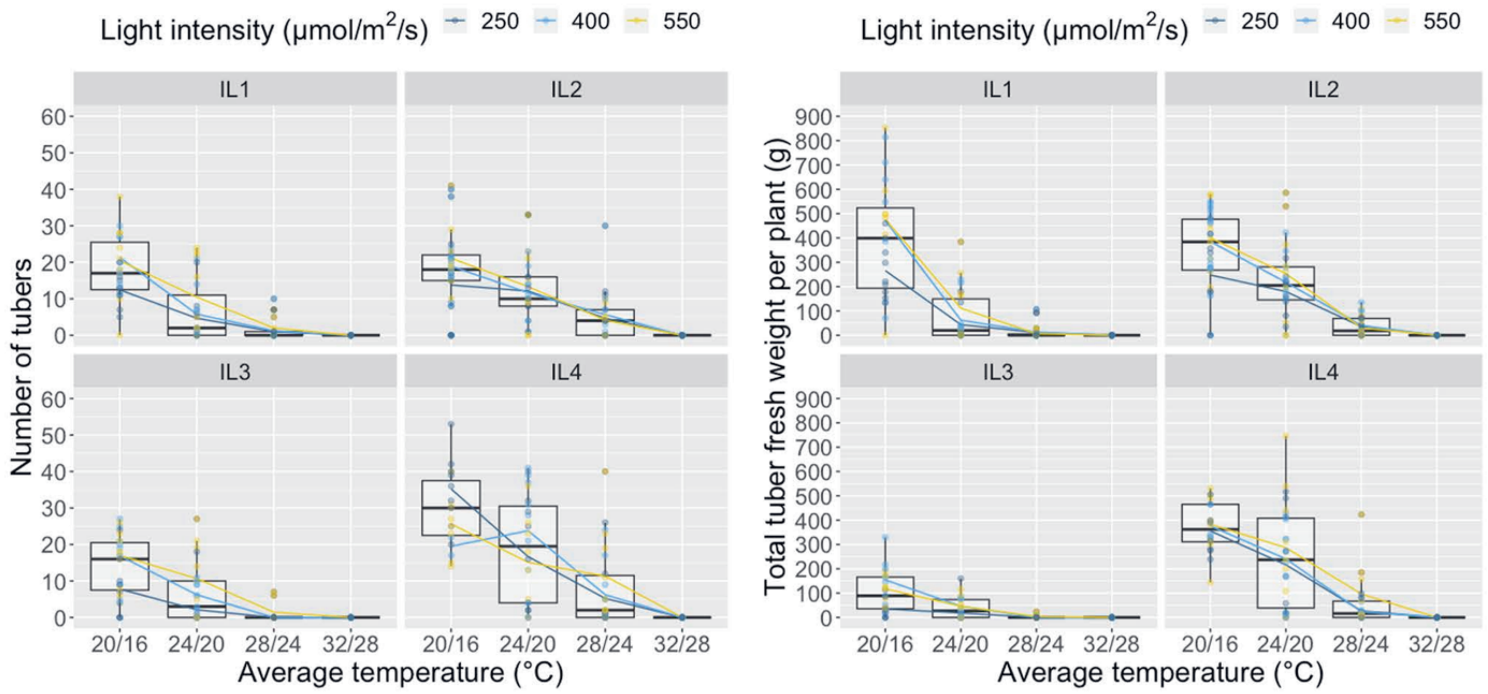

Figure 2.1: Boxplots of number of tubers per plant (left) and total tuber fresh weight per plant (g) (right) of four different breeding lines grown under four different temperature regimes and three light intensities. Boxes of the plot represent the 75th, 50th (median) and 25th percentile. Whiskers show the 1.5 × IQR (interquartile range) value. Data from Trial 1.

To test the effect of increasing average temperature on tuberisation without the additional effect of higher day and night temperature, a follow-up trial was performed in which the night temperature, the basic day temperature and the maximum day temperature were kept equal (Trial 2, Table 2.2). Different average temperatures were realised by applying different durations of 30°C during the day. Tuber weight, tuber number and as a consequence total tuber fresh weight were reduced by increasing the average temperature (Table 2.5). Furthermore, interactions between genotype and temperature were found for all traits, so the sensitivity of tuber production under increasing average temperature differed among the inbred lines (Figure 2.2).

Table 2.5: P-values of analysis of variance (ANOVA) of inbred lines grown under different durations of 30 °C during the day (Trial 2). Significant effects are indicated by points or asterisks: p value < 0.10: •, <0.01: **, <0.001: *. Data of total tuber fresh weight were square root transformed before analysis.**

Factor	Total tuber fresh weight	Average tuber weight	Number of tubers
Replicate	0.18	0.002**	0.068•
Temperature	$<2.2\cdot10^{-16}$ ***	$1.78\cdot10^{-13}$ ***	$7.85\cdot10^{-14}$ ***
Inbred line	$<2.2\cdot10^{-16}$ ***	$<2.2\cdot10^{-16}$ ***	$<2.2\cdot10^{-16}$ ***
Temperature × Inbred line	$1.47\cdot10^{-6}$ ***	0.002**	$7.48\cdot10^{-6}$ ***

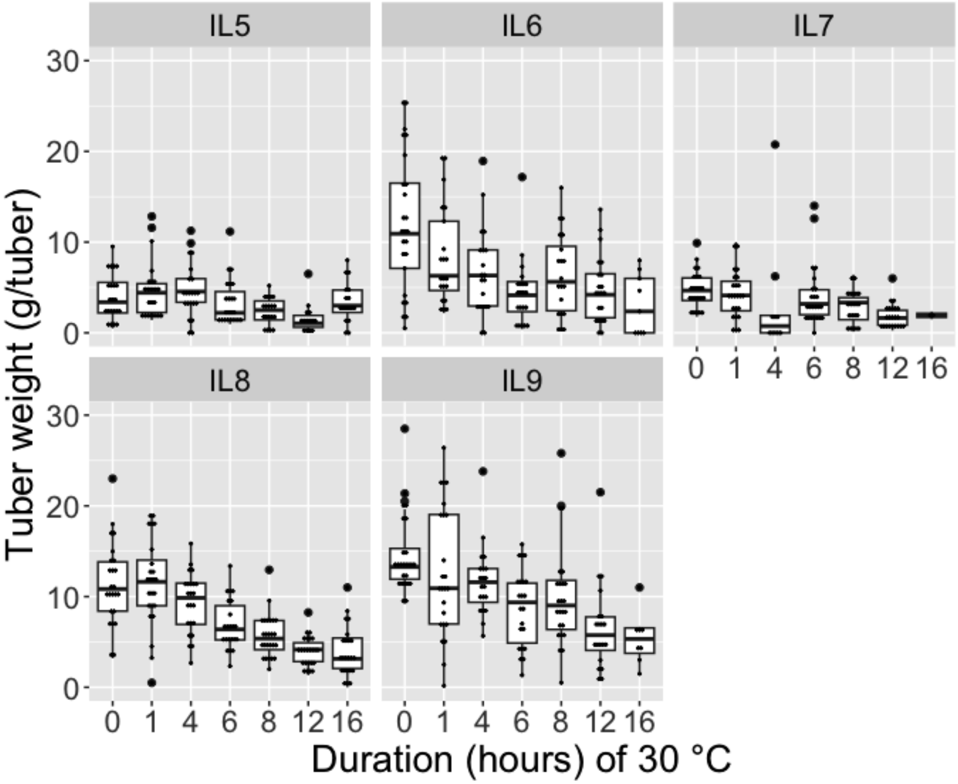

Figure 2.2: Tuber weight (g/tuber) of five different inbred lines grown under different durations of 30°C during the day (see Table 2.2 for temperature regimes). Boxes of the plot represent the 75th, 50th (median) and 25th percentile. Whiskers show the 1.5 × IQR (interquartile range) value. Data from Trial 2.

Also varying the DIF (i.e. the diurnal temperature variation, with a positive sign if the day is warmer than the night) affected the tuberisation (Trial 3). DIFs of 0°C (constant temperature of 2°C), 4°C (24/20°C) and 9°C (26/17°C) were applied. DIF mainly affected weight per tuber (Table 2.6); when grown at a constant temperature of 23°C, plants produced smaller tubers than when plants were grown at 24/20°C or 26/17°C (Figure 2.3). A larger DIF tended to increase tuber number (Table 2.6, Figure 2.3). Total tuber fresh weight was not affected (Table 2.6).

Table 2.6: P-values of the analysis of variance (ANOVA) of four different inbred lines grown under different temperature regimes and percentages of far-red light (Trial 3). Significant effects are indicated by points or asterisks: p value < 0.10: •, <0.05: *, <0.001: *". Average tuber weight was log transformed before analysis.**

Factor	Total tuber fresh weight	Average tuber weight	Number of tubers
Replicate	0.30	0.30	0.33
Temperature	0.16	0.04*	0.097 •
Spectrum	$3.26 \cdot 10^{-9}$ ***	0.80	$6.43 \cdot 10^{-5}$ ***
Temperature × Spectrum	0.45	0.80	0.15
Inbred line	$7.78 \cdot 10^{-13}$ ***	$7.49 \cdot 10^{-10}$ ***	$9.59 \cdot 10^{-9}$ ***
Inbred line × Temperature	0.16	0.056	0.086
Inbred line × Spectrum	0.64	0.82	0.70
Inbred line × Temperature × Spectrum	0.08 •	0.97	0.02 *

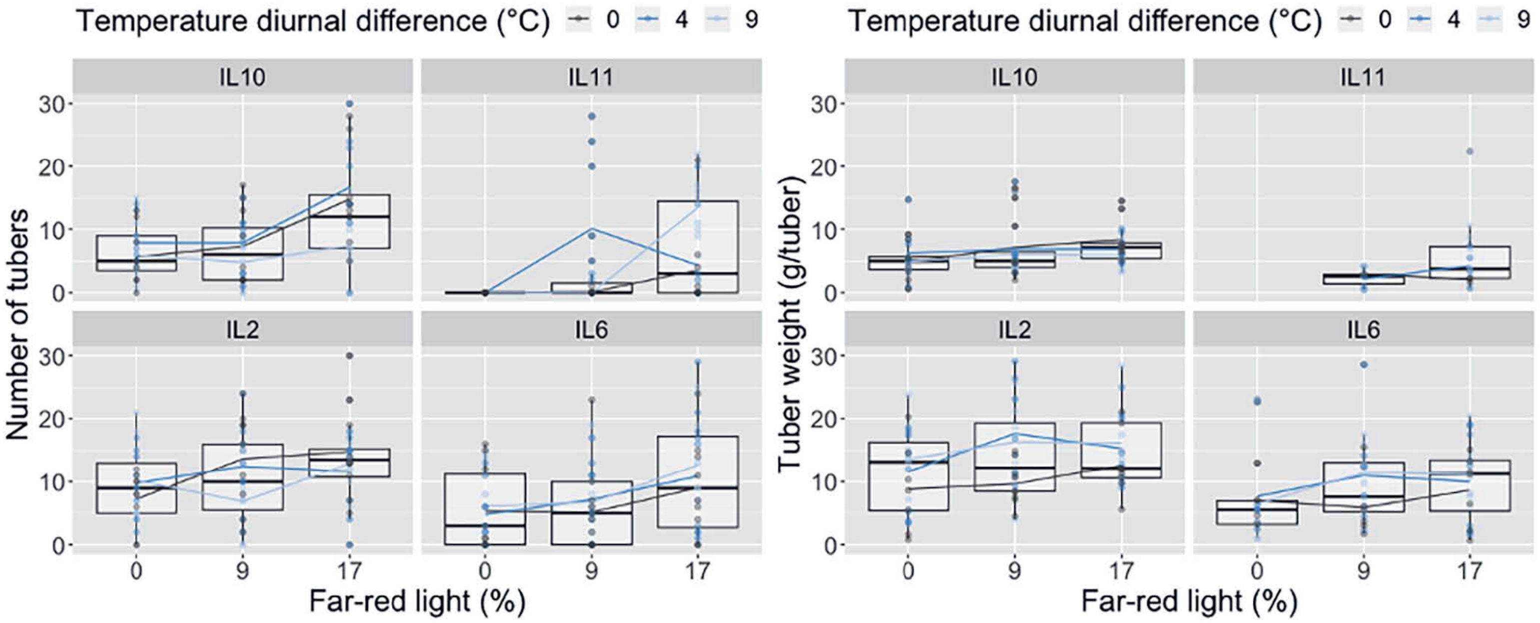

Figure 2.3: Boxplots of number of tubers per plant (left) and tuber weight in g/tuber (right) for four different breeding lines, grown under 0%, 9% and 17% far-red light, and under three different temperature regimes with equal average temperature and a contrasting day/night difference. Boxes of the plot represent the 75th, 50th (median) and 25th percentile. Whiskers show the 1.5 × IQR (interquartile range) value. Data from Trial 3.

High light intensity improves yield by increasing number of tubers

Increasing light intensity had a positive effect on tuber production in all inbred lines (Figure 2.1). Total tuber weight per plant was significantly higher when grown under 400 or 550 µmol m^{-2} s^{-1} compared to 250 µmol m^{-2} s^{-1} (Table 2.4). Depending on inbred line and temperature, the average yield increase was up to a factor of 3.6. Also number of tubers was increased by an increase in light intensity (Table 2.5), up to a factor 5. Average weight per tuber was not affected by light intensity.

Increasing far-red light improves yield by increasing number of tubers

Tuber production of all used inbred lines was improved when the percentage of far-red light in the spectrum was increased by up to 17% (Figure 2.3). Total tuber fresh weight was significantly higher when the proportion of far-red light in the spectrum was increased from 0 to 9 or 17% (Table 2.6). Average tuber weight per tuber was not significantly affected by far-red light in the spectrum (Table 2.6), despite a positive trend (Figure 2.3). Tuber number was significantly increased when increasing the proportion of far-red light (Table 2.6), both from 0% to 9% and from 9% to 17% (Figure 2.3). Depending on temperature and genotype, plants produced on average up to 2.7 times more tubers when grown under 17% far-red light compared with 0% (Figure 2.3). IL11 even barely produced tubers without far-red light in the spectrum. So, the increase in total tuber fresh weight per plant under high far-red light was the result of an increase in number of tubers.

Interactions between temperature and light and inbred line

For temperature and light intensity, an interaction was present for total tuber fresh weight; for tuber number and average tuber weight, there was no interaction (Table 2.4). Between temperature and light spectrum, no interaction was found for any of the yield traits (Table 2.6).

2.4 Discussion

The effects of high temperature, light intensity and light spectrum on tuber production of diploid potato genotypes were investigated in three climate room trials.

Temperature regime affects tuber weight and number

Three trials were performed in which different temperature treatments were applied. In the first trial, we investigated the effect of different average temperatures (20/16, 24/20, 28/24 and 32/2°C day/night) with a constant difference between day and night of 4°C. In the second trial, average temperature (between 22 and 26°C) was increased by altering the duration of high temperature during the day, so without changing day and night temperatures or the difference between day and night. In Trial 3, we investigated the effect of variation in temperature diurnal difference.

Our trials revealed that increasing the average temperature leads to lower total tuber fresh weight in diploid inbred lines. This was the result of fewer tubers as well as smaller tubers. These findings are in line with previous research on tetraploid cultivars, in which later tuber initiation and lower tuber growth rates were reported with an increase in temperature (Tengli et al. 2022; van Dam et al. 1996). Generally, assimilate partitioning shifts from the tubers towards the shoot with increasing temperature (Bodlaender, 1963; Hancock et al. 2014; van Dam et al. 1996), although different cultivars can show different responses or sensitivity to increased temperature (Ávila-Valdés et al. 2020; Marinus and Bodlaender, 1975; Struik et al. 1989b).

Also in this research, we found significant interactions between temperature and inbred line for number of tubers, tuber weight and total tuber fresh weight. These interactions were found in Trial 1, where average temperature was increased by increasing day and night temperatures, while the diurnal temperature difference was kept equal, as well as in Trial 2, where average temperature was increased by extending the period of 30°C during the day.

Although the response itself was qualitatively similar for the different inbred lines (lower tuber production at higher temperature), the sensitivity to increased temperature differed. In Trial 1, IL4 produced a total tuber weight of 96.5 g per plant under a temperature of 28/24°C, which is 21% of the total tuber weight that was produced at 20/16°C. IL1 on the other hand produced a tuber weight of 6.8 g per plant under 28/24°C, which was only 1% of the tuber production at 20/16°C. In Trial 2, IL8 produced 226 g total tuber weight per plant under 0 h of heat. This decreased to 66%, 40% and 19% of the 226 g when applying 8, 12 and 16 h of 30°C, respectively. In IL9, tuber production was 155 g without heat treatments, and it decreased to 65%, 17% and 2% of 155 g at 8, 12 and 16 h of 30°C, respectively.

Besides the average temperature, also varying the difference between day and night temperature (DIF) while keeping the average temperature equal (Trial 3) affected tuber production. Despite a higher day temperature, a difference of 9°C led to production of tubers with a higher weight than a constant day/night temperature. For example, IL2 produced tubers of 9.7 g/tuber on average at a constant temperature of 23°C, while with a diurnal difference of 9°C tuber weight was 16.3 g/tuber in the medium far-red spectrum. Although there was no significant interaction between temperature DIF and inbred line, IL10 did not produce a higher tuber weight at a larger DIF. For total fresh weight and tuber number, only positive trends of a larger day/night difference were found. These findings are in line with earlier performed research on tetraploid cultivars grown from seed tubers that showed higher number of tubers in low night temperatures, and a larger negative effect on tuber number of high night temperature than of high day temperature (Bodlaender, 1963).

Increasing light intensity improves yield by increasing number of tubers

Increasing light intensity enhanced tuber number, and therefore led to increased yield in our trials. Up to a certain maximum, increasing light intensity leads to increased CO2 assimilation rates in plants (Taiz and Zeiger 2006). Also in potato, increased assimilation rates were found under high light intensity compared with lower light intensity (Stutte and Yorio, 1996). Indeed, total plant and tuber dry weights were lower in a field trial with reduced light intensity by shading (Bodlaender 1963). Furthermore, Singh (1988) reported lower dry matter production in a shaded field trial compared with full sunlight. The current trial also shows that diploid inbred lines produce a higher total tuber fresh weight under high light intensity compared with low light intensity. In this case, the higher total fresh weight was the result of a higher number of tubers. This was contrasting to the result of Singh (1988), who found increased tuber number as well as increased tuber weight with higher light intensity. The difference could be explained by the type of the trial, Singh performed a field experiment, while our plants were grown in 3 L-pots with limited space. Moreover, the high light intensity in the climate room of 550 µmol m^{-2} s^{-1} is not comparable with light intensities in field conditions that can be more than three times higher (Marcelis et al. 2018).

No interactions between light intensity and inbred line were found in our research, all inbred lines produced a higher total tuber weight under a high light intensity. The inbred lines differed in total tuber weight production, with IL1 producing on average 578 g per plant while IL3 produced only 118 g per plant, in the high light intensity treatment in the lowest temperature of Trial 1. Furthermore, in some other trials, no differences between genotypes were found (Bodlaender, 1963), while in other research, large differences in response to shading were discovered (Menzel, 1985).

Increasing far-red light improves yield by increasing number of tubers

In this study, we varied the ratio of far-red light in relation to the other light colours. Far-red light is known to affect plant growth and development, plants can perceive the ratio of red and far-red light via the photoreceptors phytochromes. One of the processes that is affected by the red:far-red ratio is the time until flowing in Arabidopsis and several other species (Demotes-Mainard et al. 2016). Although the pathway is not fully understood yet, the initiation of flowering and tuberisation in potato is connected (Navarro et al. 2011). Indeed, Plantenga et al. (2016) found accelerated tuberisation in potato under increased far-red light conditions.

In this research, we did not measure earliness of tuberisation, but we did find more tubers produced under high far-red light. In the research of Plantenga et al. (2016) in which in vitro-derived plantlets were used, an increased number was found in one of the two tuber producing genotypes when the far-red levels were increased, while both genotypes had increased total tuber weight. Although tuber weight was not determined, this indicates that tuber weight was also increased at least in one genotype. In this trial, we did not find a genotype effect on the response to far-red light: all inbred lines showed increased total fresh weight with more tubers and similar tuber size for light treatments. The highest far-red percentage in this study was 17%, which is close to the far-red level of 19% in the sunlight spectrum (Zhen et al. 2019). Still, the climate room conditions probably are not comparable with field conditions, as factors like time of the day and reflection of far-red light from neighbouring plants play a role.

Interaction between light and temperature

A low light intensity as well as a high temperature led to lower total tuber fresh weight. An interaction was found between light intensity and temperature, where a high temperature had a smaller negative impact on tuber yield at a high light intensity than at a low light intensity. Indeed, earlier performed research has shown that the optimal temperature for tuber yield is higher under a high light intensity compared with a low light intensity (Bodlaender, 1963). Also Menzel (1985) found a larger effect of shading when the temperature was high in one cultivar. Although we did find interaction between light and temperature for total yield, no interaction was found for tuber number or tuber weight. As total yield is a product of tuber number and weight, we would expect an interaction for these traits, especially because varying in temperature did lead to significant differences in tuber weight and number, and light intensity affected the tuber number.

Conclusions and implication

In this research, we have shown the effects of light intensity, proportion of far-red light and temperature on tuber number, tuber weight and total yield in diploid potato inbred lines. Increase in light intensity or percentage of far-red light in the spectrum affected the total tuber fresh weight by enhancing the number of tubers produced per plant. The average temperature affected both tuber number and tuber weight, while the difference between day and night temperature only affected tuber weight (Figure 2.4). Increase in average temperature led to a decrease in tuber number and average weight, and a higher DIF led to heavier tubers. The used inbred lines were differently sensitive to high temperatures, but no interactions were found between inbred line and light intensity.

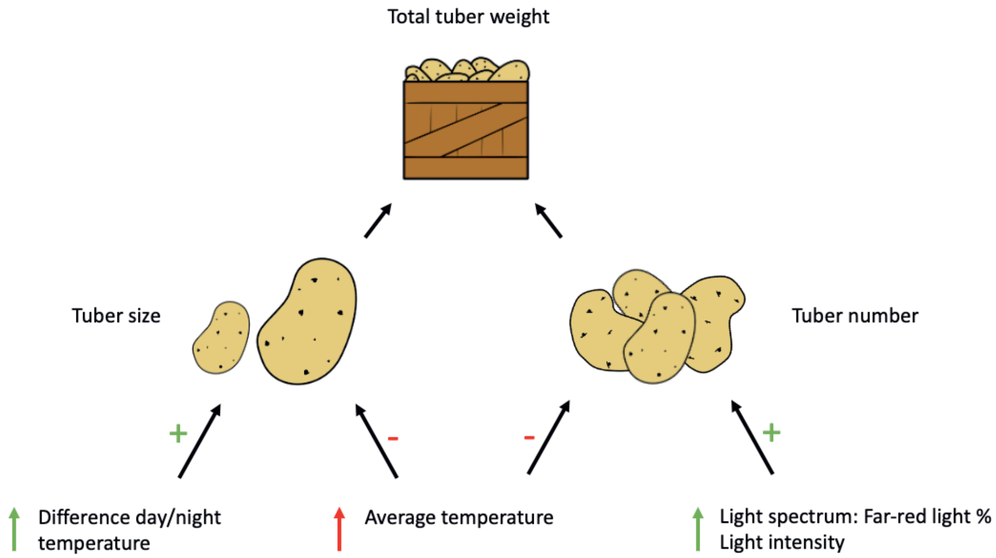

Figure 2.4: Schematic overview of how tuber size, tuber number and indirectly total tuber weight were affected by light and temperature, with '+' indicating an increasing effect on the tuber trait, and '–' indicating a decreasing effect. Increasing the difference in day/night temperature led to an increase in tuber size, while increasing the average temperature reduced tuber size as well as tuber number. Changes in the light spectrum affected tuber number: increasing the percentage of far-red light in the spectrum led to a higher tuber number. Also increasing the light intensity led to an increase in tuber number.

Variation in sensitivity to high temperature can be used to breed for temperature stress tolerance. By crossing susceptible and tolerant inbred lines, a mapping population can be created. For subsequent research, these mapping populations can be used for molecular studies to unravel the mechanisms of tuberisation under high temperatures (Campbell et al. 2022). When screening such a mapping population for high temperature tolerance, it is important to choose and control the climate conditions carefully because the plants can respond differently to high temperature under different light conditions. In these trials, we found variation for sensitivity to high average temperatures between the inbred lines for tuber weight as well as tuber number. This variation can be used for breeding heat tolerant hybrids. With climate change, breeding for stress tolerant crops becomes increasingly important (Bhadouria et al. 2019; Li et al. 2009), and heat-tolerant potato hybrids will help increase food security.

Besides this use for breeding, the results of this research show a great potential of steering the development of potato plants using light intensity, temperature and proportion of far-red light in the spectrum. Especially for production of mini or micro tubers, which generally is done indoors to prevent infection with pests or diseases, this can be relevant. The goal in these production systems is to produce as many tubers of a minimum weight as possible, and using the right climate for that can make the production more efficient. Follow-up research could be interesting to investigate whether tuber numbers increase more at a higher far-red level.

Chapter 3

Opening eyes on seedling tuber quality in potato: size matters

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Abstract

For potato, diploid hybrid breeding is a novel breeding technique that speeds up the development of new varieties. A consequence of hybrid breeding is the introduction of hybrid true potato seeds as starting material. From these seeds, seedling tubers can be produced in one field season, to use as starting material for a seed or a ware crop in the following year. For breeding purposes as well as for seed crop and ware crop production it is essential to produce seedling tubers of high quality. The production of seedling tubers is a new step in the potato production chain, therefore, we investigated the effect of tuber quality traits on plant development and yield. With similar seedling tuber weight, more eyes per seedling tuber led to more stems per plant. This was compensated by a lower number of tubers per stem resulting in an equal total tuber number and weight per plant at the end of the growing season. A higher seedling tuber weight led to a higher soil cover in the field. Therefore, all hybrids produced a higher total tuber weight per plant when grown from larger seedling tubers than when grown from smaller seedling tubers, while number of eyes and stems per tuber has no effect on final yield when using equal seedling tuber weight.

3.1 Introduction

Recently, potato breeding was revolutionized by the introduction of diploid hybrid breeding (Stokstad 2019). Whereas genetic gain in conventional breeding is low and targeted breeding is difficult due to large variation in tetraploid material, diploid hybrid breeding enables stacking of desired traits and a more predictable outcome of the breeding process (Lindhout et al. 2011; Jansky et al. 2016; Lindhout and Struik 2023). Through self-compatibility and the simpler genetics of diploids, homozygous parent lines can be produced by performing self-pollinations (Lindhout et al. 2018; Eggers et al. 2021;). By crossing two of these homozygous parent lines, a diploid hybrid is produced that can be planted in the field (de Vries et al. 2023). Besides more efficient breeding, parent lines as well as hybrids can be used in research to understand traits and perform for example mapping studies (Meijer et al. 2018; Prinzenberg et al. 2018; Endelman and Jansky 2019; Korontzis et al. 2020).

A hybrid variety of potato is the result of the cross between two homozygous parent lines, therefore, the starting material is hybrid true potato seed (HTPS). Advantages of HTPS compared to conventional seed tubers are the small size of the seeds which makes them easy to store and to transport. In cool conditions they can be stored for more than 40 years when dried and stored in good conditions (Pallais 1987), and, contrary to seed tubers, HTPS are usually disease-free. Moreover, starting material can be scaled up very fast and made available to end-users (Kacheyo et al. 2023). In one crossing season, millions of HTPS can be produced, which can in turn be used to produce seedling tubers in the following season (de Vries et al. 2023). When starting with a single seed tuber, multiplication takes many years until a new variety can be released.

With HTPS as starting material, there are different cultivation pathways to grow ware tubers (van Dijk et al. 2021). Seedlings can be produced in greenhouse conditions from HTPS, after which they can be transplanted into the field for ware tuber production. A disadvantage of starting a ware crop from HTPS is a longer growing season compared with starting from seed tubers (van Dijk et al. 2022). This can lead to lower yield in temperate climates such as the Netherlands. In East-Afrika, however, using HTPS as starting material can be an advantage due to high disease pressure when grown from seed tubers (de Vries et al. 2016; den Braber et al. 2023). Another pathway is to grow seedling tubers from seedlings produced in a greenhouse and use these as starting material for a ware crop or another generation of seed tubers in the subsequent year (Stockem et al. 2020; van Dijk et al. 2021). Especially in medium or high tech-cropping systems the production of seedling tubers would be a desirable step, as the whole production chain is optimized for growing from seed tubers.

To introduce diploid hybrids into the potato production chain with seedling tubers as starting material, it is essential to produce high quality seedling tubers. High-quality starting material is needed to produce a vigorous crop in the field with high potential yield (Caldiz 2009). Additionally in breeding programmes it is important to have high-quality starting material, because selections are based on the results of field trials. As breeders want to select the hybrids with the highest genetic potential, high-quality trials where yield differences are the result of genetics rather than of other sources of variation such as field gradients (Stockem et al. 2022) or seed-tuber quality are important.

Important quality traits in seed tubers that affect number and vigour of stems, plant development and yield are the physiological age, seed tuber weight and number of eyes per seed tuber. These traits are interrelated (Struik and Wiersema, 1999). Physiological age of seed tubers is affected by chronological age, as well as by environmental conditions during the growing season of the seed, the conditions during storage and by genotype (Struik and Wiersema, 1999; Struik 2007a; Kwambai et al. 2023). During the growing season, an important determinant for physiological age of the produced seed tubers is temperature, and besides that factors such as water availability and light conditions can play a role (Caldiz 2009; Struik and Wiersema 1999). This means that seed tuber lots produced in different locations are often of different physiological age (Kwambai et al. 2023), resulting in variation in plant development and yield when planted together in one field.

Development of the crop in the field is affected by the physiological age of the mother tuber (Caldiz 2009). An important difference between seedling tubers and conventional seed tubers is the physiological age of the material. Conventional seed tubers usually are multiplied over several generations where mother and grandparent tubers affect the development of the subsequent crop (Went 1959). Seedling tubers on the other hand are produced in one single season from true seed, as a result the physiological age only is affected by the conditions of one growing and storage season.

Besides physiological age, the tuber weight and number of eyes per tuber are important quality traits of seed tubers. Larger seed tubers usually lead to earlier emergence, more stems and faster ground cover, and yields are higher due to more tubers produced per plant (Struik and Wiersema 1999; Ebrahim et al. 2018). In tetraploid varieties, positive effects of larger seed tubers produced from seedlings were found even after the first and second clonal generation (Maris 1986; Brown 1988). Moreover, there is a positive relation between seed tuber size and eye number (Reeves and Hunter 1980; Struik and Wiersema 1999). Each eye can develop into one or more sprouts, affecting the number of stems per plant in the field. Number of stems per plant is one of the components that determines the yield of the crop, and often farmers adjust plant density to seed tuber size to achieve a stem density that is optimal for the crop's purpose or market outlet. As the proportion of eyes producing a sprout, the number of sprouts per eye, and the proportion of sprouts that develops into tuber-bearing stems, are all affected by the physiological quality of a seed tuber, it is important to assess how seedling tubers behave that have been produced by TPS-grown plants and therefore are not affected by the physiological age of seed tubers as is the case for seed tubers from seed-tuber grown plants.

In this research, we aimed to determine the effect of quality traits in seedling tubers on plant development in the field and on yield parameters, to understand to which extent selections in a breeding programme are the result of seedling tuber quality rather than genetics. We formulated the following sub-questions:

1) What is the variation in number of eyes and weight of tubers in diploid hybrid seedling tubers?
2) What is the effect of number of eyes, plant and crop development and production origin on plant development and yield?

It is expected that more eyes per seedling tuber will result in more stems per plant, and with that in more tubers and higher yield per plant. Also higher seedling tuber weight probably will result in higher yield per plant. With the results of this research we will be able to improve the selection process in hybrid potato breeding to select for highest yieldinggenotypes. Moreover, we will improve the cropping system for a ware crop grown from seedling tubers by understanding the effect of seedling tuber traits on ware yield.

To answer the above-described questions, seedling tubers of four different hybrids and different origins were selected for number of eyes and weight of individual seedling tubers. The different tuber classes were used to perform two field trials in which we compared the tubers differing in number of eyes, weight and production origin.

3.2 Material and methods

Two trials were performed to determine the effect of quality traits in seedling tubers on development of potato plants in the field and on yield parameters. In Trial 1, the effect of number of eyes was examined in four different diploid hybrids (H1, H2, H3, H4). In Trial 2, we investigated the effect of seedling tuber weight and production location of seedling tubers of the same hybrids as in Trial 1.
Trials 1 and 2 were performed on the same field back-to-back. The field was located at Grebbedijk (Wageningen, NL on a light clay soil (Table 3.1). Seedling tubers of both trials were planted on 02.05.2022, haulm killing was done on 29.08.2022 and the trials were harvested on 23.09.2022.

Planting material

Both trials were performed with four diploid test-hybrids (H1, H2, H3, H4) that were produced in a diploid hybrid breeding program as described by Lindhout et al. (2011, 2018). All hybrids produce medium – high yields. They contrasted for tuber shape, with H1 and H2 producing long-oval tubers, H3 producing long tubers and H4 producing round-oval tubers. Seed production of hybrid seeds was performed in 2020 in a greenhouse (Ressen, NL). Seedling tubers were produced in 2021 under field conditions as described by Stockem et al. (2020). For Trial 1, all seedling tubers were produced in Garsthuizen (NL). In Trial 2, production location of the seedling tubers was one of the experimental factors. Seedling tubers for this trial were produced in Emmeloord (EMM), Garsthuizen (GAR) and Anna Paulowna (ANN), all located in the Netherlands. Number of growing days in the field was 128, 93 and 110 days for Emmeloord, Garsthuizen and Anna Paulowna, respectively. Soil characteristics of these sites can be found in Table 3.1. The crop was irrigated adequately and kept disease-free by preventive spraying with fungicides. To avoid virus infection insecticides were applied, and roguing was done. Tubers were sent for testing for presence of viruses, ring rot and brown rot, and found disease-free.

Table 3.1: Physical composition of the soil at the production sites of the seedling tubers

Location	Clay (%) <2 µm	Silt (%) <2-50 µm	Sand (%)>50 µm	pH
Emmeloord	11	31	49	7.4
Garsthuizen	13	38	45	7.4
Anna Paulowna	19	30	39	7.5

<u>Quality of starting material</u>

To compare the physiological quality of seedling tubers at the moment of planting, a sprouting test was performed with seedling tubers of all treatments. Tubers for these trials were selected based on the same criteria as for the field trials. The sprouting test was designed as a complete randomized block design with 10 replicates and one tuber per replicate.

Sprouting tests were performed as described by Van der Zaag and Van Loon (1987). Seedling tubers were placed in a dark climate room in the same week as the field trials were planted.

Temperature was set at constant 18°C with a relative humidity of 85%. The sprouts were measured after 24 days in these conditions.

At the start of the test, seed tubers were de-sprouted, and tuber weight and number of eyes were assessed per tuber. At the end of the sprouting test, the number, length (mm) and fresh weight (g) of the sprouts was recorded per seedling tuber. The sprouting capacity was calculated by dividing the fresh weight of the sprout by the initial fresh weight of the tuber.

Treatments and trial design

Trial 1

The goal of Trial 1 was to determine the effect of number of eyes in seedling tubers on plant development in the field and on yield parameters. This was done in the diploid hybrids H1, H2, H3 and H4. Seedling tubers were divided into a treatment with a high and a low number of eyes per tuber, while seedling tuber weight was kept as equal as possible between the eye number classes. This was done for each hybrid separately because the ranges differed among the hybrids. The selection procedure of seedling tubers is explained below.

The trial was designed as a split-plot trial with hybrid as main plot, and number of eyes as sub-plot with three replicates. Each net plot consisted of 20 plants planted on two ridges, with a planting distance of 20 cm within the ridge and 75 cm between the ridges. On either side of the plot, one border row was placed containing the same genotype in the same planting arrangement.

Trial 2

In Trial 2, we investigated the effect of production origin and tuber weight of seedling tubers from the hybrids H1, H2, H3 and H4. Seedling tubers were divided into a high and a low weight while keeping number of eyes as equal as possible (Table 3.2) between the two treatments. Origin of the seedling tubers were the production locations Emmeloord, Garsthuizen and Anna Paulowna (Table 3.1). The trial was laid out as a split-split-plot trial with hybrid as main-plot, origin as sub-plot and seedling tuber weight as sub-sub-plot, with a net plot size of 20 plants per plot and three replicates. Plot layout was equal to plots in Trial 1.

Selection of seedling tubers

To determine the variation for number of eyes and tuber weight, a batch of 420 seedling tubers per hybrid in the size class 35-45 mm was scored for these traits. Additionally, seedling tuber weight of 200 tubers per hybrid was scored for a batch of size class 28-35 mm. Based on the variation that was found we determined the treatments for both trials.

For Trial 1, we selected seedling tubers with the largest possible difference between high and low number of eyes. This was done per hybrid, and within each eye class not more than a difference of one eyes was allowed (Table 3.2). Seedling tuber weight was kept as equal as possible within the hybrid.

Table 3.*2*: Properties of seedling tubers per hybrid in the different treatments in Trial 1.

Hybrid	Eye class	Average number of eyes	Range of number of eyes per class	Average seedling tuber weight (g)	Range of seedling tuber weight in treatment (g)
H1	High	5.3	5-6	47.9	40-55
H1	Low	2.8	2-3	46.1	40-58
H2	High	7.3	7-8	51.9	40-62
H2	Low	4.8	4-5	50.6	44-61
H3	High	6.3	6-7	54.9	41-66
H3	Low	3.8	3-4	54.4	41-70
H4	High	6.3	6-7	41.7	35-49
H4	Low	4.6	4-5	40.8	36-48

For Trial 2, seedling tubers of the size class 28-35 mm and 35-45 mm were used. Per size class and per hybrid seedling tubers were collected that had a similar tuber weight to reduce variation within the treatment (Table 3.3). So for these treatments, seedling tuber size as well as seedling tuber weight is known. For Replicate 1, number of eyes was counted for all treatments.

Table 3.3: Average number of eyes per tuber and seedling tuber weight (g/tuber) of Replicate 1 in Trial 2 (n=20). Abbreviations of production origins are: GAR = Garsthuizen, ANN = Anna Paulowna, EMM = Emmeloord.

Hybrid	Origin	Size class (mm)	Number of eyes per seedling tuber	Seedling tuber weight (g)
H1	ANN	28-35	3.2	24.5
H1	ANN	35-45	3.3	38.2
H2	ANN	28-35	3.7	26.9
H2	ANN	35-45	4.5	39.8
H3	ANN	28-35	4.1	29.0
H3	ANN	35-45	4.0	54.0
H4	ANN	28-35	3.9	22.5
H4	ANN	35-45	4.4	45.9
H1	EMM	28-35	3.8	25.7
H1	EMM	35-45	4.0	39.8
H2	EMM	28-35	4.7	26.3
H2	EMM	35-45	5.0	41.5
H3	EMM	28-35	3.9	29.6
H3	EMM	35-45	4.6	57.1
H4	EMM	28-35	3.9	21.8
H4	EMM	35-45	4.7	46.4
H1	GAR	28-35	3.9	25.3
H1	GAR	35-45	4.4	39.6
H2	GAR	28-35	5.0	26.8
H2	GAR	35-45	5.2	39.5
H3	GAR	28-35	4.3	28.6
H3	GAR	35-45	4.8	56.3
H4	GAR	28-35	5.0	22.4
H4	GAR	35-45	5.2	46.5

Measurements and statistical analysis

The same measurements were done in both trials. During the growing period, we measured emergence and soil cover over time, number of stems was counted at 65 days after planting (DAP). Ground cover was measured thirteen times between 28 and 119 DAP. This was done using a grid (75 × 75 cm), which was divided into 100 squares (7.5 x 7.5 cm). A square was counted as 1% groundcover if it was filled at least 50% with canopy. At harvest, tuber weight and tuber number were measured per plot. To compare soil cover among the treatments, the area under the canopy cover progress curve (AUC) was calculated using the R package DescTools (Signorell et al. 2017).

In Trial 1, yield was decomposed into different components that determine yield to reveal the effects of number of eyes on plant development and yield. This was done using the following function: Yield (g/plant) = number of eyes/seed tuber × number of stems/eye × number of tubers/stem × average single tuber weight.

For statistical analysis, R (R Core Team 2021) was used. For Trial 1, the ANOVA for split-plot trials was used from the Agricolae package (Felipe de Mendiburu 2020) and for Trial 2 the ANOVA for split-split-plot trials. For these trials Fisher's LSD was used as post-hoc test. In all trials, residuals were tested for normal distribution. Log or square root transformations were applied when residuals were not distributed normally. The relation between yield components in Trial 2 was calculated using Pearson's chi-squared tests.

Data of the sprouting tests partly were not distributed normally, even after transformation. Therefore, the non-parametric Kruskal Wallis test was performed. This was done per hybrid separately, the treatments were separated according to Trial 1 and Trial 2. For the treatments of Trial 2, Dunn's test was used as post-hoc test. Here, we compared per hybrid separately the effect of number of eyes on the one hand, and the effect of seedling tuber size and production origin on the other hand.

3.4 Results

3.4.1 Sprouting test

A sprouting test was performed to compare the physiological age of the starting material. Tubers were collected using the same criteria as tuber selection for Trial 1 and 2, and data were analyzed per hybrid separately.

Effect of number of eyes

Number of eyes affected only sprout length in hybrid H3, where more eyes led to a higher sprout length (Table 3.4). The rest of the traits that were measured in the sprouting test was not affected by number of eyes; as a result the physiological age in tubers with high or low number of eyes was similar.

Effect of size and origin

Several traits in the sprouting test were affected by tuber size and production origin. Number of sprouts and total sprout fresh weight were higher in the larger seedling tubers in hybrid H3 produced in ANN. Seedling tubers of hybrid H4 of size class 35-45 mm produced in EMM produced more sprouts and had a higher total sprout fresh weight than those in the size class 28-35 mm (Table 3.4). Also in H4, larger seedling tubers produced in ANN led to a higher sprout fresh weight. Sprout length was not affected by any of the tuber size or origin treatments. Number of sprouts per eye was only affected in hybrid H3 from EMM, where larger tubers produced more sprouts per eye.

As an indication of physiological age, sprouting capacity (total sprout fresh weight/tuber fresh weight) was calculated from seedling tubers in the different treatments. In the hybrids H1 and H3, sprouting capacity was higher in seedling tubers with a lower tuber weight (Table 3.4), in the other two hybrids no effect was found.

Table 3.4: Averages values of traits measured in the sprouting test. Significant differences calculated with the Kruskal Wallis test, followed by Dunn's posthoc test, are indicated with letters. When no letters are present, the value differs from no other value in that group. Significant differences were calculated separately per hybrid and for the eyes and the size and origin treatments, so values presented between horizontal lines were statistically compared with each other. Abbreviations of production origins are: GAR = Garsthuizen, ANN = Anna Paulowna, EMM = Emmeloord.

Hybrid	Origin	Treatment	Sprouting capacity	Number of sprouts	Sprout length (mm)	Sprout fresh weight (g)	Sprouts per eye
H1	GAR	eyes-high	0.021	4.3	176.7	0.98	0.95
H1	GAR	eyes-low	0.017	4.4	133.9	0.76	1.32
H2	GAR	eyes-high	0.017	3.7	140.3	0.87	0.57
H2	GAR	eyes-low	0.022	2.8	159.4	1.14	0.64
H3	GAR	eyes-high	0.013	5.3	149.3 a	0.74	0.98
H3	GAR	eyes-low	0.011	4.5	108.5 b	0.61	1.14
H4	GAR	eyes-high	0.014	4.4	136.3	0.61	0.78
H4	GAR	eyes-low	0.015	5.1	143.5	0.62	1.16
H1	ANN	size_28-35	0.024 a	3.7	126.6	0.59	1.21
H1	ANN	size_35-45	0.014 b	3.4	93.6	0.58	1.13
H1	EMM	size_28-35	0.026 ab	3.7	162.0	0.66	0.94
H1	EMM	size_35-45	0.020 ab	4.5	152.3	0.77	1.26
H1	GAR	size_28-35	0.024 ab	2.9	122.0	0.64	0.77
H1	GAR	size_35-45	0.013 b	3.3	110.3	0.50	0.80
H2	ANN	size_28-35	0.024	2.7	114.0	0.61	0.67 ab
H2	ANN	size_35-45	0.017	3.0	112.7	0.72	0.75 ab
H2	EMM	size_28-35	0.031	2.5	152.2	0.87	0.46 b
H2	EMM	size_35-45	0.024	3.3	183.0	1.00	0.85 a
H2	GAR	size_28-35	0.027	2.4	139.7	0.68	0.55 ab
H2	GAR	size_35-45	0.024	3.5	191.4	0.94	0.63 ab
H3	ANN	size_28-35	0.012 ab	3.4 b	77.3	0.35 b	1.01
H3	ANN	size_35-45	0.011 ab	5.6 a	120.3	0.61 a	1.20
H3	EMM	size_28-35	0.013 ab	3.9 ab	102.4	0.42 ab	1.06
H3	EMM	size_35-45	0.010 b	4.7 ab	126.2	0.57 ab	1.05
H3	GAR	size_28-35	0.015 a	4.3 ab	108.2	0.43 ab	1.00
H3	GAR	size_35-45	0.010 b	5.4 ab	131.3	0.60 ab	1.05
H4	ANN	size_28-35	0.019	4.2 ab	122.5	0.42 bc	1.08
H4	ANN	size_35-45	0.017	4.7 ab	126.2	0.85 a	1.13
H4	EMM	size_28-35	0.014	3.3 b	95.5	0.32 c	0.76
H4	EMM	size_35-45	0.017	5.4 a	147.1	0.82 a	0.99
H4	GAR	size_28-35	0.019	3.5 ab	106.5	0.43 bc	0.76
H4	GAR	size_35-45	0.014	4.6 ab	130.5	0.65 ab	0.82

3.4.2 Variation within seedling tuber batches

In seed tuber production, it is common practice to sort seed tubers based on square measure of the tubers. The size class 35-45 mm is often used for seed tubers. To determine the variation of tuber weight and number of eyes within this size class, these traits were measured in 420 tubers of each hybrid in the size class 35-45 mm (Figure 3.1). Range in number of eyes per seedling tuber was different among the hybrids. The largest range was found in H3 with a minimum of 2, and a maximum of 14 eyes per tuber. H1 had the smallest range with a number of eyes between 1 and 8. H3 also had the largest difference in tuber weight, with values between 31 and 143 g. In H4 the smallest difference was found with seedling tuber weights between 28 and 106 g.

So, although seedling tubers were sorted based on square measure, large variations for number of eyes (factor 4-8 difference between high and low eye number) and seedling tuber weight (factor 3.8 – 7 difference between high and low seedling tuber weight) were found.

3.4.3 Field results Trial 1: Number of eyes

In this trial, we investigated the effect of number of eyes in seedling tubers on plant development in the field and on yield parameters.

Total tuber weight per plant as well as number of tubers per plant were only affected by genotype, not by number of eyes in the seedling tuber. To reveal the effect of number of eyes on plant development and yield, a calculation of the contribution of different components to total yield was made, and in these components significant differences between the high and low eye-classes were found.

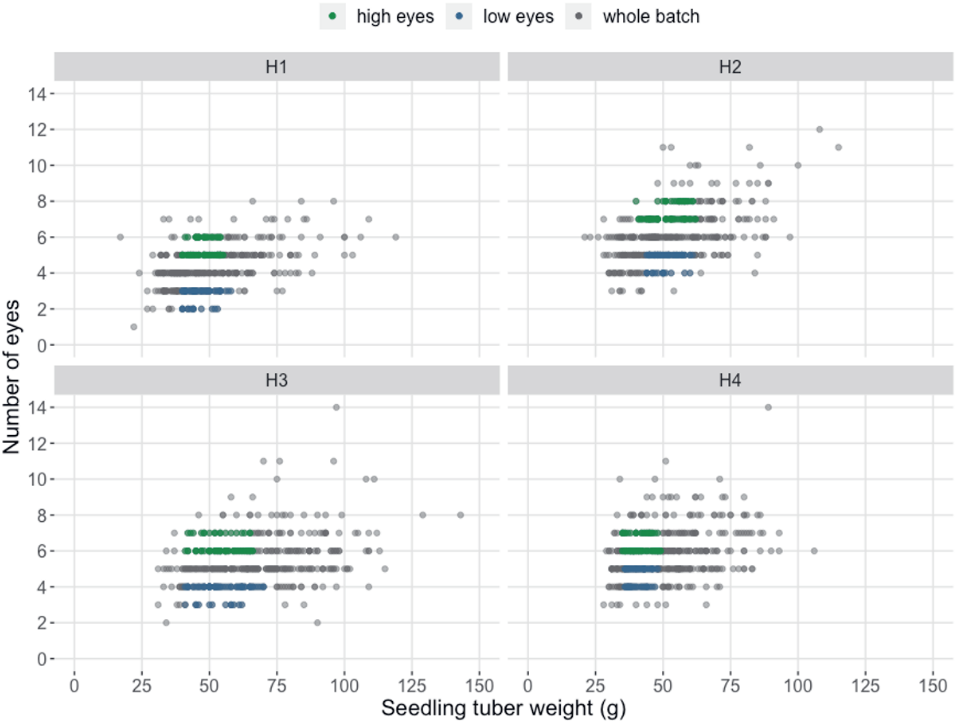

Figure 3.1: Seedling tuber weight and number of eyes per tuber of a batch of 420 tubers per hybrid in the size class 35-45 mm of the hybrids H1, H2, H3 and H4. Tubers that were selected for the high and low eyes treatments of Trial 1 are shown in yellow (high) and blue (low).

All genotypes produced more stems per eye in the low eye class than in the high eye class. Number of stems per eye was also affected by genotype, and an interaction between eye number and genotype was found, where H1 produced 1.6 times more stems per eye in the low eye class compared to the high eye class, and H4 only 1.1 times more. (Table 3.5, Figure 3.2). In total, however, seedling tubers with more eyes resulted in more stems per plant, and this was only affected by number of eyes, not by genotype (Table 3.5, Figure 3.2). Despite the higher number of stems per plant, no difference between treatments or genotypes was found for groundcover when comparing the area under the curve (AUC) of groundcover measurements.

Number of tubers per stem was higher (on average up to 0.4 tubers extra) in plants grown from seedling tubers in the low eye-class; this variable was also affected by genotype with H1 and H2 producing more tubers than H3 and H4. Together with the lower stems per plant, the higher number of tubers per stem in the low eye class led to a lack of difference in number of tubers per plant between seedling tubers with high and low number of eyes (Table 3.5). Tuber size was slightly higher in plants grown from seedling tubers with a low number of eyes (Table 3.5). Moreover, tuber size was affected by genotype. Overall, a higher number of stems per eye, combined with more tubers per stem and a slightly larger tuber size in seedling tubers in the low eye-class resulted in no difference in total tuber yield or number of tubers per plant between the high and low eye class. So, for total yield and number of tubers, the difference in number of eyes between the seedling tubers was compensated for by other yield components.

Table 3.5: P-values of ANOVAs on yield and yield components from Trial 1, AUC is area under the canopy cover progress curve

	Tuber weight per plant	No. of Stems per eye	No. of stems per plant	No. of tubers per stem	No. of tubers per plant	Tuber size	AUC
Hybrid	0.001**	2.3 x10^-5***	0.17	0.003**	0.001**	0.0008***	0.17
Eye class	0.73	9.1x10^-6***	0.0098**	0.03*	0.24	0.17	0.10
Hybrid × eye class	0.75	0.004**	0.84	0.52	0.56	0.82	0.40

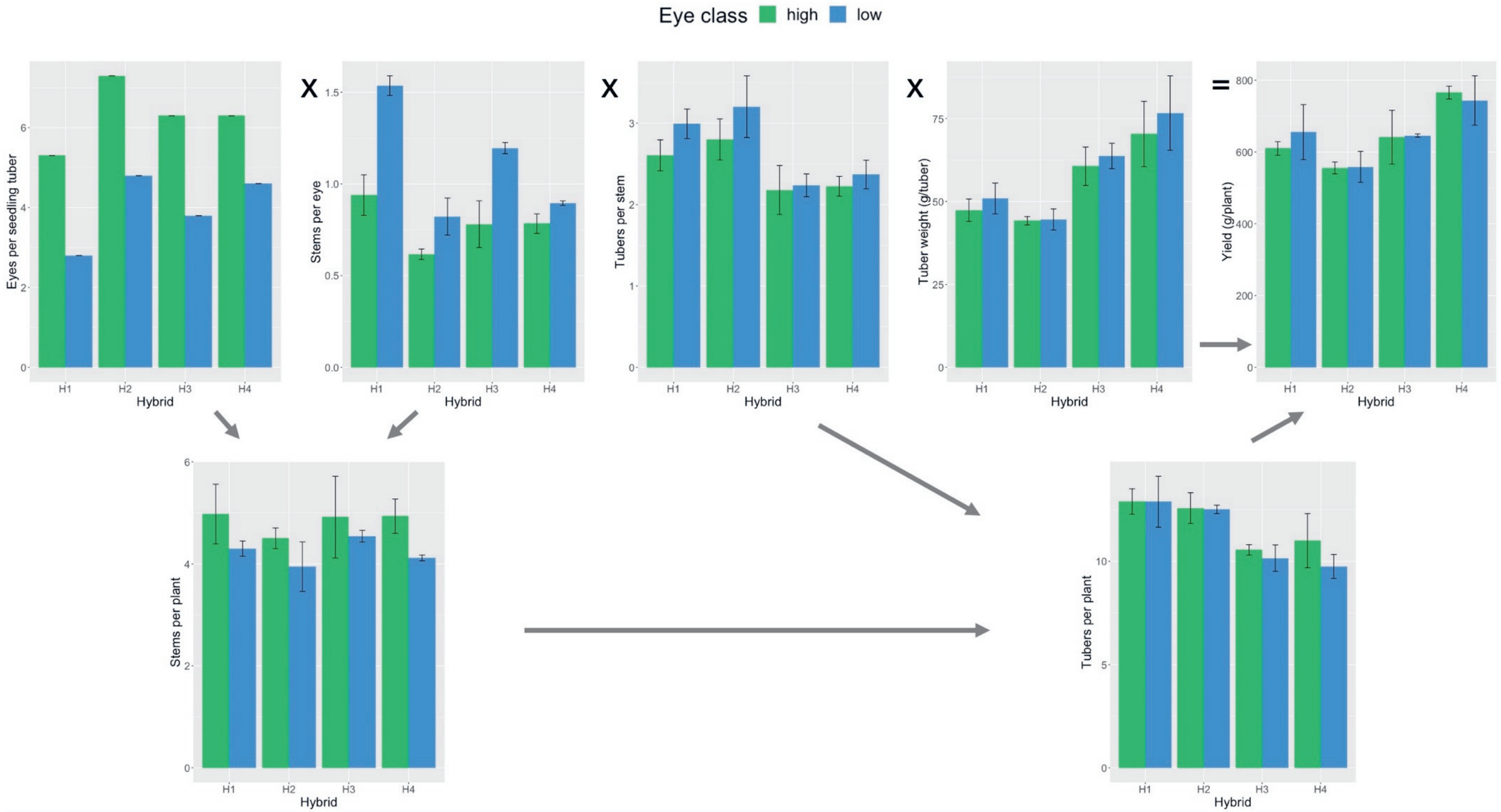

Figure 3.2: Bar plots of yield and yield components of four hybrids grown from seedling tubers with high or low eye number per seedling tuber.

3.4.4 Field results Trial 2: seedling tuber size and origin

In the second trial, the effects of seedling tuber size and production origin on plant development and yield were investigated. Seedling tubers of size class 28-35 and 35-45 mm were selected.

Table 3.6: P-values of ANOVAs performed for Trial 2, size and origin, AUC is area under the canopy cover progress curve.

	Tuber weight per plant	Tuber weight per tuber	No. of tubers per plant	No. of tubers per stem	No. of stems per plant	Yield per seedling tuber weight	AUC
Name	8.4 x10^-5 ***	3.4x10^-6 ***	0.0009***	0.1	0.2	4.1 x 10^-5 ***	0.3
Origin	0.2	0.07	0.7	0.7	0.9	0.5	0.1
Hybrid × origin	0.07	0.07	0.2	0.06	0.1	0.05	0.3
Size class	0.0008***	0.3	2.2 x 10^-6***	0.1	1	2.2* 10^-16	4.7 x 10^-7***
Size class × Hybrid	0.7	0.9	0.5	0.8	0.8	2.8*10^-7	0.5
Size class × Origin	0.09	0.2	0.4	0.4	0.4	0.03	0.9
Size class × Hybrid × origin	0.6	0.05*	0.09	0.2	0.3	0.5	0.7

A larger seedling tuber size led to an increase in number of tubers per plant, while there was no effect on average weight per tuber. This resulted in a higher total tuber weight per plant. (Table 3.6). Total tuber weight, average weight per tuber and number of tubers per plant all were affected by genotype, and no significant interactions between seedling tuber size and genotype were found. (Table 3.6). Despite the effect of seedling tuber weight on total tuber number, no significant effects of genotype, production origin or seedling tuber size on number of tubers per stems or number of stems per plant were found. The area under the curve (AUC) of ground cover was used as a measure for total ground cover during the season. AUC was significantly higher when larger seedling tubers were used.

Also for this trial, we calculated different yield components that contributed to total yield. Figure 3.3 shows the interrelations between these yield components of both seedling tuber size classes of H1. A negative correlation was found between number of tubers per stem and number of stems per plant for both seedling tuber size classes. No significant relation was found between number of tubers per stem and number of tubers per plant for both size classes. In the size class 35-45 mm, a positive relation was found between number of tubers per plant and tuber weight per plant, while this relation was not significant in the size class 28-35 mm (Figure 3.3). In both size classes, no relation was found between tuber weight per plant and number of stems per plant.

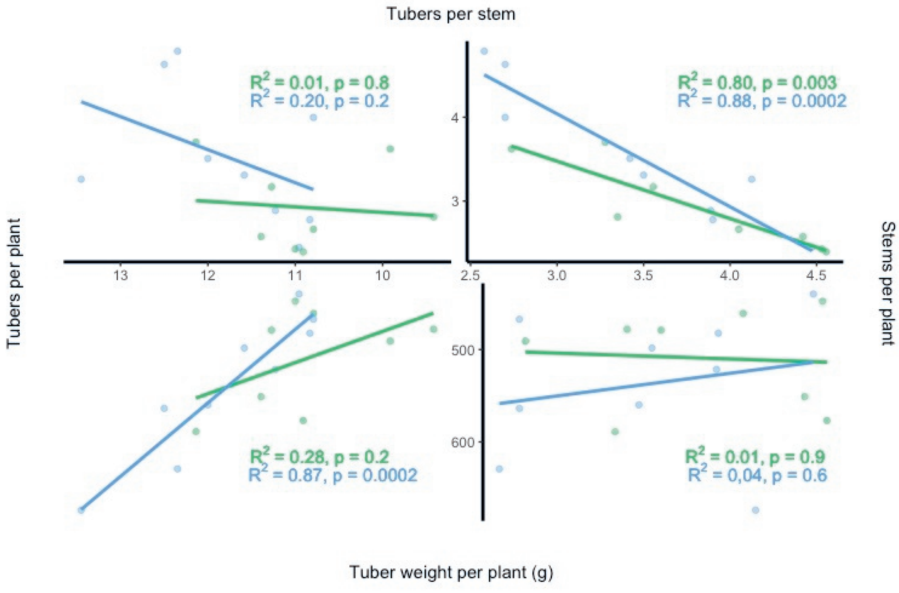

Figure 3.3: Interactions between yield components for seedling tubers of size class 28-35 and 35-45 of H1. R^2 and P-values, calculated using Pearson's chi-squared test, are indicated corresponding to the color of the plots.

To estimate the impact of differences in seedling tuber weight on yield, the increase in tuber yield per extra gram of seedling tuber weight was calculated (Table 3.7). To determine the possible variation in yield due to seedling tuber weight when sorting seedling tubers based on square measure, we used the variation that was found in the batch of 420 seedling tubers in size class 35-45 mm (Figure 3.1) for multiplying with the tuber yield increase per extra gram of seedling tuber (Table 3.7). Based on this calculation, the variation in seedling tuber weight within the size class 35-45 mm can lead to variation in yield of up to 16.1 Mg/ha, depending on the genotype (Table 3.7).

Table 3.7: Calculation of potential variation in yield due to differences in seedling tuber weight in the size class 35-45, based on measured variation in seedling tuber batch (Figure 3.1) and yield difference due to seedling tuber weight in Trial 2.

Genotype	Size class	Average seedling tuber weight (g)	Difference in seedling tuber weight between size classes (g)	Maximum difference measured in seedling tuber weight in size class 35-45 (Figure 1)	Tuber yield Trial 2 (Mg/ha)	Difference in yield between size classes (Mg/ha)	Tuber yield increase (Mg/ha) per extra gram seedling tuber	Potential variation in Mg/ha in size class 35/45
H1	28-35	25.2			32.0			
	35-45	39.2	14.0	102	34.2	2.2	0.16	16.1
H2	28-35	26.7			29.5			
	35-45	40.3	13.6	94	31.2	1.8	0.13	12.2
H3	28-35	29.1			35.0			
	35-45	55.8	26.8	112	38.1	3.1	0.12	13.0
H4	28-35	22.2			45.0			
	35-45	46.3	24.1	78	47.8	2.7	0.11	8.8

3.5 Discussion

To understand the impact of seedling tuber quality on plant and crop development and tuber yield, we performed two field trials in which we determined the effects of number of eyes, tuber size and production origin. Moreover, a sprouting test was performed to compare the physiological age of seedling tubers of the different treatments that were used.

3.5.1 Effect of number of eyes, production origin and size of seedling tubers

In Trial 1, we determined the effect of number of eyes in seedling tubers on plant and crop development and yield. As sprouts and eventually stems develop from the eyes of a seed tuber (Struik and Wiersema 1999), a tuber with more eyes potentially can develop more stems per plant. Indeed, we found that a higher number of eyes resulted in a higher number of stems per plant in the field. Previous research in tetraploid as well as diploid potato has shown that a higher number of stems lead to a higher number of stolons, and more stolons result in more tubers (Haverkort et al. 1990, Stockem et al. 2020).

In our research, the higher number of stems per plant did not result in more tubers per plant. An explanation might be the competition for resources among the stems. Previous research has shown that number of eyes per tuber and seed tuber size are positively related (Struik and Wiersema 1999). In the beginning of the growing period the stems share the resources from the mother tuber (Struik 2007b), where plants with more stems probably came from a larger seed tuber, with more resources. Later in the season the stems become independent units that compete for resources such as light and nutrients (Struik 2007b).

In our study, we selected seedling tubers that only differed in number of eyes, and not in tuber weight, so plants from tubers with more eyes that developed more stems did not have the advantage of larger initial resources. Indeed, no difference in ground cover was found between the two groups, so the plants with more stems were not able to capture more light than the plants with fewer stems. In our trial, no difference in total tuber weight or in number of tubers per plant was found between plants grown from seedling tubers with high or low number of eyes. The yield component analysis has shown that the higher number of stems was compensated for by a lower number of tubers per stem, resulting in equal number of tubers between the two groups.

In a second field trial, we investigated the effect of seedling tuber weight with similar number of eyes between the two tuber size classes. Seedling tubers of the size class 28-35 mm and 35-45 mm were selected as two treatments in Trial 2. A higher seedling tuber weight resulted in a higher total tuber weight and more tubers per plant. Also ground cover was higher in the size class 35-45mm, while the number of stems per plant and the number of tubers per stem did not differ between the size classes. So with a higher seedling tuber weight, rather than with more stems, plants develop faster which results in a higher ground cover, more tubers per plant and a higher total tuber weight. These results suggest that a higher number of eyes in seedling tuber only is advantageous when accompanied by a higher seedling tuber weight to result in a higher ground cover and in more yield.

Production origin from three locations in The Netherlands did not affect the yield or yield components that were measured, and also soil cover was not affected by production origin of the seedling tubers. Differences would be expected as growing days differed in the different locations. Also trials with seed tubers have shown that the production location affects the seed tuber quality (Kwambai 2013). Differences may be small because the production locations all were in the Netherlands and relatively close together. Also the soil compositions and pH values of the fields were similar. Moreover, seedling tubers that were used in the trial were selected for similar weight, this selection could have played a role as well.

3.5.2 Implications for breeding and ware crop production

Understanding how yield components interact and which component is limiting to gain higher yield is important for breeding as well as for ware crop production (Stockem et al. 2020). For breeding it gives direction on which traits need to be improved, and in ware crop production it can help optimizing the crop management for higher yield. A higher number of eyes did result in more stems per plant, but not in more tubers or a higher yield. Therefore, when making selections for tuber number or yield, number of eyes in seedling tubers is not an important quality trait that affects the selections. When the number of stems per plant, however, are part of the selection criteria, variation in number of eyes can lead to unwanted variation in the data.

A higher seedling tuber weight resulted in increased yield (Table 3.6). Within the seedling tuber lots, large variation in weight was found for all hybrids in the size class 35-45 mm, as shown in Figure 3.1. Tuber shape affects the variation in tuber weight within a size class, because the square measure of a size class was measured on the lowest diameter of the tuber.So, longer tubers can have a larger tuber weight than shorter tubers, while belonging to the same size class.
We calculated that the tuber weight variation that was found in size class 35-45 mm can lead to yield differences up to 8.9 to 16.1 Mg/ha for the hybrids that were used in this study. This is between 18 and 45% of the average yield of the hybrids in size class 35-45 mm over all locations.

In breeding, this can lead to selecting varieties for high yield that is the result of a higher seedling tuber weight rather than better genetics. Therefore, it is important to use similar seedling tuber weights rather than the same size class of seedling tubers in breeding trials, especially because different tuber shapes are present in breeding trials. Moreover, seedling tuber weight could be considered as a selection trait itself, as the seedling tubers of hybrids that produce large tubers have a higher yield potential.

In ware crop production, planting density partly is based on the size class of the seed tubers to optimize crop management for high yield. However, the variation that we found in seedling tuber weight and the large effect on yield, leads to the question whether plant density can be optimized better for high yields when using tuber weight instead of size class to classify seed(ling) tubers.

3.5.3 Conclusions

In this research, we investigated the effect of number of eyes, tuber size and production origin of seedling tubers on plant growth and tuber production in the field. No effect of production origin was found, this might be due to the relatively small distance between the fields, or because we selected seedling tubers for the trials based on similar tuber traits.

A higher number of eyes per tuber led to a higher number of stems per plant, however, this did not result in a higher soil cover. Besides that, the higher number of stems was compensated for by a lower number of tubers per stem, resulting in no difference in total number of tubers per plant or tuber weight per plant beween plants grown from seedling tubers with a high or low number of eyes.

In all hybrids, a higher seedling tuber weight led to higher yield. A variation in seedling tuber weight up to factor seven between low and high weight was found within the size class 35-45 mm. This variation can lead to a yield difference up to 16.1 Mg/ha, which is 45% of the average yield, based on the results of Trial 2. With these results we can design trials with lower variation that result in more precise results and lead to better selections.

Chapter 4

Optimal plot dimensions for performance testing of hybrid potato in the field

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Abstract

Field trials to evaluate the performance of new varieties are an essential component of potato breeding. Besides the genetic differences, environmental factors can lead to variation in a trial. In variety trials, the observed differences amongst varieties should reflect genetic differences, without a large impact of the random or systematic variation in the field. One way to reduce within-field variation is to adjust the plot size and its shape in a trial. Two years of field trials in which individual plants in 90-plant plots of both diploid hybrid and tetraploid varieties were measured provided data to derive relationships between LSD% and plot size and shape. We provide a method to estimate the equations to calculate the expected variation when using different plot dimensions in a relatively homogeneous trial field for tuber yield, tuber volume, tuber count, tuber shape and the standard deviations of tuber volume and shape. Compared with the yield traits, the variation for tuber shape was relatively small. The effect of plot shape was minor. With these equations, breeders can determine what plot dimensions are needed to reach the desired precision for each trait.

4.1 Introduction

Breeding of crop varieties with improved yield is an important route to keep up with the increasing demand for sustainably produced food in the world. Although potato is one of the most important staple food crops in the world (Zaheer and Akhtar 2016a), breeding progress for potato grown for human consumption has been notoriously slow (Douches et al. 1996; Rijk et al. 2013). Due to complex genetics and a large number of relevant selection criteria, little genetic gain for yield traits was realised in many developed countries during the last century (Douches et al. 1996; Jansky et al. 2009). Recently, self-compatibility at the diploid level made hybrid breeding possible in potato (Lindhout et al. 2011, 2018; Jansky et al. 2016; De Vries et al. 2016; Eggers et al. 2021). Using diploid potato in hybrid breeding, QTLs can be detected efficiently and traits can be stacked in a short time resulting in an acceleration of the breeding process (Meijer et al. 2018; Korontzis et al. 2020; Su et al. 2020).

In the process of variety selection in potato, field trials are essential to evaluate the expression of genetic traits under diverse agronomic conditions. To assess such expression of different traits well, promising new genotypes need to be benchmarked against widely grown cultivars in different environments for their yield and stability across and interaction with diverse environments. A recent example for the hybrid potato breeding system was shown by Stockem et al. (2020) who compared a large number of cultivars and hybrid genotypes at different locations in NW Europe. Plot size is an important decision in plant breeding trials, as plots should be large enough to allow identification of superior genotypes, but small enough to not waste resources that could be used differently, such as for evaluating more genotypes. Plot size has been a topic of interest in potato as well as in other crops for a long time (Caligari et al. 1985; Vallejo and Mendoza 1992; Bisognin et al. 2006; Schmildt et al. 2016; Khan et al. 2017; Lavezo et al. 2017; Lohmor et al. 2017; Donato et al. 2018). Error variances and standard errors for phenotypic traits will be a function of the number of plants within a plot and the correlations between plants within a plot as a consequence of soil heterogeneity and inter plant competition. When there is no soil heterogeneity nor inter plant competition and correlations between neighbouring plants are zero, the error variance of a plot will be the error variance of a single plant divided by the number of plants in the plot: $V_s = \frac{V_0}{s}$, with V_s the error variance for a plot of size *s*, for example a size of *s* plants, V_0 the maximum error variance for a plot, in this case a plot with a single plant, and *s* the plot size, here equal to the number of plants. However, typically, plants within a plot do influence each other and then the error variance for a plot does not follow simply from the error variance for single plants. Smith (1938) introduced a modification of the above rule for deriving the error variance for a plot of size s from the maximum error variance: $V_s = \frac{V_0}{s^b}$, where *b* is a heterogeneity factor that is equal to 1 for independent, or uncorrelated plants, and that becomes larger than 1 for correlated plants within a plot. Positive correlation between plants within plots will make the plot error to reduce less fast with increasing plant numbers than expected for independent plants. The heterogeneity factor, b, can be estimated from the slope of the regression of the logarithm of V_s on the logarithm of V_0

Not only size per se but also the shape of plots has been shown to affect the accuracy of field trials in several experiments (Zhang et al. 1994). In an anisotropic field, where soil physical properties differ along different directions, the plot shape that gives the most accurate results is rectangular with the highest number of plants in the direction of the largest heterogeneity of the soil whilst in a homogeneous field a square plot shape can be more advantageous (Zhang et al. 1994). Therefore, it can be advantageous to determine soil heterogeneity of a field before variety trials are performed, for example as described by Gomez and Gomez (1984). Indeed, effects of the shape of the plot on variation in yield data were found in Indian mustard and sunflower (Khan et al. 2017; Lohmor et al. 2017).

In potato, the plot shape is partly determined by the common practice to plant the seed tubers in ridges. The plot shape changes in different ways when adding extra plants within a row versus adding more rows, as inter-row spaces are larger, typically 75–90 cm, than intra-row spaces, ranging from 19 to 33 cm (Haverkort 2018).

In variety trials in the open field, it is important that differences between varieties can be attributed to genetic differences and are not obscured by field variation. Within a location, there can be multiple sources of variation, for example variation in nutrient distribution (Haefele and Wopereis 2005; Allaire et al. 2014), soil particle size (Santra et al. 2008) and in soil organisms (Lupatini et al. 2017), all leading to soil heterogeneity. This field variation can result in even more unexplained variation due to the interactions between the soil environment and the genotype (Portman and Ketata 1997). Also inter-plot interference is a major source of bias at plot level (Kempton 1997). Inter-plot interference occurs when neighbouring plots affect each other, for example by competing for resources (either above-ground or below-ground). In potato, inter-plot competition between cultivars was shown for plant height, tuber yield and dry matter content (Connolly et al. 1993; Bradshaw 1994). It can be minimised by using larger plots, placing similar varieties close to each other and using border plants around each plot (Bradshaw 1994, 2021; Kempton 1997), but these strategies either affect costs per plot or reduce randomness.

Variation in a field trial can also be present at individual plant level. In potato, growth of the plants is affected by the characteristics of the seed tubers. For example, a larger seed tuber results in a higher yield and in more stems per plant (Struik and Wiersema 1999). Moreover, plants are exposed to a specific and variable micro-environment (both above-ground and below-ground), whilst plant-plant interference can enlarge variation. For potato breeders, besides the gross yield, tuber traits like size or shape and variation in these traits are important traits of a new variety. For processing, tuber characteristics should be within well-defined and rather narrow ranges. For nitrate and dry matter content, it was shown that the major part of the variation amongst tubers of the same (uniform) field could be allocated to variation amongst tubers produced by the same plant and even by the same stem (Veerman et al. 1996).

In traditional tetraploid potato breeding, plot sizes in field trials are often limited by availability of starting material. These breeding trials always start from seed tubers that have been multiplied clonally, starting with a single tuber. This clonal multiplication is very slow, and generally tuber availability determines the plot size of variety trials, with multilocation performance trials taking place in year four of the selection cycle (Tiemens-Hulscher et al. 2013). With hybrid potato breeding, it becomes feasible to perform variety trials with an optimal plot size at an early stage of the selection or testing programme because starting material is not limiting in hybrid breeding. In contrast to traditionally bred potato, the starting material of hybrid potato is true seed. In hybrid breeding, it is possible to produce thousands of true seeds in the first year of selection. In the second year, these true seeds can be used to produce as many seed tubers as needed as described by Stockem et al. (2020). Hence, plot size can be optimised, without the limitation of starting material availability in variety trials to assess test hybrids.

The benefits and disadvantages of increasing plot size combined with the possibility of performing field trials that are not constrained by availability of planting material determine the need to assess the optimal plot size for testing hybrid varieties of potato, grown from seedling tubers. We therefore carried out two field trials testing hybrid potato genotypes, quantified in detail the sources of variation and analysed the effects of plot size and plot shape on accuracy of estimates of differences between genotypes.

Our paper addresses the following aims:

1)
To analyse the effect of plot size on the error variation of a trait that is measured at plot level. This will be done by analysing the least significant difference between cultivar means as a percentage of the trait mean (LSD%).

2)
To analyse the effect of plot shape on error variation amongst plots, and to define a plot shape that leads to the smallest error variation amongst plots for different plot sizes.

4.2 Material and methods

Planting material

Diploid test hybrids were produced in a hybrid potato breeding programme as described by Lindhout et al. (2011; 2018). True hybrid seeds were produced in the winter of 2015–2016 and in the winter of 2016–2017 for the field trials in 2017 and 2018, respectively. Seedling tubers were produced in the field season after the true seed production (2016 and 2017). Seedling tuber production was performed on heavy marine clay in Zeeland (The Netherlands), as described by Stockem et al. (2020). After harvest, the seedling tubers were stored at 4 °C until the end of February. The parents of the diploid hybrids were inbred lines that were self-pollinated for 4–7 generations. At this stage, the parent lines were not completely homozygous, so some variation will be present within the hybrids. Different hybrids were used in 2017 and 2018. The tetraploid cultivar Hermes was used as check in both years. Seed tubers from Hermes were produced and stored under optimal conditions for the cultivar, the seed tubers were classified in class E by the NAK (Nederlandse AlgemeneKeuringsdienst voor zaaizaad en pootgoed van landbouwgewassen, The Dutch General Inspection Service) classification system.

Design and measurements

Two field trials were performed in Est, The Netherlands, on clay soil (33% clay) in two subsequent years (2017 and 2018). The field chosen was flat and relatively homogeneous, which was checked with a soil scan in 2018 (data not shown). The field was used for a 1:4 potato rotation for at least 20 years, with the ridges for potato cultivation always drawn in the same direction that was also used for this trial. In both years, seedling tubers of four different diploid hybrids and seed tubers of the tetraploid cultivar Hermes were planted in a randomised complete block design with three replicates. Each plot consisted of 90 plants that were grown on 6 ridges of 15 plants each. Ridges had a width of 75 cm and the planting distance within the ridge was 30 cm. On both sides, each plot was bordered by a row of the tetraploid cultivar Bergerac grown from seed tubers. In 2017, planting was done by hand, and in 2018, it was done by machine (Macon, Kraggenburg, the Netherlands). Weight of each seed(ling) tuber was determined before planting. The trials were planted in a farmer's potato field and the crop management was according to farmer's best practice. The tubers of each plant were harvested separately by hand. Fresh tuber yield was measured per plant. The number of tubers and dimensions were measured automatically using a 3D camera (RMA Techniek, 's Heer Arendskerke, the Netherlands).

Data analysis

Volume and shape of each individual tuber were calculated using Eqs. 1 and 2, respectively. To determine the plot size that is needed to estimate the variation for tuber volume and shape present in a hybrid, the standard deviation of these traits was treated like a plant trait in the analysis. Plant characteristics of cv. Hermes and the diploid hybrids were compared by using an ANOVA and Fisher's LSD test. Tuber count, tuber volume and the standard deviation of tuber volume and shape were analysed after logarithmic transformation.

$$Equation\ 1: Tuber\ volume\ (cm^3) = \frac{\frac{1}{6} \times pi \times length(mm) \times width(mm) \times height(mm)}{1000}$$

$$Equation\ 2: Tuber\ shape = \frac{1}{length/\sqrt{pi \times (0.5 \times width) \times (0.5 \times height)}}$$

Estimating effect of plot size and shape on error variation

Data for plots of varying size were created by subsampling ridges and plants within ridges from the complete plots of 90 plants, where the subsampled plots should be rectangular and consist of neighbouring ridges and consecutive plants within ridges. Therefore, plots contained from one to six ridges combined with one to 15 plants per ridge. Each combination of ridges and plants per ridge was sampled 100 times. Each data set was analysed by the same linear model that was used for the complete plot data, a randomised complete block design with three replicates and five genotypes; response = block + genotype. From this analysis, the least significant difference was retained for each data set, expressed as a percentage of the trial mean (LSD%). Subsequently, the LSD% was related to plot size following a method similar to the method of Smith (1938), using the statistical model described by Eq. 3, in which the logarithm of LSD% was regressed on the logarithm of plot size. Smith's heterogeneity coefficient of 1 corresponds to a very homogeneous field with independent observations and a steep decrease in variance with an increase in plot size. A lower heterogeneity coefficient can be interpreted as lower independence of observations and slower decrease in variance with an increase in plot size. However, it should be noted that the estimated coefficient in this case will not be equal to Smith's coefficient but half of it (dependent observations corresponding to 0.5).

The experimenter can visually inspect the curve to identify the plot size beyond which the variance does not decrease greatly. However, visual inspection is challenging because it depends on the plotting scale. One method to estimate optimal plot size is to include cost estimates in the formula. In the absence of cost estimates, an alternative method to standardise estimation of optimal plot size is to use the coefficient of variation instead of the variance in the linear regression model (Meier and Lessman 1971). Simple linear regression equations similar to Smith's can be derived for other measures of variation. In this study, we recommend the use of the LSD as a more intuitive measure. This allows the experimenter to choose the plot size that will allow identification of genotypic differences that have a magnitude relevant for the breeding programme.

The estimated intercept and slope are inserted in Eq. 4 to predict LSD% for any plot size. Moreover, these predictions can be used to estimate the optimal plot size by the maximum curvature method provided by Meier and Lessman (1971) (Eq. 5). In this paper, the point of maximum curvature was used as a convenient point to compare traits with respect to the dependence of precision on plot size rather than considering it as optimal plot size.

$$Equation\ 3{:}\ log_{10}(LSD\%)_{ij} = Intercept + Slope \times log_{10}(N)_{ij} + e_{ij}$$

$$where\ N\ is\ the\ plot\ size\ and\ LSD\%\ the\ least\ significant\ difference\ between\ two\ genotypes$$
$$for\ i = \{1\ to\ 6\}\ ridges\ and\ j = \{1\ to\ 15\}\ plants\ on\ the\ ridge$$

$$Equation\ 4: LSD\% = \frac{10^{Intercept}}{N^{-Slope}}$$

$$Equation\ 5: Curvature^{max} = \left[\frac{A^2 \times B^2 \times (2B+1)}{(B+2)}\right]^{\frac{1}{(2B+2)}}$$

$$Where\ A\ is\ 10^{Intercept}\ and\ B\ is - slope$$

<u>Plot shape</u>

The effect of plot shape on LSD% was studied to determine the relative contribution of ridges versus plants per ridge to LSD%. For this purpose, the simple regression model of Eq. (3) was altered into a multiple regression model with as predictors the number of ridges (N_R) and the number of plants per ridge (N_P) (Eq. 6). The use of this model is similar to the method of Zhang et al. (1994) and offers two slopes that can be used to assess the relative contributions of ridges and plants on the ridges to LSD%.

$$Equation\ 6: log_{10}(LSD\%)_{ij} = Intercept + Slope_R \times log_{10}(N_R\ )_i + Slope_P \times log_{10}(N_P)_j + e_{ij}$$

$$where\ N_R\ is\ the\ number\ of\ ridges\ and\ N_P\ the\ number\ of\ plants\ per\ ridge$$

As a consequence of the use of this multiple regression model, LSD% can be predicted by:

$$Equation\ 7: LSD\% = \frac{10^{Intercept}}{{N_R}^{-Slope_R} * {N_P}^{-Slope_P}}$$

4.3 Results

Plant traits

To obtain an impression of the performance and characteristics of the tetraploid cv. Hermes and the diploid hybrids, we first analysed general plant performance. For that, we compared the yield, yield per seed(ling) tuber, number of stems per plant, number of tubers per plant and per stem, tuber volume, tuber shape and the standard deviations (SD) of tuber volume and shape as a measure of variation of the trait within the plant (Tables 4.1 and 4.2).

In both years, Hermes out-yielded the diploid hybrids (Table 4.2). Also, for yield per g seed(ling) tuber and for tuber volume Hermes out-performed the diploid hybrids. For stems per plant, tuber count, tubers per stem and tuber shape there were diploid hybrids with similar values as Hermes. Amongst the hybrids, significant differences were found for all traits in both years. In a breeding programme a lot of variation is present as well. Moreover, for different markets, the desired values for the tuber traits are different. To make this dataset representative for a breeding programme that breeds for different market types, this variation between the hybrids was needed.

Besides the tuber traits themselves, the within-plant-variation in tuber volume and shape, which are important determinants of number of marketable tubers for chips and fries, was analysed. As plants were harvested individually, we were able to measure the tuber characteristics per plant. This allowed us to calculate the standard deviation within one plant. Also, the standard deviation of tuber volume and shape differed amongst the hybrids and Hermes. Variation in the tubers should be as low as possible to enable industrial processing.

Table 4.1: F values of ANOVAs for yield, yield per g seed tuber, number of stems, number of tubers, tuber volume, tuber shape and standard deviation of tuber volume and tuber shape for 2017 and 2018. Significant effects are indicated by points or asterisks: p value < 0.1: “.”, <0.05: “*”, <0.01: “”, <0.001: “***”. Data of tuber count, tuber volume and SD of tuber volume and tuber shape were log transformed.**

Year	ANOVA factor	Tuber weight (g/plant)	Yield per g seed tuber	Number of stems per plant	Tuber count	Number of tubers per stem	Tuber volume	Tuber volume SD	Tuber shape	Tuber shape SD
2017	block	0.03	2.06	3.41.	0.28	3.37.	0.23	0.62	2.76	3.14.
	genotype	45.24***	102.51***	45.48***	27.39***	28.52***	162.26***	79.34***	49.04***	7.14**
2018	block	12.86**	1.26	7.32*	7.58*	0.56	0.09	0.53	0.87	2.06
	genotype	438.28***	14.43***	46.88***	55.73***	12.24**	207.16***	168.54***	71.70***	45.05***

Table 4.2: Means and results of Fisher's LSD post hoc tests of yield, number of stems, number of tubers, tuber volume and shape and yield per seed tuber for 2017 and 2018. Means followed by a common letter are not significantly different at 5% level of significance. Data of tuber count, tuber volume and SD of tuber volume and shape were log transformed.

Year	Hybrid/ cultivar	Tuber weight (g/plant)		Yield per g seed tuber		Number of stems per plant		Tuber count		Number of tubers per stem		Tuber volume		Tuber volume SD		Tuber shape		Tuber shape SD	
2017	Hermes	1121	a	57	a	2.2	d	5.5	d	2.9	a	128	a	93	a	0.66	b	0.09	bc
	Hybrid 1	686	b	27	b	5.8	c	9.6	bc	2.2	b	54	b	42	b	0.53	e	0.10	ab
	Hybrid 2	579	bc	25	b	8.8	a	14.3	a	2.0	b	29	d	23	d	0.62	c	0.10	a
	Hybrid 3	591	b	14	c	7.3	b	9.9	b	1.6	c	43	c	31	c	0.59	d	0.10	a
	Hybrid 4	457	c	17	c	6.3	bc	7.8	c	1.5	c	39	c	27	cd	0.70	a	0.09	c
2018	Hermes	1225	a	23	a	3.2	c	10.9	d	4.3	a	98	a	63	a	0.74	a	0.06	c
	Hybrid 5	601	b	15	b	4.3	c	12.7	c	3.3	b	43	b	30	b	0.51	d	0.10	a
	Hybrid 6	551	c	16	b	6.4	b	16.5	b	3.1	bc	26	d	20	d	0.69	b	0.09	ab
	Hybrid 7	542	cd	13	b	8.8	a	20.0	a	2.7	c	21	e	13	e	0.60	c	0.09	ab
	Hybrid 8	496	d	16	b	3.7	c	12.7	c	4.3	a	32	c	24	c	0.67	b	0.09	b

Plot size

The optimal plot size was compared using the log–log regression method for tuber number, tuber weight, tuber volume and tuber shape, and for the standard deviation of tuber volume and tuber shape. For each trait, an equation was created to calculate the LSD% for different plot sizes. The decrease in LSD% for each trait when increasing the plot size is shown in Fig. 4.1. The dashed lines show the location of the point of maximum curvature for each trait. This point corresponds to the optimal plot size according to Meier and Lessman (1971), it is mainly useful to compare the LSD% between different traits.

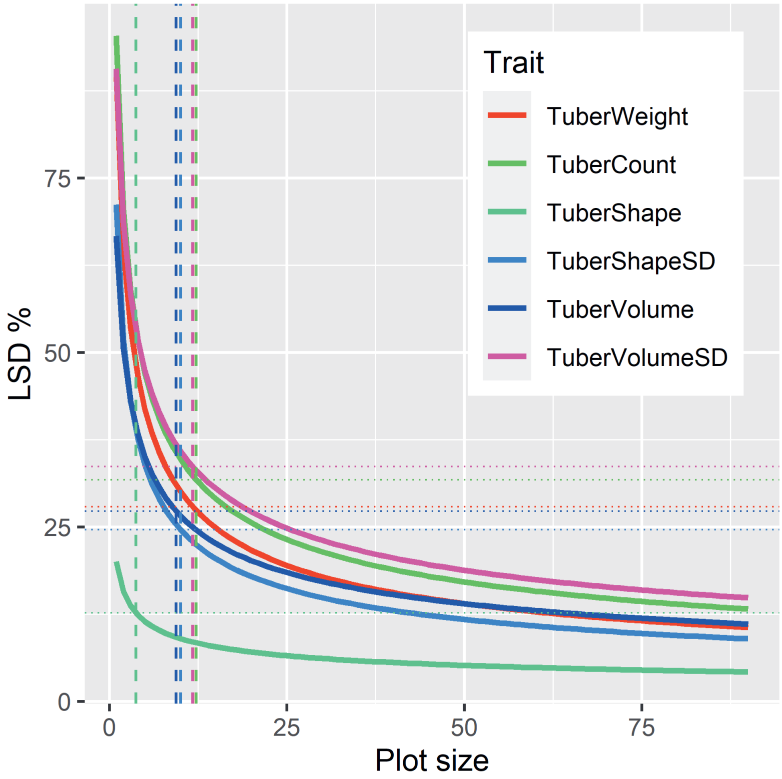

Figure 4.1: Least significant difference between cultivar means as a percentage of the trait mean (LSD%) in its dependence on plot size in number of plants per plot. Traits include tuber weight, tuber count, tuber volume, SD of tuber volume, tuber shape and SD of tuber shape. The dashed line indicates the plot size corresponding to the point of maximum curvature and the dotted line the LSD% achieved at that plot size.

The trait with the lowest LSD% was tuber shape, with also the point of maximum curvature at the smallest plot size (3.8 plants). This shows that there was less variation amongst plants for tuber shape compared with the other traits. The LSD% itself, as well as the decrease of LSD%, of the yield traits (tuber weight, count and volume) showed a similar trend, and the points of maximum curvature were very close to each other (11.8, 12.2 and 9.4 plants respectively). The LSD% for the standard deviation of tuber volume and shape was higher than that of the tuber traits themselves, and the point of maximum curvature was at a plot size of 10 plants for SD of tuber shape and 11.7 plants for SD of tuber volume.

Figure 4.1, together with the corresponding equations (Table 4.3), can be used to design a field trial with three replicates. For other numbers of replicates, the LSD% can be calculated by multiplying the LSD% determined with an equation of Table 4.3 with the square root of (3/nr), in which nr is the number of replicates. If a breeder is planning a variety trial to compare yield amongst varieties with a plot size of 4 plants Fig. 4.1 can be used to estimate the LSD%. The LSD% can also be calculated with the equation for tuber weight in Table 4.3: LSD% = 90.27/(4^0.48) = 46%. This means that the breeder can determine the difference in yield between two varieties when they differ at least 46%. When increasing the plot size to 10 plants, the LSD% decreases to 30%, so smaller differences amongst varieties can be assessed.

For tuber shape, the LSD% at a plot size of 4 plants is 12%, with a plot size of 10 plants it is 9%. This figure and the associated equations can be used by breeders as tools to design field trials that give the desired precision to compare the trait of interest amongst varieties.

Plot shape

To determine whether plot shape is an important variable in decreasing variation, the number of rows per plot and number of plants per row were included in the model as separate factors. This was done for tuber weight, tuber count, tuber volume and tuber shape, and for the SD of tuber volume and shape.

Table 4.3: Equations to calculate least significant difference between cultivar means as a percentage of the trait mean (LSD%) for different traits when using a certain plot size (number of plants) or shape. Nr = number of ridges, Np = number of plants per ridge.

Trait	Plot size	Plot shape
Tuber weight	LSD% = 90.27/(plot size)^0.48	LSD% = 90.72/(Nr^0.42*Np^0.51)
Tuber count	LSD% = 95.41/(plot size)^0.44	LSD% = 95.57/(Nr^0.42*Np^0.45)
Tuber shape	LSD% = 20.05/(plot size)^0.35	LSD% = 20.02/(Nr^0.36*Np^0.34)
Tuber volume	LSD% = 66.70/(plot size)^0.40	LSD% = 66.75/(Nr^0.39*Np^0.40)
Tuber volume SD	LSD% = 90.68/(plot size)^0.40	LSD% = 90.78/(Nr^0.39*Np^0.41)
Tuber shape SD	LSD% = 71.19/(plot size)^0.46	LSD% = 71.19/(Nr^0.46*Np^0.46)

This analysis revealed that the plot shape only had a limited effect on the LSD. Figure 4.2 shows the LSD% for different plot shapes for all traits, the corresponding equations are given in Table 4.3. In both directions (within and along the ridge), the LSD% mainly decreased with an increase in the number of plants, where it did not matter whether the increase of the number of plants came from more rows per plot or more plants per row. Only for tuber weight and count it was slightly better to increase the number of plants per row rather than number of rows per plot. For example, with a plot size of 10 plants two possibilities could be two rows per plot with five plants per row or five rows per plot with two plants per row. When using two rows per plot with five plants per row the LSD% for tuber weight would be 30%, for tuber shape it would be 9%. With five rows per plot with two plants per row, it would be 32% for tuber weight and 9% for tuber shape.

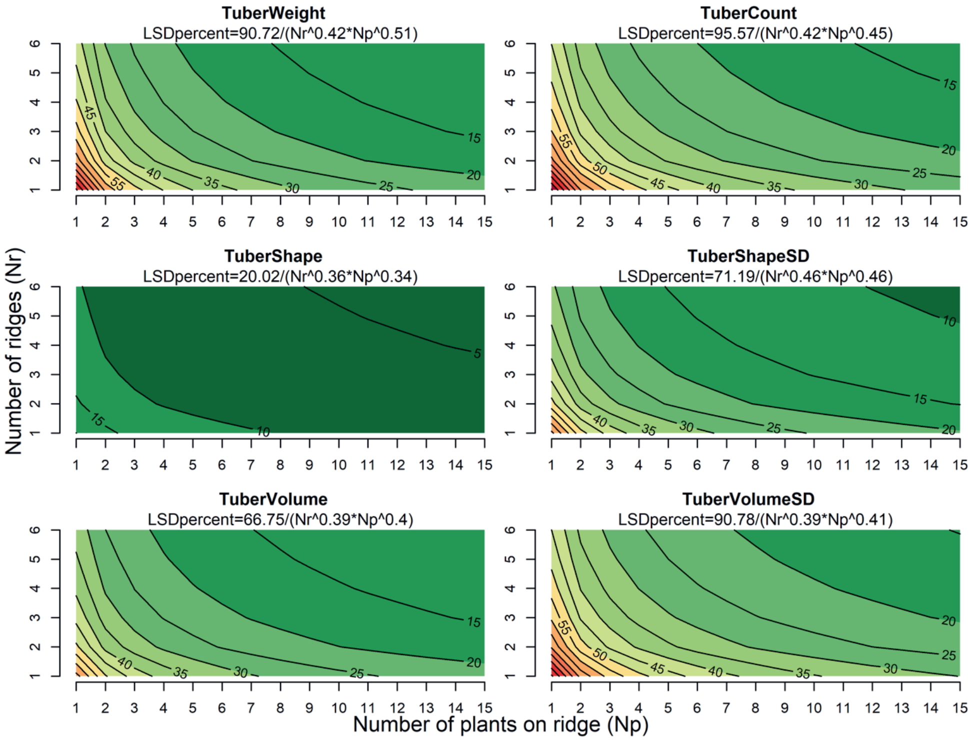

Figure 4.2: Least significant difference between cultivar means as a percentage of the trait mean (LSD%) for different plot sizes when using different plot shapes

4.4 Discussion

In this study, we aimed to determine the plot size and shape that are needed to evaluate the performance of different diploid hybrids or tetraploid cultivars. This was done by creating a linear regression model between the logarithm of LSD% and plot size. The point of maximum curvature on the back transformed relationship between LSD% and plot size (Bisognin et al. 2006; Donato et al. 2018) was used as a convenient point to compare traits with respect to the dependence of precision on plot size. To assess optimal plot size, we provide equations to calculate LSD% for different plot sizes because plot size should be determined by the need of precision for a given trial or set of trials. The user can decide upon a wanted precision and use the plot of LSD% versus plot size to determine the required plot size.

Various statistical methods were used to determine the optimal plot dimensions on this dataset (unpublished results). A disadvantage of the used method, in which we used smaller subsamples in large plots, is that small plots were further apart than large plots. This could have led to an overestimation of LSD% in small plot sizes. Adjusting the results for spatial trends, for example using Spatial Analysis of Field Trials with Splines (SpATS) (Rodríguez-Álvarez et al. 2018), could have helped to decrease this over estimation. The authors intend to design a series of spatial models which will assist in quantifying the overestimation of the LSD%s.

To select the best hybrids, it is important that measured characteristics of the plants are reliable and based on genetics, not the result of random field variation. The results of this study should be applicable to variety trials with many different hybrids with different characteristics. To make sure that different characteristics were represented in this study, nine different genotypes, a tetraploid cultivar and eight diploid test hybrids, were used for the field trials. All measured plant and yield characteristics differed amongst the genotypes, with for example a low yield of hybrid 9 (469 g/plant) and a more than twice as high yield of Hermes in that year (1225 g/plant). These large differences are typical for varieties that are tested early in a breeding programme.

Optimal plot size differs for different traits

In a variety trial, the optimal plot size and number of replications should be defined by the minimal difference that the breeder needs to detect for a certain trait. In this research, the number of replicates was not included as a variable. It is obvious that in addition to plot size, experimental design and the number of replicates will affect the plot error (Sripathi et al. 2017). Research by Caligari et al. (1985) and Bisognin et al. (2006) shows that increasing the number of replicates will be more effective for a higher precision of the trial than increasing plot size. Although the LSD% for different numbers of replicates can be estimated by multiplying the LSD% determined with an equation from Table 4.3 with the square root of (3/nr), in which nr is the number of replicates, it would be advisable to include different numbers of replicates in the analyses in future research.

To determine the plot size with three replications that is needed for a certain LSD% between cultivars, models were made for each trait that show the expected variation when using different plot sizes. The model for tuber count, tuber volume and tuber weight per plant showed that the error variation and the decrease in error variation with increasing plot size in these traits followed a similar slope. The variation in yield traits was high with an LSD% of still 21% at a plot size of 20 plants, although Talbot (1984) described high variation in potato yield trials compared with other crops. For tuber weight, the point of maximum curvature was a plot size of 12 plants, which is slightly higher than the optimal plot size found by Bisognin et al. (2006) using the same method of up to 10 plants, depending on the genotype.

The total variation found for tuber shape was lower than for the yield traits. In earlier research, it was shown that tuber shape is less affected by environmental conditions than tuber yield and number of tubers (Yildirim and Celal 1985; Stockem et al. 2020), which could explain the lower variation. The slope of decrease in variation, however, was similar to the slopes for the yield traits. One of the factors that leads to variation in potato plants is the quality of the seed tubers (Struik and Wiersema 1999). When testing hybrids, an alternative to using seed tubers as starting material is to use seedlings from true seed to prevent variation due to seed tuber quality, also from seedlings from true seeds relatively high yields up to 32 Mg/ha can be reached (Van Dijk et al. 2021).

Besides the tuber traits themselves, the LSD% of the standard deviation for tuber volume and tuber shape was analysed as a function of plot size (Fig. 4.2). The variation of these tuber traits is an important selection criterion for breeders and can be seen as a significant trait in itself. Tubers that are cultivated for processing, for example for chips, such as the cultivar Hermes, can only be used in a limited size and shape range to enable efficient mechanical processing. Variation in tuber traits within one plant can be the result of environmental conditions during the growing season (Struik et al. 1991). Moreover, stolon characteristics and timing of stolon and tuber formation can result in variation in tuber characteristics (Struik et al. 1991; Van Ittersum and Struik 1992; Kacheyo et al. 2021). The variation of the tuber traits was more difficult to estimate than the tuber traits themselves: the plot size and LSD% on the point of maximum curvature were higher for the models of SD of tuber volume and shape than for tuber volume and shape themselves.

Plot shape had a limited effect

Several studies have dealt with the comparison of plot shapes (Gomez and Gomez 1984; Zhang et al. 1994). Christidis argued that rectangular shape is always better than square (Cristidis 1931). This is based on the idea that most fields are anisotropic (spatial correlation is dependent on the direction in the field) and that plots are chosen to be elongated in the direction of the field gradient. Zhang extended Smith's method to anisotropic fields. He estimated two heterogeneity coefficients (slopes) in the same rationale with Smith: one coefficient for the decrease in variability when the number of columns is increased and number of rows is 1 and another coefficient when the number of rows is increased and number of columns is 1. He compared the sum of those 2 with a third coefficient estimated from plots with equal numbers of columns and rows to evaluate the adequacy of his method and showed that there was no interaction between number of rows and number of columns. Here, we applied Zhang's method in a simpler way, by estimating the coefficients on all plot shapes and not only when the other dimension is 1.

Potato generally is grown in rows of 75- or 90-cm width, whilst space between plants within a row generally is between 19 and 33 cm (Haverkort 2018). Potentially this could result in a higher variation in a plot when adding more rows per plot compared with adding more plants per row. In our trials, plot size rather than plot shape determined the variance in the plot. For tuber volume, shape and the SD of these traits, the LSD of the different possible plot shapes were similar; for tuber weight and count, only a small effect of plot shape was found.

This confirmed that we conducted the trials in a homogeneous field as was intended. According to Zhang et al. (1994), the optimal shape in an isotropic field is a square. As we found no large effects of plot shape, we could not confirm this for the current trials. Therefore, the results from these trials probably are most useful for trials that will be performed in homogeneous fields as well. Further research in more heterogeneous fields would be useful to investigate whether shape has an effect and to provide tools to determine the most efficient plot shape for such fields as well.

Indeed, earlier research has shown that depending on the direction of the plot in the field, the coefficient of variation decreased (Khan et al. 2017; Lohmor et al. 2017). When including practical considerations in planning an optimal plot shape in a homogeneous field, probably a long-shaped plot is the most efficient. As potatoes are grown in rows, it is easier and faster to plant more tubers or plants in a few rows than making more rows.

4.5 Practical implications

There is a trade-off between accuracy and costs when determining the ideal plot size to perform a field trial (Koch and Rigney 1951). Besides the costs for the land and the labour, costs of a field trial are for a large part determined by the production of starting material. On the other hand, the accuracy of a trial increased when the plot size increased. For example, in a breeding programme, it is important to perform variety trials in an efficient way. This means that the results need to be accurate enough to make selections, without making plots unnecessarily large resulting in higher costs. In this paper, we provide tools to determine the minimum needed plot size to determine tuber count, weight, volume and shape as well as the variation of tuber volume and shape in potato variety trials.

4.6 Conclusion

In conclusion, we present a method to derive equations, as well as the equations themselves, to calculate an LSD% for tuber weight per plant, tuber count per plant, tuber volume, tuber shape and the standard deviations of tuber volume and shape when using different plot sizes and shapes in a trial with three replicates. In fields that are similarly homogeneous as the one in our trials, the equations can be used in designing field trials to determine the optimal plot size for the required degree of precision for different traits. In more heterogeneous trials, the method can be used to define similar equations for plot size and shape.

Chapter 5

Contribution and stability of yield components of diploid hybrid potato

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Abstract

Recently, a hybrid breeding system was developed for diploid potato. We compared performance of diploid hybrids with commercially available tetraploid cultivars. Therefore, seedling tubers were produced from true hybrid seeds in field conditions. In the subsequent year, diploid hybrids grown from seedling tubers showed a yield potential comparable with commercial tetraploid cultivars: the highest yielding diploid hybrids showed a yield comparable with the lower yielding tetraploid cultivars. Yields of hybrids and commercial tetraploid cultivars were broken down into different yield components and the interactions with growing conditions were quantified. The stability of yield and other relevant traits in different growing conditions was similar between hybrids and commercial cultivars. The contribution of the different yield components to total yield over different environments was compared between diploid hybrids and tetraploid cultivars. In diploid hybrids as well as tetraploid cultivars, more tubers per stem resulted in the highest yield gain, while an increase in tuber size resulted in a relatively smaller increase of total yield.

5.1 Introduction

Potato (*Solanum tuberosum* L. and its tuber bearing crossable species) is the third food crop after rice and wheat (Zaheer and Akhtar 2016b). A large proportion of the production is used for other purposes such as seed, industrial processing and feed (Haverkort and Struik 2015). Despite its importance, yield increase by genetic gain has been limited for the last century, especially in cultivars for consumption (Douches et al. 1996; Piepho et al. 2014). The most important factor limiting genetic gain is the lack of an efficient breeding system. An advanced system is hybrid breeding based on crosses between two homozygous inbred lines (Lindhout et al. 2011). By choosing contrasting parents, yield contributing alleles can be combined (Lindhout et al. 2011). The segregation of potato traits after several generations of inbreeding was described by Meijer et al. (2018). Until now, the absence of a self-compatibility system hampered the creation of homozygous inbred lines in potato. Since the discovery of the Sli-gene (Hosaka and Hanneman 1998), it has become possible to develop a diploid hybrid breeding system (Lindhout et al. 2018). The discovery not only creates opportunities for commercial breeding programmes, it also greatly facilitates genetic research by using advanced segregating populations such as introgression libraries and backcross populations (Jeuken and Lindhout 2004; Endelman and Jansky 2016; Prinzenberg et al. 2018). Diploid germplasm allows high throughput phenotyping and efficient QTL detection, accelerating the breeding process (Meijer et al. 2018; Prinzenberg et al. 2018; Su et al. 2019).

With the development of this hybrid breeding system, which is new for potato, using diploid hybrids instead of tetraploid clones, a system of testing new cultivars needs to be developed. Commonly in breeding systems, new cultivars are grown at several locations from tubers and their performance is benchmarked against a set of already existing commercial tetraploid cultivars (Tiemens-Hulscher et al. 2013). To determine relevant genetic gain, performance of diploid hybrids needs to be benchmarked against tetraploid cultivars commonly used by farmers. Assessing the genotype by environment interactions in farmers' fields is an important aspect of acceptance of new (types of) cultivars.

Besides the use of new genetic material in diploid breeding, the starting material is also different from the traditional breeding germplasm. In tetraploid breeding, a cross is made only once and the true seeds derived from this cross are grown to seedling tubers (Tiemens-Hulscher et al. 2013). After that, genotypes are clonally propagated, which means that starting material of tetraploid cultivars always consists of seed tubers. Hybrid breeding in potato results in true (botanical) seeds. Plant development is highly affected by the type of starting material (true seed or seed tuber) and by its physiological, physical, genetic and phytosanitary quality. Size and physiological age of seed tubers have large effects on ware crop yield (Arsenault et al. 2001; Struik and Wiersema 1999). Therefore, seedling tubers need to be produced from diploid hybrids to evaluate the performance of hybrids in comparison to existing benchmark cultivars.

Although for most field crops, dry matter yield is the most important economic output, for potato fresh tuber yield, tuber size distribution, dry matter content and tuber shape are main output determinants. These traits are important criteria whether a cultivar is suitable for fry or chips production (Tiemens-Hulscher et al. 2013). Dry matter percentage should range between 22 and 25% for chips (crisps) and 21 and 23% for fries. Tubers that are processed into chips should be round, while tuber shape of frying cultivars should be oblong for optimal processing (Tiemens-Hulscher et al. 2013). Potato yield can be dissected into different components and be expressed as the product of tuber weight, tubers per stem and stems per unit area (Haverkort et al. 1990; Lynch et al. 1995; Struik and Wiersema 1999; Asghari-Zakaria et al. 2007). Potato production is highly affected by the environment (Haverkort 1990; Kooman et al. 1996b). To investigate the specific development aspects of diploid hybrids in different environments, the contribution of these components that make up potato yield was investigated.

In this paper, we show the first results of such a hybrid breeding programme focusing on yield and yield components. As testing environments, we have chosen farmers' fields in The Netherlands, Belgium and France, representative for the northwestern European potato cultivation environment. This region is known for its high technological level of potato cultivation and its conducive climate, resulting in high yields.

The objectives of this study were to (1) evaluate the performance of diploid hybrids in comparison to existing tetraploid cultivars, (2) to compare the environmental effect on yield and yield components between diploid hybrids and tetraploid cultivars and (3) to evaluate the contribution of different yield components to total yield.

5.2 Materials and methods

Genetic material

Diploid heterozygous *Solanum tuberosum* accessions were crossed with the selfcompatibility donor *S. chacoense* (Hosaka and Hanneman 1998). Inbred lines of diploid potato were developed in a dedicated diploid breeding programme as described by Lindhout et al. (2011, 2018). The inbred parents were crossed after 5 to 7 generations of self-pollinations to produce the test hybrids. These inbreds should theoretically be more than 95% homozygous, but this was not tested (Lindhout et al. 2011). In winter 2015–2016, crosses between parent lines were made manually to produce diploid hybrid seeds and 572 hybrids were grown in one location in a first year trial to select the most uniform hybrids. This resulted in a panel of 65 diploid hybrids that was used in the present study. These hybrids were selected based on a wide variation in yield and yield components to represent the genetic variation among these hybrids and to allow a detailed analysis.

Nineteen commercial tetraploid cultivars were used as controls for the different potato product types, representing the frying, chip and fresh consumption markets: Annabelle, Lady Cristil, Lady Claire, Pirol, Innovator, Lady Rosetta, Agata, Arsenal, Hermes, Brooke, Nicola, Bintje, Markies, Russet Burbank, Fontane, Spunta, Mozart, Agria and Milva. The main differences between these cultivars are dry matter content, shape and tuber size. The diploid hybrids were not yet differentiated for the different market segments. These control cultivars are commonly grown in the regions where the trials took place. Hence, these controls are a realistic representation of farmers' practice.

Seed tubers of commercial cultivars were classified in class A in the NAK (Nederlandse Algemene Keuringsdienst voor zaaizaad en pootgoed van landbouwgewassen, The Dutch General Inspection Service) classification system (NAK 2018). Seed tubers were produced and stored in the conditions optimal for the specific cultivar for a realistic representation of the cultivar’s potential, so storage conditions were not identical for all cultivars. Diploid hybrids were all stored at 4 °C as optimal conditions for the specific hybrids were yet unknown. Seed tubers of equal size and sprout distribution were selected for the trial. To assess the suitability of the seedling tubers for a ware crop trial, sprouting was scored after 6 months of storage (data not shown) (Tiemens-Hulscher et al. 2013). Generally, seed tubers should be planted when dormancy has been broken (Struik and Wiersema 1999). Seedling tubers with a sprouting score between 3 and 7 were used in the trial; sprouting of seed tubers of tetraploid cultivars was comparable with the sprouting score of 5 of the diploid hybrids.

Seedling tuber production

Field trial description

Seedling tuber production from true diploid hybrid seeds was performed on a heavy marine clay in Wolphaartsdijk [NL], in 2016. The botanical seeds of the hybrids were extracted from the berries and sown into 104 plug trays (2.5 × 2.5 × 3.8 cm) filled with soil (Horticoop) in a greenhouse compartment in the first week of April 2016. Germination after 2 weeks was between 54 and 98%. Six weeks after sowing the seedlings were mechanically transplanted into the open field, into ridges of 75 cm width and a plant distance of 30 cm within the row. The crop was treated as a standard seed-tuber crop, according to farmers’ practices. Irrigation was applied at 1 and 3 days after planting to prevent the seedlings from drying out. Pests and diseases were chemically controlled. Both mechanical weeding and hand-weeding were applied twice. After 90 growing days in the field, the haulm was chemically killed. Three weeks after haulm killing, the seed tubers of all diploid hybrids were lifted by a harvesting machine. The seed tubers were stored in a climate-controlled storage room at 4 °C from October 1st onwards until the end of February.

Design and measurement

Seedling tuber production was done in a non-replicated design with a plot size between 90 and 488 plants, depending on hybrid seed availability. Seedlings were transplanted semi-mechanically with an adjusted 4-row cabbage planter with a planting distance of 30 cm between plants. After harvest, seedling tubers were sorted by size and yield was determined for tubers > 28 mm. Phytosanitary control on quarantine diseases was routinely carried out by the national inspection service (NAK at Emmeloord, NL). To assess the quality of the seedling tubers, sprouting was scored in February, at the preparation of the yield trials according to a 1–9 scale, with 9 being completely dormant and 1 abundantly sprouting (Tiemens-Hulscher et al. 2013).

Yield trials

Field trial description

In 2017, yield trials were conducted using the seedling tubers as starting material that were produced in 2016. Certified seed tubers (class A) of the tetraploid cultivars were obtained to serve as control cultivars in the trials. The field trials were conducted at five different sites (Warmeriville [FR], Berthem [BE], Hilvarenbeek [NL], Est [NL], Giethoorn [NL]). These sites differed in soil type, climate and crop management (Table 5.1). Two of the sites were typical sandy soils, one a loamy soil, one a light clay and one a heavy clay soil, covering the full range of physical soil types on which potatoes are grown in NW Europe. At all sites, the trial was laid out within a farmers' field, hence crop management was according to farmers' practice. Typically, the tubers were planted in ridges, spaced at 75 cm, except for the experiment in Warmeriville, where the distance between ridges was 90 cm (Table 5.1). Spacing between seed(ling) tubers in all locations was 25 cm. In Hilvarenbeek, the farmer had adopted a bed system, which was better adapted to his conditions. Due to the fine sand at this location, ridges would be prone to erosion.

Table 5.1: Location, soil characteristics, crop management system, planting and haulm killing dates of the five experimental sites.

Site	Longitude (E)	Latitude (N)	% clay	% org C	pH	Ridge / Bed system	Planting date	Haulm killing date
Warmeriville [FR]	4.219	49.345	13	4.2	7.6	90 cm ridge	19-4-2017	29-8-2017
Hilvarenbeek [NL]	5.086	51.483	2	3.1	6.8	150 cm beds	21-4-2017	6-10-2017
Est [NL]	5.329	51.845	33	3.7	6.1	75 cm ridge	14-4-2017	15-9-2017
Berthem [BE]	4.633	50.871	13	2.2	6.4	75 cm ridge	25-4-2017	2-10-2017
Giethoorn [NL]	6.054	52.671	1	7.4	5.4	75 cm ridge	24-4-2017	5-10-2017

Design and measurements

At preparation of the field trials, the weight of the seed(ling) tubers for each plot was recorded. Trials were planted mechanically in a randomized complete block design with three repetitions and 20 plants per plot, divided over two rows. Between each plot, two border plants of the cultivar Miss Blush were planted to obtain an equal border effect over all plots. The bi-colored cultivar Miss Blush was chosen to avoid mixture at harvest, as test cultivars and diploid hybrids showed uniform skin color.

The whole experiment was bordered by two ridges of Miss Blush at the sides and one full plot of Miss Blush at the beginning and end of each ridge. During the growing season, stem number was counted per plot. After harvest, tuber number and size were measured automatically using a 3D camera (RMA Techniek, ‘s Heer Arendskerke, Netherlands). Total tuber weight was determined per plot. A subsample of approximately 5 kg was used to determine dry matter content (see below for formulas).

Data analysis

Data of the yield trials were statistically analyzed using R (R Core Team 2017). A component analysis was done on total yield (Formula 1) to determine how these components contribute to total yield in each cultivar. Yield and yield components were adjusted for spatial field trends with Spatial Analysis of Field Trials with Splines (SpATS) (Rodríguez-Álvarez et al. 2018). In this model, differences in plant development due to environmental variation within the field are distinguished from differences in the genetic compositions between the cultivars. In SpATS, variation in the field is analyzed in two directions (rows and columns of the trial) using P-splines; effects of spatial variation are described by a standard mixed model (Rodríguez-Álvarez et al. 2018). By adjusting the data using SpATS, effects of environmental variation within trial fields were reduced, as the focus of this analysis was the genetic differences and the differences between trial fields. To meet the assumptions of normality of the SpATS model, data were log transformed, means and standard errors were back-transformed to linear data for graphical presentation. To examine to what extent the different yield components contribute to total yield, the variation in each yield component was compared with variation in total yield by linear regression. The contribution was defined as the change in yield by changing one unit of the yield component. This was determined by the slope of a linear model, which was fitted through the data of all sites. Genotype by environment interaction was calculated with the AMMI analysis (Additive Main effects and Multiplicative Interaction models), using the Agricolae package in R (Crossa 1990). Results of the AMMI analysis are presented in Figure 5.2 and in Table 5.2. For the AMMI analysis, the raw data were used; the rest of the presented data of yield, stems/m2, tubers/stem and tuber size were adjusted for spatial effects within the field using SpATS. Under water weight was used to determine dry matter content (Formula 3, 4) (Tiemens-Hulscher et al. 2013). Tuber shape was determined using Formula 5.

Table 5.2: Analysis of variance table, calculated using the AMMI analysis (Additive Main effects and Multiplicative Interaction models)

Factor	*df*	Sum of squares	*F* value	*p* value
Location	4	160537	239.0	<0.001***
Replicate	10	1679	1.8	0.05
Genotype	83	448198	59.0	< 0.001***
Genotype x Location (GxE)	332	104915	3.5	< 0.001***
Residuals	789	72212		

$$Formula\ 1: yield\ (g/m^2) = tubers/stem\ \times stems/m^2 \times gram/tuber$$

$$Formula\ 2: y_{ij} = Block + \boldsymbol{row_{r(j)}} + \boldsymbol{col_{c(j)}} + f(row, col) + \boldsymbol{g_i} + \boldsymbol{e_{ij}}$$

$$Formula\ 3: Under\ water\ weight = \frac{5050}{weight\ above\ water} \times weight\ under\ water$$

$$\begin{aligned} Formula\ 4: & Dry\ matter\ content\ (\%) \\ & = under\ water\ weight \times 4.90713\ \times 10^{-2} + 2.054 \end{aligned}$$

$$Formula\ 5: Tuber\ shape = 1/\ \frac{tuber\ length}{\sqrt{(\pi \times (0.5\ \times tuber\ width)) \times (0.5 \times tuber\ height))}}$$

5.3 Results

Seedling tuber production from seedlings

In contrast to regular potato seed tuber productions, seedling tubers were produced from diploid hybrid seedlings. After transplanting to the field, seedlings grew rapidly and hardly any plant died. For most hybrids, the canopy was more or less closed at the end of June. After harvest of the seedling-tuber production, the number of seedling tubers was assessed (Figure 5.1). Although diploid hybrids were not selected for yielding capacity, a good seedling tuber production was achieved.

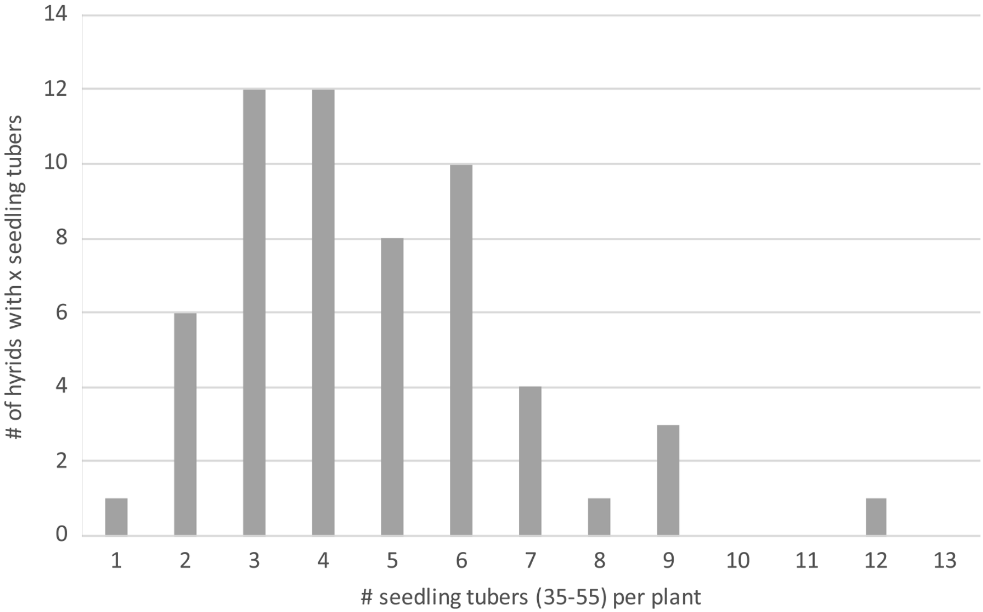


Figure 5.1: Frequencies of number of seedling tubers (35–55 mm) produced per plant.

Yield trials

Seedling tubers produced from true hybrid seeds in 2016 were used to conduct multilocation yield trials in 2017. In these trials, the performance of diploid hybrids grown from tubers was compared to the performance of tetraploid cultivars in different environments. Diploid hybrids that were used in this experiment were the first test hybrids derived from a hybrid breeding system based on homozygous inbred lines (Lindhout et al. 2011). It is known that the correlation between yield of single greenhouse-grown plants and larger blocks in the field of the same cultivar is low. Therefore, traits for high yield were not considered as selection criteria during the selection of parent lines for these hybrids.

Yield

Among the different sites, significant differences ($p < 0.001$) in yield were found (Table 5.2). Highest yielding site was Hilvarenbeek with on average 54 Mg/ha, while lowest average yield was measured in Berthem with 27 Mg/ha. Average yield for the Netherlands and France was 43 Mg/ha, in Belgium 50 Mg/ha (Eurostat), so differences in production between sites were due to local environmental and field management differences.

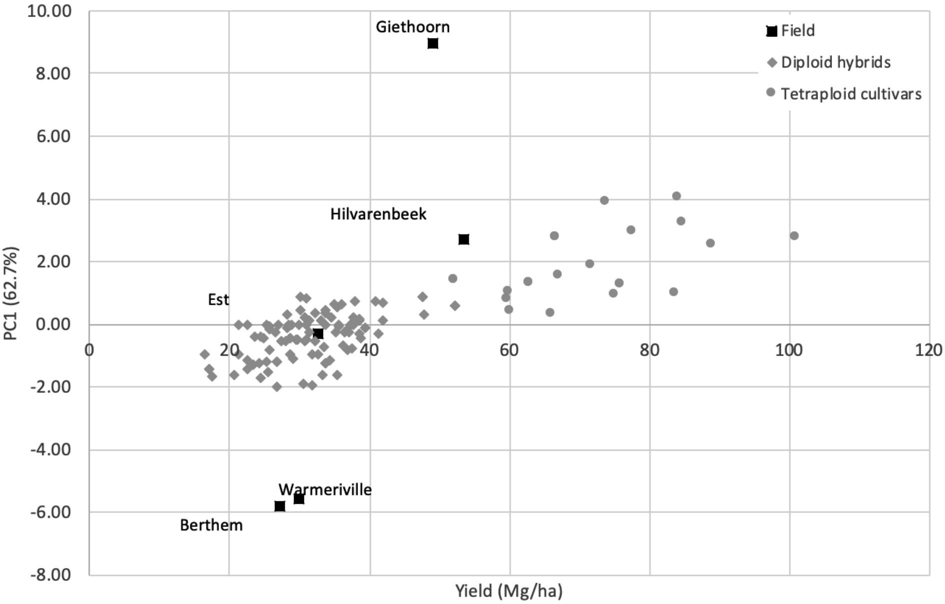


Figure 5.2: Biplot of yield and PC1 of the AMMI analysis (Additive Main effects and Multiplicative Interaction models)

Fresh tuber yield differed significantly among cultivars ($p < 0.001$). Diploid hybrids yielded on average between 16 and 52 Mg/ha. For the tetraploid cultivars, average yield was between 52 and 101 Mg/ha. Performance of cultivars among the different sites varied; indeed, a significant interaction (GxE, $p < 0.001$) in yield between cultivar and site was found (Table 5.2).

The genotype × environment interaction of the hybrids and cultivars was analyzed using the AMMI analysis. The AMMI parameters can be used to make a biplot in which the interaction effects of genotype and environment are shown (Gauch and Zobel 1997). In the biplot (Figure 5.2), 62.9% of the GxE interaction is explained in the principal component axis PC1. Also, the principal component axis PC2 explained a significant part of the G × E interaction (23.6%); the rest of the PC axes were not significant. On average, the yield of diploid hybrids was lower than the yield of tetraploid cultivars; however, the highest yielding hybrids were similar to the lowest yielding tetraploid cultivars. For these higher yielding hybrids, the interaction with the environment was also similar to several tetraploid cultivars (Figure 5.2). The large distance between the trial locations in Figure 5.2 shows that they differed in environmental effect, except for Warmeriville and Berthem that were very close to each other (Figure 5.2).

Dry matter

Dry matter percentage (DM%) was measured in each genotype on all fields separately. A wide genetic variation for DM% was observed, as well as variation in DM% among the different sites. Diploid hybrids showed a broad range of DM%, between 15.8 and 21% (Figure 5.3). This was in range with most of the tetraploid cultivars, except for three chip cultivars with a DM% of more than 22.5% (Brooke, Pirol and Lady Rosetta). The rest of the chip and frying cultivars had a DM% between 19.2 and 21.3%, 12 of the diploid hybrids also had a DM% within this range. The rest of the hybrids had a DM% comparable with the tetraploid table cultivars that produced a DM% between 15.6 and 19.2. So in general, in this test panel, the diploid hybrids had similar DM% to the tetraploid cultivars.

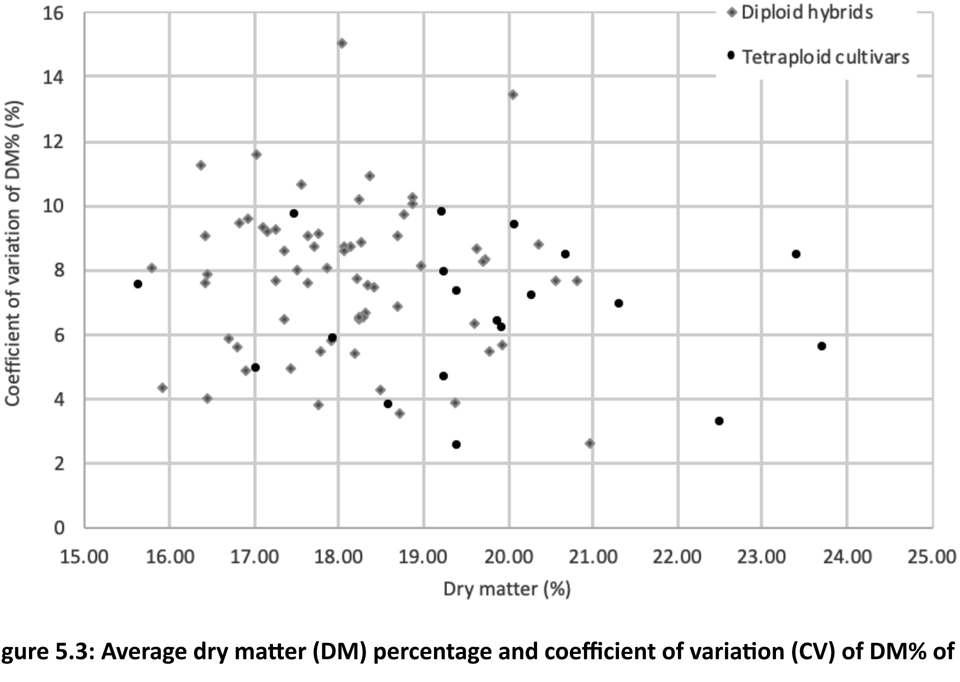


Figure 5.3: Average dry matter (DM) percentage and coefficient of variation (CV) of DM% of diploid hybrids and tetraploid cultivars

To compare the extent of the environmental effect on genotypes, the coefficient of variation (CV) of DM% over the sites was calculated for each diploid hybrid and tetraploid cultivar (Figure 5.3). Variation of DM% between fields in most diploid hybrids was in the same range as the variation of DM% in tetraploid cultivars. The most stable diploid hybrid (Hybrid 1) had a CV of 2.7%, which was similar to the CV of the tetraploid cultivar Innovator, while the DM% in hybrid 1 was even higher than in Innovator (21% and 19.4%, respectively). So, dry matter percentage and stability in different environments were as good as, and sometimes even better in hybrids than in tetraploid cultivars.

Tuber shape

Tuber shape is an important trait for cultivars in the chip and the frying industry, because tubers need to be suitable for the processing machines (Tiemens-Hulscher et al. 2013). Tuber shape is here expressed by Formula 4, in which the length, width and height of individual tubers are taken into account. The result is a dimensionless number between 1 and 0, with 1 representing a perfect sphere. Without selection for specific tuber shape in the diploid hybrids during the breeding process, the same range from round to long was found in hybrids as in tetraploid cultivars (Figure 5.4). The variation for long tubers for fries represented by frying cultivars like Innovator and Russet Burbank was similar to the variation of several hybrids, as well as a round shape represented by several chip cultivars like Hermes and Lady Rosetta.

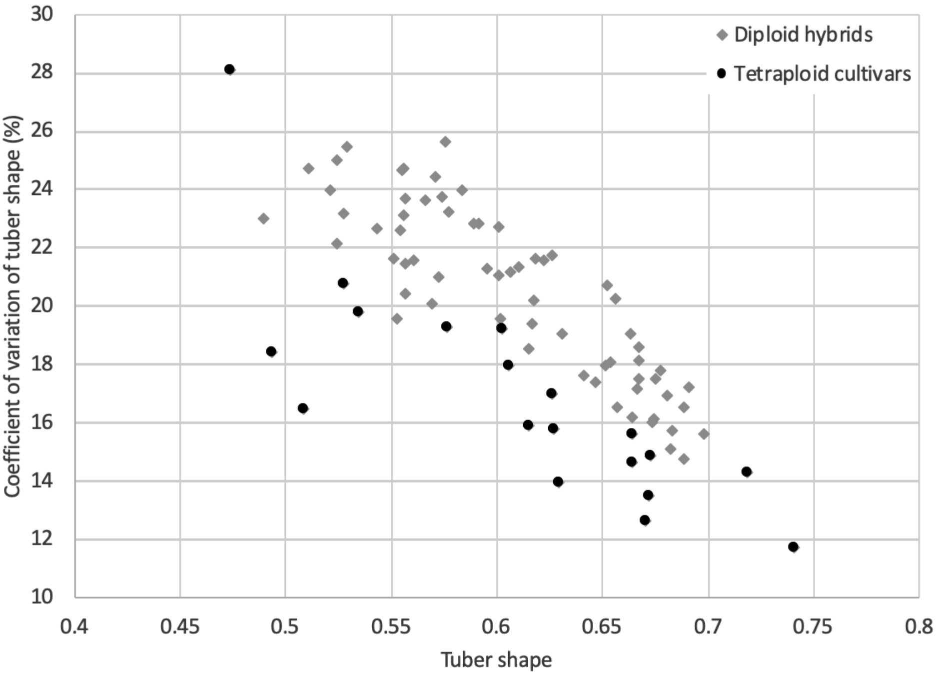


Figure 5.4: Average tuber shape and coefficient of variation (CV) of tuber shape of diploid hybrids and tetraploid cultivars. Tuber shape is a dimensionless number between 1 and 0, with 1 representing a perfect sphere.

The uniformity of the shape is important for mechanical processing. Therefore, the coefficient of variation of the shape data of the individual tubers was calculated for each hybrid and cultivar (Figure 5.4). Most diploid hybrids had a higher CV than the tetraploid cultivars. On average, the CV for the hybrids was 20%, and for the tetraploid cultivars, it was 17%; however, the most stable hybrids showed overlap with the least stable tetraploid cultivars. To gain insight into the effects of the different environments on the distribution of shape, we show boxplots of two cultivars (Figure 5.5). Both Hybrid 9 and Bintje had a stable shape across the different environments. The differences in mean shape and variation between the five locations were smaller for Hybrid 9 than for cultivar Bintje.

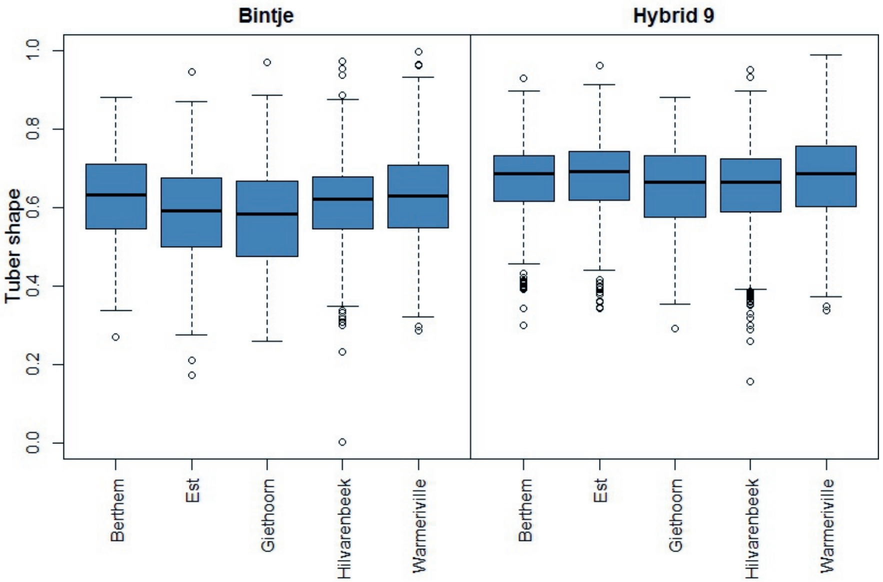


Figure 5.5: Boxplots of shape of individual tubers of tetraploid cultivar Bintje (n = 3484) and diploid hybrid 9 (n = 2782) grown at five different locations in 2017.

Yield components

Potato yield was analyzed as the product of different plant components that can contribute to yield. Individual tuber weight, number of tubers per stem and number of stems per surface unit were chosen as relevant yield components to compare the growth of diploid hybrids and tetraploid cultivars. These yield components were compared between tuber grown diploid hybrids and commercial tetraploid cultivars that grew in yield trials on five different locations.

Generally, the yield components differed between the tetraploid cultivars and the diploid hybrids. The mass of the individual tubers of diploid hybrids was lower than that of the tetraploid controls (71 vs. 177 g on average). The hybrids had on average a higher number of stems (25.6 stems/m^2) than the tetraploids (14.9 stems/m^2). The number of tubers per stem was higher in the tetraploids, compared to the diploids (2.9 and 1.8, respectively). This resulted in approximately equal number of tubers per unit area (45.2 and 43.5 tubers/m^2 for diploids and tetraploids, respectively) and all hybrids performed with the number of tubers/m^2 within the range of the tetraploid cultivars.

Table 5.3: Average values of adjusted yield and adjusted yield components of a selection of representative hybrids and tetraploid cultivars and adjusted yield, adjusted yield components and effect of yield components on total yield per cultivar (regression coefficient). Average values of yield components and yield were calculated using the full dataset of diploid hybrids and tetraploid cultivars

		Yield		Yield components			Contribution yield components to yield		
Cultivar, hybrid	Seed(ling) tuber weight (kg/plot)	kg/m^2	Tubers/m^2	Stems/m^2	Tubers/stem	g/tuber	Contribution per stem [g/m^2/(stems/m^2)]	Contribution tuber size [g/m^2/(g/tuber)]	Contribution tuber number [g/m^2/(tubers/stem)]
Hybrid 1	0.72	1.6	31.2	29.9	1.0	48	84	104	3161
Hybrid 2	0.91	2.8	31.1	17.9	1.7	89	− 20	90	1822
Hybrid 3	0.71	2.9	51.9	34.1	1.5	56	− 174	145	2020
Hybrid 4	1.00	3.3	36.1	19.6	1.8	89	160	62	2124
Hybrid 5	0.82	3.8	54.7	31.3	1.7	71	− 2	119	2033
Hybrid 6	0.97	3.8	43.7	22.4	2.0	91	96	1	426
Hybrid 7	0.76	3.8	52.0	19.8	2.6	68	867	179	2642
Hybrid 8	0.83	4.8	56.0	25.5	2.2	85	127	126	2305
Hybrid 9	0.73	5.2	60.6	26.4	2.3	93	− 111	− 8	1617
Annabelle	1.14	5.2	44.6	17.4	2.6	117	165	5	2123
Innovator	1.44	6.4	29.8	16.0	1.9	222	322	− 41	3387
Hermes	0.45	6.9	43.1	11.6	3.7	159	956	− 101	2153
Bintje	0.51	7.2	60.3	16.0	3.8	125	257	− 42	1164
Site									
Berthem	–	3.0	38.6	27.6	1.4	93	–	–	–
Warmeriville	–	3.1	37.4	20.9	1.8	99	–	–	–
Est	–	3.3	36.6	18.5	2.0	98	–	–	–
Giethoorn	–	5.3	60.8	25.3	2.4	90	–	–	–
Hilvarenbeek	–	5.5	63.7	23.7	2.7	96	–	–	–

When comparing yield components of individual tetraploid cultivars and diploid hybrids, the differences were small. A positive correlation was found between yield and tubers per stem ($p < 0.001$). Indeed, the higher yielding hybrids had a relatively high number of tubers per stem. Hybrid 9 for example produced 2.3 tubers per stem on average, which was nearly as much as Annabelle (2.6) and more than Innovator (1.9). Tuber size also was positively correlated with yield ($p < 0.001$); also for the diploid hybrids, the larger tubers resulted in higher yield, even though all hybrid tubers were relatively small.

The cultivars with higher yield also had a relatively high number of tubers per m2. For example, Bintje showed a high number of tubers/m2 (60.3) and the highest yield (7.2 kg/m^2) across all cultivars, while Hybrid 1 had the lowest yield (1.6 kg/m^2) and a low number of tubers m^{-2} (31.2). In higher yielding hybrids, the number of tubers/m^2 was also high, with both Hybrid 9 and tetraploid cultivar Bintje having the largest number of tubers/m^2.

Contribution of yield components to yield

Variation in yield was found across sites as well as between cultivars. The variation in yield was due to the difference in responses of individual genotypes to the environments. Although there are trade-offs and interdependencies between yield components, they explain differences in yield between cultivars and environments.

The number of tubers per stem showed the largest variation across sites (CV = 24.8%); between Berthem and Hilvarenbeek, the difference was almost a factor two. For stems/m2, the variation was much lower (CV = 15.3%) and the weight of the tubers was rather stable (CV = 3.7%). In conclusion, the yield component that was affected most by the environment was the number of tubers per stem.

To compare the extent to which different yield components were affected by the environment between cultivars, the contribution of individual yield components to total yield over the different sites was determined by calculating the slope between total yield and yield component across sites (Table 5.3). It estimated the yield gain for a single yield component for each cultivar, with a positive value indicating a contribution to higher yield when the specific yield component increases, and a negative value indicating a loss in total yield when increasing a specific yield component.

To compare the relative effects of yield components on yield between the tetraploid cultivars and the diploid hybrids, correlations between yield components and yield were calculated. Every pair of average yield and yield component value of each location was used in the regression analysis, data are shown in the last three columns of Table 5.3. It compared the difference in contribution to yield of yield components. Number of tubers per stem explained 80% of the variation in yield in tetraploids and 90% in the diploid hybrids. The relative effect of number of stems per m^2 on yield was much smaller: 18% in tetraploid cultivars and 5% in hybrids. Tuber size affected yield least in tetraploids and hybrids (2% and 5%, respectively). So, on average, hybrids and tetraploid commercial cultivars shared the most important contributing factor to yield.

A higher number of tubers per stem contributed positively to total yield in all cultivars (Table 5.3). For diploid hybrids in the selection of Table 5.3, the contribution was between 426 and 3161 g yield increase per m^2 for each extra tuber per stem. In the tetraploids, this was between 1164 and 3387 g/ m^2 yield increase/(tuber/stem). More stems per m^2 led in some hybrids to a decrease in total yield while in others, total yield increased with more stems per m^2 (contribution between − 174 and 867 g/ m^2 yield increase/(stems/ m^2) in Table 5.3). In the tetraploid cultivars, higher stem numbers were positively correlated with yield with contributions between 165 and 956 yield g/ m^2 yield increase/(stems/ m^2). There was a large variation in the contribution of larger tubers to total yield between diploid hybrids as well as in tetraploid cultivars, with values between − 8 and 175 g/m^2 yield increase/(g/tuber) for hybrids and − 101 and 5 g/ m^2 yield increase/(g/tuber) for tetraploid cultivars. Overall, the range of yield contribution by the different yield components was overlapped between diploid hybrids and tetraploid cultivars.

Variation was found between the different cultivars in total yield as well as in the contribution of the separate yield components to yield. To examine whether there is an optimal value for each yield component for high yield, the gain in yield with an increase of the yield component was compared with the average value of that yield component for each cultivar. Although yield gain and increase in yield component are not completely independent, it provides insight into whether there are optimal yield component values.

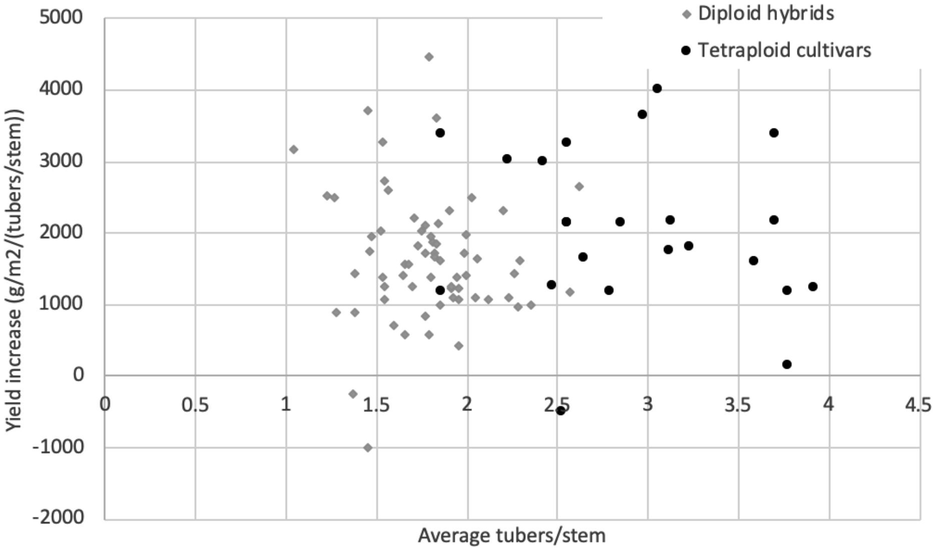


Figure 5.6: Contribution of more tubers per stem to total yield, expressed in yield increase per extra tuber/m^2, compared to average number of tubers per stem over five sites (Berthem, Giethoorn, Hilvarenbeek, Est, Warmeriville).

Genotypic variation was found for the contribution of yield components to total yield. The effect of differences in yield components between genotypes on the correlation between yield component and yield was examined (Figures 5.6, 5.7 and 5.8). Most genotypes showed a positive correlation between yield and tubers/stem when the average number of tubers/stem was between 1.0 and 3.8, so for this dataset, more tubers per stem contributed positively to total yield in the whole range.

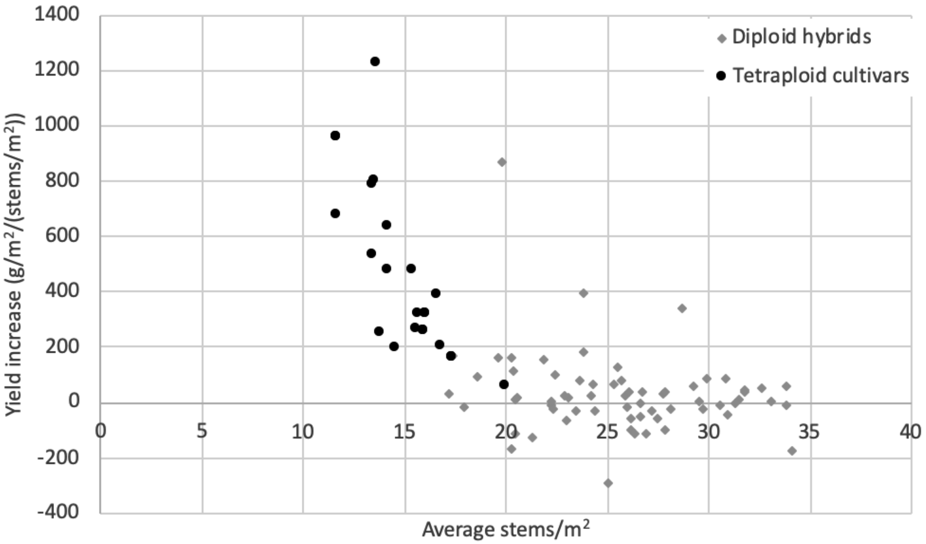


Figure 5.7: Contribution of more stems per m^2 to total yield, expressed in total yield increase per extra stem/m^2, compared to average number of stems/m^2 over five sites (Berthem, Giethoorn, Hilvarenbeek, Est, Warmeriville).

In contrast to the number of tubers/stem, larger tubers also affected yield negatively in some cultivars (Figure 5.8). A negative relation was found between contribution of larger tubers to total yield and average tuber size of a genotype (Figure 5.8). Until a tuber size of about 90 g, the production of larger tubers resulted in a higher total yield. When a genotype already produced large tubers on average, larger tubers decreased total yield. So, with increasing average tuber size of a genotype contribution of even larger tubers to total yield decreased.

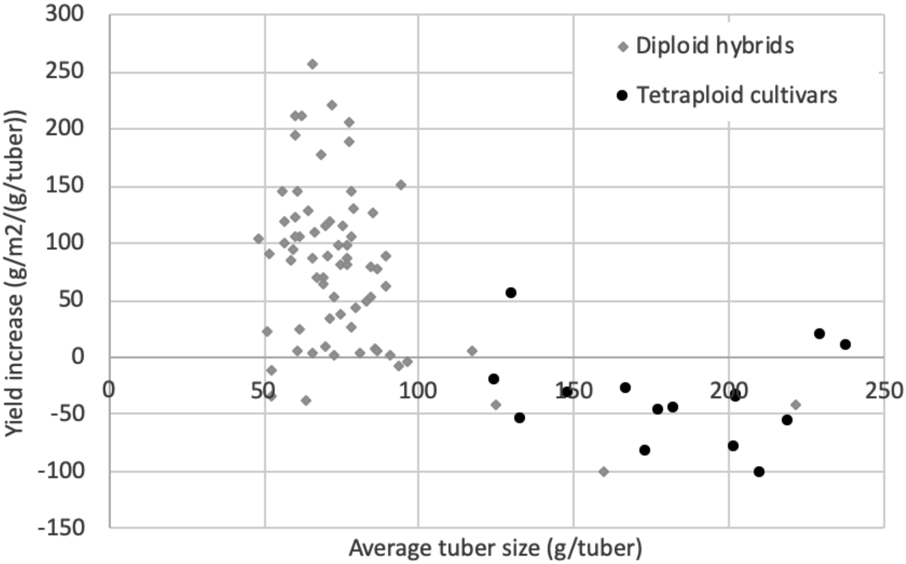


Figure 5.8: Contribution of larger tubers to total yield, expressed in total yield increase per extra g tuber (tuber size), compared to average tuber size over five sites (Berthem, Giethoorn, Hilvarenbeek, Est, Warmeriville).

5.4 Discussion

In this research, we have presented results of replicated field trials across multiple locations of diploid hybrids in comparison with tetraploid cultivars. We have analyzed the yield components to explain differences in yield level and stability.

Hybrid seedling tuber production

To perform yield trials in which diploid hybrids could be benchmarked against tetraploid commercial cultivars, we first produced seedling tubers from diploid hybrid seeds. This was done to decrease the difference in starting material, as plant performance is largely affected by the starting material (Arsenault et al. 2001; Struik and Wiersema 1999). The breeding process of the test hybrids that were used in this trial was done exclusively in the greenhouse, so no selection for yield was applied.

Seedling tubers that were produced were used in the subsequent year for yield trials. The physiological age of seed tubers is one of the main determinants for seed tuber quality (Struik and Wiersema 1999) and it is affected by many genetic and environmental factors such as management of the seed tuber crop, storage conditions, seed tuber size and the state of dormancy (Struik 2007a). Before the seed tubers were selected for the ware crop trial, sprouting was scored. The results showed that dormancy was broken in all tubers; however, there was some variation probably due to different genetic background of the diploid hybrids.

To minimise the experimental error, caused by differences in the physiological conditions of the tubers, seed tubers were stored under optimal conditions and equally sprouting tubers were selected for the yield trial. If present, the largest unexplained error would occur in the diploid hybrids, due to differences in physiological conditions as the tubers of the different hybrids were not stored under optimal conditions for each hybrid. In addition, some genetic variation within the hybrids may still occur as the parents were not 100% homozygous. However, the within-plot variation of the hybrids was not higher than this variation within commercial cultivars (data not shown). So, this supports the conclusion that the differences between the hybrids and the commercial cultivars were due to genetic differences.

Yield trials

Yield trials were conducted to examine yield potential of diploid hybrids, the GxE interaction and to compare yield components between diploid hybrids and tetraploid cultivars. The trials were conducted at five sites in France, Belgium and Netherlands (3 sites) from which insight into the variation due to genetics, the different environments and their interactions was obtained.

Performance of diploid hybrids and tetraploid cultivars

For a long time, the assumption that tetraploid potatoes are inherently higher yielding than diploid potatoes was based in a large part on the untested hypothesis that having four allelic variants per locus contributes to yield superiority (Bani-Aameur et al. 1991; Jong and Tai 1991; Werner and Peloquin 1991; Buso et al. 1999). When grown in a farmer-managed ware crop, we have shown that two of the diploid hybrids and cultivar Annabelle show similar average yields with 48, 52 and 52 Mg/ha, respectively, thereby proving the yield potential of diploid hybrids. This was suprising; as diploid hybrids were never selected for high yielding under field conditions, they were not expected to keep up with yield of tetraploid commercial cultivars in which selection for high yield has been performed for hundreds of years. These results are supported by trials of Hutten (1994) and Jansky et al. (2016) in which they demonstrated yield potential of diploid potato.

Next to fresh tuber yield, other important criteria that determine the economic value of a cultivar were assessed. The diploid hybrids that were used in this trial had a range for DM% as well as for tuber shape that overlapped with the tetraploid cultivars for chips and fries (Hermes and Innovator). As heritability of tuber shape is high (Bisognin et al. 2012; Lindqvist-Kreuze et al. 2015; Meijer et al. 2018), it is a trait that can be selected for efficiently.

Environmental effect on plant performance

Insight into the stability of desired traits across different environments is important to be able to select for desired trait properties efficiently in the breeding process. These diploid hybrids were a set of first test hybrids, developed while the breeding programme was still immature. Therefore, more variation was expected in the hybrids than in the tetraploid cultivars, which were clones. This expected variation was investigated for yield as well as a number of relevant traits.

Yield of diploid hybrids and tetraploid cultivars was affected by the environment. In line with the results found in this study, potato tuber yield is known to be highly influenced by different environments (Haverkort 1990). Several approaches of measuring yield stability are possible (Lin et al. 1986). In this study, the AMMI analysis was used as a useful method to distinguish the effects of genotype, environment and their interaction (Gauch et al. 2008). The first principal component axis PC1 explained 62.9% of the GxE interaction and is therefore used to discuss the interaction. Although we cannot attribute differences in performance between hybrids or tetraploid commercial cultivars to specific environmental conditions, we were able to determine that yields of both diploid hybrids and tetraploid cultivars were affected by GxE. The magnitude on the PC1 axis was comparable between hybrids and cultivars; however, the scores of the tetraploid cultivars generally were higher. A higher score of a cultivar in a specific environment indicates a better performance in that environment, while genotypes that score close to zero perform more constant across environments (Gauch and Zobel 1997). Even though the hybrids generally had lower yields and performed less stable across the environments than cultivars, there were also hybrids that perform similar to tetraploid cultivars in both yield and stability in this set of unselected test-hybrids.

Dry matter content of both diploid hybrids and tetraploid cultivars was affected similarly by the environment. When stability of a cultivar for DM% is high, efficient selection for specific dry matter percentages is possible (Wang et al. 2017). Although there was still genetic variability within the diploid hybrids and the hybrids were never tested in the field before, overall stability of the performance was similar to clonally propagated tetraploid cultivars. This genetic variability may have led to the slightly higher CV of shape in diploid hybrids compared to tetraploid cultivars; however, it was small in effect, which is in line with earlier reports (Yildirim and Celal 1985; Love et al. 1997).

The variation of yield components in the different environments was compared between the diploid hybrids and the tetraploid cultivars. The relation of the three different yield components was similar for tetraploids and diploids: for both, the number of tubers that was produced per stem was affected most by the environment, followed by number of stems per m2 and finally tuber size.

No difference was found in the average effect of environment on yield components between hybrids and tetraploid cultivars. This comparison shows that diploid hybrid cultivars express similar variation to various environments as commercial tetraploid cultivars despite the genetic variation that is still present in these first test hybrids, and the difference in starting material (seedling tubers compared to normal seed tubers). Moreover, it shows that yield and dry matter are highly affected by the environment, so multiple environments are needed for selection, while tuber shape is more stable across different environments.

Contribution of yield components to total yield

The variation of yield components in different environments was compared to the difference in total yield between those environments to evaluate the contribution of each yield component to the total yield. An increase in number of tubers produced per stem showed the strongest positive effect on yield in diploid hybrids as well as in tetraploid cultivars. For tetraploid cultivars, a similar effect of number of tubers per plant was found by Asghari-Zakaria et al. (2007). In contrast to the effect of the number of stems, the effect of tuber size on yield differed between tetraploid cultivars and hybrids. The commercial tetraploid cultivars already produced relatively large tubers and larger tubers did not result in more yield or affected yield even negatively. This finding is in contrast to the results of Asghari-Zakaria et al. (2007) who showed a positive correlation between tuber size and yield. However, as both studies contained only 1 year of field trials, different findings could be explained by year effects. The results suggest that there is an optimum in tuber size above which larger tuber size has a neutral or negative effect on total yield.

The distribution of yield components was different between diploid hybrid cultivars and tetraploid cultivars. Diploids had smaller tubers and fewer tubers per stem, but more stems/m2 than tetraploids. The large number of stems/m2 in diploid hybrids even affected total yield negatively, while in all tetraploid cultivars, an increase of stem density had a positive effect on yield. Firman and Allen (2007) showed that increasing stem density up to 10 stems/m2 resulted in a higher total yield, but higher densities did not affect total yield, and in our study, we found an optimum of 18 stems/m2. With larger stem densities, the tuber size distribution can be steered: higher density results in more smaller tubers (Firman and Allen

2007). Indeed, in this experiment, a negative correlation was found between stem density and tuber size.

As most commercial tetraploids cultivars produced larger tubers than diploid hybrids, the effect of increased tuber weight was larger in hybrids than in tetraploid cultivars. The estimated optimal tuber size was 100 g/tuber and larger tubers did not contribute to higher total yield or even resulted in lower yield. A larger number of tubers per stem resulted in a higher total yield in most diploid hybrids and tetraploid commercial cultivars. This comparison shows the similarity of distribution of yield components contributing to yield in diploid hybrids and tetraploid cultivars. In addition, it gives insight in which yield components need most focus in the diploid hybrid cultivars to improve yield potential.

Implications for breeding

The analysis of yield components was performed for different environments and the variation of specific plant properties in different environments was evaluated. With this information, we determined traits that need to be tested in several environments to make reliable selections, for example yield, dry matter and tuber number were affected by the environment. Specific yield components that can be used as selection criteria in a breeding program were identified. In this trial, optimal configurations of yield components for the tested environments were found: a tuber size of approximately 100 g/tuber and a stem density of about 18 stems/m2, with as many tubers as possible as more tubers always increased total yield. These configurations can be used as breeding targets. Moreover, we were able to identify more stable hybrids over more variable ones, and hence we can start selecting for trait stability.

Conclusions

In this study, we compared the performance of diploid test-hybrid cultivars to tetraploid commercial ones in field trials at representative locations in the NW-European potatogrowing area. In these trials, some diploid hybrids have shown a yield potential comparable to the tetraploid cultivars. For major economically relevant traits such as dry matter percentage, tuber number/m2 and tuber size, the diploid hybrids only slightly underperformed compared to the tetraploid cultivars. For yield, the best hybrids performed as good as the lower yielding tetraploid cultivars, the diploid hybrid cultivars having never been selected under field conditions. Stability of these traits across different locations was remarkably similar to the that of tetraploid cultivars. Moreover, the stability was different for the measured traits. For making selections in breeding, this information helps to determine the number of environments that is needed to test a certain trait. There was a large overlap in performance of the diploid hybrids and tetraploid cultivars for all the yield components analyzed (stems per m2, tubers per stem and tuber size). In both tetraploid cultivars and diploid hybrids, the number of tubers per stem affected the total yield most, followed by stems/m2. Tuber size had the smallest effect on total yield. With the variation for these traits present in diploids, desired properties can be combined in a cultivar through hybrid breeding which is competitive with the current commercial tetraploid cultivars.

Chapter 6

General discussion

For potato, diploid hybrid breeding is a new technology that will lead to improved cultivars in the near future. Compared to a tetraploid traditional breeding system, diploid hybrid breeding is faster, and, because of simpler genetics, more targeted breeding is possible. Hybrid potato breeding is enabled by the development of inbred parent lines, that become more homozygous with each generation of self-pollination. Hybrids are produced by making a cross between two of these inbred parent lines, resulting in hybrid true potato seed. The production of inbred lines and hybrids not only means a new way of breeding for potato, but also leads to a new seed system and a new way of growing the crop. Therefore, the success of a hybrid potato breeding programme, not only depends on genetics but also on non-genetic aspects.

In this thesis, I investigated critical, non-genetic aspects of a breeding programme, which influence the success rate in the breeding programme by making it more precise and efficient. First, I investigated the influence of environmental factors on the tuber production of the inbred parent lines. These parent lines are used to make sexual crosses; therefore, it is relevant to produce many tubers as parent material for hybrid production. I studied the effect of light intensity, percentage of far-red light in the light spectrum, and temperature on tuber production under controlled conditions. The number of tubers per plant, the size of the single tubers and the tuber-size distribution are all relevant. Second, I focused on improving the quality of field trials that are performed during the selection and testing phase of a breeding process by managing various sources of variation. I investigated the effect of seedling tuber quality on the quality of the results of the selection and testing trials. To be specific, I assessed the effect of single seed tuber weight, number of eyes and production origin of seedling tubers on plant growth in the field as well as on yield parameters. I also developed a method to determine the plot size that is needed to reach the required precision in selection and testing trials. Third, variety trials were carried out in which I compared the performance of test-hybrids with the performance of tetraploid cultivars in several contrasting locations.
The overall aim of this thesis is to provide non-genetic tools to make selections in the breeding programme more precise, efficient, and successful.

In this general discussion to the thesis, I start with a section on the major findings for each of the four research chapters. A short description on the work that was done is given per chapter, followed by the main results obtained and the conclusions that were drawn. Subsequently, I discuss how the results could be used to strengthen a hybrid potato breeding programme. I particularly describe the sources and importance of environmental variation in the breeding programme. On the one hand, this variation can lead to a lower accuracy of selections or trials results. In this case, I present options to deal with this source of variation. On the other hand, I describe opportunities for using environmental variation to speed up the breeding process.

6.1 Major findings

In this section, an overview of the work performed, and the main findings per chapter will be given (Figure 6.1).

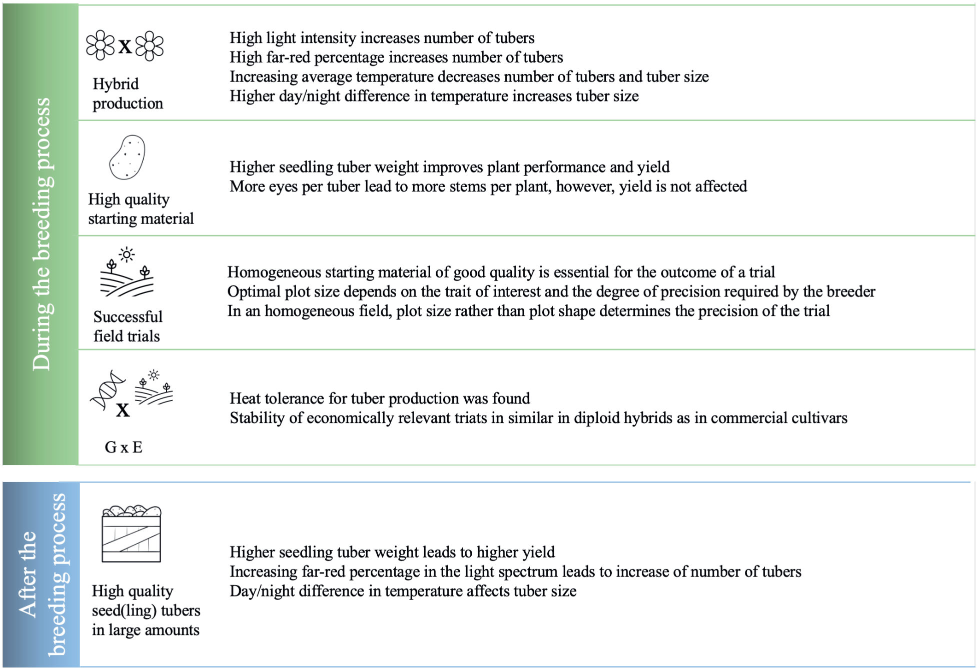
6.1.1 Increasing tuber production by changing light and temperature regimes

Chapter 2 focused on the effect of environmental conditions in tuber production of diploid inbreeding lines. Earlier research in tetraploid cultivars had shown that both, temperature and light, affect the tuber production and that different cultivars can respond differently to these factors (Struik 2007b). A temperature of 17°C is considered optimal for high tuber yield and increasing the temperature up to 28°C enhances shoot growth while impeding tuberization and dry matter allocation to tubers and thus reducing tuber production (Ewing 1981; Struik 2007b; Trapero-Mozos et al., 2018). For light, the photoperiod, light intensity, and light spectrum can all affect growth and development of the potato plant (Plantenga 2016). In this research, I focused on light intensity and light spectrum, while keeping the photoperiod constant. Earlier research had shown that increased light intensity results in increased assimilation rates (Stutte et al. 1996), thus increasing the assimilate pool available for tuber growth, and that extra far-red light led to earlier start of tuberization (Plantenga et al. 2016). In this **Chapter 2**, I investigated the effects of light intensity, percentage of far-red light, average temperature, and the day/night difference in temperature on tuber production of diploid parent lines under controlled conditions. The results showed that light affects the total tuber weight that a plant is able to produce by affecting the number of tubers per plant.

Increasing the light intensity led to more tubers per plant, while no effect on tuber size was found. Increasing the percentage of far-red light in the light spectrum also led to more tubers per plant, without affecting tuber size. This means that a light regime with a high light intensity and a high proportion of far-red light is able to produce a relatively high yield and a relatively high rate of multiplication.

The average temperature during a 24-h period affected tuber size as well as number of tubers per plant. Two climate room trials were conducted in which the effect of average temperature was investigated. In the first trial, different temperature levels were realized by applying different day and night temperatures, keeping the difference between day and night temperature constant at 4°C. In the second trial, we altered the average temperature by varying the duration of a period of high temperature during the day. Base day and night temperatures of 24 and 18°C were imposed, and periods between 0 and 16 hours of 30°C during the day were implemented to create different average temperatures, while keeping the minimum and maximum temperature constant (Table 2.2). In both trials, an increase in average temperature led to a decrease in number of tubers per plant, as well as a lower weight per tuber. Together, this led to a lower total tuber weight per plant when plants were grown at higher average temperature. In these trials, different parent lines were used, and significant interactions between the factors genotype and average temperature were found. That means that the parent lines differed in sensitivity to temperature regarding tuber production. Indeed, one of the parent lines used was relatively tolerant to high average temperature regarding tuberization. The lowest average temperature that I used was 18.7°C, and no decrease in total tuber weight due to a decrease in temperature was found yet, so the optimal temperature for tuber production might be even lower.

In a third climate room trial, I varied the difference between day and night temperature, while keeping the average temperature constant. This only affected the weight per tuber, not the number of tubers per plant. Increasing the difference in day/night temperature from 0 to 9°C led to an increase in weight per tuber.



During the breeding process	Hybrid production	High light intensity increases number of tubers High far-red percentage increases number of tubers Increasing average temperature decreases number of tubers and tuber size Higher day/night difference in temperature increases tuber size
	High quality starting material	Higher seedling tuber weight improves plant performance and yield More eyes per tuber lead to more stems per plant, however, yield is not affected
	Successful field trials	Homogeneous starting material of good quality is essential for the outcome of a trial Optimal plot size depends on the trait of interest and the degree of precision required by the breeder In an homogeneous field, plot size rather than plot shape determines the precision of the trial
	G x E	Heat tolerance for tuber production was found Stability of economically relevant triats in similar in diploid hybrids as in commercial cultivars
After the breeding process	High quality seed(ling) tubers in large amounts	Higher seedling tuber weight leads to higher yield Increasing far-red percentage in the light spectrum leads to increase of number of tubers Day/night difference in temperature affects tuber size

Figure 6.1: Main conclusions from the different chapters in this thesis. The breeding process entails the development and selection of parent lines, as well as testing and selection of hybrids, until a hybrid is ready for registration.

6.1.2 Seedling tuber quality of diploid hybrids

Chapter 3 focused on the quality of diploid hybrid seedling tubers. It is known that in tetraploid cultivars, the quality of seed tubers can affect plant development and tuber yield of the crop grown from these seed tubers. In tetraploids, seed tuber size and number of eyes per seed tuber have been shown to affect yield (Struik & Wiersema, 1999). Production origin can affect physiological age, and with that the plant performance in the field after planting such seed tubers. For diploid seedling tubers, the effects of these tuber quality traits were unknown. Also, the variation that is present in a seedling tuber batch was not known yet. I therefore investigated the variation in quality traits present in a batch of seedling tubers and the effects of these quality traits on plant performance and tuber yield of such seedling tubers in the field.

Two trials were conducted. In the first trial, the effect of number of eyes was determined by using seedling tubers with high or low number of eyes, but with similar single-tuber weight. The results showed that a higher number of eyes per seedling tuber gives more stems per plant, without affecting ground cover and total tuber yield. The higher number of stems was compensated for by a less vigorous individual stem and a lower number of tubers per stem, resulting in similar number of tubers and total tuber weight per plant.

In the second trial, I determined the effect of production origin and of tuber weight of seedling tubers on crop development and yield of the crops grown from such seedling tubers. Tubers produced in three locations in the Netherlands were used (Emmeloord, Garsthuizen and Anna Paulowna, Table 3.1). From all three locations, tubers from the size class 28 − 35 mm and 35 − 45 mm were collected for the trial. Weight of tubers from both size classes was measured before planting. In our trial, production origin had no effect on crop development or on tuber yield. Seedling tuber weight, however, did affect both. Higher seedling tuber weight led to higher ground cover, while no effect on number of stems was present. Total tuber weight per plant as well as number of tubers per plant was higher when heavier seedling tubers were used. These data were used to calculate the increase in yield per gram increase in seedling tuber weight. Moreover, the variation in tuber weight in a batch of 420 seedling tubers in the size class 35 − 45 mm was measured. Within this size class, variation in tuber weight up to factor 4.6 was found. When combining this variation with the increase in tuber yield per extra gram seedling tuber, we found that the variation in seedling tuber weight in size class 35 − 45 mm potentially can lead to variation in yield up to 16.1 Mg/ha in the hybrids that we used. Therefore, I concluded that a tuber grading system based on seed(ling) tuber weight rather than one based on size would be better for uniform growth and optimal crop management. This also applies to a breeding programme, where uniform growth is important for efficient selection and where yield is one of the selection criteria. So in a breeding programme it would lead to less variation in the selection trials, and with that higher quality of the results and better selections.

6.1.3 How to calculate optimal plot dimensions

Performance trials are an important part of a breeding programme, and to perform conclusive trials the variation should not be limited. Besides the experimental design, plot shape and an adequate number of replicates, plot size is an important factor to decrease variation in a field trial (Bisognin et al. 2006). In the early generations of the breeding process in a conventional tetraploid breeding programme usually plot sizes are relatively small because the amount of starting material (i.e., seed tubers) is limited (Bradshaw 2021). In hybrid breeding, this limitation is no issue because larger amounts of starting material can be produced through hybrid true potato seed (i.e., true potato seed or seedling tubers). The question what plot size is optimal for testing hybrids has become more relevant, because there is more room for maneuvering.

In **Chapter 4**, I developed a method to calculate the required plot size when conducting performance trials with diploid hybrid potato. Two field trials were conducted in two subsequent years. In both trials, four different diploid hybrids and one tetraploid cultivar were planted in a randomized block design with three replicates. Plot size was 90 plants per plot, and each plant was harvested individually. With this design, I could compare yield results of plot sizes ranging from 1 plant to 90 plants per plot.

Based on these data, I calculated the least significant difference, expressed as a percentage of the trial mean (the so-called LSD%). This LSD% was linked to different plot sizes and shapes to develop equations that can be used to calculate the LSD% for a certain plot size and shape (Table 4.3). This was done for the traits tuber weight, number of tubers, tuber shape, tuber volume and as a measure of variation in the tuber batch the standard deviation of tuber shape and tuber volume. These are relevant traits for breeders, and in relatively homogeneous fields the equations can be used to calculate the plot size that is needed for the required precision per trait.

6.1.4 Stability of yield components

In **Chapter 5**, I describe the results of performance trials of test hybrids conducted in five different locations. In these trials, I benchmarked hybrids against commercial cultivars. Moreover, genotype × environment (G × E) interactions of yield and yield components were investigated for both, hybrids, and commercial cultivars. Sites were chosen in France (FR), in the Netherlands (NL) and in Belgium (BE). Experiments were conducted in Warmeriville [FR], Hilvarenbeek [NL], Est [NL], Berthem [BE] and Giethoorn [NL]. The locations were different in crop management, climate, and soil type.

I found that the diploid test-hybrids only slightly underperformed against the tetraploid cultivars: the lowest yielding tetraploids were comparable with the highest yielding hybrids. Stability of total yield, shape and dry matter percentage was remarkably similar for hybrids and commercial cultivars.

A yield component analysis was performed to analyze the contribution of the different yield components to total yield for hybrids and commercial cultivars. Number of tubers per stem, number of stems/m^2 and tuber size in g/tuber, resulting in the tuber yield (g/m^2) were considered in the analysis. In both groups, hybrids and commercial cultivars, number of tubers per stem had the highest effect on total yield, followed by number of stems per m^2. Tuber size had the smallest effect on total yield.

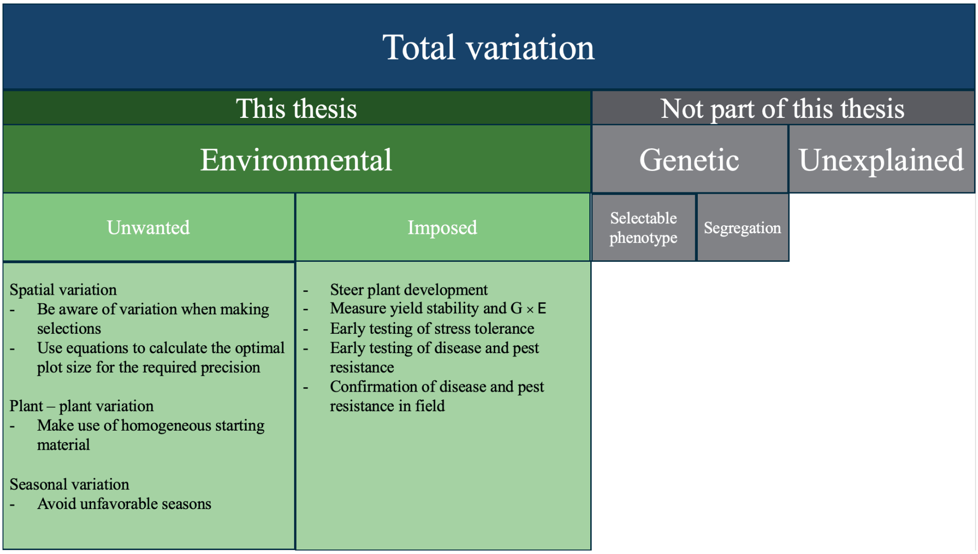
6.2 Variation in a hybrid potato breeding programme

The goal of a hybrid potato breeding programme is to develop new cultivars that perform better than old, existing cultivars. There are many ways how the plants can perform better. For example, yield can be higher, but plants could also be more tolerant to diseases and pests or to abiotic stresses (ter Steeg et al. 2022). Breeders try to reach these goals by developing plants with an improved genetic composition (Adams et al. 2022). To that end crosses are made that combine gene variants (alleles) that contribute positively to plant performance in different ways. For this process, the presence of genetic variation is a prerequisite. Breeders use the natural genetic variation that is present in the crossable germplasm to combine the best alleles and develop superior cultivars (Bradshaw et al. 2006).

In this process, it is essential that new genotypes can be tested, for breeders to make selections based on their performance (ter Steeg et al. 2022). In these trials, genetic variation can also be a source of unwanted variation. Test-crosses are made while the parent lines are still in the breeding process. That means they are still relatively heterozygous, and hybrids often are segregating for many traits, making the selection process harder.
To select the best genotypes, differences between plants should be the result of the different genetics, and not due to environmental variation, or due to the interaction between the genotype and its environment, at least during the early stages of the breeding programme. Therefore, it is important to know how to deal with unwanted variation in breeding trials. So, environmental variation is often unwanted in breeding trials, as it makes the selections less precise.

On the other hand, breeders can make use of environmental variation to speed up the breeding process. Part of testing new cultivars is testing how plants perform in different environments and under biotic and abiotic stress conditions (Bradshaw 2021). By exposing the plants to different conditions early in the process, selection for tolerance can be done earlier, which will prevent the disappointment of poor performance under stressful conditions during later stages of cultivar development or commercialization.

In the following sections, I will discuss possible sources of non-genetic variation, and how to deal with it in a hybrid potato breeding programme. The variation can be unwanted because it disturbs the progress in and efficiency of the breeding and selection process, or variation can be applied to make the breeding and selection process more efficient. An overview is given in Figure 6.2.



Spatial variation
- Be aware of variation when making selections
- Use equations to calculate the optimal plot size for the required precision

Plant – plant variation
- Make use of homogeneous starting material

Seasonal variation
- Avoid unfavorable seasons

- Steer plant development
- Measure yield stability and G × E
- Early testing of stress tolerance
- Early testing of disease and pest resistance
- Confirmation of disease and pest resistance in field

Figure 6.2: Scheme with results from this thesis on variation that can be present in a breeding programme. In this thesis, I focused on environmental variation. On the one hand, I investigated how to decrease the impact of unwanted variation in selections, and with that make the breeding programme more precise. On the other hand, I used variation to make the breeding programme more efficient.

6.2.1 Variation in parent line and hybrid development

In this section, I will discuss variation that can be present in the phase of parent line and hybrid development of a hybrid potato breeding programme, usually performed in a greenhouse.

Unwanted variation

The development of parent lines as well as the production of hybrids is usually executed inside a greenhouse in a hybrid potato breeding programme. In this way, the plants can grow in a more protected environment than when grown outdoors. This is relevant as plant vigour in inbreeding lines is often relatively low and plant performance of inbreeding lines will greatly benefit from a protected environment. Moreover, by growing plants indoors, the chance on unwanted cross-pollinations by insects is strongly reduced. Compared with field conditions, growing conditions inside a greenhouse are relatively controlled and therefore can be used to manipulate plant growth and development. For example, temperature can be increased by heating, additional lighting can be applied, and soil can be irrigated. A disadvantage of making selections in parent lines inside a greenhouse is that the product of the selections (the hybrids) needs to grow under field circumstances, and that the correlation between greenhouse and field can be low. For example, low correlations between yield of greenhouse-grown potato plants in pots were found with the yield of the same cultivars grown in the field (Köhl et al. 2021).

Despite the attempt to control environmental conditions in a greenhouse to protect plants, usually a lot of variation is still present within a greenhouse. Partly this is spatial variation, for example shades from the greenhouse construction, or variation due to outdoor light and wind because of the orientation of the greenhouse (Tang et al. 2018). Also ventilation in greenhouses, and even in climate rooms, can cause spatial variation in the climate because the ventilation tubes always are in the same position, and distribution of temperature, humidity or CO_2 are regulated by ventilation (Bournet & Boulard 2010). Besides the spatial variation that is present inside a greenhouse, influences from outside that affect the whole greenhouse cannot be ruled out completely. For example, on a warm and sunny day it often becomes very hot in the greenhouse, possibly leading to heat stress in plants, thus for example impeding pollination and fertilization.

When making selections in parent lines, for example for tuber characteristics or plant vigour, using material that is grown in a greenhouse, it is important to be aware of the spatial variation that is present, because plants should not be selected because they can profit from a better microenvironment in the greenhouse and therefore perform better than their counterparts exposed to a less conducive micro-environment. Also, productions carried out in summer or winter season cannot be compared with each other, and the variation in expression of certain traits can vary depending on whether the material is grown in a winter season or in a summer season. For example, as shown in **Chapter 2**, variation in light conditions mainly affect number of tubers, while differences in temperature affect the number of tubers as well as the single tuber size.

Application of environmental variation in parent line development

Environmental variation can also be used intentionally as a tool in the breeding programme to speed up the multiplication or selection process. One way is to steer the development of plants as needed, for example, by applying conditions that enhance the formation of tubers when seedling tubers need to be produced, or by applying conditions that enhance branching, sympodial growth and therefore stimulated early and abundant flowering, thus increasing true seed production. Another reason for applying specific environmental variation is to perform stress tests, by exposing the plants or crops to biotic or abiotic stresses, thus testing, for example, their disease resistance, drought tolerance or heat tolerance, in an early stage of the breeding process. Below, I will discuss both aspects.

Parent line and hybrid production, and bulking of starting material

In hybrid breeding, parent lines are produced by performing self-pollinations and selecting the best performing individual inbred lines to advance to the next generation (Lindhout et al. 2018; Bradshaw 2022). Test-hybrids are made to evaluate the potential of the parent lines and to determine which parent lines are a good combination. This general combining ability can be used for prediction models to improve future crosses (Adams et al. 2022). Further in the breeding process, the advanced hybrids will be tested in different locations to compare their individual yield stability and the genotype × environment (G×E) interaction of a population of hybrids. For these trials, larger amounts of starting material need to be produced. In one season, true hybrid seeds are produced in the greenhouse by crossing two parent lines. One way to produce seed of advanced hybrids, is to use seedling tubers of parent lines that were produced in the greenhouse as starting material (Kacheyo et al. 2023). Higher vigour of tuber-grown plants as compared with true-seed grown plants (de Vries et al. 2023) is a reason to follow this route.

For these purposes, it is most efficient to steer the development of parent line plants first towards producing many seedling tubers which can be used as starting material for seed production in the subsequent season. In this thesis, I have shown that this can be done by adjusting temperature and light regimes (**Chapter 2**). Decreasing the average temperature led to increased tuber weight in inbreeding lines. In **Chapter 3**, it has been demonstrated that a higher seedling tuber weight leads to faster plant development and higher tuber yield in the field. It can be surmised that also for greenhouse grown seedling tubers, a larger tuber weight gives more vigorous plants. The conditions during seed production as well as the architecture of the mother plant affect quality and quantity of the seeds (Pallais 1987; Almekinders & Struik 1996), so more vigorous mother plants might be advantageous for true-seed production as well. For the difference in temperature between day and night, I have demonstrated that a larger difference leads to the production of more tubers (**Chapter 2**). Depending on the region where the production is situated, and depending on how technologically advanced the greenhouse is, such a temperature regime can be realized relatively easy in winter, spring, and autumn when the nights are cool outside by heating the greenhouse less during the night. For both, average temperature, and the day/night difference in temperature, it would be interesting to test a wider range of temperature regimes to assess whether conditions can be optimized even more for high tuber production, as the results in **Chapter 2** suggest that I did not find the optimal values for average temperature and day/night difference in temperature yet.

Tuber production can also be steered by adjusting light conditions. Increasing the light intensity results in an increase in the number of tubers produced by parent lines (**Chapter 2**). In a greenhouse production context, this can be taken into account by planning the tuber production in a season with high natural light intensity, although this can prove to be contradictive with the need for reasonably low temperatures, especially during the night. Another possibility is to use artificial lighting in the greenhouse to increase tuber production. Besides by increasing light intensity, the number of tubers that a parent line produces, can also be improved by increasing the proportion of far-red light in the light spectrum. This result can be applied by installing lighting that contains a relatively high proportion of far-red light in the light spectrum, for example using LED light.

So, for future research, it would be interesting to determine the optimal values of average temperature and of the day/night temperature differential, as well as light intensity and percentage of far-red light in the light spectrum with regards to tuber development of parent lines, both in terms of number of tubers above a certain minimum (usable) size, average weight of usable tubers and total weight of usable tubers. Furthermore, the effect of other light conditions such as day length and different wavelengths other than far-red could be further investigated. Also, the effect of CO_2 concentration in the ambient air would be an interesting factor that is relatively easy to adjust in a greenhouse production and is likely to be able to affect the number of tubers per plant and their size or weight.

Moreover, the effect of environment on the hybrid true seed production could be studied. In many species, light and temperature are steering factors in the progress of a plant towards flowering and in the abundance of flower production, the success rate of flowers, the number of fruits per inflorescence and the number of seeds per fruit. It is plausible that also true seed production of potato can be optimized by improving the environmental conditions.

Early testing of traits

Growing plants in a greenhouse or in climate rooms can be an advantage because plants can be protected against some naturally occurring stress. Under controlled growing conditions, specific stress can also be applied intentionally to test resistances or tolerances to certain abiotic and biotic stress factors. In this way, it is possible to let genetic variation come to expression very early in the breeding process and in a predictable manner (in terms of timing, intensity, and duration) that otherwise could have been expressed much later in the breeding process or even in the period of commercialization of the cultivar. Moreover, in field trials the desired stress is not always present at the right time, in the right intensity or for the right duration. For example, in a greenhouse it is simple to apply drought treatments to test drought tolerance of plants. The stress can even be applied in different intensities, for different durations or at different developmental stages of the plant. In a field trial, it depends on the weather if, when, for how long and how intense the stress will be present.

Also heat stress can be applied when growing plants under controlled conditions. In this thesis, I performed heat stress trials with parent lines to test the effect of high temperature on tuber production of different parent lines. These trials were performed in climate rooms (**Chapter 2**). I found that high average temperature leads to fewer tubers, and the tubers that were produced had a lower tuber weight. I also found a significant interaction between the factors parent line and temperature for tuber production. This means that the different lines differed in their ability to tuberize at high temperatures. Breeders can use this information in the development of parent lines for hybrids that need to be grown in hot environments, for example in certain parts of India where heat stress during potato cultivation is common. In future, one would also need to test experimentally whether the hybrids of stress-tolerant parents are indeed tolerant to the same stress as well.

Besides tolerance to abiotic stress, it is also possible to perform specific tests for pest or disease resistance at an early stage of a breeding programme. Usually, different disease tests are developed for different pests or diseases, and they can be performed on whole plants or parts of plants, such as detached leaf assays for Phytophthora (Jansky 2000). However, the resistance in hybrids should be confirmed in field trials as well (Bradshaw 2021). This can be performed by inoculation under conditions that are conducive for infection and disease development but requires proper measures for the governance of the spread of the disease.

6.2.2 Variation in field trials

In this paragraph, I will discuss environmental variation that can be present when conducting performance trials of hybrid potato in the field.

Unwanted variation

In the process of inbreeding of the parent lines, test-crosses are made to evaluate the potential of these parents in test-hybrids. With these hybrids, field trials are conducted to evaluate the performance of the hybrids. Differences that are measured between hybrids should represent the genetic potential of the hybrids and of the parent lines. Therefore, other variation or interactions with other sources of variation should be avoided as much as possible.

Sources of variation that complicate the selections are, amongst others, spatial and environmental conditions in the field. Field trials in this stage of the breeding programme are usually relatively large, and the scale of field trials often involves creates variation in conditions in the soil in which the trial is laid out. This variation can be the result of landscape factors as well as physical (e.g., soil physical structure), chemical (organic matter content and nutrient content) and biological (beneficial organisms and harmful organisms and their interactions) soil factors.

For example, aphids or Colorado potato beetles can enter the field on one side from ecological niches in that part of the environment and start feeding on the plants. Even if they eventually spread across the entire field, the plants on the side from which they entered would be damaged first and suffered longer than plants in other parts of the field, resulting in variation in damage and therefore variation on yield within the field.

As stated above, another factor that often leads to unwanted variation within a trial is variation in the soil. Physical, chemical, and biological soil fertility are not evenly spread through the field, and there can be gradients or patches of different levels of these three types of soil fertility (Haefele and Wopereis 2005; Stantra et al. 2008; Allaire et al. 2014; Lupatini et al. 2017). The variation in soil compaction and composition affects the availability of nutrients, and also the water holding capacity can differ. Moreover, plant management is less precise compared to management in a greenhouse or climate room. For example, in a greenhouse it is possible to water all plants in very similar amounts. In a field this is possible as well using drip irrigation, however, the type of irrigation that is used often is chosen based on practical reasons. When a pivot is used, distribution of the water often is less precise due to the scale and external influences such as wind. Besides the spatial variation in a field due to the large scale, also the micro-climate between plants can lead to differences in growth, for example, fast growing plant can successfully compete for light and other resources against neighbouring plants which reduces the growth of these neighbours (Connolly et al. 1993; Bradshaw 1994). This can be within plots when test-hybrids are still segregating, or between plots, especially when the canopy architecture of the different plots varies. Especially in performance trials of a breeding programme usually many different hybrids are tested that differ from each other in dynamics of growth and development. These border effects can be present at plot level when different hybrids affect each other, but also at trial level, for example when plots at one side of a trial are more exposed to wind and light than plants on another side, while in the middle of a trial conditions might also differ, for example the temperature can be higher as well as the relative humidity and the leaf wetness.

In a trial, variation in a field can lead to differences in growth of plants between and within plots. To measure the differences between hybrids that are the result of their different genetic background and not due to variation in the field, the trial design should be adjusted to the field and to the requirements of the breeder. To a certain extent, field variation can be compensated for by choosing a proper trial design. Border rows around the trial can be used to decrease the effect of factors such as wind on the sides of the trial (Kempton 1997). By using border plants of one particular cultivar around each plot the different hybrids have less effect on each other, and they all are influenced by the presence of the same cultivar. Moreover, the precision of a trial can be increased by increasing the plot size. After performing the trial, it is possible to decrease the effect of spatial variation in the data by using models for spatial analysis such as the Spatial Analysis of Field Trials with the Splines package (SpATS) (Rodriguez-Alvarez et al. 2018). In this model, field variation is analyzed and the effects of the spatial variation are described in mixed models, with the goal to distinguish variation in results due to spatial trends in the field from those that are caused by genetic differences. Adams et al. (2022) successfully applied this method in hybrid potato trials for different traits, such as tuber yield, number of tubers and tuber volume.

Before starting a performance trial, the breeder needs to decide which traits are important, and what level of precision is required. The response of different traits to variation in environment may differ. For example, tuber shape is much more stable than tuber yield (**Chapter 5**), and therefore the plot size can be smaller when evaluating only tuber shape to reach the same level of precision than when evaluating tuber yield (**Chapter 4**). In this thesis, I developed equations to calculate the least significant difference in percentage of the mean (coded as LSD%) when using different plot shapes and sizes. This was done for total tuber weight per plant, number of tubers per plant, tuber shape, tuber volume, and as a measure of within plant variation the standard deviation of tuber shape and volume. So, when a breeder decided on the precision that is needed for the traits of interest, the equations can be used to determine the matching plot size. Plot shape was also included in the equations; however, a relatively homogeneous field was used for these trials. Therefore, only a minor effect of plot shape on LSD% was found. In future trials, it would be interesting to perform similar trials as I did and reported in **Chapter 4** for a field with much greater heterogeneity to improve the equations of plot size and shape for such fields.

Although LSD% steeply decreased with increasing plot size, variation remained high for the yield traits that were included in the analysis of **Chapter 4**. Even with a plot size of 90 plants per plot the LSD% for total tuber weight per plant was still 10%. That means that the yield of two hybrids needs to differ at least 10% to be significantly different from each other. In practice, often small plot sizes of 8 or 16 plants are used in breeding performance trials during the early stages of a breeding programme. This would correspond to LSD% values of 33 and 24%. So, when selecting the ten highest yielding hybrids in a performance trial it does not necessarily mean that really the ten best hybrids are selected, when yield difference is below the LSD% of the used plot size. This information is important to be aware of for breeders when making selections based on the results of a performance trial.

To improve the precision of performance trials, there is an additional source of variation that could be decreased. In hybrid potato breeding the starting material of a trial can be hybrid true potato seed, seedling tubers, or seed tubers produced from seedling tubers. When comparing the performance of hybrids with existing tetraploid cultivars, the starting material should be similar to compare the performance based on genetic properties alone and not starting material differences. In this thesis, seedling tubers were therefore used as starting material for performance trials. Further research is needed to investigate the differences between seedling tubers and seed tubers, and which type of starting material is most suitable to compare with tetraploid cultivars.

In tetraploid cultivars as well as in diploid hybrids the quality of seed(ling) tubers has a major effect on the outcome of a trial (**Chapter 3**). The number of eyes in seedling tubers affects the number of stems per plant, where more eyes per tuber give more stems per plant. Number of tubers per plant and tuber yield per plant, however, were not affected: more stems were compensated by fewer tubers per stem. The seedling tubers with high and low number of eyes were of similar tuber weight. A possible explanation is that without extra seed tuber weight as energy for initial growth, the plant cannot exploit the extra stems to produce more tubers per plant. This interaction between number of eyes and seedling tuber weight needs further investigation, to eventually create a that describes on how to optimize plant management in the field based on seedling tuber weight per eye.

Another source of unwanted variation in a trial could be the result of seedling tuber weight. We found that a higher seedling tuber weight gives more tubers per plant and a higher total tuber weight per plant. We also found that in a seedling tuber batch in the size class 35 − 45 mm tuber weight varied up to factor 4.6, potentially leading to variation in yield up to 16.1 Mg/ha. This was 47% of the average yield of that cultivar, so the use of seedling tubers of similar weight could increase the precision of a trial significantly. In the plot size trials of **Chapter 4** seedling tubers were not selected based on weight, but on size class. To determine how precise a trial can be made, it would be interesting to repeat the trials described in that **Chapter 4** with seedling tubers of equal weight. Moreover, the effect of dry matter percentage was not investigated yet. When determining the sources of variation and how to deal with them, this variable should be included as well as a higher dry matter percentage in a seed(ling) tuber potentially gives the plant more energy for initial growth.

Application of variation in field trials

Similar as in parent line development, environmental variation can be used as a tool in field trials to speed up the breeding process. Besides selecting hybrids for yield and tuber traits, stress tolerance and yield stability across environments are important properties that need to be tested. Genotypes can respond differently to different environments; this is called genotype × environment (G × E) interaction (Bradshaw 2021). A hybrid can be very stable, so without much variation in performance across different environments. High stability of yield can be advantageous, as it makes the hybrid more reliable. Hybrids can also be very responsive to the environment, performing very well in one type of environment while doing poor in another. These hybrids can be used to grow in specific regions that are very conducive to a high performance of the hybrid. In **Chapter 5**, I described experiments in which it was observed that the highest yields among hybrids as well as for tetraploid cultivars were found on sandy soil, in Hilvarenbeek (2% clay) followed by Giethoorn (1% clay), while the lowest yields were found in Berthem on light clay soil (13% clay). Probably, several local environmental or field management factors and characteristics affected yield, as other locations contained more clay while yielding more. Moreover, average yields in Belgium were higher than in the Netherlands and in France in that year (**Chapter 5**).

Different traits of a hybrids can show G × E interaction to a different extent. For example, tuber shape is a more stable trait than tuber weight (**Chapter 5**).

In tetraploid breeding, the number of locations that can be tested early in the breeding process is limited because of the low availability of starting material, as multiplication rates with clonal reproduction are low (Bonierbale et al. 2020). In hybrid breeding, it is possible to produce thousands of seedling tubers in one field season very early in the breeding programme. This gives the opportunity to already test the hybrids in multiple locations in their first field season based on experiments conducted with seedling tubers, or even in their first field season based on experiments conducted with hybrid true potato seed. Of course, it takes time and considerable financial and skilled human resources to produce enough starting material and to manage field trials. Also, producing seedling tubers from hybrid true potato seed costs an extra year (or at least an extra growing season) and resources to produce. Therefore, the question rises what the optimal number of locations is to test a hybrid in order to obtain enough information on G × E interaction and stability of performance. Moreover, it would be interesting to determine whether G × E of a crop grown from hybrid true potato seed is comparable with a seed(ling) tuber grown crop.

Furthermore, hybrids can be tested in the field for specific stress tolerance such as tolerance to drought or heat, as well as for resistance against or tolerance to pests and diseases. As a field environment always is different from a greenhouse environment, it is important to test these traits in a field environment. In these trials, it is important to have a uniform field, and to control the stress factor (Blum 2011). For example, for a trial on drought tolerance, one could choose for a location or season with high chance on drought. Another possibility would be the use of rainout shelters in field or semi-field conditions (Blum 2011; Svane et al. 2019).

Another type of stress that can be tested under field conditions as well is biotic stress, i.e., from infection or infestation by diseases or pests. Also after screening lines for resistance or tolerance (or even recovery after the stress) in controlled conditions during the inbreeding process, it is important to confirm the resistance, tolerance, or ability to recover under field conditions (Bradshaw 2021). An example in hybrid potato is the resistance against late blight, caused by the oomycete *Phytophthora infestans*. Su et al. (2020) introgressed resistance genes into diploid parent lines via marker assisted backcrossing. Crosses between these lines were made to produce hybrids with stacked resistance genes, and in a field trial that was inoculated with *Phytophthora infestans* they demonstrated the resistance (Lindhout & Struik, 2023).

Future research in field trials

There are several topics that can be investigated further to improve the quality or efficiency of the trials. First, it would be helpful to extend the equations of optimal plot size and shape for usability in heterogeneous fields. It is expected that in these fields the shape of the plots and of the trial becomes more relevant than in our trials of **Chapter 4**. For trials in which G × E is measured, an addition to these equations would be the optimal number of locations to test G × E reliably. Furthermore, it would be interesting to make the plot size equations using seedling tubers of similar weight as starting material, to determine how precise a trial can be made. Another unknown potential source of variation is the dry matter percentage (DM%) of seedling tubers. Seedling tubers of similar weight could vary in DM%, and with that have a different amount of energy available for initial growth during the onset of the growing season. Also, the effect of starting material remains an open question: would the same hybrids be selected from a field cropped with potato from hybrid true potato seed as from a field cropped with potato from seedling tubers? Is G × E interaction similar in crops grown from hybrid true potato seed and crops grown from seedling tubers?

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Summary

For potato, diploid hybrid breeding is a new way of creating new genotypes that enables faster development of superior cultivars. Some elements in diploid hybrid breeding are significantly different from the conventional way of potato breeding. These novel elements include the development of inbreeding lines, the production of true hybrid seeds, and growing a crop starting from seedlings or seedling tubers. As these processes are new for potato, many questions are raised on how to execute these steps in the best possible way, for the breeding as well as for commercial crop production of seed and ware. An important question in such a new breeding programme is how the programme and the selection process can be done in an efficient way. One of the factors that decrease the efficiency tremendously is variation, at many different levels. On the other hand, the presence of variation is the basis of a breeding programme, as breeders use the natural genetic variation that is present in crossable germplasm to develop superior cultivars.

In this thesis, I focused on non-genetic variation that can be present in a breeding programme. Herein, I made a distinction between unwanted and wanted variation. Variation is considered unwanted when it disturbs the selection process, for example by affecting results, making the selections less precise, or requiring more and larger experiments for selection and testing. In this thesis, I investigated the impact of important sources of non-genetic variation on the results of breeding trials, and present tools for breeders to deal with this variation. Wanted variation is variation that is applied by breeders to speed up the selection process, for example, during the performance of stress tests in hybrids. For this type of variation, I describe opportunities for using environmental variation to speed up the breeding process.

Overall, the goal of this thesis was to provide non-genetic tools to make selections in the breeding programme more precise, efficient, and successful.

In Chapter 2, I focused on applying variation under controlled conditions on parent lines. The goal was to determine the effects of light and temperature on tuber production. For light, I investigated the intensity and the percentage of far-red light in the light spectrum. For temperature, the average temperature and the differential between day and night was investigated. Tuber production was determined as the total tuber weight per plant, which is a product of the average weight per tuber and the number of tubers per plant. Increasing light

intensity and increasing the percentage of far-red light in the light spectrum led to an increase in number of tubers. Increasing the average temperature led to a decrease in number of tubers as well as tuber weight, and a larger day/night differential in temperature resulted in a higher average weight per tuber. In these trials, parent lines were discovered that are tolerant for heat stress. Moreover, the results can be used to enhance tuber production in a seedling tuber production phase.

Chapter 3 deals with the quality of diploid hybrid seedling tubers. Diploid hybrid seedling tubers are a new type of starting material that arise from this new way of breeding. Therefore, the knowledge on how to measure the quality of these seedling tubers, and the effect of quality of the seedling tubers on subsequent plant performance, is limited.
To determine the effect of variation in tuber quality traits on plant performance and yield, two field trials were conducted. The variation in number of eyes and in tuber weight of four different hybrids in a batch of seedling tubers was measured, and in the field the effects of production origin, number of eyes and seedling tuber weight was assessed. No effects of tuber origin (three different locations within The Netherlands) were found. A higher number of eyes, without difference in seedling tuber weight, led to an increase in the number of stems without affecting the ground cover or yield. A higher seedling tuber weight led to an increase in ground cover and in yield. In combination with the variation in tuber weight that is present in a batch of seedling tubers, this can result in significant yield variation in breeding trials. So, for reliable breeding trials, it is important to use seedling tubers of similar weight.

In Chapter 4, a tool was developed to determine the required plot size when conducting performance trials with diploid hybrid potato. Based on two field trials, equations were developed to calculate the least significant difference, expressed as a percentage of the trial mean (the so-called LSD%) for different plot sized and shapes. This was done for the following traits: total tuber weight, number of tubers, tuber shape, standard deviation of tuber shape, tuber volume, and standard deviation of tuber volume. These are relevant traits for breeders, and in relatively homogeneous fields the equations can be used to calculate the plot size that is needed for the required precision per trait.

In Chapter 5, environmental variation was applied in multilocation field trials to measure genotype × environment (G × E) and yield stability in test hybrids. In these trials, hybrids were benchmarked against commercial cultivars. Diploid test-hybrids only slightly underperformed against the tetraploid cultivars; the lowest yielding tetraploids were comparable with the highest yielding hybrids. Stability of total yield, shape and dry matter percentage was remarkably similar for hybrids and commercial cultivars. Moreover, a yield- component analysis was performed to determine which trait contributes most to total yield. For both, hybrids and commercial cultivars, number of tubers per stem had the highest effect on total yield, followed by number of stems per m^2. Tuber size had the smallest effect on total yield.

In Chapter 6, the main findings of the thesis were summarized, discussed and put in a wider perspective. Possible sources of unwanted variation that can be encountered during the breeding process were described, and results of this thesis were presented as tools to deal with the variation. Moreover, possibilities were shown to apply variation during the inbreeding process as well as when testing hybrids.

Overall, this thesis provided non-genetic tools for breeders to improve their breeding programme by making the selections more precise, efficient, and successful.

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About the Author

Julia was born in Aachen, Germany, on 14th July 1990. Her parents moved to Landgraaf, Netherlands, when Julia was two years old. In Landgraaf, she finished primary school at OBS de Harlekijn and secondary school at Eijkhagen College. In 2009, she moved to Nijmegen to study biology at Radboud Universiteit. In 2015, she finished the Master Biology in Nijmegen, after which she immediately started working as a researcher at Solynta in Wageningen. Here, Julia worked on various topics related to the agronomy of hybrid potato and on how to produce hybrid true potato seeds (HTPS) in inbred lines. After several years of research in potato, the unique possibility was given to start a PhD trajectory, while continuing with the research for Solynta. The results of this PhD project are described in this thesis. She currently works at Solynta as a program scientist.

PE&RC Training and Education Statement

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

Review/project proposal (6 ECTS)

- Effect of environmental conditions on tuberization in diploid hybrid potato

Post-graduate courses (4.8 ECTS)

- Fundamentals of crop physiology; WUR (2021)
- Environmental signalling in plants; Utrecht University & EPS (2021)
- Linear models; PE&RC & WIMEK (2017)
- Statistical analysis for new phenotyping techniques; WUR, Biometris (2018)

Laboratory training and working visits (5.6 ECTS)

- Growth of hybrid potato in the field under drought stress; CREA Bologna (2019)
- Root development of hybrid potato under combined nutrient and drought stress; Copenhagen University (2020)
- High throughput phenotyping of hybrid potato in aeroponics; UCLouvain (2021)

Competence strengthening/skills courses (6.3 ECTS)

- Talent development training; Solynta (2018-2019)
- Solynta development afternoons; Solynta (2018-2019)
- Resultaatgericht zelf leiderschap; Evolution logique (2022)

Scientific integrity/ethics in science activities (0.3 ECTS)

- Ethics in plant and environmental sciences; WUR (2023)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

- PE&RC Last year weekend (2021)
- PE&RC Day (2021, 2022)

Discussion groups/local seminars or scientific meetings (12 ECTS)

- Flowerpot meetings (2015-2019)
- Research council Solynta (2019-2021)
- Literature discussion (2019-2021)
- Member of user committee HIP project getting to the roots of stress resilience (2019-2020)

International symposia, workshops and conferences (7.6 ECTS)

- EAPR; oral presentation; Versailles, France (2016)
- Wintertagung; oral presentation; Göttingen, Germany (2017)
- Solace; poster presentation; Dundee, Scotland (2020)
- Solace; oral presentation; online (2020)

Societally relevant exposure (1.3 ECTS)

- Presentation at Radboud university
- Bedrijvendag Semper Florens
- Represented Solynta at several potato days, aardappeldemodagen, potato Europe, tuinbouwdagen, Seed meets technology

Lecturing/supervision of practicals/tutorials (5.1 ECTS)

- Begeleiden van groepsprojecten op de HAS (2015-2020)
- Guest lecture HAS hybrid potato breeding (2017)

BSc/MSc thesis supervision (11.67 ECTS)

- Effect of pollination frequency on true seed production of inbred lines
- Effect of inflorescence location on seed vigour
- Effect of plant architecture and pot size on pollen production on diploid inbred potato

- Effect of starting material on plant development and yield
- Seedling root development from hybrid true potato seed in two cultivation systems
- Effect of soil temperature on flower and tuber development in diploid potato
- Effect of environmental factors on fertility of potato plants
- Optimizing growth and development of potato seedlings with nutrients and soil temperature in the greenhouse
- Effect of GA and IAA on berry production in diploid potato
- Drought tolerance in hybrid potato
- Effect of GA and CCC on berry production in diploid potato
- Tuberization period in the field of diploid hybrid potato grown from seedlings and tubers

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