Physiology and genetics of root growth, resource capture and resource use efficiency in lettuce (*Lactuca sativa* L.)

Pauline J. Kerbiriou

Thesis committee

Promotors

Prof. Dr E.T. Lammerts van Bueren
Professor of Organic Plant Breeding
Wageningen University

Prof. Dr P.C. Struik
Professor of Crop Physiology
Wageningen University

Co-promotor

Dr T.J. Stomph
Assistant professor, Centre for Crop Systems Analysis
Wageningen University

Other members

Prof. Dr M.E. Schranz, Wageningen University
Prof. Dr L.F.M. Marcelis, Wageningen University
Prof. Dr E. Hoffland, Wageningen University
Prof. Dr K. Thorup-Kristensen, University of Copenhagen, Denmark

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Pauline J. Kerbiriou

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Pauline J. Kerbiriou

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Preface

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Abstract

Modern cultivars of lettuce (*Lactuca sativa* L.) are not well adapted to organic and low-input cropping systems. Current agricultural practices in conventional systems use high amounts of water and nutrients to provide the market with a continuous and reliable flow of high quality fresh product. The unavailability of below-ground resources, even temporary, during growth, can impair the marketable yield of a short-cycle leafy crop such as lettuce. For the supply of organic markets this proves to be a major stumbling stone. There is thus a need to provide organic growers with new cultivars of improved robustness, defined as the ability to display stable yields over a range of environments even when the availability of inputs is irregular and on average low. We hypothesized that improved below-ground traits, such as an improved root system architecture, and improved ability to capture water and nutrients could confer robustness in lettuce. Below-ground traits are recently receiving more attention in research but are to a large extent still an unexplored area for practical breeders as these traits are complex, and many (unknown) component traits contribute to resource capture and resource use efficiency.

In order to identify meaningful traits to select for when breeding for robustness, it is essential to understand the internal physiological mechanisms regulating resource capture in the plant, especially in a resource-limiting context. Not much was known about such mechanisms, and the genetic variation in these mechanisms or traits regulating these mechanisms was not yet assessed or analysed. The objective of this study was therefore to develop a breeding strategy to improve robustness in lettuce, based on improved below-ground traits.

In this view we developed four component studies allowing us to explore different aspects of the strategy. Firstly, the physiological mechanisms regulating resource capture below-ground and the resource use efficiency above-ground in a resource-limiting context were studied in a controlled environment. Secondly, the contribution of below-ground traits to field performance of lettuce, and the genetic variation therein were assessed in in-depth field studies with a limited set of contrasting cultivars. The third component included a study on the genetic variation in resource capture below-ground, and the impact of the environment on such traits was quantified

using a large population of 148 lettuce cultivars in four different organic field conditions. The results of these studies were combined to develop a conceptual model which could help the breeding process when breeding for robustness. Finally, in the fourth study the genetic control of below-ground traits was tested with this large set of cultivars with the objective to detect quantitative trait loci (QTL) for the below-ground traits and associated above-ground traits.

The main findings of these four component studies highlight that the relationship between root traits and temporal and spatial patterns of resource acquisition were influenced by internal factors such as the physiological status of the plants, as well as external factors such as the type of most limiting resource being foraged. In controlled conditions, additional root length does not lead to additional resource being taken up, except when the resource is limited. Indeed, it appeared that when localized drought was applied, additional root growth occurred in the dry zone (while no additional root growth was measured in the rest of the soil profile); when localized nutrient limitation was applied, additional root growth was observed in the zone were more nutrients were available for uptake. Under field conditions the relationship between root traits and resource capture is much less clear due to numerous environmental influences and variations; genetic variation was observed in the way different lettuce genotypes capture and use nitrogen, but variation differed for each individual field trial as these trials were exposed to contrasting weather conditions. However, overall, the field trials with a population of 148 lettuce cultivars showed that the impact of the environment on resource capture was larger than that of the genotypic variation present in the population, highlighting the need for a model able to cope with the large environmental influence and the large genotype-by-environment interactions. Only after such modelling will it be possible to assist breeders in selecting the best traits to breed for in a certain environment, as well as in identifying the best selection environment to allow the best expression of the traits they are interested in. The association mapping analysis carried out using 1170 SNP markers showed that resource capture below-ground is controlled by numerous QTL located on different genomic regions, of which the combination and effect vary largely with the environment.

This thesis highlights that the complexity of the relationships between root traits and resource capture could be analysed by improved phenotyping through technological innovations. Furthermore, it questions if investigating the plasticity in the below-ground traits – and not the traits themselves – would also not be of interest when breeding for robustness. Finally, this thesis shows that close collaborations between physiologists and breeders is needed to tackle the challenge of breeding for complex traits such as resource capture and resource use efficiency.

Keywords

Lettuce; Low input and organic farming systems; Breeding; Modelling; Root system architecture; Resource capture; Resource use efficiency.

Chapter 1

General introduction

Abstract

This chapter provides an overview of lettuce (*Lactuca sativa* L.) production with particular emphasis on below-ground factors influencing lettuce yield in sustainable cropping systems, such as low-input and organic systems. While exploring and breeding for below-ground traits are new areas for breeders, options to realize high yields of lettuce under low input or organic horticultural systems are addressed and knowledge gaps are highlighted. The aim of the thesis, the methodological framework and the overall structure of this dissertation are outlined in this chapter.

1.1 Background of the project

1.1.1 Economic importance of lettuce production

With about 25 million tonnes of heads produced in 2012, lettuce (*Lactuca sativa* L.) is one of the most widely grown vegetables in the world, after tomatoes (162 million tonnes), watermelons (105 million tonnes), onions (82 million tonnes), cabbages and other brassicas (70 million tonnes), cucumbers and gherkins (65 million tonnes), eggplants (48 million tonnes), carrots and turnips (37 million tonnes), chilies and peppers (31 million tonnes) and melons (32 million tonnes) (FAOSTAT). China dominates the world production with approximately 14 million tonnes produced in 2012, followed by the United States (3.9 million tonnes), India (1.1 million tonnes) and Spain (0.9 million tonnes), which is the main European supplier of lettuce (FAOSTAT). Other large European lettuce producers include Germany (0.42 million tonnes), France (0.33 million tonnes), Italy (0.32 million tonnes), Greece (0.13 million tonnes) and United Kingdom (0.12 million tonnes).

Lettuce production consists of five main head types: crisphead (iceberg, Batavia), butterhead, romaine (cos), leaf (cuttings), and Latin (Mou, 2007). In 2006, the North American production was partitioned between crisphead type (62%), romaine (23%) and leaf and butterhead types (15%) and characterized by large open fields being cropped uniformly (using direct sowing) (Mou, 2007). On the other hand, in Europe the market is more scattered and fragmented, with different types of lettuce being produced in each country, smaller production areas and higher level of technology (use of transplants, glasshouse production, hydroponics, etc.). In 2006, the iceberg type of lettuce dominated the export market in Spain and France, but other types of lettuce were preferred for domestic consumption, such as romaine and Latin lettuces in Spain and butterhead and Batavia types in France (Mou, 2007). The dynamics of the market, however, can change rapidly. In Great Britain for instance, 75% of the production was of crisphead type in 2006, 10% was of romaine type and 1% was of butterhead type – which is a great change compared to the 1970s and earlier, when the production was dominated by butterhead types (Mou, 2007). In Germany, approximately 70% of the lettuce production was of butterhead type in 2006, and the remaining 30% were of

crisphead type (Mou, 2007). In 2006, lettuce production in the Netherlands and Belgium was of butterhead type for glasshouse production, and of butterhead, crisphead and leaf lettuces grown outdoors for summer production (Mou, 2007). The total lettuce seed market in Europe has a yearly value of over €60 million and the Dutch seed companies are in the lead in this market.

1.1.2 Lettuce production systems: intensive and high inputs

Lettuce is a short cycle crop consisting of green leaves forming a more (e.g. Iceberg) or less (e.g. butterhead or other leaf types) compact head. To ensure good yields (based on head weight and the percentage of heads per surface harvested) and good head quality (maturity, leaf colour, texture and shape, diameter) (Mou, 2007), water and nitrogen – the primary resources ensuring adequate vegetative growth – are intensely managed in lettuce cultivation (Bumgarner et al., 2010). The significance of water and nitrogen impact on plant growth has led to an important increase in the use of N fertilizers in lettuce cultivation since the 1980s (Gallardo et al., 1996a,b; Broadley et al., 2000; Frantz, 2004; Bottoms et al., 2012). Nowadays in the Salinas Valley (California, USA), where more than 50% of the US lettuce production is taking place, production systems include two or three crops a year with frequent irrigation and heavy mineral N fertilization with rates or nitrogen application ranging from 100 to 220 $kg.ha^{-1}$ (Bottoms et al., 2012). These systems use standard fertilisation programmes with scarce adaption to specific field conditions (Bottoms et al., 2012).

In lettuce, fertilizer costs are low compared to the return value. Besides, extra yields in lettuce have high economic value which does not encourage lettuce growers to risk lower yields caused by nutrient shortage (Saleh et al., 2010). Moreover, because on the one hand little is known about the interactions between the effect of field conditions (precipitation, irrigation frequency, soil N mineralization potential) and crop yield potential (plant population, genotype-specific nitrogen use efficiency), and because on the other hand lettuce as a fresh product is a high-value crop subjected to strict market standards for product quality (e.g. fresh appearance, colour, head size, shelf life), growers are also not keen on changing or modifying their current cultivation practices (Bottoms et al., 2012).

1.1.3 Organic lettuce production

However, the excessive use of fertilizer and water in intensive vegetable production systems such as lettuce in the Salinas valley (USA) has raised concerns about potential adverse environmental effects on water quality, leaching and runoff (Hoquet et al., 2010). In California, the nitrate concentration in surface water and groundwater often exceeds the standard threshold for drinking water (10 $mg.L^{-1}$) (Bottoms et al., 2012). The alarming health effects of excessive nitrate absorption by contaminated drinking water may include among others, methemoglobinemia, cancer, adverse reproductive outcomes, and thyroid hypertrophy (Ward et al., 2005). Growers are therefore under increasing pressure to improve their cultivation practices to align the amounts of nitrogen brought to the crop through applied fertilizer with the amount of nitrogen taken up by the plants (Bottoms et al., 2012).

In parallel, because consumers are increasingly aware of the adverse health and environmental effects of intensive cropping systems, they are more and more demanding for crops grown in a more sustainable manner, e.g. under low input and/or organic farming systems. According to Saleh (2010) consumers of vegetables are health-conscious and favour high quality produces (improved mineral and vitamin contents) as well as vegetables free of chemical residues. In this perspective the demand for organically grown food rose tremendously over the last decade with a worldwide market size for organic food increasing from about 15 billion US dollars in 1999 to 59 billion US dollars in 2012. The highest penetration of market shares was recorded for fresh fruits and vegetables in the US (Zhang et al., 2011). In Europe vegetable production still was a small part of the total organic production with only about 1.2% of the total area dedicated to organic production being devoted exclusively to the production of organic vegetables in 2013 (EU, 2013). The Netherlands is, after Malta, the European country where the largest portion of the organic area is dedicated to vegetables (EU, 2013).

Among the other fresh produces included in the study by Zhang et al. (2011) (potato, tomato and onion) lettuce appeared as the fresh produce for which the share of organic sales was the most important with about 4% market share for the period 1999-2003. As processed lettuce (washed, cut, and mixed) is becoming more and more popular

worldwide, the demand for organically-grown lettuce will surely increase in the near future, following the general trend of increasing market shares for sustainably produced, high-quality produce. Butterhead lettuce will surely benefit from this trend with its soft texture and good taste.

Nevertheless, in the Netherlands, although lettuce is an important crop, it is mainly sold in specialized health food stores, but only to a limited extent in supermarkets as the organic sector is not yet able to provide supermarkets with a year round constant and sufficient supply of lettuce of adequate quality. This is due to the fact that organic farming aims at optimizing the production system more than the individual crop and thus uses organic manure instead of inorganic fertilizer. Moreover, irrigation is often less frequent in sustainable production systems. Nutrient supply and water supply are therefore less regular, less abundant, and more depending on (variable) environmental conditions, including the physical, chemical, and biological conditions in the soil. For example, mineralization of organic matter and uptake of nutrients depend on availability of adequate amounts of soil moisture, thus increasing variation in growth, both within and among seasons. As organic agriculture has fewer means to adjust the environment to the genotype, it needs cultivars that are better adapted to variable low-input (organic) growing conditions (Lammerts van Bueren et al., 2002; Wolfe et al., 2008).

1.2 Problem statement

To optimise sustainable cropping systems to increase yield security and stability both improved agronomic measures and cultivars better adapted to low-input systems are required. With the objective to gain a better understanding of the factors limiting lettuce yield under low-input and organic cultivation, recently several studies investigated lettuce production under low-input and organic fertilization. For instance, Mogren et al. (2010) assessed the possibility to reduce total nitrogen fertilization by the application of a starter fertilizer strategy. Montemurro (2010) studied the effect of diverse organic bio-products based fertilizers on lettuce field performance. Ribeiro et al. (2010) investigated the dynamics of the mineralization of diverse organic fertilization

programmes on lettuce nitrogen uptake. Promising outcomes of these studies included no significant difference between using organic vs. mineral fertilizer on lettuce yield (Montemurro 2010). Moreover, equivalent yields were obtained when using a starter fertilizer strategy (Mogren et al., 2010) as when using a "conventional approach" – highlighting the promises of low-input lettuce production. Eventually, a positive effect on lettuce yield was measured when using a mixture of compost and hen manure on baby leaf production instead of mineral fertilizer, underlining the possibility to reduce fertilization costs while maintaining yields.

Yet the economic reality of organic lettuce production in open field conditions shows that yields are not stable, and often lower than in conventional systems. For instance Polat et al. (2008) found that the yield of lettuce grown under organic management was 20% lower than under conventional management while all treatments received equivalent amounts of N fertilization. In a study by Moccia et al. (2006) the yield of lettuce grown in a conventional system (where the plants received about 80 $kg.ha^{-1}$ N), was significantly higher than the yield of lettuce grown in an organically managed system (where the plants received about a maximum of 40 $kg.ha^{-1}$ N).

In organic production systems, nutrient availability depends on the soil processes affecting the mineralization of the organic fertilizer. For instance, the decomposition of compost manure, releasing nitrogen, depends on the soil chemical, physical, hydrological and biological properties – properties, which, in return, are improved by the release of organic matter contained in the compost manure (Montemurro, 2010). Therefore the availability of nutrients in organic farming systems is more variable than in conventional systems, and organically-grown lettuce may be more affected by temporary abiotic stresses. As lettuce is a short cycle crop, such fluctuations may at the end affect marketable yields as modern lettuce cultivars have been shown not to be able to fully recover from temporary stress during growth (Gallardo et al., 1996a). Moreover, variability in nutrient availability during growth may also affect product quality as nitrogen supply impacts the shoot greenness as shown by Ozgen (2014). Lack of nitrogen may result in dull green colour and drought may impact leaf turgor.

Compared to agronomic studies, less research has been carried out to improve cultivars for production under low-input cropping systems. As in most organic farming systems,

growers have been using seeds originating from conventional seed companies (i.e. not organically certified) or organically-produced seeds of cultivars bred and selected under conventional, high input environments (Lammerts van Bueren et al., 2002; Wolfe et al., 2008). As shown by Reid et al. (2009) in cereals, breeding under conventional conditions while targeting adaptation to organic conditions may not result in the advancement of the best possible lines. This may be explained by the fact that traits that confer field performance under low-input, organic conditions may not be the same traits that confer good field performance in a conventional system. Because organically grown lettuce may be more exposed to temporary abiotic stresses, organic growers are in need of more robust lettuce cultivars, possessing traits that ensure a good and stable field performance despite variable and low-input growing conditions.

1.3 Problem analysis

1.3.1 Breeding lettuce for improved root system architecture

Most of the lettuce cultivars currently used by growers have been bred under high levels of input in conventional systems (Jackson and Striver, 1993) where water is abundant and nitrogen is provided in mineral form, and therefore always readily available for uptake by the plant. Conventionally bred lettuce cultivars consequently have limited demands on the root system: their root systems are often rather shallow and mainly concentrated in the top layers (0.0-0.20 m) of the soil profile (Jackson, 1995), see Figure 1.1. As roots are the main organ controlling resource capture below-ground and the major determinants for a balanced nutrition (Giehl et al., 2014), such a root system architectural feature can impair resource capture for lettuce grown in systems where soil resources (water, nitrogen) can be less uniformly available over space (heterogeneous distribution of nitrate over the soil profile) and time (variation in rate of mineralization and in nitrate availability). Such a scenario happens frequently in organically-managed systems. In lettuce, even temporary resource shortage can affect yield as it was shown that the growth rate of lettuce is reduced under temporary nutrient deficiency and is not able to fully recover when availability of nitrogen increases again (Burns et al. 1991).

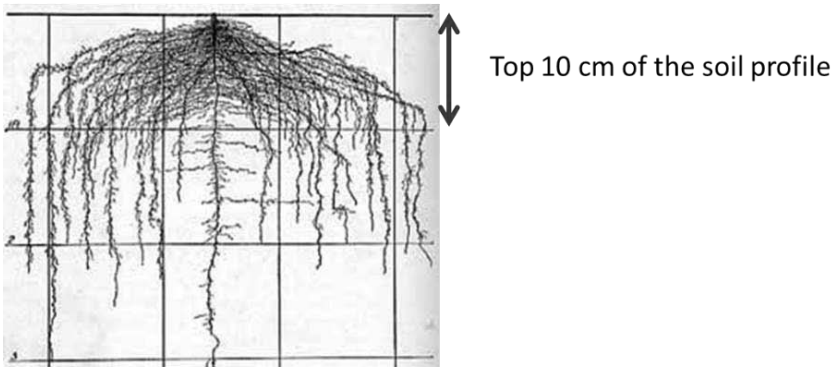

Figure 1.1 Root system of cultivated lettuce localized in the upper soil layers of the soil profile (0.0-0.2 m) (From: Weaver and Bruner, 1927)

Johnson et al. (2000) argue that improved root systems can more efficiently capture water and nutrients from the soil and found that the taproot of wild lettuce (*Lactuca serriola* L.) is longer and has more lateral roots than those of cultivated lettuce (*Lactuca sativa* L.). Johnson et al. (2000) detected 13 quantitative trait loci (QTL) for root architectural traits and water capture in the interspecific cross *L. sativa* × *L. serriola* that each accounted for 28–83% of the phenotypic variation. They concluded that *L. serriola* is a potential source of important root traits for optimal resource capture in lettuce production systems applying direct sowing as is usual in the USA. However, in Europe lettuce production systems are based on transplants. Transplanting causes loss of taproots making the lettuce crop even more dependent on capturing nutrients and water from upper soil layers and thus even more sensitive to drought and nutrient stress. Also among cultivated lettuce varieties genetic variation has been found. Within a group of cultivated lettuces, Moccia et al. (2006) found that different lettuce types (romaine vs. butterhead) showed different behaviours under organic management (more disease susceptible and more prone to nutrient deficiency for the romaine and butterhead types, respectively); moreover, their study showed that overall, the plants grown under organic conditions had a higher root mass than the plants grown under conventional conditions. Besides, variation in overall root mass (Ryder and Waycott, 1993) and in onset of lateral root formation (Van der Post and Groenewegen, 1990; MacIsaac et al., 1989) have been reported in lettuce. In a study by Den Otter and

Lammerts van Bueren (2007), different cultivars showed different root mass distribution over the soil profile, shedding light on the potential genetic variation existing in root system architecture of lettuce with respect to more efficient uptake of resources from deeper layers.

The findings of above studies suggest that breeding for an improved root system architecture in lettuce may confer robustness, defined as the ability to display stable field performance in a wide range of environments. The concept of robustness was developed in the framework of the organic movement and refers to the ability for a crop ideotype to display an acceptable level of field tolerance against stress conditions (Lammerts van Bueren, 2006; De Goede et al., 2013). In this view, the ability to display stable yields is more important than the yield *per se*, and robust varieties require flexibility and tolerance to a wide range of environmental stressors, such as nutrient shortage and mild levels of drought, which can occur in organic and low input systems. The potential differences in root characteristics that can impact robustness of cultivars has not yet been fully exploited by breeders. To be able to incorporate relevant root traits as selection criteria in a practical breeding programme an efficient selection method needs to be developed. Therefore a better understanding of the physiological and genetic backgrounds of the relationship between root system architecture, resource capture, stress resistance, growth pattern, quality and yield is needed. However, as most of the genetic variation for root system architecture is regulated by small-effect loci that interact with the environment, De Dorlodot et al. (2007) concluded that there is a need to integrate efficient and accurate phenotyping, modelling and genomics to define optimal root system architecture.

Recent research provides us the opportunity to develop such a breeding strategy also for root characteristics as King et al. (2003) developed a theoretical framework for the relationship between the root length density (RLD) or root mass distribution (RMD) and the capturing of water and nutrients in cereals. We will extend this theoretical framework by including spatial and temporal aspects of variable water and nutrient availability in organic and low-input production systems.

1.3.2 Linking root traits and resource capture

As highlighted by Lynch and Brown (2012), water and nutrients are the main yield limiting factors in agriculture and there is a need to investigate the traits controlling resource capture and the traits involved in the physiological utilization of the captured resources. As phenotyping root traits can be labour-intensive and expensive, many studies have been focusing on the modelling of this mechanism as described by Van Noordwijk and Van de Geijn (1996). Currently tremendous efforts are being made worldwide to tackle the complexity of the mechanisms regulating resource capture and root traits dynamics in the three-dimensional space, as reviewed by Dunbabin et al. (2013). Also, the molecular aspects of such mechanisms are being investigated as reviewed by Nacry et al. (2013).

Modelling has proved to be a valid tool for predicting root traits in relation to resource capture in Arabidopsis (Gruber et al., 2013), to nitrogen capture in oil-seed rape (Malagoli and Le Deunff, 2014), to phosphate capture in maize (Leitner et al., 2010; Zygalakis and Roose, 2012) and to nitrate capture in rice (Coudert et al., 2013). In lettuce, models were able to predict nitrogen capture (Linker et al., 2004; Zhang et al., 2008) but neither root system architecture nor genetic variation in the model parameters were investigated in these models. Crop models are usually calibrated based on at best a few cultivars; the range of values for the model parameters thus often do not reflect the genetic diversity existing among genotypes.

1.3.3 Integrating physiological aspects into a breeding strategy

Giehl et al. (2014) showed that the characteristics of the root system architecture result from both the genetic background and the environmental conditions prevalent at a particular moment for a particular plant, highlighting that the response of the root system to a particular below-ground situation is determined by changes in the nutritional status of the plant and the external nutrient supply below-ground in time. Moreover, genetic variation in root system architecture has been found to be controlled by loci of small effect which interact with the environment (De Dorlodot et al., 2007). Therefore as Lynch (2013) concludes: models would be a useful tool to understand and predict root phenome and its interaction with the biotic- and abiotic environment.

Yin et al. (2004) and Van Eeuwijk et al. (2005) showed that combining crop physiological models and genetic analyses of complex traits could lead to the development of an essential tool which would enable a better understanding of the phenotype-genotype gaps. In the case of root phenes, such a model would mean a better insight into the morphological and physiological mechanisms controlling resource capture over time and space, as well as an improved comprehension of the genetic control of such traits. Postma et al. (2014) emphasized this statement by exploring the current modelling approaches studying resource capture aspects related to root traits in a low-input context (nitrogen and phosphorus limitation). They conclude that breeding for below-ground traits linked to improved resource acquisition requires a better understanding of the root architectural, anatomical and physiological characteristics, and the interactions among them. They also highlight that not only is it important for new models to take into account the temporal and spatial heterogeneity of nutrient availability but also the developmental stage of the plant to get a better insight of the functional utility of the root traits.

1.4 Objectives and research questions

This thesis aims at developing a physiology-based breeding strategy to increase stress-tolerance in lettuce, by analysing below-ground traits and physiological mechanisms that could confer robustness. Therefore this research has multiple objectives:

- to identify the physiological mechanisms involved in resource capture and use efficiency in lettuce under temporary or localized below-ground resource limitation, and
- to understand the importance of root phenes for field performance of lettuce, how they are genetically controlled and what are the interactions with the environment and how can we integrate all these elements into an eco-physiological modelling approach to lettuce breeding for robustness.

This dissertation encompasses the following specific research questions:

- ➔ What are the physiological mechanisms regulating resource capture and use efficiency at the root and the shoot level in the context of temporal/localized resource shortage? (Chapter 2)
- ➔ What is the contribution of root morphological (root system spatial architecture) and physiological traits (spatial and temporal resource capture) to shoot performance in field conditions? (Chapter 3)
- ➔ What are the main physiological mechanisms to be included in a new eco-physiological model that is able to help breeders to breed for robustness, and what is the importance of the environment and the genetic background in the regulation of such mechanisms? (Chapter 4)
- ➔ Can we find significant marker-trait associations in a population butterhead lettuce for below-ground traits associated with resource capture, and how large is the influence of the environment on these associations? (Chapter 5)

1.5 Methodological framework

This thesis is based on experimental field work and greenhouse trials and data analysis. To explore the physiological mechanisms regulating resource capture and use efficiency at the root and the shoot level in the context of temporal/localized resource shortage, two greenhouse trials were designed using pots of 0.20 m diameter and 0.40 m height; the first trial conducted in 2009 investigated the effect of temporary drought applied at different growth stages on resource capture and use efficiency as well as on root and shoot development of two lettuce cultivars. The second trial, conducted in 2011, simulated the effect of continuous, localized drought, nutrient shortage, and the combination of both on resource capture and use efficiency, as well as on root and shoot development of the same two lettuce cultivars used in the 2009 trial.

To understand the contribution of the root morphological (temporal dynamics of the root system architecture) and physiological traits (spatial and temporal resource capture) to shoot performance in field conditions and the genetic variation thereof, three field trials using four lettuce cultivars were carried out under organic conditions in two

locations: Wageningen, the Netherlands in 2009 and 2010, and Voorst, the Netherlands, in 2009.

Finally, to assess the potential genetic variation existing in the processes identified in the pot trials, and to investigate the potential of breeding for below-ground traits four field trials were carried out using a population of 148 lettuce cultivars in the Netherlands: Wageningen (early spring 2010 and late spring/early summer 2010) and in Voorst (early spring and late spring/early summer 2011).

The methodological framework on this thesis and the associated chapters is illustrated in **Error! Reference source not found.**.

1.6 Outline of the thesis

This thesis consists of six chapters including this introduction (Chapter 1). Chapter 2 proposes to identify physiological mechanisms regulating root growth and nitrogen and water capture and use efficiency under temporal and localized resource availability. Chapter 3 investigates the importance of root traits to field performance of lettuce and the genetic variability thereof. Chapter 4 offers to integrate the physiological mechanisms identified in Chapter 2 into a new eco-physiological model concept to help breeding efforts targeting robustness. Chapter 5 studies the genetic background of resource capture dynamics below-ground and the potential thereof for breeding. Chapter 6, finally, discusses the main findings contained in this thesis, and contains reflections on the potential of the methodology used in the chapters and the challenges below-ground traits represent for accurate phenotyping, the implications of the identified physiological mechanisms for future research and the use of modelling for breeding purposes.

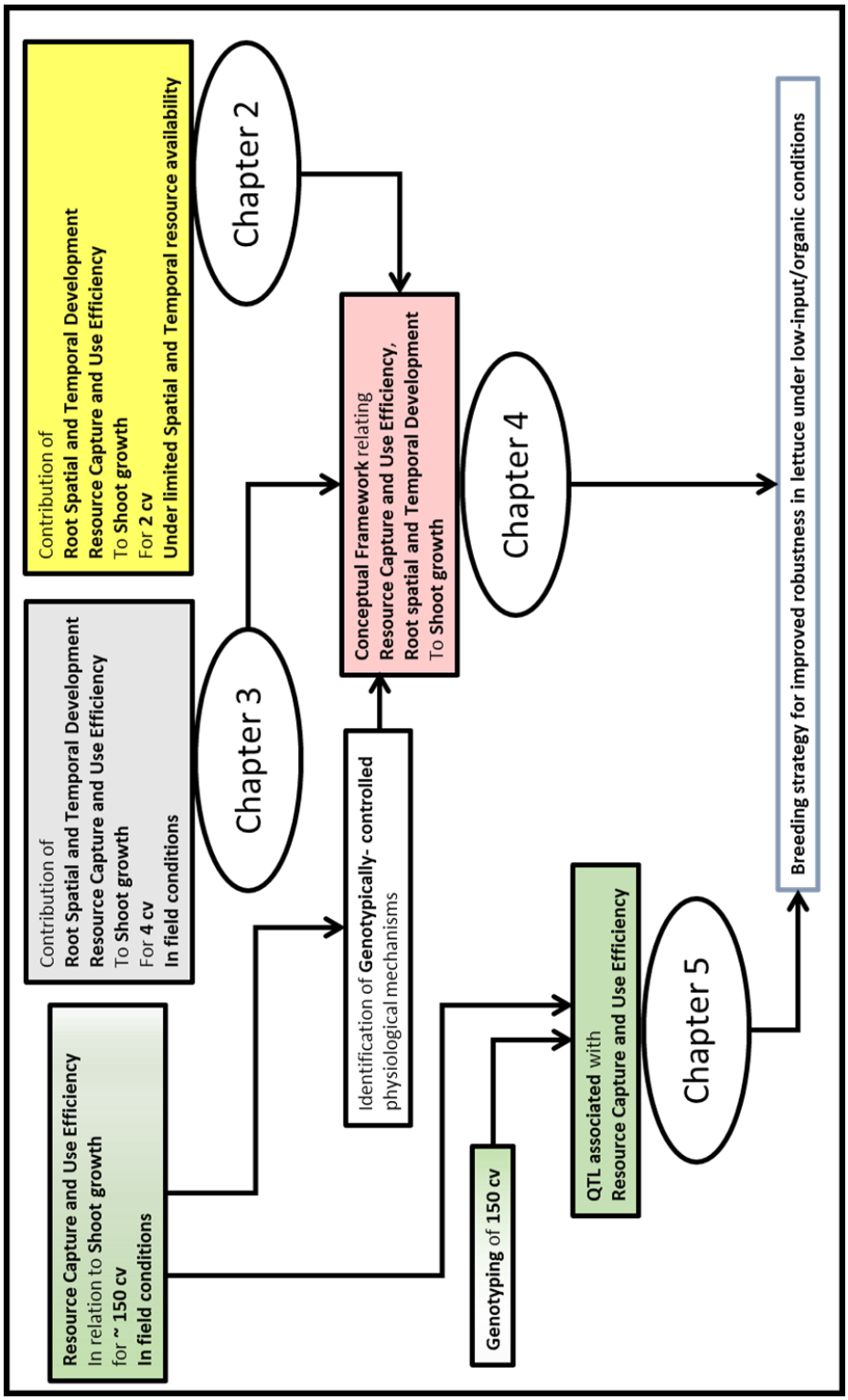

Figure 1.2 Methodological framework of this thesis

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Chapter 2

Shoot growth, root growth and resource capture under limiting water and N supply for two cultivars of lettuce (*Lactuca sativa L.*)

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Kerbiriou PJ, Stomph TJ, Van Der Putten PEL, Lammerts van Bueren ET, Struik PC. 2013. Shoot growth, root growth and resource capture under limiting water and N supply for two cultivars of lettuce (*Lactuca sativa* L.). Plant and Soil 371: 281–297

Abstract

To improve vegetable crops adapted to low input and variable resource availability, better understanding is needed of root system functioning, including nitrogen and water capture. This study quantified shoot and root development and patterns of water and nitrate capture of two lettuce cultivars subjected to temporary drought at two development stages (Trial 1) or to continuous, localized drought and/or nitrate shortage (Trial 2). In Trial 1, early drought slowed down shoot and root growth, whereas late drought enhanced root proliferation in the top 0.1 m. Nitrate capture during drought was sustained by increased nitrate inflow from deeper layers. Plants did not recover fully from drought after re-watering. In Trial 2, root proliferation was stimulated in the drier soil compartment partially compensating reduced water availability and nitrate mobility. Under nitrate shortage, root proliferation was enhanced in the compartment where nitrate was more abundant, irrespective of water availability. Changes observed in the root system are 'feed-forward' mechanisms to sustain resource capture in a limiting growing environment. The type of stress (drought or nitrate shortage) affects coping strategies; nitrate concentration in the soil solution, combined with the nutritional status of the plant will determine the stress response.

Keywords: lettuce; transplanting; root activity; nutrient use efficiency

Abbreviations

DST	Drought Stress applied in the Top compartment	NST+DSB	Nutrient Stress applied in the Top compartment combined with Drought Stress applied in the Bottom compartment
DST+NSB	Drought Stress applied in the Top compartment combined with Nutrient Stress applied in the Bottom compartment	NUE	Nitrogen Use Efficiency (g DM g^{-1} N per plant)
DW	Dry Weight (g)	QTL	Quantitative Trait Loci
ED	Early Drought	SLA	Specific Leaf Area (m^2 g^{-1})
FW	Fresh Weight (g)	LA	Total Leaf Area (m^2 per plant)
L	Litre	TLN	Total Leaf Number per plant
LD	Late Drought	TRL	Total Root Length per plant
NST	Nutrient Stress applied in the Top compartment	WUE	Water Use Efficiency (g DM L^{-1} water)

2.1 Introduction

Because of growing concerns on environmental effects of high-input vegetable production, declining availability of external resources and increasing water scarcity, sustainable crop production systems need to be designed. For instance, there is evidence that the cost of applying nitrogen, an input with major impact on crop yield, will increase because of rising costs of fertilizer production (Witcombe et al., 2008), probably leading to a reduction in amounts applied. Drought constitutes a major threat for crop yield worldwide: as transpiration is directly associated with gas exchange required for photosynthetic reactions, water shortage considerably lowers plant dry matter production and thus final yield (Wu et al., 2008).

Organic fertilizers are used in sustainable farming systems as only soil fertility amendment, while the use of irrigation is restricted. Mineralization of organic fertilizers depends on soil chemical, physical and biological processes –which are in turn influenced by environmental conditions (temperature, precipitation, etc.) – leading to temporal and spatial variability in resource availability. Therefore, sustainable systems require more robust cultivars, i.e. cultivars that can perform well under limiting growing conditions, for example (combinations of) temporary and/or localized shortage of water and nutrients.

Plant robustness can be defined as the ability to perform well despite fluctuating growing conditions (Kitano, 2007). Among others traits, an improved root system, displaying morphological and/or physiological adaptations that optimize the temporal and spatial capture of soil resources, may contribute to robustness (Lammerts van Bueren et al., 2002). In lettuce (*Lactuca sativa* L.), breeding programmes have been focusing mainly on disease resistance and high yield, achieved in conventional systems characterised by high input of nutrients and frequent application of irrigation (Gallardo et al., 1996). Conventional systems have thus considerably reduced the demand on the roots, resulting in the release onto the market of lettuce cultivars with a shallow root system (Burns, 1980) that maximizes nutrient and water uptake from the top soil, where irrigation water is provided. In organic, low-input farming systems aiming at reducing frequency of irrigation, these lettuce cultivars are thus more subject to drought and

nutrient stress because a superficial root system mainly located in the top soil (0-0.20 m) is not able to capture resources when mainly available in deeper layers (Johnson et al., 2000).

Understanding the role of root system architecture for a better resource capture and use efficiency is a key step to develop innovative models that can predict root development based on resource capture measurements. Such models may help to identify genetic variation in temporal and spatial root foraging and resource capture strategies, and therefore propose traits of interest when selecting robust lettuce cultivars.

Multiple attempts have been made to design models, assisting in the interpretation of limited data (among others: Burns, 1980; Johnson, 1983; Jarvis, 1989; Dunbabin, 2011) that could predict root development as a function of resource capture. Indeed, resource capture can be assessed more easily than root development, and has been demonstrated to associate closely with root activity (e.g. Robinson, 1996; Lynch & Brown, 2012). However, these models often overlook the relationship between below- and above-ground processes or are too complex or too demanding in terms of input to be suitable for use in field studies on large sets of genotypes. Little is known about the effects of localized and/or temporal shortages of water and/or nutrient on root system architecture, hence models do not account for such effects. To improve our knowledge of (the limits to) resource efficiency, there is thus a need to examine root responses to heterogeneous spatial and temporal water and nitrogen availability either as single limitation or/and in combination. The goal of this study, as a first step, was therefore to provide more insight in underlying processes and to assess:

- What the impact is of the type of resource limitation, and its occurrence in time, on root growth.
- What the endogenous and exogenous conditions are that trigger such responses.
- Whether different types of responses can be observed at the root level in reaction to type and timing of resource limitation.
- How root responses to soil-borne resource limitations contribute to maintain shoot development.

To test how robust outcomes are, they were tested for two commercially relevant cultivars that, in preliminary studies, had shown potentially different responses.

Two greenhouse pot trials using two butterhead lettuce cultivars were designed in order to simulate the effect of temporary and localized drought and/or nitrogen shortage on the plants' shoot and root development. These experiments intended to provide background information about the strategies developed by lettuce at the root level to cope with drought, nitrate depletion and the interactions thereof, and to identify new input traits to be included in an improved crop model.

2.2 Materials and methods

2.2.1 Cultivar choice and transplant raising

Two commercial cultivars, 'Matilda' and 'Pronto', were used, both commonly sold to either conventional or organic growers for the spring, summer and autumn seasons. Both cultivars have shown a consistent performance over many years in their prevailing growing conditions (Northern Europe summer season for 'Matilda' and Southern Italy summer for 'Pronto').

Seeds of both cultivars originated from seed lots produced under the same environmental conditions. Seeds were sown in 0.04 m cubic organic peat blocks (Jongerius, Houten, the Netherlands). Seed dormancy was broken by exposure to 4˚C for 24 hours. Subsequently, transplants were raised in a greenhouse set at 20˚C during the day (12 h) and 15˚C during the night (12 h). Transplanting to the pots was done at the 5-leaf stage, which lettuce growers consider an 'optimal' seedling stage for field transplanting.

2.2.2 Plant management

After transplanting plants were grown in a greenhouse in PVC tubes of 0.20 m diameter and 0.40 m length. The tubes were wrapped in reflecting isolating material to avoid excessive warming. In Trial 2, discs of isolating material were also put around the plant base on top of the soil to prevent evaporation. Pots were filled with a 40%:60% (v:v) mixture of river sand and field soil, taken from an organically managed field (Wageningen, the Netherlands) excluding the upper 0.05 m. Both soils were dried at 40˚C and were sieved using a 3 mm sieve prior to pot filling (except for the river sand in Trial 1, which was not sieved). The mixture of the two soils + organic fertilizer (9% N,

3% P, 3% K + 3% MgO, EcoFertiel, EcoStyle, Appelscha, The Netherlands) + water was prepared for each pot separately (and in Trial 2 for each compartment within a pot, Figure 2.1) ensuring uniform distribution of water and nutrients.

The pots were placed in a fully conditioned greenhouse with a night temperature (12 h) set at 15 ˚C and a day temperature (12 h) set at 25 ˚C (Trial 1) or 20˚C (Trial 2). The actual data recorded in the greenhouse for Trial 1 showed a mean value for the night temperature of 15.7±1.06 ºC (mean±one standard deviation) and for the day temperature of 24.8±2.21 ºC; for Trial 2 night temperatures were on average 15.8±1.11 ºC and the day temperature were on average 21.5±1.11 ºC. Air humidity was on average 73±9.0% in Trial 1 and 57±6.1% in Trial 2.

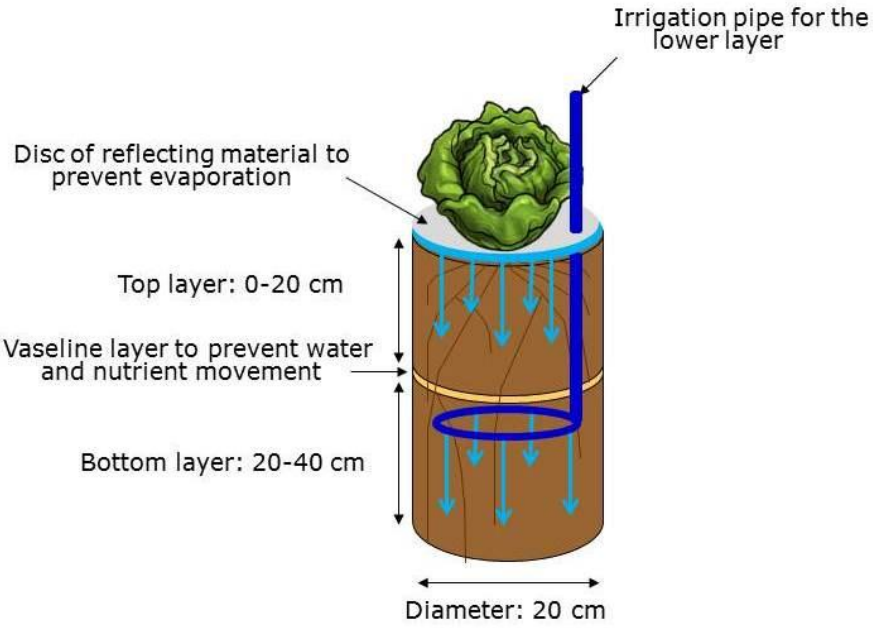

Figure 2.1 Pot design (Trial 2)

Radiation and air temperature were recorded; soil temperatures were also monitored during the whole experiment at various depths (0-0.10 m, 0.10-0.20 m, 0.20-0.30 m and 0.30-0.40 m). Cumulated thermal time was calculated using a base temperature of 4 ˚C and recorded air temperatures. Individual pots were weighed twice a week, and watered

to bring pot weights back to the required level, while compensating for changes in plant fresh weight. The difference between initial weight (P_0) and weight at time t (P_t) was surmised to be due to water uptake (W_t), evaporation at the soil surface (E_l) and plant shoot growth (L_t). Shoot fresh weight was measured on separate pots (small weight variation due to root development was neglected):

$$P_t = P_0 - W_t - E_l + L_t$$

The soil moisture levels for the stressed and control treatments were based on a pF curve specific to the soil mixture used in the pots. In both experiments, the control treatment had a pF value of 2.6; for the drought treatment pF was 3.3.

2.2.3 Treatments

Treatments included early drought and late drought in Trial 1 and various combinations of drought and nitrogen shortage in different compartments of the pots throughout the duration of the experiment in Trial 2. In Trial 1, plants were sampled before and after the drought period, and during the recovery period for both the control and the drought treatment. 'Early Drought' was applied between 320 and 432 ºCd and followed by recovery during a period comprised between 432 and 656 ºCd; 'Late Drought' was applied between 432 and 544 ºCd and followed by recovery during a period comprised between 544 and 656 ºCd. In Trial 2, treatments were applied from transplanting onwards. Plants of all treatments were sampled 2, 4 and 6 weeks after transplanting in the greenhouse, corresponding to 288, 512 and 768 ºCd, respectively. Treatments are detailed in Table 2.1 and Table 2.2. Experiments were set up in a complete randomized block design. There were 5 (Trial 1) or 4 (Trial 2) replicates.

Table 2.1 Treatments and sampling scheme in Trial 1.

	Transplanting	0–14 days AT[1]	**Sampling**	14–21 days AT[1]	**Sampling**	21–28 days AT[1]	**Sampling**	28–35 days AT[1]	**Final sampling**
Total radiation received at plant level (Mmol m^{-2})		1.042		1.325		1.601		1.946	
Cumulated Degree-days (˚C d)		320		432		544		656	
Control	**x**		**x**		**x**		**x**		**X**
ED Stress[2]				**Drought stress**		1st week recovery		2nd week recovery	
					x		**x**		**X**
LD Stress[3]						**Drought stress**		1st week recovery	
							x		**X**

[1] After Transplanting
[2] Early Drought stress
[3] Late Drought stress

Table 2.2 Treatments in Trial 2.

		Treatments				
		Control	DST[1]	NST[2]	DST+NSB[3]	NST+DSB[4]
Top compartment (0-0.20 m)	Fertilizer (g NO_3-N)	0.625	0.625	0.178	0.625	0.178
	Water status (v:v; %)	14	6	14	6	14
Bottom Compartment (0.20-0.40 m)	Fertilizer (g NO_3-N)	0.625	0.625	0.625	0.178	0.625
	Water status (v:v; %)	14	14	14	14	6

[1] Drought Stress in Top compartment
[2] Nutrient Stress in Top compartment
[3] Drought Stress in Top compartment combined with Nutrient Stress in Bottom compartment
[4] Nutrient Stress in Top compartment combined with Drought Stress in Bottom compartment

2.2.4 Measurements

Fresh weight (FW), dry weight (DW), total number of leaves (TLN) and total leaf area (TLA) were recorded on the shoots at each harvest. Specific leaf area (SLA, $m^2 g^{-1}$) was calculated as TLA/DW. At final harvest, plant total nitrogen was measured using the Kjeldahl method. Nitrogen use efficiency was calculated as g DM/g total nitrogen present in the plant. Water use efficiency was calculated as g DM/L water added to the

pot. At each harvest, the content of each pot was divided into four layers of 0.10 m each (0-0.10 m, 0.10-0.20 m, 0.20-0.30 m, 0.30-0.40 m). Roots inside the peat block of the original transplant were ignored. The roots in each layer were rinsed and cleaned from organic matter manually, and subsequently scanned and analysed for total root length (TRL, m) using WinRhizo Pro 2007 (v2005b, Regent Instruments, Québec, Canada). Once scanned, the root samples were dried at 105 ºC for 16 h for dry weight assessment. In addition, for each layer, a soil sample was taken to measure soil moisture content (after drying at 40 ˚C for 48 h) and NO_3-N content. NO_3-N content was measured using an Ion Selective Electrode (ThermoFisher, Waltham, MA, USA). NO_3-N was extracted using 30 g dry soil mixed in 100 mL deionized water for one minute. NO_3-N uptake from a soil sample was calculated as the difference with the NO_3-N content in a soil sample taken from a pot without a plant.

2.2.5 Statistical analyses

Data of each harvest of Trial 1 were analysed by a two-way ANOVA. Data of each harvest of Trial 2 were analysed by two-way ANOVA followed by the Bonferroni test at *p-value* ≤ 0.05 to determine the statistical significance of the differences between treatments. Statistical analyses were performed with Genstat 14th Edition (Hempstead, UK).

2.3 Results

2.3.1 Effect of temporary drought stress (Trial 1)

Early drought

When drought was applied early, total root length and thus root length density (km m^{-3}) was reduced by approx. 40% compared with the control in all layers of the pot for both cultivars. The total root weight, however, was only reduced by 15% for both cultivars. Figure 2.2 shows that early drought reduced root length in all layers more than it reduced root weight.

The pattern of nitrate inflow into the roots (amount of nitrate captured from the soil per m of root length) changed (Figure 2.3a): it was reduced in the top layers where some

water was still provided but where water and part of the nitrate was depleted, and dramatically increased at the same time in layers where water and nitrate resources remained relatively abundant (by more than 200% in the 0.20-0.40 m layer for both cultivars).

However, the improved nitrate inflow in lower layers did not fully compensate for the reduced nitrate capture in the top layer, as the total nitrate capture was reduced by 10% for both cultivars under stress. This impacted on the plant total nitrogen content, which was reduced by 24 and 29% for cv. Matilda and cv. Pronto, respectively (Table 2.3).

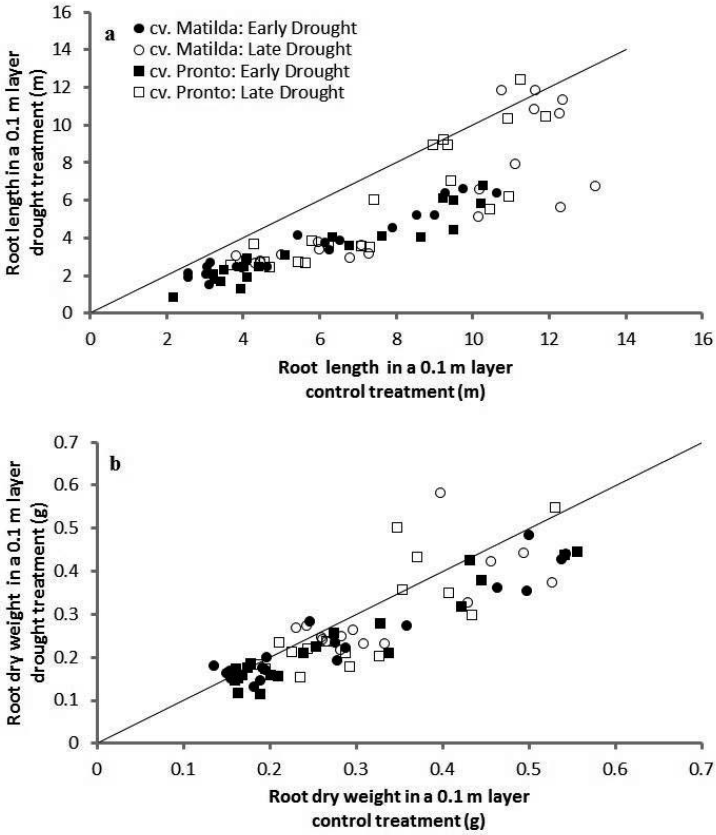

Figure 2.2 Relationship between root length of the control and root length of the drought treatment (measured just after Early Drought termination at 320 ˚Cd and just after Late Drought termination at 432 ˚Cd) in a layer for cv. Matilda and cv. Pronto (a), and between root dry weight of the control and root dry weight of the drought treatment (measured just after Early Drought termination at 320 ˚Cd and just after Late Drought termination at 432 ˚Cd) in a layer for cv. Matilda and cv. Pronto (b).

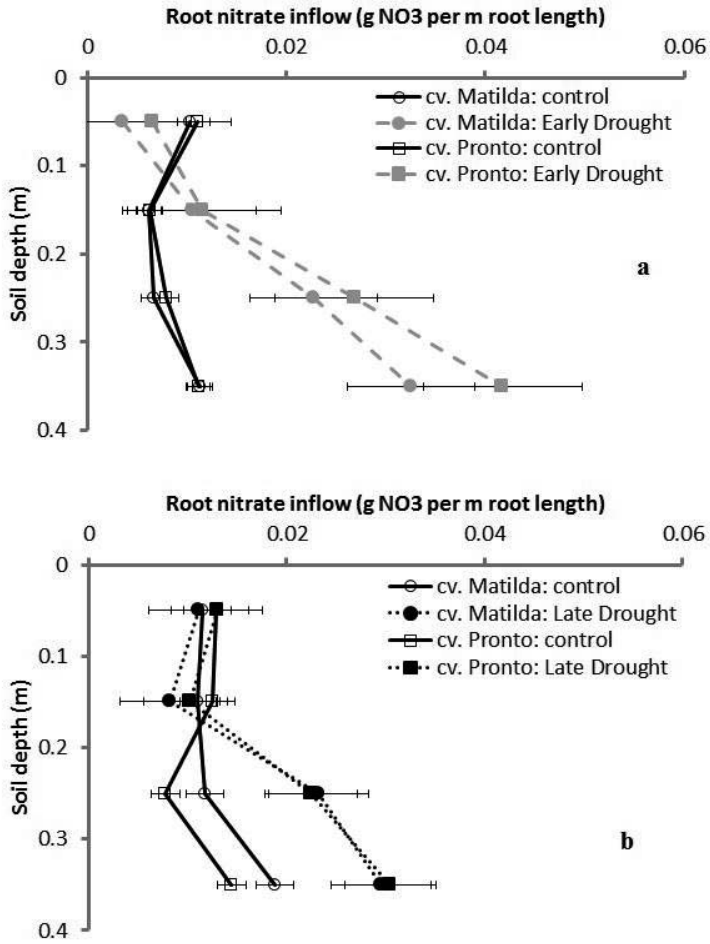

Figure 2.3 Nitrate inflow at the roots of cv. Matilda and cv. Pronto during Early Drought (a) (320-432 ºCd) and Late Drought (b) (432-544 ºCd) stress treatments (Trial 1). Error bars indicate ± one standard deviation.

The reduced availability of water and nitrate to the root system affected the shoot development: at the end of the drought period the shoot dry weight of the two cultivars was reduced by 25% (Figure 2.4) and the fresh weight by 40% (data not shown). The rate of leaf expansion slowed down by more than 50% during the drought period for both cultivars (Figure 2.4). However, drought did not affect the nitrogen use efficiency (NUE) (Table 2.3) or the water use efficiency (WUE) (Table 2.4).

Table 2.3 Effect of early and late drought on total nitrogen per plant, total nitrogen captured from the soil and Nitrogen Use Efficiency of cv. Matilda and cv. Pronto (Trial 1).

	Total N / plant (g)		Total N captured from the soil (g)		Nitrogen Use Efficiency (g DM g^{-1} N)	
	Stressed	Control	Stressed	Control	Stressed	Control
	Cv. Matilda					
Early Drought (ED)						
Just after ED	0.19±0.01*	0.25±0.02	0.20±0.03	0.27±0.04	24±1.20	24±0.70
After 1 week recovery	0.37±0.03	0.48±0.02	0.40±0.12	0.51±0.06	26±0.87	28±1.55
After 2 weeks recovery	0.67±0.06	0.74±0.04	0.71±0.14	0.79±0.04	30±2.33	34±1.56
	Cv. Pronto					
Early Drought (ED)						
Just after ED	0.20±0.01	0.28±0.02	0.22±0.03	0.30±0.07	24±0.95	22±0.73
After 1 week recovery	0.37±0.02	0.48±0.04	0.39±0.11	0.51±0.09	24±1.14	24±0.54
After 2 weeks recovery	0.69±0.01	0.76±0.02	0.73±0.05	0.81±0.03	31±1.29	35±2.11
	Cv. Matilda					
Late Drought (LD)						
Just after LD	0.35±0.04	0.48±0.02	0.38±0.03	0.51±0.06	27±1.52	28±1.55
After 1 week recovery	0.63±0.02	0.74±0.04	0.67±0.05	0.79±0.04	31±1.39	34±1.56
	Cv. Pronto					
Late Drought (LD)						
Just after LD	0.40±0.02	0.48±0.04	0.42±0.04	0.51±0.09	26±0.87	24±0.54
After 1 week recovery	0.64±0.03	0.76±0.02	0.68±0.04	0.81±0.03	31±0.92	35±2.11

* Standard error of the mean

Recovery from early drought

After one week recovery, the effect of early drought was still visible on the shoot of both cultivars, but was gradually reduced towards the end of the experiment (after two weeks recovery; Figure 2.4). Total root system elongation during the first week after early drought was 1.03 and 1.02 m $(^{\circ}Cd)^{-1}$ (calculated from air temperature using a base temperature for lettuce of 4 ºC, Dufault et al., 2009) for the control and the recovering plants of cv. Matilda, respectively, whereas it was only 0.61 and 0.80 m $(^{\circ}Cd)^{-1}$ for the control and recovering plants of cv. Pronto, respectively (data not shown; cultivar effect significant at $p<0.05$). For cv. Matilda, the development occurred mainly in the 0.10-0.20 m layer for the control treatment, while the elongation was most prominent in the 0-0.10 m layer for the recovering plants. For cv. Pronto, the root growth was less than for 'Matilda' in the control treatment, and soil exploration by the roots was equal over the layers for the recovering plants. For the control treatment, the overall root

expansion rate decreased drastically towards the end of the experiment for both cultivars, but recovering plants kept expanding their root systems at high rates (Figure 2.5a,b). Control plants of cv. Matilda expanded in the lower layers (0.20-0.30 and 0.30-0.40 m) while expansion had stopped in the top layers (0-0.10 and 0.10-0.20 m) (Figure 2.5a). Root expansion of the control still occurred in the layers 0-0.10 and 0.20-0.30 m for cv. Pronto, but not in the other layers (Figure 2.5b). Recovering plants showed root expansion below 0.10 m for cv. Matilda and in all layers for cv. Pronto (Figure 2.5a,b). During the first week of recovery, the nitrate inflow into the roots was still higher for the stressed plants than for the control plants in the 0.10-0.30 m layer and after two weeks recovery, there was no difference anymore with the control (data not shown). This reflected on the total nitrogen content in the plants, which was only reduced by 9% for both cultivars at the end of the experiment. Nevertheless, whereas NUE was not affected immediately after early drought, early drought significantly reduced NUE for both cultivars at final harvest (Table 2.3). Early drought did not affect WUE after recovery (Table 2.4).

Late drought

Late drought (LD) slightly increased root development of both cultivars, with increased root elongation taking place in the top 0.10 m of the pot (where some water was provided) against a decrease in all other layers (data not shown). During LD, nitrate inflow increased in the lower layers, reaching values of approx. 0.02 to 0.03 g NO_3-N per m root length for the stressed plants of both cultivars in the 0.20-0.30 and 0.30-0.40 m layers, while nitrate inflow into the roots in those layers was limited to 0.01 g NO_3-N per m on average for the control treatment in both cultivars (Figure 2.3b). The nitrate uptake from the lower layers for cv. Pronto was better than for cv. Matilda (data not shown). This reflected on shoot performance: whereas cv. Pronto showed a shoot dry weight reduction of only 10%, this reduction was 30% for cv. Matilda in comparison with the control (Figure 2.4b). Also the reduction in total plant nitrogen differed, approx. 30% for cv. Matilda against 17% for cv. Pronto. As for ED, LD did not affect NUE (Table 2.3) or WUE (Table 2.4).

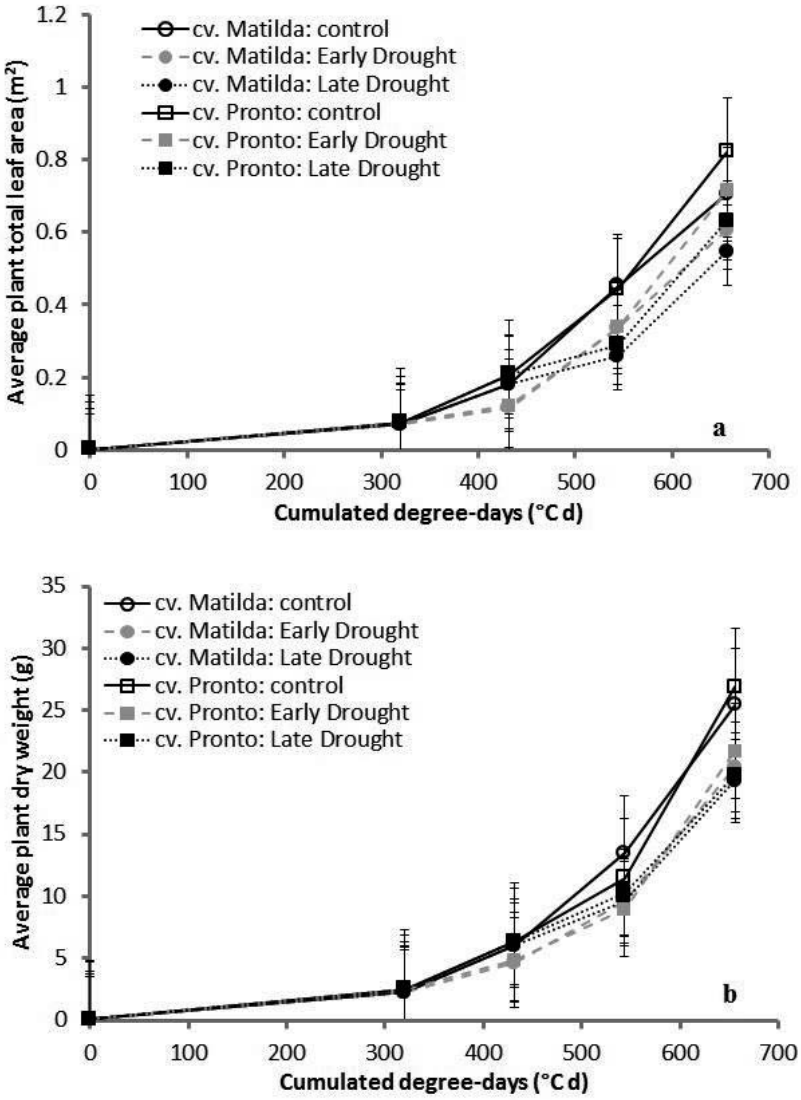

Figure 2.4 Average total leaf area (a) and average dry weight (b) of cv. Matilda and cv. Pronto under Early Drought and Late Drought stress application (Trial 1). Error bars indicate ± one standard deviation.

Table 2.4 Effects of early and late drought on Water Use Efficiency (WUE) of cv. Matilda and cv. Pronto (Trial 1).

	WUE (g DM L^{-1} water)	
	Stressed plant	Control
	Cv. Matilda	
Early Drought (ED)		
Just after ED	3.28±0.31*	3.31±0.25
After 1 week recovery	3.51±0.18	3.78±0.21
After 2 weeks recovery	3.93±0.37	4.13±0.06
Early Drought (ED)	**Cv. Pronto**	
Just after ED	3.33±0.21	3.52±0.25
After 1 week recovery	3.51±0.24	3.44±0.28
After 2 weeks recovery	4.27±0.11	4.31±0.16
	Cv. Matilda	
Late Drought (LD)		
Just after LD	3.05±0.24	3.78±0.21
After 1 week recovery	3.60±0.08	4.13±0.06
	Cv. Pronto	
Late Drought (LD)		
Just after LD	3.21±0.27	3.44±0.28
After 1 week recovery	3.79±0.29	4.31±0.16

* Standard error of the mean

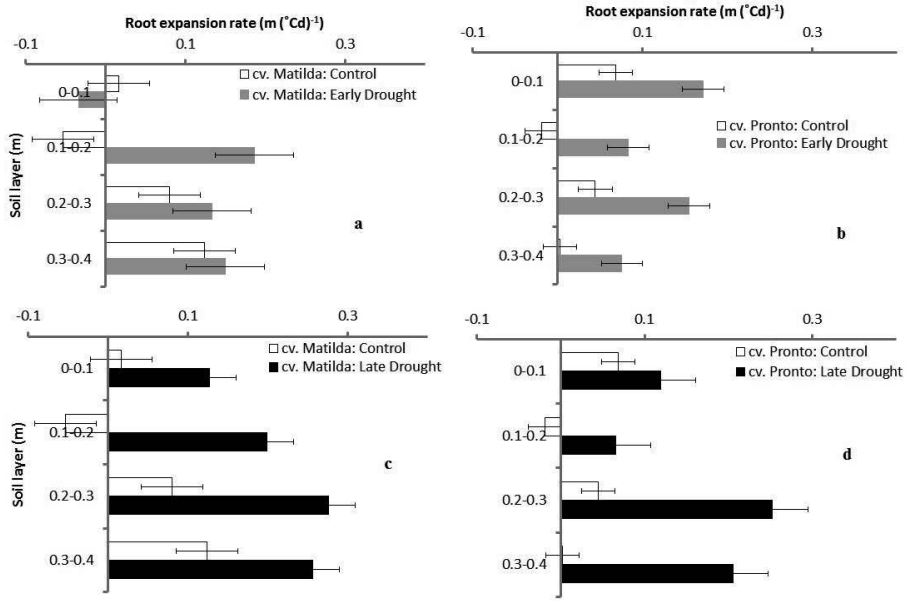

Figure 2.5 Root expansion rate of cv. Matilda (a) and cv. Pronto (b) during the second week of recovery after Early Drought application (between 544 – 656 ºCd), and of cv. Matilda (c) and cv. Pronto (d) during first week of recovery after Late Drought application (between 544 – 656 ºCd) (Trial 1). Error bars indicate ± one standard deviation.

Recovery from late drought

After a week recovery, the late drought stress effect was still visible, with a reduction of shoot dry weight of 25% for both cultivars (Figure 2.4b). Whereas control plants of both cultivars almost stopped root expansion during this phase, root expansion continued for LD plants of both cultivars, with a remarkable cultivar difference in the layer 0.10-0.20 m (Figure 2.5 c,d). Like for ED, after a week recovery, NUE of both cultivars was reduced by approx. 10%. In contrast to ED, the WUE of both cultivars was significantly reduced after recovery, but for 'Matilda' this reduction was already visible at the end of the drought period (Table 2.4).

2.3.2 Effect of continuous, localized drought and nitrogen shortage (Trial 2)

Drought stress applied to the upper compartment

Drought applied to the top compartment (DST) had a large impact on shoot development. Plant growth was reduced (reduced DM production, reduced rate of leaf appearance and lower rate of leaf expansion; Table 2.5); at final harvest (6 weeks after transplanting), no significant difference was found for total root weight and total root length between the control and the DST plants.

At two weeks after transplanting , while shoot dry matter production of both cultivars was reduced by 50% (in comparison with the control), RLD was reduced by 30 and 15% in the top 0.10 m of the pot for cv. Matilda and cv. Pronto, respectively (Figure 2.6a,b,c,d). In the lower compartment, root elongation seemed stimulated, but root length density was still very low. Water uptake and nitrate removal were reduced in the top compartment (in comparison with the control), associated with a severe reduction in nitrate uptake in the 0.10-0.20 m layer (by about 90% for both cultivars), and increased uptake in the lower compartment, where proportional nitrate uptake (e.g. proportion of nitrate captured out of the available amount) was maintained at levels similar to those of the control for both cultivars.

At 6 weeks after transplanting, only a small portion of the available nitrate was captured in the upper compartment by both cultivars whereas there were also significant amounts of nitrate left in the lower compartment (Table 2.6). Shoot development was reduced by

30% for both cultivars in comparison with the control (Table 2.5), whereas final NUE was reduced by more than 20% for both cultivars (Table 2.6) and WUE was higher than the control by about 8% and was higher than for any other treatment except the combined nitrogen stress in the top compartment and drought stress in the lower compartment (NST+DSB, Figure 2.7).

Table 2.5 Summary of treatment effects on shoot and root variables for cv. Matilda and cv. Pronto in Trial 2.

Treatment	cv.	FW[1] (g)	DW[2] (g)	LA[3] (m^2)	SLA[4] (m^2 g^{-1})	Total RW[5] (g)	Total RL[6] (m)	SRR[7]
	Sampling 2 weeks after transplanting (288 ˚Cd)							
Control	'M'[12]	24.4±4.25*	1.73±0.34	0.06±0.010	0.035±0.002	0.17±0.06	27.3±5.04	10.5±2.75
	'P'[13]	24.2±2.68	1.73±0.18	0.06±0.003	0.032±0.003	0.21±0.06	25.6±4.11	8.7±2.33
DST[8]	'M'	8.6±1.35	0.79±0.10	0.03±0.006	0.031±0.004	0.15±0.02	22.4±2.21	5.3±1.14
	'P'	8.2±0.94	0.75±0.85	0.02±0.003	0.029±0.003	0.18±0.03	24.0±4.49	4.1±0.31
DST+NSB[9]	'M'	10.8±1.59	0.95±0.07	0.03±0.006	0.034±0.004	0.14±0.03	22.6±4.73	7.1±1.44
	'P'	9.3±2.45	0.82±0.18	0.03±0.008	0.031±0.005	0.14±0.03	22.4±4.12	6.0±1.91
NST[10]	'M'	26.4±4.26	1.76±0.21	0.07±0.012	0.037±0.003	0.17±0.04	26.5±3.57	11.1±3.99
	'P'	28.2±1.66	1.79±0.10	0.07±0.007	0.037±0.003	0.19±0.05	28.7±2.92	10.2±3.18
NST+DSB[11]	'M'	27.0±1.11	1.76±0.10	0.07±0.003	0.039±0.004	0.17±0.05	27.6±6.25	11.2±2.87
	'P'	24.8±4.82	1.58±0.32	0.06±0.012	0.039±0.003	0.16±0.05	29.8±5.96	10.1±2.46
	Sampling 4 weeks after transplanting (512 ˚Cd)							
Control	'M'	167±9.4	11.8±1.17	0.35±0.051	0.030±0.007	0.80±0.14	123±37.2	15.0±3.15
	'P'	152±18.4	11.2±0.82	0.36±0.069	0.032±0.008	0.82±0.12	122±23.6	13.8±2.30
DST	'M'	60±16.7	5.6±1.02	0.17±0.041	0.030±0.004	0.65±0.10	129±5.8	8.6±0.55
	'P'	65±18.8	5.2±1.11	0.14±0.028	0.026±0.004	0.68±0.08	107±24.3	7.6±0.74
DST+NSB	'M'	93±12.8	7.2±0.83	0.22±0.012	0.031±0.003	0.58±0.15	109±9.6	13.0±3.96
	'P'	97±10.8	7.6±0.92	0.22±0.029	0.029±0.005	0.92±0.19	110±19.1	8.4±1.16
NST	'M'	161±23.7	11.9±1.44	0.37±0.068	0.031±0.006	0.83±0.15	113±20.6	14.6±2.77
	'P'	183±15.0	13.0±1.34	0.48±0.095	0.038±0.009	0.88±0.12	141±25.7	15.0±2.16
NST+DSB	'M'	135±9.6	10.7±0.90	0.31±0.037	0.029±0.005	0.86±0.22	135±12.3	13.0±2.83
	'P'	142±6.3	10.9±0.80	0.33±0.056	0.030±0.004	1.11±0.22	137±18.8	10.1±1.53
	Sampling 6 weeks after transplanting (768 ˚Cd)							
Control	'M'	396±20.7	31.5±0.94	0.69±0.096	0.022±0.004	2.16±0.65	285±56.0	15.5±4.03
	'P'	374±12.1	30.7±1.50	0.68±0.049	0.022±0.002	2.27±0.29	239±30.9	13.7±1.52
DST	'M'	259±16.1	21.2±1.95	0.60±0.041	0.028±0.002	2.01±0.34	312±61.3	10.7±1.18
	'P'	239±46.7	20.3±4.50	0.49±0.093	0.024±0.001	2.41±0.41	299±32.5	8.53±1.83
DST+NSB	'M'	265±3.6	22.9±1.06	0.62±0.008	0.027±0.001	2.63±0.32	382±49.8	8.79±0.88
	'P'	237±19.8	21.1±2.57	0.51±0.040	0.024±0.001	2.66±0.27	293±23.4	7.93±0.72
NST	'M'	347±12.8	32.1±2.76	0.70±0.033	0.022±0.001	2.93±0.30	316±39.6	11.0±0.86
	'P'	341±7.4	32.2±1.05	0.68±0.027	0.021±0.001	2.54±0.47	217±71.1	13.1±3.01
NST+DSB	'M'	309±13.1	25.5±1.87	0.68±0.074	0.027±0.002	2.67±0.09	381±38.8	9.58±0.97
	'P'	306±4.7	28.1±1.25	0.64±0.027	0.023±0.002	3.24±0.33	376±32.5	8.72±1.02

[1] Fresh Weight; [2] Dry Weight; [3] Leaf Area; [4] Specific Leaf Area; [5] Total Root Weight; [6] Total Root Length; [7] Shoot:Root Ratio; [8] Drought Stress in Top compartment; [9] Drought Stress in Top compartment combined with Nutrient Stress in Bottom compartment; [10] Nutrient Stress in Top compartment; [11] Nutrient Stress in Top compartment combined with Drought Stress in Bottom compartment; [12] cv. Matilda; [13] cv. Pronto; *Standard error of the mean

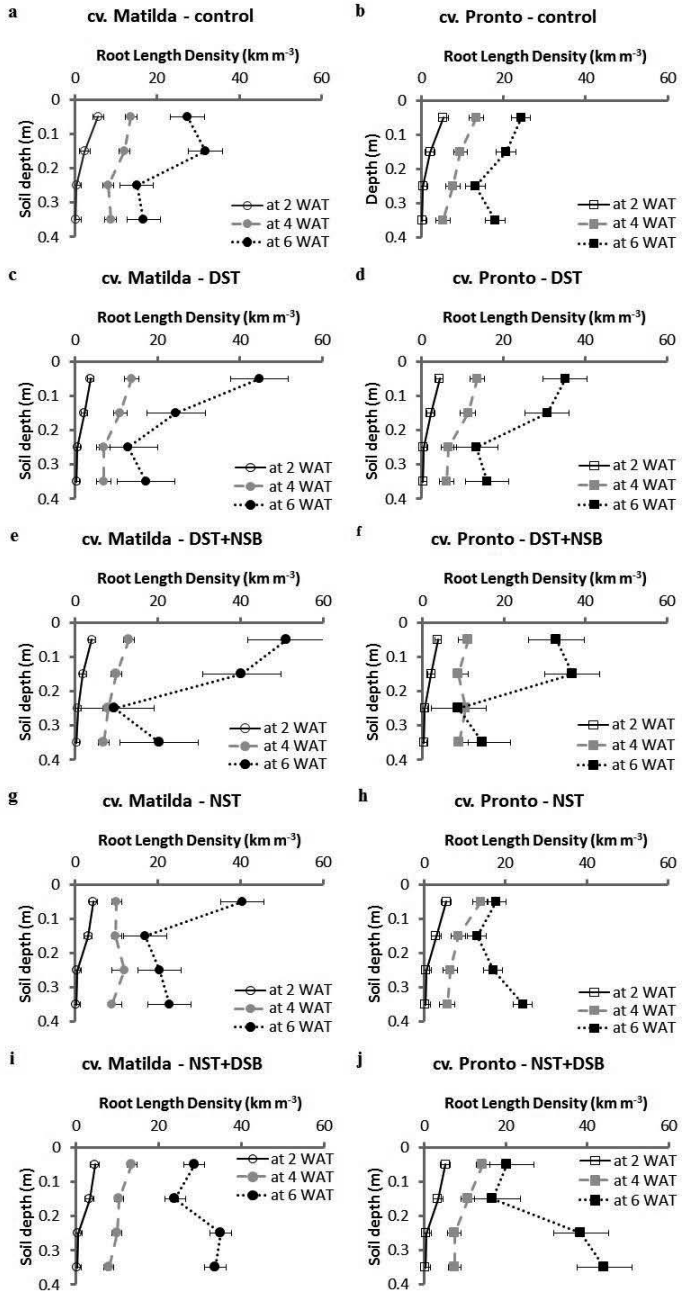

Figure 2.6 Root Length Density evolution in time [2, 4, and 6 Weeks After Transplanting (WAT) correspond to samplings done at 288, 512 and 768 ºCd, respectively] and over the soil profile for the two cultivars under the 4 treatments (Trial 2). For treatment codes, see Table 2.2. Error bars indicate ± one standard deviation.

Table 2.6 Effects of treatments on nitrogen captured in the soil, total nitrogen content per plant and Nitrogen Use Efficiency of cv. Matilda and Pronto 6 weeks after transplanting (768 ˚Cd) in Trial 2.

	N captured in the soil (g)			Total N / plant (g)	Nitrogen Use Efficiency (g DM g^{-1} N)
	Top compartment	Bottom compartment	Total		
	cv. Matilda				
Control	0.53±0.001*	0.53±0.002	1.07±0.003	0.69±0.044	46±3.27
DST[1]	0.22±0.036	0.41±0.011	0.63±0.001	0.60±0.028	36±1.27
DST+NSB[2]	0.34±0.041	0.31±0.000	0.65±0.002	0.56±0.007	41±1.88
NST[3]	0.27±0.067	0.54±0.000	0.81±0.003	0.56±0.022	57±3.45
NST+DSB[4]	0.31±0.000	0.35±0.025	0.66±0.001	0.60±0.028	43±0.75
	cv. Pronto				
Control	0.54±0.001	0.53±0.003	1.07±0.001	0.70±0.024	44±0.69
DST[1]	0.28±0.078	0.41±0.082	0.69±0.003	0.59±0.067	34±3.18
DST+NSB[2]	0.31±0.032	0.31±0.001	0.61±0.002	0.55±0.030	38±2.33
NST[3]	0.30±0.001	0.54±0.001	0.85±0.001	0.57±0.014	57±0.58
NST+DSB[4]	0.31±0.001	0.36±0.027	0.67±0.002	0.60±0.008	47±1.58

[1] Drought Stress in Top compartment
[2] Drought Stress in Top compartment combined with Nutrient Stress in Bottom compartment
[3] Nutrient Stress in Top compartment
[4] Nutrient Stress in Top compartment combined with Drought Stress in Bottom compartment
*Standard error of the mean

Drought stress in the upper compartment coupled with nitrogen shortage in the lower compartment

When DST was coupled with NSB, there was no difference in shoot development with the DST-only treatment in the first stage of growth, because there was no uptake taking place in the 0.2-0.40 m layer. At 4 weeks after transplanting, both cultivars subjected to DST+NSB showed a slight reduction in RLD in the upper compartment when compared with the DST-only treatment (Figure 2.6e,f vs. Figure 2.6c,d). For both cultivars, the limited amount of nitrogen available in the lower compartment stimulated slightly more nitrate capture in the upper compartment despite the drought limitation, when compared with the DST-only treatment. At 6 weeks after transplanting, all nitrate available in the lower compartment was captured by the plants. Huge root elongation took place in the upper compartment for both cultivars, combined with a reduction in root elongation in the lower compartment (in comparison with the control). It allowed the plants to capture approximately 70% of the available nitrate in the upper compartment, whereas only approx. 50% of the available nitrate was captured in that

same compartment by plants subjected to DST-only. As for the DST-only treatment, the dry weights of the plants under DST+NSB were reduced by 30%. Whereas for the DST-only treatment final NUE was reduced by approx. 20% for both cultivars, it was only reduced by 10-14% in DST+NSB (Table 2.6). At 2 weeks after transplanting the WUE of both cultivars was significantly increased compared to the control for plants subjected to DST+NSB (Figure 2.7). At 4 weeks after transplanting, there was no difference with the control.

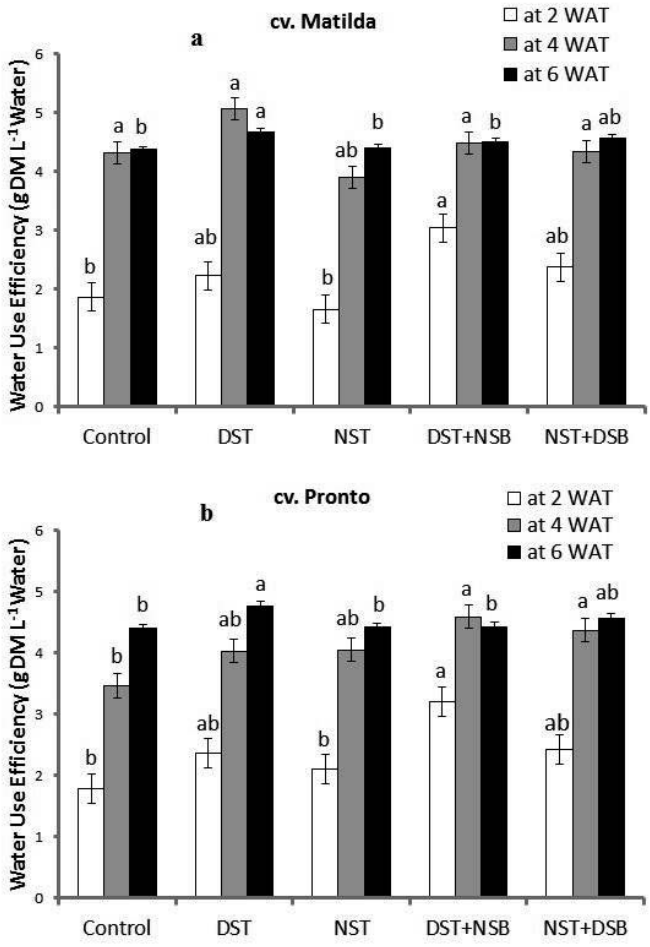

Figure 2.7 Water Use Efficiency for cv. Matilda and cv. Pronto at three sampling dates [2, 4, and 6 Weeks After Transplanting (WAT) correspond to samplings done at 288, 512 and 768 ºCd, respectively]. Comparisons as indicated by the lettering above each bar were carried out within each sampling date between treatment□ cultivar combinations. Threshold of significance was set at p-value ≤ 0.05. Error bars indicate ± one standard deviation.

Nitrogen shortage applied to the upper compartment:

When nitrogen was short in the upper compartment (NST), it had no effect on the plants' development at the first harvest as the amount of nitrate present in the soil was not yet limiting. For both cultivars, RLD was increased by about 30% in all layers except for the 0-0.10 m layer (Figure 2.6g,h vs. Figure 2.6a,b). At 4 weeks after transplanting, still no effect of the nitrogen shortage was visible on the shoot, but root development patterns changed because all nitrate available in the upper compartment was already depleted, as well as 50% of what was available in the lower compartment. At 6 weeks after transplanting, the available nitrate in the pot was entirely depleted for both cultivars. Both cultivars elongated their roots in the lower compartment in order to capture more nitrate, but as it was entirely depleted, it led to some (10%) reduction in shoot growth. The reduced availability of nitrogen in the upper compartment increased the final NUE of both cultivars (Table 2.6) but did not affect the WUE (Figure 2.7).

Nitrogen shortage in the upper compartment combined with drought stress in the lower compartment

No limitation was visible at 2 weeks after transplanting either on the shoot or on the root development. At 4 weeks after transplanting, the drought in the lower compartment combined with the total depletion of available nitrate in the upper compartment triggered extra root growth in the lower layers (NST+DSB, Figure 2.6i,j vs. Figure 2.6g,h). If root elongation enabled nitrate capture levels similar to the control, the drought limited the nitrate capture in the 0.30-0.40 m layer by 60% approximately for both cultivars. At 6 weeks after transplanting, both cultivars reduced their root development in the upper compartment and increased it in the lower compartment, leading to proportional nitrate capture performance similar to the control levels in the 0.20-0.30 m. The final NUE (Table 2.6) and WUE (Figure 2.7) were not affected compared with the control.

2.4 Discussion

2.4.1 Methodological issues

We used 0.40 m deep pots providing the lettuce plants with abundant rooting volume. However, the pot diameter was lower than the plant distance in the field forcing root growth more downwards than in the field. Under the favourable conditions (e.g. root temperature) of these experiments, plants easily exploited the entire reservoir of resources over the full growing period. The results of the intermediate harvests are crucial for adequately interpreting the data as differences between cultivars in rooting patterns were present but short-lived or did not impact final plant performance.

Using Vaseline to prevent movement of resources from one compartment to the other in Trial 2 (Figure 2.1) might have slightly affected our results. In Trial 1, where no Vaseline was used, records for nitrogen taken up from the soil matched values for nitrogen present in the plant well (Table 2.3). In Trial 2 the match was poorer: there was less nitrogen present in the plant than removed from the soil, especially for the control and NST treatment (Table 2.6). We surmise that the Vaseline was a carbon source for soil microbiota that converted nitrate into volatile nitrogenous compounds provided there was enough moisture and root activity, thus causing nitrogen loss.

2.4.2 Limitation-type related responses: drought triggered root proliferation in the dry compartment; nitrate shortage triggered root proliferation in the N-rich compartment

If the observation made in Trial 2 confirmed the existing literature on root proliferation in localized N-rich patches, it also confirmed previous studies about root proliferation in dry soil. Increased root proliferation in the upper compartment in treatment ‘DST’ was consistent with the review of Franco et al. (2011) showing increased root growth in dry soil in case of drought. Indeed, increased root length density in dry soil may have enhanced the surface area available for absorption, minimized localized reduction in soil moisture content around individual roots and helped reducing the resistance to water transport. Moreover, spatially separated combination of drought and nutrient stresses in Trial 2 led to additional effects beyond the separate treatments. The combination between drought in the top compartment and nitrogen shortage in the

bottom compartment ('DST+NSB') led to additional root growth in the top layer especially in the 0.10-0.20 m layer when compared with the 'DST' treatment. The combination of nitrogen shortage in the top compartment and drought in the bottom compartment ('NST+DSB') led to additional root growth in the bottom (0.20-0.40 m) layer when compared with the 'NST' treatment. However the interactions between soil moisture content and nitrate concentration are largely neglected in literature, whereas they may be key to understanding different rooting strategies. Indeed, changes in nitrate concentrations in the soil solution, either due to uptake or local depletion caused by leaching, have been shown to trigger morphological changes in the root response such as root branching, root hair production, root diameter, root growth angle, etc. (Fordes & Lorenzo, 2001), see below.

2.4.3 The root response was triggered by the soil nitrate concentration and the shoot nutritional status

In Trial 1, early drought increased nitrate concentration in the soil solution and reduced nitrate mobility, halting root elongation and therefore increasing shoot: root ratio of both genotypes. In contrast, drought applied at a later stage reduced shoot: root ratio of both cultivars and increased root proliferation in the top 0.10 m (Figure 2.2). These different strategies were the result of the plant's developmental stage (and therefore their nutritional status) and changes in soil nitrate concentration (the nitrate concentration in the soil was probably lower during late drought, as total nitrate capture was larger at the beginning of the late drought than of at the beginning of the early drought). These strategies might illustrate what Forde & Lorenzo (2001) called a 'trophomorphogenic' response, i.e. a "change in plant morphology arising from variations in the availability or distribution of nutrient in the environment". They claimed that "trophomorphogenic responses may be direct (localised responses resulting from changes in external nutrient status), indirect (systemic responses resulting from changes in the plant's internal nutrient status), or a combination of the two". It is likely that the changes observed in root development - partly as a function of the timing of drought application - were linked with both changes in the soil nitrate concentration and the plant nutritional status. At Early Drought, the plants were relatively small (6 g fresh weight) and their transpiration and nitrogen requirements

were relatively limited. In contrast, at late temporary drought, the plants were much bigger (85 g fresh weight) requiring a higher water and nitrate supply. Therefore, the need for water to sustain growth was more crucial during late drought than during early drought and may have triggered root proliferation in the top layer. While the overall observation is that drier soil leads to more roots (Franco et al., 2011; this study) this reaction may not occur at early, temporary drought (Trial 1).

2.4.4 Root morphological versus physiological responses

In Trial 1, nitrate inflow was stimulated in the lower part of the pot during drought (Figure 2.3). As mentioned by Vuuren et al. (1996) inflow might be stimulated as a short term response to a localized concentration of nutrients. The nitrate concentration in the lower part of the pot was probably higher than in the top because root development was less important, so less nitrate had been absorbed in that part of the pot and therefore more nitrate remained available for uptake. Moreover, because of their spatial location, these lower layers were probably subjected to less intensive drought than the top layers and therefore nitrate possibly moved towards the roots with more ease through bulk flow or by diffusion, and consequently an increased nitrate inflow was possible. According to Vuuren et al. (1996) increased nitrate inflow is a short term and transient solution which therefore may have helped lettuce to sustain the temporary drought and consequent nitrate immobilization in the top part of the pot, where all the roots were present. As Robinson (2001) mentioned, the carbon necessary for root proliferation and maintenance may be too costly and enhanced nitrate inflow may have been a preferred strategy.

2.4.5 Root morphological responses: Root morphology plasticity as a feed-forward mechanism to compensate for resource limitation

A feed-forward mechanism keeps an output steady by modifying its input course under an external disturbance (Schulze, 1994). In treatment NST of Trial 2, the prompt depletion of nitrate due to its limited amount in the upper compartment led to root proliferation in the lower compartment (Figure 2.6g,h). Root growth was further enhanced when there was less water in the bottom compartment (Figure 2.6i,j) . It might be hypothesized that the change in nitrate concentration in the soil, as well as the

difference in concentrations between the two layers might have been the external triggers to this modification in spatial root growth. This morphological change allowed the root system to maintain nitrate capture synchronous with the amount of nitrate required to sustain shoot growth, until all nitrate available from upper and lower compartments was fully depleted. Morphological changes in root development associated with exploring N-rich patches have been well documented (Vuuren et al., 1996; Miller & Cramer, 2005; Robinson, 1994, 2001; Hodge, 2004). Those studies showed that root proliferation occurred when roots growing in an N-poor environment encountered an N-rich patch, questioning the eventuality that initial development in an N-poor patch may have "primed" the root system for increased inflow and eventually later proliferation when encountering an N-rich patch (Robinson, 2001; Mingo et al., 2004). In addition to the fact that this mechanism could possibly explain the observation made in Trial 2, it might also explain observations in Trial 1, where nitrate inflow first increased during drought, and was followed by an increase in the root elongation rate (relative to the control) during the recovery following the drought, when re-watering restored nitrate availability.

2.4.6 Absence of cultivar differences

The two cultivars used in this study did not show different coping strategies in terms of root proliferation or resource capture in these pot trials. Indeed, the two cultivars did not express significant differences for any of the direct above- or below-ground measurements. The fact that their responses were also very similar in quantitative terms, contributes to the credibility of the physiological responses and of the methodology of our experimentation. Significant, although minor, cultivar differences did occur in calculated parameters (such as nitrogen use efficiency, nitrate inflow per unit of root length or root expansion rate; Table 2.3, Figs 2.3 and 2.5). Apart from those minor differences, both cultivars reacted remarkably similarly to the stress application in the two experiments, illustrating that creating stress, especially drought stress, may level genotypic differences in favour of the expression of adaptive responses (Franco et al., 2011). This suggests that the physiological mechanisms observed in abiotic stress

tolerance are conserved in pot trials. However, further research will be needed to define in which parameters genotypic variation is to be expected under field conditions.

2.5 Concluding remarks

This study aimed at investigating the effect of temporal and/or spatial heterogeneities in soil conditions on root growth and resource capture, with the objective to identify genotypic differences in responses, and at proposing new elements to be incorporated in existing models as tools to predict root distribution profiles in field trials using resource capture measurements. The results highlighted that changes in root morphology and/or activity are 'feed-forward' mechanisms that sustain shoot growth in a resource-limited environment. The type of limitation (drought or nitrate shortage), associated with the nutritional status of the shoot, triggers different root morphological and physiological responses. The small genotypic variations found in root traits in this study underlines the dominating effect of resource limitation on adaptations in below-ground traits to sustain shoot growth. Field trials have been carried out to confirm the validity of these findings and results will be published in Chapters 3 and 4. As Robinson (1994) underlined, roots do not always react to a local deficiency in nutrient supply, but when they do, the response of the root system may be predicted in general terms but the detailed patterns and their implications for resource capture, as well as the repercussions on shoot development are much more difficult to evaluate. This work will enable further improvement to the modelling of root growth and resource capture under localized stress conditions.

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Chapter 3

Influence of transplant size on the above- and below-ground performance of four contrasting field-grown lettuce cultivars

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Abstract

Modern lettuce cultivars underperform under conditions of variable temporal and spatial resource availability, common in organic or low-input production systems. Information is scarce on the impact of below-ground traits on such resource acquisition and performance of field-grown lettuce; exploring genetic variation in such traits might contribute to strategies to select for robust cultivars, i.e. cultivars that perform well in the field, even under stress. To investigate the impact of below-ground (root development and resource capture) on above-ground (shoot weight, leaf area) traits, different combinations of shoot and root growth were created using transplants of different sizes in three field experiments. Genetic variation in morphological and physiological below- and above-ground responses to transplant shocks was assessed using four cultivars. Transplanting over-developed seedlings did not affect final yield of any of the four cultivars. Small transplant size persistently impacted growth and delayed maturity. The cultivars with overall larger root weights and rooting depth, 'Matilda' and 'Pronto', displayed a slightly higher growth rate in the linear phase leading to better yields than 'Mariska' which had a smaller root system and a slower linear growth despite a higher maximal exponential growth rate. 'Nadine', which had the highest physiological nitrogen-use efficiency (g dry matter produced per g N accumulated in the head) among the four cultivars used in these trials, gave most stable yields over seasons and trial locations. Robustness was conferred by a large root system exploring deep soil layers. Additional root proliferation generally correlates with improved nitrate capture in a soil layer and cultivars with a larger root system may therefore perform better in harsh environmental conditions; increased nitrogen use efficiency can also confer robustness at low cost for the plant, and secure stable yields under a wide range of growing conditions.

Keywords: lettuce; transplanting; root activity; nutrient use efficiency

3.1 Introduction

In organic or low-input production systems, nutrient availability is more dependent on the soil's biological, chemical and physical processes that influence mineralization of organic fertilizers than in conventional, high-external input production systems. Indeed, in conventional systems fertilization is provided in a mineral form and nutrients are therefore readily available for uptake by the plants once they are sown or transplanted. In lettuce, the impact of variable temporal or spatial shortage of water and nutrients common in organic production systems may significantly reduce final yields, as shown in Chapter 2. In lettuce, like in other crop plants, breeding has mainly focused on aboveground characteristics, and modern cultivars have been bred for high-input production systems; these cultivars are characterized by large heads and small root systems (Johnson et al., 2000). The small root systems perform sufficiently in such intensive systems.

Current cultivars also have a shallow root system, concentrated in the top 0.20 m of the soil profile (Johnson et al., 2000) which limits the access to deeper soil zones rich in water and nutrients that have leached through the profile. This root morphotype can affect shoot performance under organic conditions, which entail high temporal and spatial variability of resources availability. Exploring the impact of morphological (e.g. spatial configuration) and physiological (e.g. resource capture efficiency) root traits on shoot growth of lettuce may thus be interesting when evaluating the field performance of cultivars under organic conditions. Such investigation might be valuable in breeding programmes, as a mean to select genotypes with desirable root traits increasing tolerance to abiotic stresses and consequently improved yield stability (Bengough et al., 2013). One way to study the impact of below-ground processes– i.e. root growth and resource capture – on shoot growth of lettuce in field conditions is to impact the equilibrium existing between shoot and root growth, by, for instance, altering the shoot:root ratio during the growth. An easy way to manipulate the shoot:root ratio of lettuce during growth is to use different shoot:root ratios at transplanting.

Transplanting is a common horticultural practice, which aims at increasing productivity in horticultural systems. In Western Europe, field-grown lettuce crops are established

from transplants raised in compact peat blocks in greenhouses; because seeds germinate faster and more uniformly in peat blocks than in the field, transplanted crops are more competitive towards early weed infestation (Maltais et al., 2008) and provide a more uniform stand, thus facilitating crop scheduling (Cattivello and Danielis, 2008), reducing cropping time and allowing more plantings per year in the same field. However, transplanting induces a major stress in lettuce cultivation: lettuce seedlings in the optimal stage for transplanting (5-7 leaf stage) often suffer from mechanical root pruning (decapitation of the root tip; Biddington and Dearman, 1984) when seedlings are pulled out of the tray. The loss of root tips and root hairs due to root pruning at transplanting disturbs the shoot:root ratio and induces a 'recovery phase' during which shoot growth is suppressed until the previous shoot:root ratio is restored (Bar-Tal et al., 1994a).

During this 'recovery phase' capture of water (Grossnickle, 2005) and of nutrients (Bar-Tal et al., 1994b) is impaired to levels below requirements. Moreover, there is an imbalance in root and shoot hormones (Overvoorde et al., 2010) and additional assimilates are allocated to the roots to heal root injuries and restore root growth (Bastow Wilson, 1988). Nevertheless, moderate root pruning at transplanting, despite the need for a 'recovery phase', seems to hardly affect final yields: for instance, Bar-Tal et al. (1994a) found that fruit number or total fresh fruit yield were not significantly reduced in tomato plants whose roots were mildly pruned at transplanting, compared with plants whose roots stayed intact at transplanting. In a recent study, Ros et al. (2003) found that 40% root pruning of rice seedlings at transplanting had only a small effect on shoot growth, reducing grain yield and straw dry matter at maturity by a mere 10%. These findings were established for crops like rice, that require a long field growth; it is unclear what the consequences of root pruning could be on a short cycle crop like lettuce, which is usually harvested within 100 days of field growth (Mou, 2011).

The small or short-lasting effect of root pruning on shoot growth implies that plants are plastic and able to overcome physical damage and adjust to their environment. Plants developed strategies to overcome the loss of root tips and root hairs at transplanting and to compensate for the subsequent impaired resource capture. For instance, Bar-Tal et al.

(1994b) found that root pruning in tomato temporarily increased relative growth rate of the pruned roots compared to the intact roots and that nitrogen uptake per unit root volume was larger for plants with pruned root systems than for intact ones. Cattivello and Danielis (2008) showed that chemical root pruning in a selection of vegetables (asparagus, celery, Treviso chicory, fennel, lettuce, and parsley) resulted in a more fibrous and branched root system and had no long-term impact on yield.

In lettuce, the contribution of root traits to field performance has not yet been investigated. It is not clear yet how plastic the plants are in displaying an adaptive response to stresses in the field, and what the contribution is of root morphological (changes in root spatial exploration) or root physiological (resource uptake for instance) traits to shoot development. We used different types of shocks caused by transplanting as a proxy for stress induction. By creating three levels of stress using three growth stages (i.e. differences in root:shoot ratios and in size) at transplanting, we expect to observe different responses in shoot growth that may be explained by below-ground cues, such as root growth and nitrate uptake.

Moreover, breeders assume that there might be considerable genetic variation in the capacity of lettuce plants to recover from transplanting, based on field observations (Velema and Koper, *pers. comm.*). This suggests that cultivars may develop various strategies below- and above-ground to overcome the disturbance in shoot:root ratio created by transplanting. This study also aims at identifying genetic variation in the physiological below- and above-ground responses to different types of transplant shocks.

3.2 Material and methods

3.2.1 Cultivar choice and growing transplants

Four commercial butter head cultivars, ‘Mariska’, ‘Matilda’, ‘Nadine’ and ‘Pronto’, were chosen. These were known for their robust performance in the field, but also for differences in growth pattern. In a previous pilot study they also showed contrasting rooting patterns (deep vs. superficial) (Den Otter and Lammerts van Bueren 2007). These cultivars are commonly sold to conventional and organic growers for cropping in

spring, summer and autumn seasons and have been performing consistently over many years (Enza Zaden, *pers. comm*.).

Seeds used in each of these experiments originated from seed lots produced under the same environmental conditions. Seeds were sown in 4 × 4 × 4 cm organic peat blocks (Jongerius, Houten, the Netherlands) after breaking seed dormancy by exposure to 4 ºC for 24 hours. Transplants were raised in a greenhouse with day temperature of 20 ºC and night temperature of 15 ºC.

3.2.2 Experimental design

Three trials were implemented at two different locations: Wageningen (51.97° N, 5.67° E, The Netherlands) in spring 2009 and 2010 and Voorst (52.23° N, 6.08° E, The Netherlands) in summer 2009. Each trial included three repetitions. The experimental set up was a complete randomized block design, each block consisting of 12 plots featuring all combinations of four cultivars and three transplant sizes.

3.2.3 Field conditions

For each trial, weather data (air temperature, radiation, rainfall) were recorded daily (Voorst) or hourly (Wageningen) at the nearest weather station (for the Wageningen trials, data were collected from http://www.met.wau.nl/ and for the Voorst trials, data were collected from the on-farm weather station). Soil temperatures were measured at 4 to 5 depths (0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4 and 0.4-0.5 m) using a data logger. Air and soil temperatures recorded during the growing season at Wageningen in spring 2009 were fairly conducive to crop growth, average daily air temperatures ranging from 9.5 to 20 ºC and average daily soil temperatures at -0.25 m ranging between 10 and 16 ºC. Rainfall was rather limited during the experiment (Table 3.1) but there was no drought stress. In contrast, rainfall during the early spring trial at Wageningen in 2010 was abundant, but air temperatures were rather low: during 36 days (i.e. half of the growing period) the daily mean temperature did not exceed 9.5 ºC. Average daily soil temperatures recorded at -0.25 m ranged between 6 and 15 ºC during growth, and did not exceed 10 ºC during the first month of growth. Experiment Voorst 2009 was conducted during late spring under warm weather. The average daily air and soil temperatures at -0.25 m were 16.5 ºC and 17 ºC respectively, with air temperatures

above 13 ºC during 85% of the growing period. Soil temperatures at -0.25 m ranged between 15.5 and 20 ºC. Cumulated degree-days (based on air temperatures), as well as cumulated rainfall and irrigation (in the case of Voorst 2009) at each sampling date for each trial, are shown in Table 3.1.

Table 3.1 Planting and harvesting dates and cumulated thermal time (CDD) and rainfall at the three sampling moments for each of the three field trials.

	Wageningen 2009		Voorst 2009		Wageningen 2010	
Planting date	1 April 2009		25 May 2009		23 March 2010	
	CDD[1]	Rainfall (mm)	CDD	Rainfall (mm)	CDD	Rainfall (mm)
Root sampling 1	111	7.3	152	20.0	152	35.5
Root sampling 2	224	21.5	253	77.4	252	60.4
Root sampling 3	325	32.4	420	83.4	347	91.3
Final harvest date	31 May 2009		30 June 2009		31 May 2010	

[1]Cumulated Degree-Days (ºCd) after planting at sampling date based on air temperature, using a base temperature of 4 ºC

3.2.4 Treatments

Transplanting shocks were used as a proxy for stress induction: seedlings at different growth stages at the moment of transplanting presented different qualities of transplants; three contrasting transplant sizes were obtained by staggered sowings with intervals of 2 weeks. These differences in growth duration before transplanting resulted in intertwined variations in shoot characteristics (number of leaves, and consecutive leaf area) and in root characteristics (root length and mass, not measured at transplanting because of the organic matter in the peat blocks), and associated with the latter also in different levels of damage of the root system at transplanting:

- ‘Over-Developed’ (OD) transplant size: 7-9 leaf-stage, developed root system largely emerging out of the peat block, many roots tips mechanically removed at transplanting; both changing the root:shoot ratio and causing mechanical damage, in addition to the physiological shock of rather large seedlings;
- ‘Normally Developed’ (ND) transplant size: 5-leaf stage, only few roots emerging out of the peat block, some root tips mechanically removed at transplanting, hardly any mechanical damage or root:shoot ratio change;

- 'Under-Developed' (UD) transplant size: 3-leaf stage, no visible roots emerging from the peat block except the tap root which was damaged at transplanting; the shock here was mainly the early transplanting of rather small seedlings.

Crop plants raised from these treatments are called 'OD plants', 'ND plants' and 'UD plants', respectively. In Voorst 2009 damage caused by a hail storm hastened final harvest by approximately 2 weeks, and therefore harvested plants were not fully mature; as UD plants formed heads very late they were not harvested. The final harvest date in the Wageningen trials was determined according to the marketable stage of head maturation for the ND plants. All treatments were harvested at the same date, no matter head maturation stages (which was visually not affected by the treatments at final harvest).

3.2.5 Field management

All trial fields had been organically managed and were selected for uniform management in the past and for adequate soil structure. They were fertilized prior to transplanting with 100 kg/ha nitrogen, from seaweed pellets (9% N, 3% P, 3% K + 3% MgO, EcoFertiel, EcoStyle, Appelscha, The Netherlands). Weeding was done manually every week. Irrigation was only provided at Voorst in 2009: 10 mm water was given 20 days after transplanting.

3.2.6 Measurements

Calculation of thermal time

Cumulated degree days at each sampling date were calculated as the sum, between the date of transplanting and the sampling date, of the degrees above 4 ºC (base temperature for lettuce), based on an average daily temperature:

$$CDD_{sampling\ x} = \sum_{day\ 0}^{sampling\ date\ x} \left[\frac{(T_{max}+T_{min})}{2} - T_{base}\right]$$

where T_{max} and T_{min} correspond respectively to the maximum and to the minimum temperatures recorded on a certain day, respectively.

Shoot measurements

Fresh weight, dry weight, total leaf area and total number of leaves of three plants per plot were assessed weekly. Final harvest took place 6-10 weeks after transplanting

depending on trial. For samples taken at final harvest total nitrogen in the head was measured using the Kjeldahl method. Physiological Nitrogen Use Efficiency (NUE, g DM g^{-1} N in head) was calculated based on the head [N] (g N kg^{-1} DM) extracted by the Kjeldahl method: NUE = 1/head [N].

Root measurements

Roots outside the peat block of three plants per plot were sampled at three moments during growth, and at two positions ('central' and 'peripheral') for each plant using the method described by Van Noordwijk et al. (1985) (Figure 3.1). Using a cylindrical auger of 0.07 m diameter and 0.1 m height, samples were taken every 0.1 m over a depth of 0.5 m. For each sample, roots were rinsed from soil and most organic matter using a rinsing machine and remaining organic matter was then manually removed using tweezers. Root samples were subsequently scanned and root length was measured using WinRhizo Pro 2007 (v2005b, Regent Instruments, Québec, Canada). Root dry matter was measured after drying the root samples at 105 °C for 24 hours. Root Mass Density per layer (mg root dry weight g^{-1} soil) was calculated as root dry weight measured in the sample taken with the auger, divided by the product of the volume of soil in the sample taken and the bulk density of that soil (based on dry weight).

Soil measurements

Soil samples were taken simultaneously on the opposite side of the same plants (Figure 3.1). For three plants per plot, soil samples were pooled to account for plant-to-plant variation. Soil moisture content was recorded after drying at 40 °C for 48 hours and soil nitrate content (soil $[NO_3]$) was measured using an Ion Selective Electrode (ThermoFisher, Waltham, MA, USA) using the method described previously by Sibley et al. (2009) and also used in Kerbiriou et al. (2013). As a measure for the difference between treatments in estimated NO_3 capture, the difference between the average soil $[NO_3]$, based on pooled data for all cultivar × transplant size combinations within a layer, and the soil $[NO_3]$ measured on an individual plot was expressed as percentage difference in estimated NO_3 capture. This was calculated as:

$$\% \text{ difference for sample } i = 100 \times (([NO_3]_i / [NO_3]_{avg}) - 1)$$

Where

$[NO_3]_i$ = observed $[NO_3]$ in sample *i* on sampling date *d* and for soil layer *l*

$[NO_3]_{avg}$ = the average observed $[NO_3]$ in all samples on sampling date *d* and for soil layer *l*.

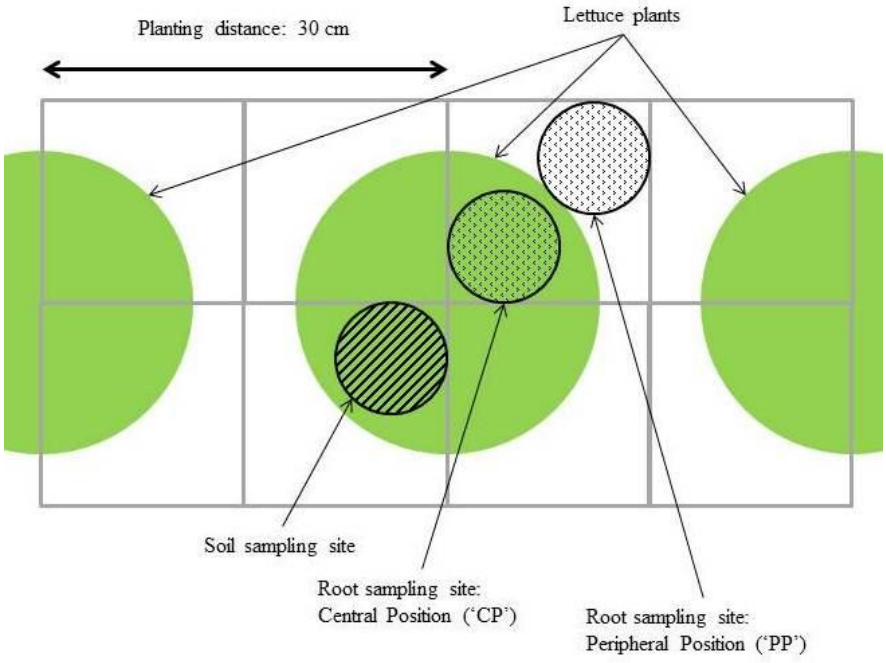

Figure 3.1 Root and soil sampling scheme, adapted from Van Noordwijk et al. (1985).

3.2.7 Statistical analyses

Dry weight and total leaf area data of all harvests for each trial were pooled per plot and a regression analysis was performed using the expolinear model of Goudriaan & Monteith (1990) to obtain estimates of the curve fit parameters for each combination of transplant size × cultivar × replicate. Then a two-way ANOVA was performed on those parameters to determine main effects of stage at transplanting (UD, ND and UD), cultivar and their interactions, followed by a Tukey test with a threshold of significance set at *p-value* ≤ 0.05 to determine the statistical significance of the differences.

Moreover, for each sampling date for each trial a two-way ANOVA was performed followed by the Tukey test at *p-value* ≤ 0.05 to determine the statistical significance of the differences for the shoot, root and soil measurements.

Curve fitting and statistical analyses were performed with Genstat 15th Edition (Hempstead, UK).

3.3 Results

3.3.1 Effect of transplant size on shoot growth and development

The overall effects of transplant size on dry matter accumulation and total number of leaves decreased in time after transplanting (cf. Figure S1, supplementary material). Differences between the Over-Developed- ('OD') or the Under-Developed ('UD') plants and the Normally-Developed ('ND') plants when expressed in percentages were larger for dry matter accumulation than for total number of leaves, and these differences disappeared faster for the OD plants than for the UD plants (cf. Fig. S1A and B). After 200 ºCd there was less than 20% difference in dry matter between the OD and the ND plants, whereas this level was reached by 500 ºCd for the UD plants. No cultivar differences were observed. The same trends were observed in all experiments.

Dry matter accumulation

Differences in growing conditions affected the dry matter accumulation of the four cultivars, independently of stage at which they were transplanted, although all followed a typical expolinear growth pattern (Goudriaan and Monteith, 1990; Figure 3.2). Overall warmer growing conditions recorded during Voorst 2009 led to a higher maximal relative growth rate during the initial exponential growth phase, a lower maximal growth rate during the linear growth phase, and a reduced 'lag phase' (time at which the asymptote of the expolinear growth curve meets the time abscissa, cf. Figure 3.2), compared to the trials conducted in Wageningen in 2009 and 2010 (Table 3.2).

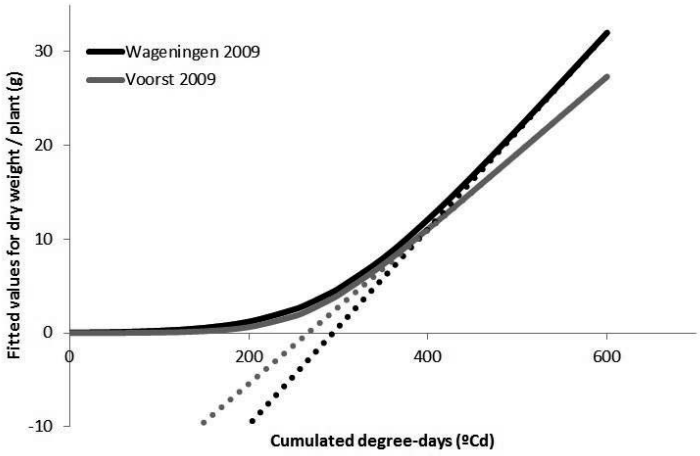

Figure 3.2 Fitted values for dry weight accumulation over thermal time (based on average curve parameters for cultivars and transplant sizes within a trial, cf. Table 3.2) in Wageningen 2009 and Voorst 2009. The asymptotes to the expolinear curves cut the x-abscissa at the values obtained for 'lag phase' which are, in this case, 293 ºC for Wageningen 2009 and 262 ºC for Voorst 2009.

Maximal relative growth rate during exponential phase

During the exponential growth phase, OD plants had a significantly smaller maximal relative growth rate than ND and UD plants, while no differences were observed between ND and UD in Wageningen 2009 and 2010. In Wageningen 2009 'Mariska' had the highest maximal relative growth rate for all transplant sizes. The two-way interaction was not significant in Wageningen 2009 and 2010, while it was in Voorst 2009. Here the same trend was observed as in the Wageningen trials but only the maximal relative growth rate of 'Mariska' ND plants was different from all other treatments.

Maximal growth rate during the linear phase

No significant effect of transplant size was recorded on the maximal growth rate during the linear phase in any of the three trials. 'Mariska' had a significantly lower growth rate than the other cultivars in the linear phase for all transplant sizes in Wageningen 2009 and Voorst 2009. The same trend was observed in Wageningen 2010, albeit not significant (p-value = 0.058). No two-way interactions were significant.

Table 3.2 Values for curve fit parameters when applying an expolinear model (Goudriaan & Monteith, 1990) for dry matter accumulation against thermal time, for combinations of transplant sizes and cultivars in each of three experiments.

	Maximal relative growth rate in the exponential phase (mg g^{-1} (°Cd)$^{-1}$)					**Maximal growth rate in the linear phase (mg DM m^{-2} (°Cd)$^{-1}$)**					**'Lag phase' (°Cd)**				
Cultivar:	Mariska	Matilda	Nadine	Pronto		Mariska	Matilda	Nadine	Pronto		Mariska	Matilda	Nadine	Pronto	
TS[6]	**Wageningen 2009**				*Tr.*[4]	**Wageningen 2009**				*Tr.*	**Wageningen 2009**				*Tr.*
OD[1]	15.9	14.4	15.4	14.7	*15.1*a	91	109	110	112	*106*a	267	306	299	304	*294*ab
ND[2]	17.7	16.5	16.8	16.8	*16.9*b	93	102	113	104	*103*a	265	292	292	283	*283*a
UD[3]	18.8	16.5	16.3	17.9	*17.4*b	93	104	116	106	*105*a	279	318	323	296	*304*b
Cv.[5]	*17.5*b[7]	*15.8*a	*16.2*ab	*16.5*ab		*92*a	*105*b	*113*b	*107*b		*271*a	*305*b	*305*b	*294*ab	
	Wageningen 2010				*Tr.*	**Wageningen 2010**				*Tr.*	**Wageningen 2010**				*Tr.*
OD	16.0	14.9	14.7	14.9	*15.1*a	103	180	145	140	*142*a	255	311	291	286	*286*a
ND	16.5	18.2	17.3	16.8	*17.2b*	132	140	125	130	*132*a	283	273	274	278	*277*a
UD	16.7	19.3	19.4	18.6	*18.5b*	126	129	123	113	*123*a	316	319	309	309	*313*b
Cv.	*16.4*a	*17.5*a	*17.1*a	*16.7*a		*121*a	*149*a	*131*a	*128*a		*285*a	*301*a	*291*a	*291*a	
	Voorst 2009				*Tr.*	**Voorst 2009**				*Tr.*	**Voorst 2009**				*Tr.*
OD	21.1a	18.5a	20.7a	16.5a	*19.2*	68	100	78	101	*87*a	208	267	222	277	*244*a
ND	41.3b	24.8a	25.2a	22.4a	*28.4*	48	105	75	80	*77*a	218	330	303	303	*288*b
UD	-	-	-	-		-	-	-	-		-	-	-	-	
Cv.	*31.2*	*21.7*	*23.0*	*19.5*		*58*a	*103*b	*76*ab	*90*b		*213*a	*299*b	*262*b	*290*b	

[1]'Over-Developed' transplant size; [2]'Normally Developed' transplant size; [3]'Under-Developed' transplant size
[4]Mean for transplant size across cultivars; [5]Mean for cultivar across transplant sizes; [6]Transplant size
[7]Means with different letters indicate a significant difference at $p \leq 0.05$ – means separation with lettering is indicated for each single parameter within an experiment and at the level of main factors cultivar or transplant size when the two-way interaction was not significant, and at the level of transplant size × cultivar when the interaction was significant

'Lag phase'

UD plants had a longer lag phase in both Wageningen trials than OD and ND plants. In Voorst 2009, OD plants had a shorter lag phase than ND plants (Table 3.2). In Wageningen 2009 and Voorst 2009, 'Mariska' had a significantly shorter lag phase than other cultivars across transplant sizes (Table 3.2). No two-way interactions were significant.

Dry weight at final harvest

While there was no significant effect of transplant size on dry weight at final harvest in Wageningen 2009, cultivar differences were visible, with 'Mariska' having the lowest dry weight at final harvest and 'Nadine' performing the best (Table 3.3). In Wageningen 2010, significant interactions between transplant size and cultivar effects were recorded. No significant difference at $p \leq 0.05$ was found between cultivars within the UD and the ND transplant size. OD plants of 'Matilda' and 'Nadine' had higher final dry weights than OD plants of 'Mariska'. Whereas UD plants of 'Matilda' and 'Pronto' had significantly smaller dry weights at final harvest compared to ND and OD plants of these cultivars, for 'Mariska' and 'Nadine' there was no significant effect of transplant size on dry weight at final harvest.

In Voorst 2009, OD plant had significantly higher dry weight at final harvest than ND plants (Table 3.3). 'Matilda' had a significantly higher final dry weight per plant than other cultivars across transplant sizes, whereas 'Mariska' had the lowest dry weight at final harvest across transplant sizes.

(Shoot dry weights measured at intermediate root samplings are presented in the supplementary materials, Tables S1, S2)

Leaf area expansion

Interestingly no significant cultivar effect was found on the curve fit parameters of an expolinear model on leaf area expansion (Table 3.4). On the other hand, size at transplanting significantly affected the leaf area expansion rates of the plants both during the exponential and the linear growth phases.

Maximal relative leaf area expansion rate during the exponential phase

In Wageningen 2009 and Wageningen 2010, UD plants of all cultivars had a significantly higher maximal relative leaf expansion rate during the exponential phase than ND and OD plants (Table 3.4). In Wageningen 2009, OD plants had a significantly lower maximal relative leaf expansion rate during the exponential phase than ND plants (Table 3.4).

Maximal leaf area expansion rate during the linear phase

In Wageningen 2009, the leaf expansion rate of the UD and ND plants of all cultivars was reduced during the linear phase compared to OD plants (Table 3.4).

'Lag phase'

A significantly longer lag phase was found for the OD plants of all cultivars compared to ND and UD plants (Table 3.4) only in Wageningen 2009.

Table 3.3 Average shoot dry weights (g per plant) of the four cultivars at final harvest, after establishment from three different transplant sizes in each of three trials.

Harvest Date	CDD[6] (°Cd)	TS[8]	Mariska	Matilda	Nadine	Pronto	
			Wageningen 2009				*Tr.*[5]
May 25th, 2009	474	OD[1]	30.3±2.3[7]	32.1±3.1	33.9±4.9	33.1±2.0	*32.3*a
		ND[2]	31.0±2.1	32.3±2.1	35.3±2.4	33.0±3.2	*32.7*a
		UD[3]	30.2±2.5	30.0±2.0	33.3±2.7	33.0±2.1	*31.6*a
		Cv.[4]	*30.5*a[9]	*31.5*ab	*34.1*c	*32.8*bc	
			Wageningen 2010				*Tr.*
May 30th, 2010	400	OD	25.4±2.2abcde	34.0±4.7g	31.3±2.6fg	29.7±3.5efg	*30.1*
		ND	29.1±2.1cdefgh	33.0±3.8fg	29.4±2.9defg	28.5±3.5bcdef	*30.0*
		UD	23.5±1.8ab	24.0±3.1abc	24.3±3.5abcd	22.4±4.7a	*23.6*
		Cv.	*26.0*	*30.4*	*28.3*	*26.9*	
			Voorst 2009				*Tr.*
June 29th, 2009	420	OD	18.5±1.9	22.6±2.7	20.5±2.5	21.5±2.7	*20.8*b
		ND	13.1±2.0	17.5±2.1	14.2±2.2	14.8±3.3	*14.9*a
		UD	-	-	-	-	
		Cv.	*15.8*a	*20.1*c	*17.4*ab	*18.2*b	

[1]'Over-developed' transplant size; [2]'Normally developed' transplant size; [3]'Under-developed' transplant size; [4]Mean for cultivar across transplant sizes; [5]Mean for transplant size across cultivars; [6]Cumulated Degree-Days; [7]Standard error of the mean; [8]Transplant Size; [9]Means with different letters indicate a significant difference at $p \leq 0.05$ – means separation with lettering is within an experiment and at the level of main factors cultivar or transplant size when the two-way interaction was not significant and at the level of transplant size × cultivar when the interaction was significant.

Table 3.4 Values for curve fit parameters when applying an expolinear model (Goudriaan & Monteith, 1990) for leaf area expansion against thermal time.

	Maximal relative growth rate in the exponential phase ($mm^2\ cm^{-2}\ (°Cd)^{-1}$)					**Maximal leaf expansion rate in the linear phase ($cm^2\ m^{-2}\ (°Cd)^{-1}$)**					**Time lost during canopy development before all radiation is intercepted (°Cd)**				
Cultivar:	Matilda	Mariska	Nadine	Pronto		Matilda	Mariska	Nadine	Pronto		Matilda	Mariska	Nadine	Pronto	
TS[6]	**Wageningen 2009**				*Tr.*[4]	**Wageningen 2009**				*Tr.*	**Wageningen 2009**				*Tr.*
OD[1]	1.46	1.36	1.51	1.36	*1.42*a	40.6	43.1	41.9	45.2	*42.7*b	309	350	330	353	*335*b
ND[2]	1.64	1.65	1.56	1.57	*1.60*b	34.9	27.3	38.5	36.3	*34.2*ab	291	285	321	307	*301*a
UD[3]	1.77	1.67	1.73	1.83	*1.75*c	37.6	24.5	27.9	35.1	*31.3*a	305	301	309	300	*304*a
Cv.[5]	*1.62*a[7]	*1.56*a	*1.60*a	*1.59*a		*37.7*a	*31.6*a	*36.1*a	*38.8*a		*301*a	*312*a	*320*a	*320*a	
	Wageningen 2010				*Tr.*	**Wageningen 2010**				*Tr.*	**Wageningen 2010**				*Tr.*
OD	1.53	1.58	1.38	1.57	*1.52*a	31.5	40.0	45.5	35.0	*38.0*a	290	304	355	302	*313*a
ND	1.54	1.63	1.56	1.65	*1.60*a	41.8	36.5	36.0	38.2	*38.1*a	321	308	325	308	*316*a
UD	1.83	1.65	1.84	1.70	*1.75*b	30.9	40.0	29.1	35.5	*33.9*a	308	366	329	352	*339*a
Cv	*1.63*a	*1.62*a	*1.60*a	*1.64*a		*34.7*a	*38.9*a	*36.9*a	*36.2*a		*307*a	*326*a	*336*a	*321*a	

[1]'Over-Developed' transplant size; [2]'Normally Developed' transplant size; [3]'Under-Developed' transplant size; [4]Mean for transplant size across cultivars; [5]Mean for cultivar across transplant size; [6]Transplant size; [7]Means with different letters indicate a significant difference at $p \leq 0.05$ – means separation with lettering is indicated for each single parameter within an experiment and only at the level of main factors cultivar and transplant size as the two-way interactions were not significant.

Table 3.5. Average estimated root dry weights (g per plant) of the four cultivars at third root sampling, after establishment from three different transplant sizes.

Harvest Date	CDD[6] (ºCd)	TS[8]	Mariska	Matilda	Nadine	Pronto	
			Wageningen 2009				*Tr.*[5]
May 11th, 2009	325	OD[1]	0.39±0.14[7]	0.47±0.27	0.44±0.12	0.48±0.21	*0.44*a
		ND[2]	0.35±0.14	0.55±0.26	0.47±0.18	0.53±0.22	*0.48*a
		UD[3]	0.36±0.10	0.42±0.15	0.46±0.17	0.47±0.17	*0.43*a
		Cv.[4]	*0.37*a	*0.48*ab	*0.46*ab	*0.49*b	
			Wageningen 2010				*Tr.*
May 25th, 2010	347	OD	0.61±0.22	0.68±0.12	0.52±0.17	0.67±0.20	*0.62*b
		ND	0.58±0.17	0.63±0.22	0.66±0.32	0.74±0.22	*0.65*b
		UD	0.40±0.07	0.48±0.19	0.57±0.22	0.55±0.17	*0.50*a
		Cv.	*0.53*a	*0.60*a	*0.59*a	*0.65*a	
			Voorst 2009				*Tr.*
June 29th, 2009	420	OD	0.18±0.03	0.28±0.06	0.24±0.12	0.29±0.09	*0.25*a
		ND	0.12±0.10	0.24±0.11	0.15±0.05	0.29±0.10	*0.20*a
		UD	-	-	-	-	-
		Cv.	*0.15*a	*0.26*bc	*0.20*ab	*0.29*c	

[1]'Over-developed' transplant size; [2]'Normally developed' transplant size; [3]'Under-developed' transplant size; [4]Mean for cultivar across transplant sizes; [5]Mean for transplant size across cultivars; [6]Cumulated Degree-Days; [7]Standard error of the mean; [8]Transplant Size; [9]Means with different letters indicate a significant difference at $p \leq 0.05$ – means separation with lettering is within an experiment and at the level of main factors cultivar or transplant size as the two-way interaction was not significant.

3.3.2 Effect of transplant size on root growth and resource capture

Root dry weights

In Voorst 2009, overall measured root dry weights were much lower than in Wageningen 2009 and Wageningen 2010 due to the precocious termination of the trial (Table 3.5).

In Wageningen 2009 and Voorst 2009, no significant transplant size effect was found on root weight at final harvest. On the other hand, significantly lower root weights were observed for all cultivars of UD plants compared to OD- and ND plants in Wageningen 2010 (Table 3.5). In this trial, no significant cultivar effect was measured, whereas these were recorded in Wageningen 2009 and Voorst 2009. In both trials, 'Mariska' had – on average for all transplant sizes – a lower total root weight per plant than 'Pronto' (Table 3.5).

(Root dry weights measured at intermediate root samplings are presented in the supplementary materials, Tables S3, S4).

Root mass densities over the soil profile

Figure 3.3 shows the root mass densities for the four cultivars under the three transplant sizes over the soil profile at the third root sampling date, both at the central- and at the peripheral sampling position (cf. Figure 3.1).

Apparently, the most important element of variation in root spatial (horizontal and vertical) exploration (as measured by root mass densities over the soil profile at the different sampling positions) was conferred by the growing season: whereas under the rather optimal conditions in Wageningen 2009 (Figure 3.3A-D), the root mass density measured in the top 0.1 m at the central sampling positions was rather identical to the root mass density measured at the peripheral position for all cultivars, with the exception of 'Nadine' (Figure 3.3C), under the much cooler conditions in Wageningen 2010 a larger root mass density was measured at the central position compared with the peripheral sampling position (Figure 3.3E-H). The same pattern was observed, although to a lesser extent, under the rather warm conditions in Voorst 2009 (Figure 3.3I-L). The transplant sizes did not influence the root mass density distribution over the soil profile in any of the three trials.

Relationship between NO_3 capture from the soil and RLD (Root Length Density)

The NO_3 capture and corresponding root proliferation data are provided in Figure 3.4. In this figure the percentage difference in Root Length Density (RLD) or in NO_3 capture between a particular combination of cultivar × transplant size in a given layer, and the average value obtained for the pooled data per layer has been plotted (cf. Materials and Methods). It is surmised that additional RLD is correlated to additional NO_3 capture in a layer.

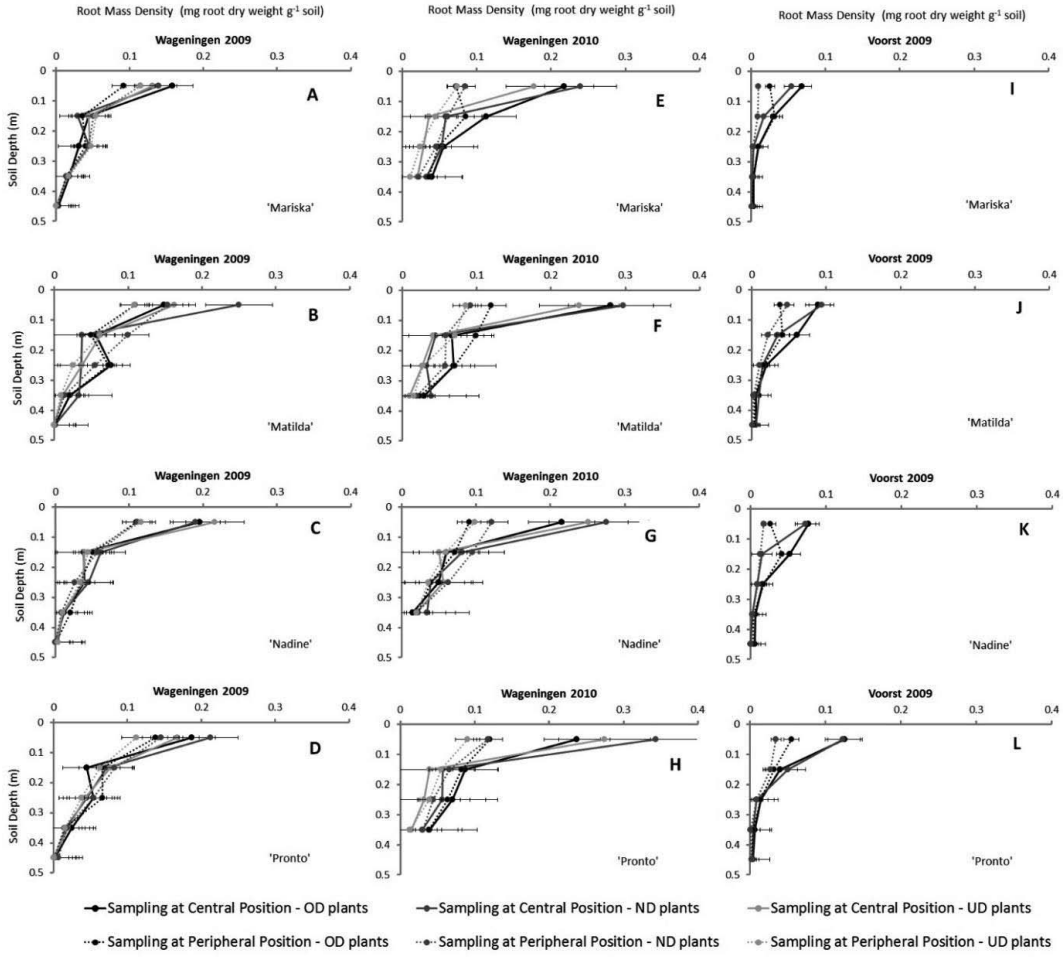

Figure 3.3 Root Mass Density measured at the Central- and Peripheral positions at the third root sampling of the four cultivars averaged over the three or two transplant sizes [Over-Developed- ('OD'), Normally Developed- ('ND') and Under-Developed- ('UD') transplant size] for the trials Wageningen 2009 ((A) to (D)), Wageningen 2010 ((E) to (H)) and Voorst ((I) to (L)) (for sampling method, cf. Figure 3.1). Error bars indicate ± one standard deviation.

Effect of OD transplant size on NO_3 capture and root proliferation

In Wageningen 2009, no clear pattern emerged showing a higher RLD being proportionally correlated with a higher NO_3 capture in a layer. Mainly only the OD plants of 'Pronto' showed a higher efficiency in NO_3 capture from the soil in all layers (Figure 3.4A), but this was not accompanied by a higher RLD than average in these layers (Figure 3.4B). Conversely, the OD plants of 'Matilda' had a higher than average

RLD in the 0.3-0.5 m layers but this was not combined with a higher relative NO_3 capture. 'Nadine's' OD plants showed an overall reduced RLD throughout the soil profile. In Wageningen 2009 the correlations were clearer, with an overall higher NO_3 capture being positively correlated with a slightly higher RLD in all layers for all cultivars (Figure 3.4C,D). In Voorst 2009, the capture of NO_3 for the OD plants in all layers did not differ from the average, although the RLD was increased compared with the average for all cultivars through the soil profile, except for 'Mariska' (Figure 3.4E,F).

Effect of UD transplant size on NO_3 capture and root proliferation

In Wageningen 2009, overall NO_3 capture was not extremely impaired by a somewhat smaller RLD (Figure 3.4G,H). 'Matilda' showed the most pronounced impaired NO_3 capture in the 0-0.4 m layers, although this was not associated with a lower RLD in these layers. In Wageningen 2010, NO_3 capture of UD plants was reduced compared with the average in all layers, and this was well correlated with a reduced RLD throughout the soil profile (Figure 3.4I,J).

Root:shoot ratios over time

Table 3.6 provides details on the average root:shoot ratios of the four cultivars at the three root sampling dates. Over time the root:shoot ratios declined in all experiments and during the entire period of measurement, except in Wageningen 2009 between the first and second sampling, associated with the low temperatures during the initial growth period in that experiment. Plants in the Voorst 2009 experiment had considerably lower root:shoot ratios than plants in the Wageningen 2009 and Wageningen 2010 experiments at all samplings, in line with the very low root mass observed in the Voorst 2009 experiment. Differences in root:shoot ratios between transplant sizes were only observed in the Voorst 2009 experiment at the second sampling: the normally developed transplants had a higher root:shoot ratio than the over-developed transplants in all cultivars. The same trend was also visible at the first sampling but could not be proven statistically. This general lack of treatment effect even at early stages shows how short-lived the effect of root damage associated with the transplanting actually was and how plastic dry matter partitioning over roots and shoots

can be. Significant differences in root:shoot ratio amongst cultivars were found at later sampling dates, but were not always consistent across experiments and were not repeatable over samplings. However, 'Pronto' showed consistently high values and 'Mariska' consistently low values when cultivar differences proved significant (Table 3.6).

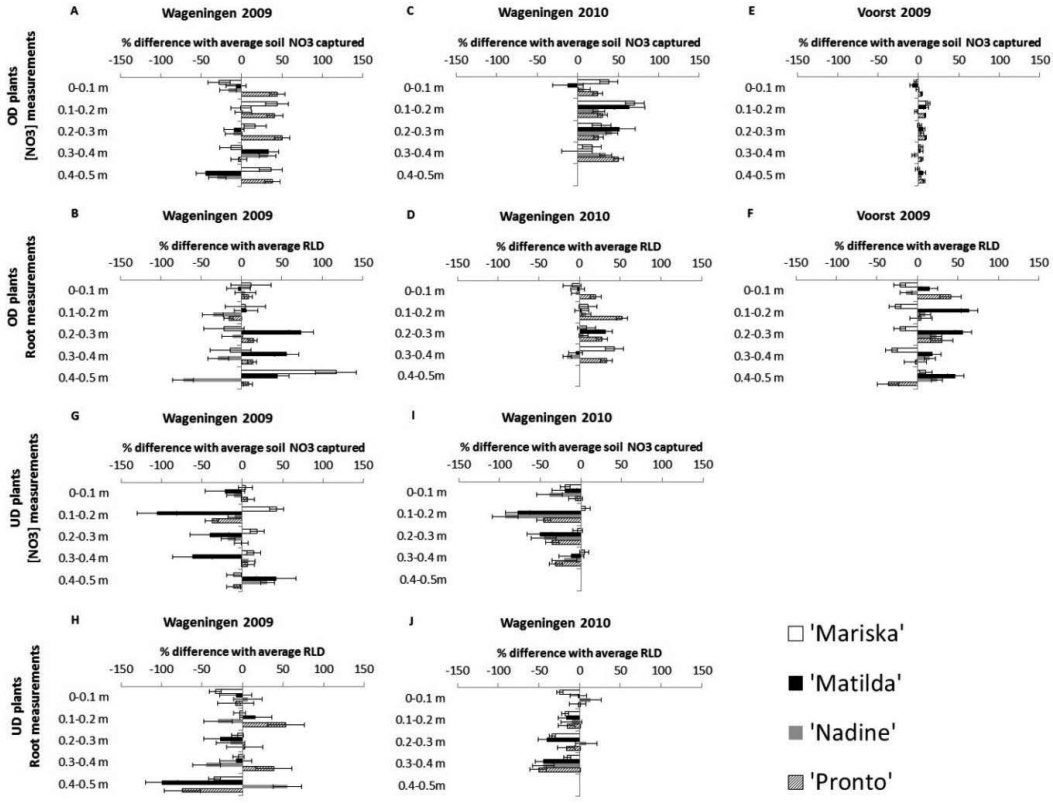

Figure 3.4 Percentage difference in NO_3 captured in a layer with the average* NO_3 captured, and percentage difference in RLD with the average RLD, for each cultivar under the 'OD' transplant size ('Over-Developed' transplant size) in trial Wageningen 2009 (A and B), in trial Wageningen 2010 (C and D) and Voorst 2009 (E and F), and under the 'UD' transplant size ('Under-Developed' transplant size) in trial Wageningen 2009 (G and H) and Wageningen 2010 (I and J) . Error bars indicate ± one standard deviation. Mean based on pooled values obtained for all cultivar × transplant size combination within a layer.

Table 3.6. Average root:shoot ratios of the four cultivars at first, second and third root sampling, after establishment from three different transplant sizes in each of three trials.

Harvest Date	CDD[6] (ºCd)	TS[8]	Mariska	Matilda	Nadine	Pronto	
First root sampling							
			Wageningen 2009				*Tr.*[5]
April 15th, 2009	111	OD[1]	0.107±0.082[7]	0.113±0.129	0.091±0.056	0.133±0.035	*0.111*a
		ND[2]	0.115±0.051	0.133±0.091	0.072±0.023	0.114±0.038	*0.108*a
		UD[3]	0.095±0.041	0.083±0.059	0.061±0.023	0.073±0.040	*0.078*a
		Cv.[4]	*0.105*a[9]	*0.110*a	*0.075*a	*0.107*a	
			Wageningen 2010				*Tr.*
April 26th, 2010	152	OD	0.111±0.106	0.089±0.025	0.114±0.041	0.086±0.022	*0.100*a
		ND	0.094±0.057	0.121±0.046	0.108±0.088	0.071±0.036	*0.099*a
		UD	0.087±0.047	0.087±0.048	0.095±0.055	0.091±0.037	*0.090*a
		Cv.	*0.097*a	*0.099*a	*0.106*a	*0.083*a	
			Voorst 2009				*Tr.*
June 8th, 2009	152	OD	0.070±0.029	0.078±0.041	0.072±0.041	0.069±0.041	*0.072*a
		ND	0.070±0.047	0.127±0.162	0.072±0.035	0.071±0.050	*0.085*a
		UD	-	-	-	-	-
		Cv.	*0.070*a	*0.102*a	*0.072*a	*0.070*a	
Second root sampling							
			Wageningen 2009				*Tr*
April 28th, 2009	224	OD	0.082±0.049	0.102±0.060	0.099±0.040	0.130±0.043	*0.103*a
		ND	0.110±0.022	0.109±0.041	0.084±0.033	0.082±0.040	*0.096*a
		UD	0.086±0.019	0.132±0.049	0.103±0.037	0.128±0.058	*0.112*a
		Cv.	*0.093*a	*0.115*a	*0.095*a	*0.113*a	
			Wageningen 2010				*Tr.*
May 10th, 2010	252	OD	0.039±0.012	0.035±0.007	0.048±0.017	0.052±0.026	*0.043*a
		ND	0.043±0.018	0.029±0.010	0.043±0.013	0.063±0.034	*0.045*a
		UD	0.049±0.018	0.043±0.009	0.062±0.017	0.057±0.018	*0.053*a
		Cv.	*0.044*ab	*0.036*a	*0.051*bc	*0.058*c	
			Voorst 2009				*Tr.*
June 17th, 2009	253	OD	0.010±0.007	0.012±0.007	0.012±0.006	0.022±0.011	*0.014*a
		ND	0.017±0.012	0.028±0.033	0.021±0.019	0.029±0.019	*0.024*b
		UD	-	-	-	-	-
		Cv.	*0.014*a	*0.020*a	*0.016*a	*0.025*a	
Third root sampling							
			Wageningen 2009				*Tr.*
May 11th, 2009	325	OD	0.028±0.011	0.038±0.027	0.033±0.008	0.040±0.019	*0.035*a
		ND	0.026±0.011	0.044±0.021	0.034±0.014	0.045±0.024	*0.037*a
		UD	0.028±0.007	0.042±0.017	0.042±0.017	0.037±0.015	*0.037*a
		Cv.	*0.028*a	*0.041*b	*0.036*ab	*0.040*ab	
			Wageningen 2010				*Tr.*
May 24th, 2010	347	OD	0.029±0.011	0.024±0.006	0.021±0.008	0.026±0.009	*0.025*a
		ND	0.023±0.006	0.026±0.011	0.028±0.014	0.029±0.009	*0.027*a
		UD	0.021±0.005	0.025±0.010	0.030±0.012	0.032±0.014	*0.027*a
		Cv.	*0.024*a	*0.025*a	*0.026*a	*0.029*a	

Table 3.6. Average root:shoot ratios of the four cultivars at first, second and third root sampling, after establishment from three different transplant sizes in each of three trials (continued).

Harvest Date	CDD[6] (ºCd)	TS[8]	Mariska	Matilda	Nadine	Pronto	
			Voorst 2009				*Tr.*
June 29th, 2009	420	OD	0.009±0.002	0.012±0.003	0.012±0.007	0.013±0.004	*0.012*a
		ND	0.009±0.007	0.014±0.007	0.010±0.003	0.019±0.007	*0.013*a
		UD	-	-	-	-	
		Cv.	*0.009*a	*0.013*b	*0.011*ab	*0.016*c	

[1]'Over-developed' transplant size; [2]'Normally developed' transplant size; [3]'Under-developed' transplant size; [4]Mean for cultivar across transplant sizes; [5]Mean for transplant size across cultivars; [6]Cumulated Degree-Days; [7]Standard error of the mean; [8]Transplant Size; [9]Means with different letters indicate a significant difference at $p \leq 0.05$ – means separation with lettering is within an experiment and at the level of main factors cultivar or transplant size when the two-way interaction was not significant and at the level of transplant size × cultivar when the interaction was significant.

Physiological Nitrogen Use Efficiency (NUE) and nutritional status of the plant

Physiological nitrogen use efficiency Significant interactions were found between transplant sizes and cultivar effects on physiological NUE (defined as g dry weight per g nitrogen found in the plant) in Wageningen 2010 and Voorst 2009 (Table 3.7). In Wageningen 2009, OD and UD plants had a significantly reduced physiological NUE compared to ND plants. Overall, 'Nadine' showed to have a higher physiological NUE whatever transplant size was applied, compared to 'Mariska'. In Wageningen 2009, this cultivar had the lowest physiological NUE. In Wageningen 2010, OD and ND plants of 'Matilda' had a significantly higher physiological NUE than OD plants of 'Mariska'.

In Voorst 2009, physiological NUE values were lower than values obtained for the Wageningen trials (Table 3.7). No significant difference in physiological values was found between transplant sizes or between cultivars. Only within the ND plants, 'Nadine' had a significantly higher physiological NUE than the other cultivars.

Nutritional status of the plant Figure 3.5 shows the relationship between the nutritional status of the plant (shoot [N]) and its estimated root dry weight for the three trials at the respective final harvests. The alignment of the data obtained for the three trials highlights that the final harvests took place at different nutritional statuses of the plants which were proportionally related to root dry weight.

Table 3.7 Average physiological NUE (g DM g^{-1} N in head) of the four cultivars at final harvest, after establishment from three different transplant sizes.

Harvest Date	CDD[6] (ºCd)	TS[8]	Mariska	Matilda	Nadine	Pronto	
			Wageningen 2009				*Tr.*[5]
May 25th, 2009	474	OD[1]	29.1±1.8[7]	29.6±1.9	32.7±3.0	31.3±3.6	*30.7a*
		ND[2]	30.6±1.5	33.0±4.5	33.1±2.5	32.0±2.3	*32.2b*
		UD[3]	30.3±1.3	30.2±2.7	31.8±1.8	29.3±1.5	*30.4a*
		Cv.[4]	*30.0*a[9]	*31.0*ab	*32.5*b	*30.9*ab	
			Wageningen 2010				*Tr.*
May 30th, 2010	400	OD	34.8±4.2a	45.1±4.9d	41.7±3.0cd	40.5±3.0abcd	*40.5*
		ND	39.2±6.2abcd	44.8±3.8d	41.5±3.8bcd	38.4±2.4abc	*41.0*
		UD	35.5±3.4ab	37.1±2.8abc	39.7±2.2abcd	36.6±2.2abc	*37.2*
		Cv.	*36.5*	*42.3*	*41.0*	*38.5*	
			Voorst 2009				*Tr.*
June 29th, 2009	420	OD	24.9±1.8ab	24.1±0.6ab	24.6±0.6ab	24.2±0.6ab	*24.5*
		ND	23.1±0.8a	22.8±1.0a	25.6±3.0b	23.0±0.7a	*23.6*
		UD	-	-	-	-	
		Cv.	*24.0*	*23.4*	*25.1*	*23.6*	

[1]'Over-developed' transplant size; [2]'Normally developed' transplant size; [3]'Under-developed' transplant size; [4]Mean for cultivar across transplant sizes; [5]Mean for transplant size across cultivars; [6]Cumulated Degree-Days; [7]Standard error of the mean; [8]Transplant Size; [9]Means with different letters indicate a significant difference at $p \leq 0.05$ – means separation with lettering is within an experiment and at the level of main factors cultivar or transplant size when the two-way interaction was not significant and at the level of transplant size × cultivar when the interaction was significant.

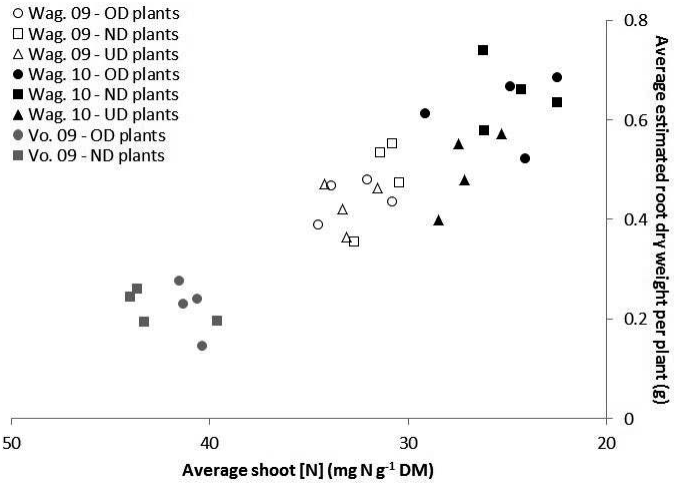

Figure 3.5 Relationship between the nutritional status of the plant (average shoot [N]) and its estimated root weight for the two or three transplant sizes [Over-Developed- ('OD'), Normally Developed- ('ND') and Under-Developed- ('UD') transplant size], measured at the final harvest in the trials Wageningen 2009 ('Wag. 09'), Wageningen 2010 ('Wag. 10') and Voorst 2009 ('Vo. 09').

3.4 Discussion

Transplanting four cultivars at three different transplant stages gave a significant insight into the impact of below-ground physiological processes developed by lettuce to overcome the stresses created by altering the shoot:root ratio and to maintain shoot growth. Strong Treatment × Environment interactions were visible in these trials.

3.4.1 Seasons and soil conditions impacted transplant size effect on shoot and root growth: Treatment × Environment interactions

The early spring growing seasons in the Wageningen 2009 and 2010 trials were to a certain extent similar in terms of photoperiod and soil conditions (texture, CEC, etc.) although the Wageningen 2010 trial experienced slightly more rainfall (Table 3.1) and a colder start (cf. Materials and Methods) than the Wageningen 2009 trial; in contrast, the Voorst 2009 trial was conducted later in the season, under higher soil and air temperatures and likely higher levels of radiation (not recorded), which led to much higher relative growth rates during the initial growth phase (Table 3.2). On the other hand, whereas maximal growth rates during the linear phase reached average values between 100 (Wageningen 2009) and 130 (Wageningen 2010) mg DM m^{-2} $(^{o}Cd)^{-1}$, these rates remained below 100 mg DM m^{-2} $(^{o}Cd)^{-1}$ for Voorst 2009 (Table 3.2). This influenced the effects of transplant sizes to a large extent, as the differences between the OD and the ND plants were significant in the Voorst 2009 trial but not in the early spring trials in Wageningen (Table 3.3). In Voorst 2009, the warm growing conditions even led to failure of UD plants, of which head formation and maturation did not occur within the time frame of the experiment, despite the higher cumulated thermal time.

Figure 3.5 shows that the root dry weight of the plants under the various transplant sizes was not driven by the transplant size and/or the cultivars, but rather a function of the nutritional status i.e. the growth stage. The higher shoot N concentration for some treatments is an indication of physiologically younger plants. Here shoot N is diluted over less biomass as shown by the smaller dry weights. Comparison of these data with the root:shoot ratio and shoot dry weight data in Tables 3.6 and 3.3 respectively shows that the harvested plants at the lower shoot N concentration also had a higher root:shoot

ratio. This may have been related to the functional equilibrium change under reduced plant nitrogen status (Poorter and Nagel, 2000).

3.4.2 Unbalanced shoot:root ratio created by root pruning at transplanting has short-lasting effects on shoot growth

Root pruning at transplanting using overdeveloped seedlings did not impact the yield at final harvest in the Wageningen trials (Table 3.3). The mechanical damage inflicted to the roots of the OD plants at transplanting did not impact root growth either, as no significant difference was found between the OD and the ND plants in total root weight at any sampling date or in RLD at any soil depth for any sampling date (data not shown). Any impact of the treatment on the root:shoot ratios had already disappeared at first sampling in the Wageningen experiments and only showed itself temporarily in Voorst 2009 (Table 3.6). For the three trials, OD plants showed an overall lower maximal relative growth rate (Table 3.2) and an overall lower maximal leaf expansion rate (Table 3.4) during the exponential phase compared with the ND plants, which was caused by their bigger size at transplanting compared to ND plants (therefore a lower amount of tissue produced per amount of existing tissue in the exponential phase). However, this did not influence the start of the linear growth phase, as no significant difference in lag phase was found for dry weight accumulation (Table 3.2) or leaf expansion (Table 3.4), except in Wageningen 2009. These results suggest that for the lettuce cultivars used in this study, a mild root pruning at transplanting is not a large stress for shoot growth and does not affect final yield in the early spring season. The moderate soil and air temperatures, light intensity and radiation (not recorded) in the Wageningen trials led to a slower shoot growth, especially in the exponential phase (Table 3.2), and consequently required less from the roots to sustain the growth. This may explain why the stress created by root pruning was not crucial for shoot growth for these trials. In contrast, the higher air and soil temperatures recorded in the Voorst trial (late spring/early summer) increased the shoot growth rates in the exponential phase (Table 3.2) and emphasized the important role of a larger root system in this trial to sustain the growth of larger shoots such as the OD plants. This was very visible in the results, as the cultivars with the largest root weight (‘Matilda’ and ‘Pronto’, Table 3.5)

under both the OD and the ND transplant size, performed better in terms of shoot weight (Table 3.3) than 'Mariska' which had the smallest root weight (Table 3.5).

3.4.3 Transplanting underdeveloped plants impacts roots and shoot growth to a large extent

Transplanting UD seedlings in open field conditions imposes considerable physiological stress on growth and development of the plant. UD plants were not able to recover from transplant shock and to catch up with ND plants during the experiments in terms of dry weight accumulation, especially for Wageningen 2010 (Supplementary materials, Tables S1, S2 and Table 3.3). Vos et al. (1996) showed that leaf initiation and potential leaf size are largely determined before leaves actually appear, i.e. the number of leaves and the size of the leaves are determined already in the apex. They hypothesized that stress at an early growth stage may disturb the physiological mechanisms controlling leaf initiation in the apex, and may therefore affect later field performance over a longer time, as observed in our experiments. The smaller size at transplanting impacted shoot growth: the UD plants' smaller leaf area at transplanting increased the maximal relative growth rate/leaf expansion rate during the exponential phase (Table 3.2) which increased the lag phase, as the UD plants required more time to finalize the exponential growth period. As a result UD plants had slightly smaller heads and delayed maturity (data not shown). In practice, transplanting smaller plants, delaying maturity, translates into a longer period in the field and consequently some financial loss for the grower.

The transplanting shock did not only affect shoot growth and development. We surmise that the shock imposed on the plants by transplanting underdeveloped seedlings also disturbs root initiation and leads to a smaller root system for the UD plants compared to the ND plants, as observed in Wageningen 2010 (Table 3.5), the trial with lowest soil temperatures. The smaller root system was not compensated by an improved NO_3 capture capacity, as shown clearly for Wageningen 2010 in Figure 3.4.

3.4.4 Genetic variation in shoot:root growth strategies

The four cultivars were chosen according to their different growth patterns in the field as well as their specific root mass distributions over the soil profile as observed

previously by Den Otter and Lammerts van Bueren (2007). The diverse strategies exhibited by the cultivars to overcome the transplant shock seemed rather consistent across years.

'Mariska' was a cultivar which had the smaller root system overall (Figure 3.5A,E,I). For this cultivar, root pruning tended to increase total root mass consistently in Wageningen 2009 and 2010 (Table 3.5) which underlines a powerful root regeneration capacity. In practice, the cultivar Mariska is often preferred for the early spring growing season, when weather conditions force growers to delay the planned planting date. They are then faced with overdeveloped transplants, a situation from which the cultivar is known to recover easily (K. de Jong, *pers. comm*.). This research shows that for 'Mariska' this high root regeneration capacity is however a trade-off for shoot growth, as the larger assimilate allocation to the roots was at the expense of the shoot, which tended to be lighter than that of the other cultivars at final harvest (Table 3.3).

In contrast to 'Mariska', 'Matilda' and 'Pronto' were the two cultivars which had the largest root system (Table 3.5), whereas Pronto often had the highest root:shoot ratio (Table 3.6). Such a large root system may have contributed to their steady good field performance across transplant size, locations and years (Table 3.3); indeed developing more roots, especially in deeper soil layers (as it was measured for these cultivars in layers 0.1-0.2 and 0.3-0.4 m, Fig. 3.3B,F,J for 'Matilda' and Fig. 3.3D,F,L for 'Pronto') increased resource capture quantitatively and consequently conferred a proportional advantage for shoot performance. Besides, the results of this study suggest that these cultivars are relatively robust, as their response to transplant shock (either root pruning or underdeveloped transplant size) was consistent over locations and seasons. In practice, these cultivars are often preferred by 'hobby' gardeners as robust cultivars when growing conditions are less controlled and less optimal, which confirms our findings. However, it must be underlined that the field conditions under which the trials were carried out in this study were rather optimal, as no strong drought or nitrate leaching occurred. It might be that a larger proportion of assimilates allocated to root proliferation as displayed by 'Matilda' and Pronto' could be a trade-off for final yield in case of less optimal field conditions, e.g. temporary drought or spatial limitation in nitrate availability. Other physiological mechanisms involved in nitrate capture e.g.

improved nitrate inflow per unit root length (Vuuren et al., 1996) may then confer robustness.

Finally, 'Nadine' is a cultivar that had a relatively smaller root system but had a higher physiological NUE than the other cultivars (Table 3.7). This cultivar performed consistently in all three experiments under all transplant sizes, underlining the fact that not only the capacity to take up resources from the soil is important, but also the internal ability to use these resources in order to ensure adequate shoot growth despite environmental stresses.

3.5 Concluding remarks

This study investigated the effect of different types of transplant shocks, created by root pruning or underdeveloped transplant size, on field performance of lettuce, and the role of below-ground traits in overcoming such disturbances. The results of three field experiments showed that the mechanical damage inflicted at transplanting to the roots of overdeveloped transplants has short-lasting effects on shoot growth and does not impact final yield. This suggests that the plants respond quickly to such a shock by adaptive responses at the root level, and are able to restore the initial root:shoot ratio fast enough not to impact final yield. Strategies to overcome the mechanical damage at the root level include high root regeneration capacity, which however, can be trade-off for shoot yield as shown for 'Mariska'.

On the other hand, a large transplant shock, created by transplanting underdeveloped seedlings, cannot be overcome by lettuce; the results showed that transplanting undeveloped seedlings has lasting effects on overall root and shoot growth: slower growth results in smaller plants that mature later.

Overall, more roots in deeper layers, as observed for 'Matilda' and 'Pronto', was linked to stable field performance despite transplant shock across trials, locations and seasons, and may therefore constitute a trait of robustness for lettuce, as we hypothesized. If a more developed root system enables the plants to sustain growth during temporary periods of drought or nitrate shortage by capturing resources from deeper soil layers, the ability to efficiently transform the captured resources into shoot mass is also an important trait for robustness, as found for 'Nadine' in these trials.

Monitoring spatial and temporal changes in below-ground cues and measuring their effects on above-ground parameters were only feasible in this study by using a limited set of cultivars, selected on the basis of specific criteria. In no way do we suggest that our results are fully representative for the genetic variation present among the numerous lettuce varieties. Instead, this study, together with a previous paper reporting on the spatial and temporal dynamics of root development and resource capture in lettuce (Chapter 2), will provide the basis for a conceptual framework to design a strategy to breed lettuce for robustness, which will be used to interpret results obtained from a large set of lettuce varieties trialled in diverse environmental conditions.

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Appendix

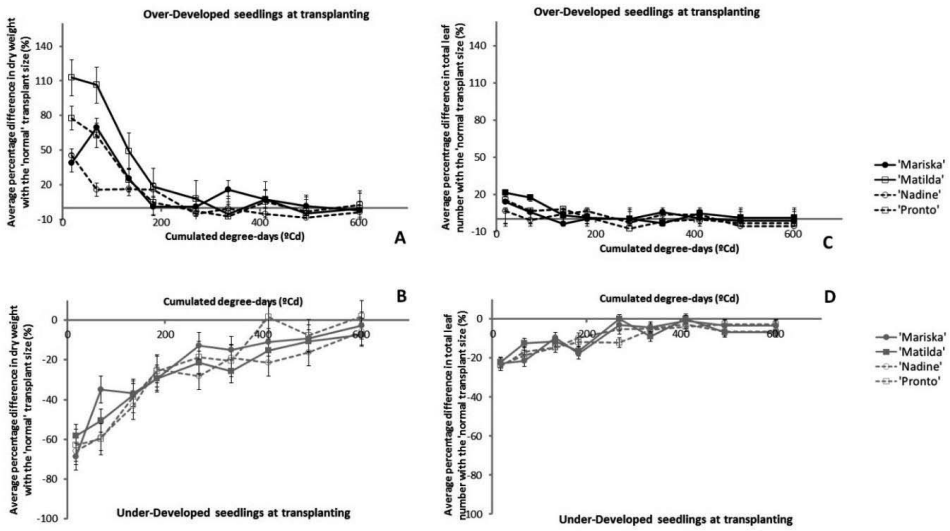

Figure A1 Average percentage difference in dry weight of plants originating from Over-Developed (A) and Under-Developed (B) seedlings, in comparison with the dry weights of plants originating from Normally Developed seedlings, and average percentage difference in total number of leaves of plants originating from Over-Developed (C) and Under-Developed (D) seedlings in comparison with the number of leaves counted on plants originating from Normally Developed seedlings for the four cultivars (trial Wageningen 2009). Error bars indicate ± one standard deviation

Supplementary material

Table S1. Average shoot dry weights (g per plant) of the four cultivars at first root sampling, after establishment from three different transplant sizes in each of three trials.

Harvest Date	CDD[6] (°Cd)	TS[8]	Mariska	Matilda	Nadine	Pronto	
			Wageningen 2009				*Tr.*[5]
April 15th, 2009	111	OD[1]	0.58±0.08[7]	0.62±0.05	0.49±0.06	0.57±0.15	*0.56*c
		ND[2]	0.47±0.05	0.45±0.05	0.46±0.11	0.48±0.05	*0.46*b
		UD[3]	0.30±0.03	0.24±0.06	0.27±0.04	0.32±0.04	*0.28*a
		Cv.[4]	*0.45*a[9]	*0.44*a	*0.41*a	*0.45*a	
			Wageningen 2010				*Tr.*
April 26th, 2010	152	OD	2.48±0.31	2.43±0.44	2.93±0.37	2.69±0.26	*2.63*c
		ND	2.28±0.41	2.04±0.51	2.11±0.29	2.42±0.46	*2.21*b
		UD	1.18±0.28	0.79±0.11	0.99±0.15	0.93±0.41	*0.97*a
		Cv.	*1.98*a	*1.75*a	*2.01*a	*2.02*a	
			Voorst 2009				*Tr.*
June 8th, 2009	152	OD	1.46±0.30	1.10±0.42	1.48±0.36	1.34±0.28	*1.34*b
		ND	0.51±0.10	0.30±0.20	0.36±0.07	0.49±0.09	*0.43*a
		UD	-	-	-	-	
		Cv.	*0.99*b	*0.73*a	*0.92*ab	*0.91*ab	

[1]'Over-developed' transplant size; [2]'Normally developed' transplant size; [3]'Under-developed' transplant size; [4]Mean for cultivar across transplant sizes; [5]Mean for transplant size across cultivars; [6]Cumulated Degree-Days; [7]Standard error of the mean; [8]Transplant Size; [9]Means with different letters indicate a significant difference at $p \leq 0.05$ – means separation with lettering is within an experiment and at the level of main factors cultivar or transplant size when the two-way interaction was not significant and at the level of transplant size × cultivar when the interaction was significant.

Table S2. Average shoot dry weights (g per plant) of the four cultivars at second root sampling, after establishment from three different transplant sizes in each of three trials.

Harvest Date	CDD[6] (°Cd)	TS[8]	Mariska	Matilda	Nadine	Pronto	
			Wageningen 2009				*Tr.*[5]
April 28th, 2009	224	OD[1]	3.83±0.35[7]	3.23±0.74	3.41±0.47	3.33±0.39	*3.45*b
		ND[2]	3.52±0.47	2.95±0.26	3.49±0.57	3.36±0.47	*3.33*b
		UD[3]	3.06±0.83	2.37±0.35	2.44±0.28	2.71±0.49	*2.64*a
		Cv.[4]	*3.47*b[9]	*2.85*a	*3.11*a	*3.13*ab	
			Wageningen 2010				*Tr.*
May 10th, 2010	252	OD	8.58±1.65	8.67±1.18	8.14±1.14	8.20±2.06	*8.40*b
		ND	7.98±1.18	8.78±1.42	7.83±1.03	7.17±1.39	*7.94*b
		UD	4.72±0.82	4.54±0.95	4.19±1.31	4.57±1.00	*4.50*a
		Cv.	*7.09*a	*7.33*a	*6.72*a	*6.65*a	
			Voorst 2009				*Tr.*
June 17th, 2009	253	OD	10.55±1.27	8.55±1.08	11.23±1.88	8.19±2.02	*9.63*b
		ND	5.80±1.92	3.80±1.45	3.14±0.61	3.45±0.70	*4.05*a
		UD	-	-	-	-	
		Cv.	*8.18*c	*6.18*ab	*7.18*bc	*5.82*a	

[1]'Over-developed' transplant size; [2]'Normally developed' transplant size; [3]'Under-developed' transplant size; [4]Mean for cultivar across transplant sizes; [5]Mean for transplant size across cultivars; [6]Cumulated Degree-Days; [7]Standard error of the mean; [8]Transplant Size; [9]Means with different letters indicate a significant difference at $p \leq 0.05$ – means separation with lettering is within an experiment and at the level of main factors cultivar or transplant size when the two-way interaction was not significant and at the level of transplant size × cultivar when the interaction was significant.

Table S3. Average estimated root dry weights (g per plant) of the four cultivars at first root sampling, after establishment from three different transplant sizes in each of three trials.

Harvest Date	CDD[6] (°Cd)	TS[8]	Mariska	Matilda	Nadine	Pronto	
			Wageningen 2009				*Tr.*[5]
April 15th, 2009	111	OD[1]	0.06±0.05[7]	0.07±0.09	0.04±0.03	0.07±0.03	*0.06*b
		ND[2]	0.05±0.02	0.06±0.05	0.03±0.01	0.05±0.01	*0.05*b
		UD[3]	0.03±0.01	0.02±0.01	0.02±0.01	0.02±0.01	*0.02*a
		Cv.[4]	*0.05*a[9]	*0.05*a	*0.03*a	*0.05*a	
			Wageningen 2010				*Tr.*
April 26th, 2010	152	OD	0.30±0.34	0.21±0.06	0.34±0.13	0.23±0.06	*0.27*b
		ND	0.21±0.14	0.24±0.13	0.23±0.20	0.17±0.08	*0.21*b
		UD	0.10±0.04	0.07±0.04	0.09±0.05	0.09±0.05	*0.09*a
		Cv.	*0.20*a	*0.18*a	*0.22*a	*0.16*a	
			Voorst 2009				*Tr.*
June 8th, 2009	152	OD	0.09±0.02	0.08±0.06	0.10±0.05	0.08±0.03	*0.09*b
		ND	0.04±0.03	0.03±0.02	0.02±0.01	0.03±0.02	*0.03*a
		UD	-	-	-	-	
		Cv.	*0.07*a	*0.06*a	*0.06*a	*0.06*a	

[1]'Over-developed' transplant size; [2]'Normally developed' transplant size; [3]'Under-developed' transplant size; [4]Mean for cultivar across transplant sizes; [5]Mean for transplant size across cultivars; [6]Cumulated Degree-Days; [7]Standard error of the mean; [8]Transplant Size; [9]Means with different letters indicate a significant difference at $p \leq 0.05$ – means separation with lettering is within an experiment and at the level of main factors cultivar or transplant size when the two-way interaction was not significant and at the level of transplant size × cultivar when the interaction was significant.

Table S4. Average estimated root dry weights (g per plant) of the four cultivars at second root sampling, after establishment from three different transplant sizes in each of three trials.

Harvest Date	CDD[6] (°Cd)	TS[8]	Mariska	Matilda	Nadine	Pronto	
			Wageningen 2009				*Tr.*[5]
April 28th, 2009	224	OD[1]	0.32±0.20[7]	0.30±0.14	0.34±0.16	0.43±0.13	*0.34*a
		ND[2]	0.38±0.08	0.32±0.11	0.28±0.11	0.27±0.13	*0.31*a
		UD[3]	0.26±0.01	0.30±0.09	0.26±0.12	0.33±0.12	*0.29*a
		Cv.[4]	*0.32*a[9]	*0.31*a	*0.29*a	*0.34*a	
			Wageningen 2010				*Tr.*
May 10th, 2010	252	OD	0.33±0.12	0.31±0.08	0.37±0.10	0.40±0.17	*0.35*b
		ND	0.34±0.15	0.25±0.09	0.33±0.10	0.43±0.22	*0.34*b
		UD	0.23±0.09	0.19±0.06	0.26±0.08	0.25±0.08	*0.23*a
		Cv.	*0.30*ab	*0.25*a	*0.32*ab	*0.36*b	
			Voorst 2009				*Tr.*
June 17th, 2009	253	OD	0.11±0.06	0.10±0.05	0.13±0.07	0.16±0.07	*0.12*b
		ND	0.08±0.05	0.08±0.07	0.06±0.05	0.10±0.05	*0.08*a
		UD	-	-	-	-	
		Cv.	*0.10*a	*0.09*a	*0.09*a	*0.13*a	

[1]'Over-developed' transplant size; [2]'Normally developed' transplant size; [3]'Under-developed' transplant size; [4]Mean for cultivar across transplant sizes; [5]Mean for transplant size across cultivars; [6]Cumulated Degree-Days; [7]Standard error of the mean; [8]Transplant Size; [9]Means with different letters indicate a significant difference at $p \leq 0.05$ – means separation with lettering is within an experiment and at the level of main factors cultivar or transplant size when the two-way interaction was not significant and at the level of transplant size × cultivar when the interaction was significant.

Chapter 4

Modelling concept of lettuce breeding for nutrient efficiency

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Abstract

Modern lettuce cultivars are bred for use under high levels of input of water and nutrients, and therefore less adapted to low-input or organic conditions in which nitrate availability varies over time and within the soil profile. To create robust cultivars it is necessary to assess which traits contribute to optimal resource capture and maximum resource use efficiency. We therefore revisited earlier published results on root growth, resource capture and resource use efficiency of lettuce exposed to localized drought and nitrate shortage in a pot experiment. Root growth in a soil profile with localised resource shortage depended on the resource that was in short supply. We conceptualised a model describing nitrogen uptake and use efficiency. We also investigated the genetic variation among 148 cultivars in resource capture over time and soil depth and in resource use efficiency in four (two locations × two planting dates) field experiments. Cultivars proved to be highly diverse in their ability to capture and use resources. This ability, however, was strongly affected by other sources of variance, stressing the need for an eco-physiological model capable of reducing the residual variance and improving the expression and evaluation of cultivar differences in relation to both resource capture and use efficiency in lettuce. We showed that genetic variation was best expressed under limiting conditions. To improve the conceptualised model further we identified issues requiring further analysis, e.g. the physiological reasons why certain cultivars are capable of quickly responding to changes in the environment to maintain optimal resource capture.

Keywords: Drought stress; Modelling concept; Nitrogen Use Efficiency; Organic; Root growth; Resource capture

4.1 Introduction

With increasing awareness of health benefits of vegetables, world-wide demand and supply of lettuce have risen tremendously since 1960, making it nowadays one of the leading vegetables in terms of crop value (Boriss and Brunke 2005). Lettuce breeding has focused on increasing yield of marketable head size, targeting leaf development, leaf shape, and head formation (Pua and Davey 2007). As vegetative growth in lettuce, a crop with a short cycle, strongly depends on availability of water and nitrogen (Ouzounidou et al. 2013), the abundant supply of these two resources in a sustainable way is crucial. Lettuce is usually very responsive to growth-limiting factors but not always efficient in capturing all the resources available or converting them into harvestable produce (Zhang et al. 2008). Nitrogen shortage, even when only temporary, can limit lettuce growth as the physiological or morphological mechanisms compensating for an impaired resource uptake may require some time before being triggered (Mou et al. 2013).

The availability of water and nitrogen over time and space largely depends on variable soil factors (Curtin et al. 2006). The role of the soil biological, physical, and chemical characteristics in making nitrogen and water available is even more important in organic and low-input systems than in conventional systems (Nautiyal et al. 2010). Indeed in the former systems the release of nutrients provided by organic fertilisers relies on soil characteristics such as temperature, moisture content, pH, texture, etc. (Mele and Crowley 2008). Enhanced soil life and improved organic matter content buffer processes in the soil-water-plant interface of organically managed soils (Masciandaro et al. 2013). Resource availability in soils under organic management can therefore be less rapidly and timely influenced than in conventionally managed soils where mineral fertilisation and the use of chemicals can have prompt effects on crop growth (Clark et al. 1999). Organically grown crops may consequently be more prone to temporary water or nutrient shortage which may easily lead to yield reduction (De Ponti et al. 2012). In lettuce, yields in low-input and organic systems are often lower than in conventional systems: for instance Leogrande et al. (2013) found that lettuce head weight (fresh matter) in fields fertilised organically can be 16 to 17% lower compared to fields where mineral fertilisation was applied.

One way to secure stable yields over a wide range of environmental conditions may be to breed robust lettuce cultivars (cf. Ceccarelli et al. 1991). Robustness is defined as the ability of the cultivar to perform well despite the presence of various environmental stressors (Kerbiriou et al. 2013a). Plasticity in morphological traits or physiological processes supporting continued nutrient capture, flexible internal storage and transport regimes, and improved nutrient use efficiency could create robustness, as such characteristics may enable the plants to withstand short periods of mild stress by conserving growth rates (Liao et al. 2001). In woody and herbaceous species, for instance, Mou et al. (2013) demonstrated that stable reduction in nutrient availability triggered morphological changes in root and shoot mass, and that physiological plasticity in nutrient foraging at the root level was less predictable, especially in temporally variable nutrient availability. In pot experiments with lettuce, Chapter 2 showed that the resource that was in short supply and the timing of the shortage determined the response.

In Europe, commercial lettuce cultivation entails the transplanting of seedlings grown in root blocks, consequently breaking the taproot; as a result, plants have a shallow root system mostly located in the top soil layers (0.0-0.2 m). Moreover, as lettuce breeding has mostly been focusing on improved head characteristics, roots morphological and physiological traits have not yet been fully exploited.
Compared to modern commercial lettuce cultivars, wild lettuce species have a strong taproot (up to 0.5 m deep in the soil profile) (Johnson et al. 2000). This morphological feature enables wild lettuce species to cope with drought stress as they can extract water from deeper soil layers (Johnson et al. 2000). Breeding lettuce for improved root system architecture may then be one of the strategies to increase the capture in space and time of soil-bound resources such as water and nitrogen.

In field experiments using four lettuce cultivars, Chapter 3 showed that larger root mass was in general positively associated with larger nitrogen capture throughout the soil profile, and that cultivars which had a larger root mass also had a larger shoot weight. On the other hand, a cultivar with a smaller root system but better nitrogen use

efficiency than the other cultivars displayed stable yields across experiments, highlighting that the use of the resources captured below-ground is also important to secure stable yields across environments (Barlow 2010). Water and nitrogen capture and use efficiency are complex traits which are strongly affected by large genotype × environment (G × E) interactions (Jackson et al. 1996); their influence on crop performance and the genetic control of their expression can therefore be difficult to assess. Understanding the physiological mechanisms underlying water and nitrogen capture and use efficiency, as well as dissecting such traits into simpler, biologically meaningful component traits, is a major challenge which can be tackled by eco-physiological modelling approaches (Yin et al. 2004; Hammer et al. 2006; Yin and Struik 2008; Yin and Struik 2010).

Because models can predict crop performance, account for G × E, and capture spatial and temporal dimensions of processes, they provide a valuable insight into the traits involved in diverse physiological and morphological mechanisms (Yin et al. 2004; Hammer et al. 2006; Yin and Struik 2008; Yin and Struik 2010); models can thus be used for breeding purposes, by pointing out which traits are biologically relevant, less influenced by G × E and amenable for selection (Hammer et al. 2006; Postma et al., 2014). Models can also help to assess which markers account for the largest proportion in variance of a trait in a certain environment (Yin and Struik 2012; Gu et al. 2014). They can also test ideotypes, predict which environments will be very suitable for specific genotypes, and evaluate which genotypes are needed for specific environments (Yin and Struik 2012; Gu et al. 2014).

Several studies attempted to understand physiological mechanisms underlying responses to temporary or spatial limitations in water and nutrient supply (Kerbiriou et al. 2013a). To the best of our knowledge, no model is currently available that can include genetic information, physiological and morphological mechanisms involved in water and nitrogen capture and use efficiency above- and below-ground, their dynamics in space and time, and eventually, the influence of environmental conditions thereon. As a strategic decision tool, such a model would teach the breeder which trait should be targeted in the considered breeding environment.

However, the understanding of all G × E interactions and their integration into existing crop models is very tedious, requires a specific model design with strong heuristic power (Yin et al. 2004; Yin and Struik 2008) and requires numerous empirical and theoretical steps for proper calibration and validation. As a step towards the design of such a model, we propose in this study to:

1- Investigate the physiological and morphological mechanisms involved in water and nitrogen capture and use efficiency in lettuce;
2- Design a conceptual model based on these investigations;
3- Assess the genetic variation in traits related to resource capture and use as indicated by in-depth phenotyping studies and the model;
4- Assess the (relative) importance of G × E interactions.

In order to examine the elements above, we build on a previously published pot trial (Chapter 2) and four additional field experiments with a set of 148 commercial cultivars.

4.2 Materials and Methods

Two types of experiments will be described. A pot experiment ('pot trial'), published by Kerbiriou et al. (2013a), which had been designed to observe the effects of localized nitrogen shortage or drought on lettuce shoot and root growth, was re-analysed. To investigate the role of below-ground morphological and physiological mechanisms involved in shoot performance, water and nitrogen capture, as well as root length and mass in each 0.10 m layer over a 0.40 m soil profile was measured during shoot growth. These measurements were related to nitrogen and water use efficiency calculated based on shoot measurements during growth.

A population of 148 commercial lettuce cultivars was phenotyped for resource capture and yield in four experiments, by planting them at two locations in the spring or summer season of two consecutive years ('field trials'). Water and nitrogen in each 0.10 m layer over a 0.40 m soil profile were measured during growth, and related to water and nitrogen use efficiency at harvest (based on marketable yield). These data have not been

published before and therefore materials and methods of these trials will be described in detail in this paper.

4.2.1 Pot trial

The materials and methods used in this experiment are described in detail in Kerbiriou et al. (2013a, cf. Chapter 2). In brief, seeds of butterhead cultivars 'Pronto' and 'Matilda' were raised in a greenhouse and transplanted at the 5-leaf stage to PVC tubes of 0.2 m diameter and 0.4 m length. The tubes were placed in a fully conditioned greenhouse. Individual pots were weighed twice a week, and watered to bring pot weights back to the required level, while compensating for changes in plant fresh weight.

Treatments included various combinations of drought and nitrogen shortage in the upper and the lower pot compartment (cf. Table 4.1). Measurements were made 2, 4 and 6 weeks after transplanting in the greenhouse, corresponding to 288, 512 and 768 ºCd, respectively.

At each harvest, the content of each pot was divided into four layers of 0.1 m each. The roots in each layer were dried at 105 °C for 16 h for dry weight assessment. For each layer, a soil sample was taken to measure NO_3-N content using an Ion Selective Electrode (ThermoFisher, Waltham, MA, USA). NO_3-N uptake from a soil sample was calculated as the difference with the NO_3-N content in a soil sample taken from a pot without a plant. Data were analysed by a two-way ANOVA using Genstat 14th Edition (Hempstead, UK).

4.2.2 Field trials

The large-scale field trials using a population of 148 lettuce cultivars grown in four different environments enabled to assess the potential genetic variation existing in the physiological mechanisms regulating resource capture and use efficiency identified in the pot experiment and conceptualized in the model design. Hundred and forty eight commercial butterhead cultivars suitable for field spring/summer conditions were selected for this study.

Table 4.1 Treatments applied in the pot trial for both cultivars (Source: Kerbiriou et al. 2013a, cf. Chapter 2, Table 2.2).

		Treatments				
		Control	DST[1]	NST[2]	DST+NSB[3]	NST+DSB[4]
Upper compartment (0.00-0.20 m)	Fertiliser (g NO_3-N)	0.625	0.625	0.178	0.625	0.178
	Water status (v:v; %)	14	6	14	6	14
Lower Compartment (0.20-0.40 m)	Fertiliser (g NO_3-N)	0.625	0.625	0.625	0.178	0.625
	Water status (v:v; %)	14	14	14	14	6

[1] Drought Stress in Top compartment
[2] Nitrogen Stress in Top compartment
[3] Drought Stress in Top compartment combined with Nitrogen Stress in Bottom compartment
[4] Nitrogen Stress in Top compartment combined with Drought Stress in Bottom compartment

Seeds used for the trials originated from seed lots produced under the same environmental conditions and were sown in 0.04 m × 0.04 m × 0.04 m organic peat blocks (Jongerius, Houten, the Netherlands) after breaking seed dormancy by exposure to 4 ºC for 24 hours. Transplants were raised in a greenhouse with day temperature of 20 ºC and night temperature of 15 ºC. Transplanting was done when the transplants had 5-7 leaves and few roots emerged out of the peat block. In the field, plant arrangement was 0.3 m × 0.3 m.

Two field trials were carried out at each of two different locations: Wageningen (51.97° N, 5.67° E, The Netherlands) in spring and summer 2010, and Voorst (52.23° N, 6.08° E, The Netherlands) in spring and summer 2011 (see Table 4.2 for exact planting dates). Both locations had a uniform, sandy soil profile up to 0.5 m depth and adequate structure, but relatively low organic matter content and water retention capability. The sites had been cropped uniformly in the previous 5 years on a larger surface than the area covered by the trials. They were certified organic and managed according to organic standards during the experiments.

Each trial included two repetitions. The experimental set up was a complete randomized block design, each block consisting of 150 plots to which a cultivar was randomly assigned. Two plots per block were left empty for measurements in bare soil. A plot

with plants consisted of 25 individuals (5 × 5 plants) of the same cultivar. Measurements were done on the nine inner plants.

All trial fields were uniform, certified organic and managed according to organic standards during the experiments. Fertilisation was provided by applying 100 kg/ha nitrogen, from seaweed pellets (9% N, 3% P, 3% K + 3% MgO, EcoFertiel, EcoStyle, Appelscha, the Netherlands) on the day before transplanting. Irrigation was not provided.

For each trial, weather data (air temperature, radiation, rainfall) were recorded daily (Voorst) or hourly (Wageningen) at the nearest weather station. Cumulated degree-days (based on air temperatures), as well as cumulated rainfall at each sampling date for each trial, are shown in Table 4.2. Cumulated degree days at each sampling date were calculated as the sum, between the date of transplanting and the sampling date, of the degrees above 4 ºC (base temperature for lettuce), based on an average daily temperature.

Table 4.2 summarizes the characteristics of the contrasting environments in the four trials. During Trial 2, 2010 the environment was apparently the most conducive to lettuce growth; during this trial, the plants received about 800 ºCd and more than 100 mm rainfall (Table 4.2). In Trial 1, 2010 and Trial 1, 2011, conditions were relatively dry with only 48 and 27 mm cumulative rainfall received over the whole trial period, respectively. This poor rainfall was associated with relatively mild temperatures in the case of Trial 1, 2010, where the temperature sum reached a final value of 793º Cd, but temperatures were lower during Trial 1, 2011, where temperature sum only reached a final value of 500 ºCd. Trial 2, 2011 had the wettest conditions, with 150 mm rainfall received during the trial period, but especially concentrated shortly before final harvest (25-07-2011).

Soil samples were taken every 0.1 m over a depth of 0.4 m outside of the peat block, using a 0.06 m diameter and 0.4 m long auger, during growth (‘intermediate sampling’) and at final harvest (cumulated degree days at the moment of sampling are detailed in Table 4.2). For three plants per plot, soil samples taken in each soil layer were pooled to account for plant-to-plant variation. Volumetric soil moisture content in each layer (soil

[H_2O], v:v) was recorded after drying the sample at 40 °C for 48 hours. Soil samples were taken every 0.1 m over a depth of 0.4 m outside of the peat block, using a 0.06 m diameter and 0.4 m long auger, during growth ('intermediate sampling') and at final harvest (cumulated degree days at the moment of sampling are detailed in Table 4.2).

Water left over the 0.4 m soil profile (mL) was calculated based on the soil [H_2O] measurement in each layer over a soil column of 0.1 m radius (R) and 0.4 m depth. Nitrate content (soil [NO_3], ppm) in each 0.1 m soil layer was measured using an Ion Selective Electrode (ThermoFisher, Waltham, MA, USA) using the method described previously by Sibley et al. (2009) and also used in Kerbiriou et al. (2013a,b; cf. Chapters 2 and 3). The total nitrate left over the 0.4 m soil profile (g) was calculated based on the nitrate concentration in each layer over a soil column of 0.1 m radius (R) and 0.4 m depth.

Shoot measurements were done only at final stage of the growth. Fresh weight and dry weight (g per plant) were assessed based on three plants per plot at final harvest, which took place 5 to 9 weeks after transplanting depending on trial. Plant [N] (g N g^{-1} DM) was measured using the Kjeldahl method, based on the grinded material of three plants per cultivar and per replicate within a trial. Physiological Nitrogen Use Efficiency (NUE, g DM g^{-1} N in heads) was calculated based on the head [N]: NUE = 1 / (head [N]). Plant N was calculated as average head dry weight × head [N]. Plant H_2O was calculated as plant fresh – plant dry weight. Plant [H_2O] was calculated as plant H_2O/plant dry weight.

Data were statistically analysed by a one way ANOVA using the statistical package Genstat 15th Edition (Hempstead, UK). To calculate the variance components, we used the REML procedure in Genstat 15th Edition (Hempstead, UK) with the following model:
Genotype by (Year/Trial/Sampling) with all terms of the model as random terms.

Table 4.2 Transplanting- and final harvest dates, and weather conditions during the four field trials.

Year	*2010*				*2011*			
Location	*Wageningen*				*Voorst*			
	Trial 1		Trial 2		Trial 1		Trial 2	
Planting date	20-05-2010		22-06-2010		22-03-2011		09-06-2011	
	Intermediate sampling	Final harvest	Intermediate sampling	Final harvest	Intermediate sampling	Final harvest	Intermediate sampling	Final harvest
Sampling date	14-06-2010	05-07-2010	19-07-2010	28-07-2010	19-04-2011	17-05-2011	05-07-2011	25-07-2011
Cumulative rainfall (mm)	18	48	90	104	16	27	49	145
CDD* (°Cd)	357	793	607	782	174	481	329	590

*Cumulated Degree-Days (using 4 °C as base temperature)

This equals the following model for the soil measurements ([NO_3] in each 0.1 m layer of the 0.4 m soil profile and total NO_3 of the whole 0.4 m soil profile, and soil moisture content in each 0.1 m layer of the 0.4 m soil profile and the volume of water left over the whole 0.4 m soil profile):

response = var(genotype) + var(year) + var(trial within year) + var(sampling within trial within year) + var(genotype by trial within year) + var(genotype by sampling within trial within year) + var(residual)

For the shoot measurements, as they were made only at final harvest (plant fresh and dry weight, plant [N], plant N, plant NUE, plant [H_2O], plant H_2O), it equals to the model:

response = var(genotype) + var(year) + var (trial within year) + var (genotype by trial within year) + var(residual)

with response being the total variance observed for a variable, var(genotype) the proportion of the total variance due to the genotypic effect, var(year) the proportion of the total variance due to year effect (confounded with location as trials within a year were carried out at the same location), var(trial within year) the proportion of the total variance due to trial effect (each year counted two trials), var(sampling within trial year) the proportion of the total variance due to sampling effect (two sampling dates within a trial) and var(residual) the residual variance. The other variance components were variances associated with interactions. Block effects were not statistically significant and therefore block effect was not accounted for in the analyses to enhance model power.

4.3 Results and Discussion

4.3.1 Assessing physiological mechanisms regarding resource capture and use

General physiological mechanisms regulating root growth and nitrogen capture and use efficiency in relation to shoot growth were assessed by carrying out the pot trial. This

section focuses on the processes involved in spatial root growth and resource capture in the soil.

Spatial root proliferation is resource-specific

Both cultivars reacted very similarly to the treatments; mainly the results for 'Pronto' are presented in this section. Figure 4.1 shows the fraction of the total root mass present in each layer at different sampling dates for this cultivar. In the control treatment (Figure 4.1C), on average 64% of the total root mass was allocated to the upper compartment at the third sampling (768 ºCd). When drought was applied in the upper compartment, this fraction increased: on average 73% of the total root mass was present in the upper compartment at the third sampling (768 ºCd) (Figure 4.1A). When drought stress in the upper compartment was combined with nitrogen stress in the lower compartment (Figure1B) this fraction increased even more, with 77% of the total root mass being allocated to the upper compartment. Only 23% of the total root mass developed in the lower compartment, compared to 36% for the control treatment.

The pattern was opposite when nitrogen stress was applied in the upper compartment: at the third sampling (768 ºCd), the fraction of roots allocated to the upper compartment was lower than in the control: 54% for the 'NST' treatment (Figure 4.1D); but in the lower compartment it was higher than in the control (46% for the 'NST' treatment). This pattern was reinforced when nitrogen stress application in the upper layer was combined with drought stress in the lower compartment (Figure 4.1E): the fraction of total root mass present in the upper compartment decreased to 36% and the fraction of total root mass present in the lower compartment increased to 64%.

Solely in dry soil additional root proliferation increases nitrate capture to a limited extent

Figure 4.2 shows the relationship between the root mass in a 0.1 m layer and the fraction of total nitrate present in the layer which was captured in the upper compartment (0.0-0.1 m and 0.1-0.2 m layers) and in the lower compartment (0.2-0.3 m and 0.3-0.4 m layers) in the five treatments (as 'Pronto' and 'Matilda' exhibited the same behaviour in this pot trial, data of these two cultivars were pooled together in the graphs). This figure shows that in this pot trial, a significant fraction of the total amount of nitrate

available could be captured with little root mass: in the control treatment for instance less than 0.1 g of roots in either of the compartments were able to capture more than 40% of the nitrate available in the soil layer (Figure 4.2A and B). Moreover, this figure shows that roots kept growing in a layer although no more nitrate was available for uptake: this is clear in Figure 4.2G where 100% of the total amount of available nitrate in the layer was captured already between Sampling 1 (288 ºCd) and Sampling 2 (512 ºCd) but root mass in the top layers increased from 0.2 g at Sampling 1 up to almost 1.5 g at Sampling 3 (768 ºCd).

When drought was applied in the upper compartment (Figures 4.2C, D) nitrate capture was impaired at Sampling 1 (288 ºCd): whereas about 70% of all nitrate available in a layer could be captured in the control treatment (Figure 4.2A), in the drought treatment, only 40% or less was captured by approximately the same root mass. At Sampling 2, while 100% of the available nitrate was captured in the top layers in the control treatment, in the drought treatment this percentage was only approximately 60%. At the last sampling (Sampling 3, 768 ºCd), although root mass was increased significantly in the dry compartment compared to the control, only up to 80% of the nitrate present in the layer was captured by the roots. The same results were obtained when drought stress was applied in the lower compartment in combination to nitrogen stress in the upper compartment (Figure 4.2J).

4.3.2 Model design

Based on the results obtained from the pot trial and analysed above and in Chapter 2, a model concept was developed, shown in Figure 4.3. This model was built on the assumption that water or nitrogen shortage in the soil leads to different root responses and that temporal and spatial dimensions influence the physiological mechanisms regulating resource capture and use efficiency.

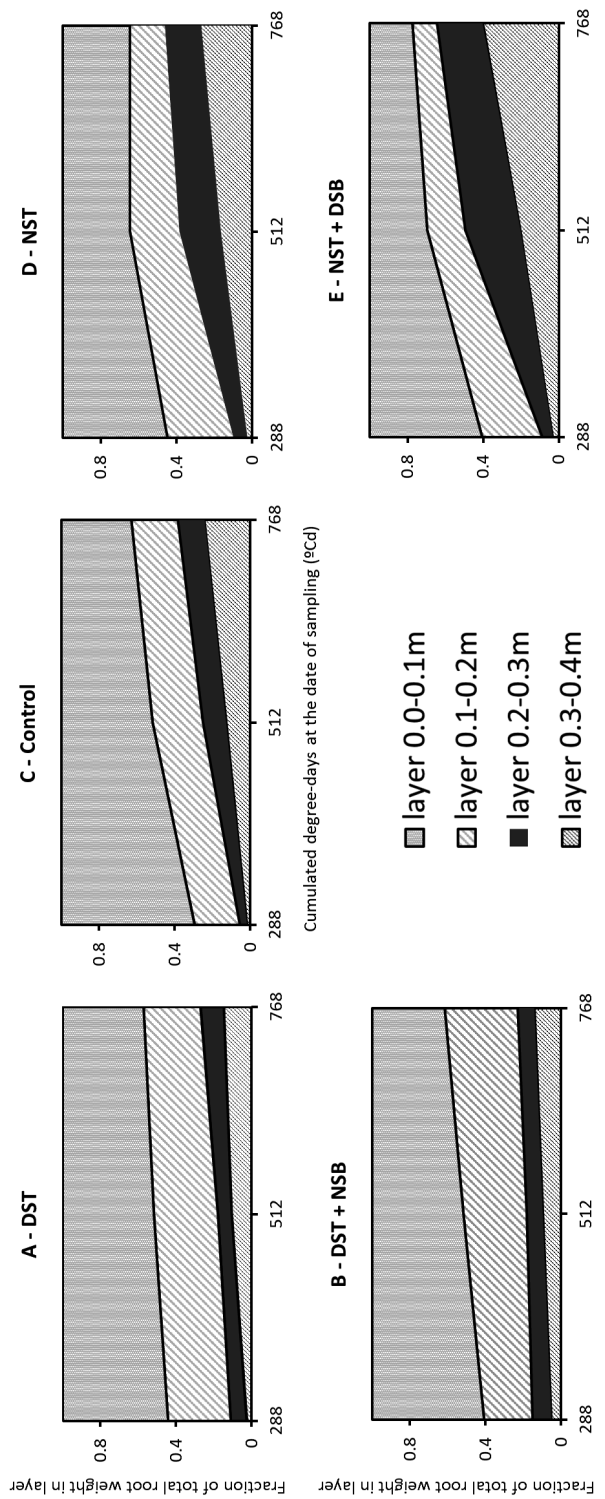

Figure 4.1 Fraction of total root weight allocated to each layer (layer 0.0-0.1m, layer 0.1-0.2m, layer 0.2-0.3m and layer 0.3-0.4m) at different sampling moments during the trial (228 ºCd, 512 ºCd and 768 ºCd) for each treatment (A: drought applied in the upper compartment ('DST'), B: drought stress applied in the upper compartment combined with nitrogen stress applied in the lower compartment ('DST+NSB'), C: control treatment, D: nitrogen stress applied in the upper compartment ('NST'), E: nitrogen stress applied in the upper compartment combined with drought stress applied in the lower compartment ('NST+DSB')) for the cultivar 'Pronto' in the pot experiment.

External conditions as well as the internal status of the plant determine the partitioning of assimilates between the shoot and the root

In the model concept, the pool of assimilates produced by photosynthesis is influenced by environmental conditions and the ratio between the actual and potential plant transpiration. Besides, the nutritional status of the plant, measured as shoot [N] also influences the partitioning of assimilates as young plants vs. mature plants do not have the same nutritional requirements; more developed plants may require higher levels of nitrogen to maintain their growth rate and would therefore invest more assimilates into root growth to sustain their needs.

Spatial root growth throughout the soil profile is influenced by the local soil nitrate concentration

The portion of assimilates allocated to total root growth indirectly determines root proliferation into different soil layers. A fixed fraction of them are allocated to vertical soil exploration. In each soil layer, the nitrate concentration is determined by the amount of nitrate present in the layer and the moisture content of that layer. Nitrate concentration in a soil layer varies over time due to nitrate and water capture by the plant, and potential leaching to a lower soil layer. The partitioning of the total root mass in different soil layers depends on the nitrate concentration in the layer; as observed previously in the pot trial, root growth may occur in an N-rich layer (as opposed to an N-poor layer) when the plant requires nitrogen capture to sustain its growth rate; based on the same amount of nitrate present in two layers, root growth may increase in the driest layer as its nitrate concentration increases when its moisture content decreases.

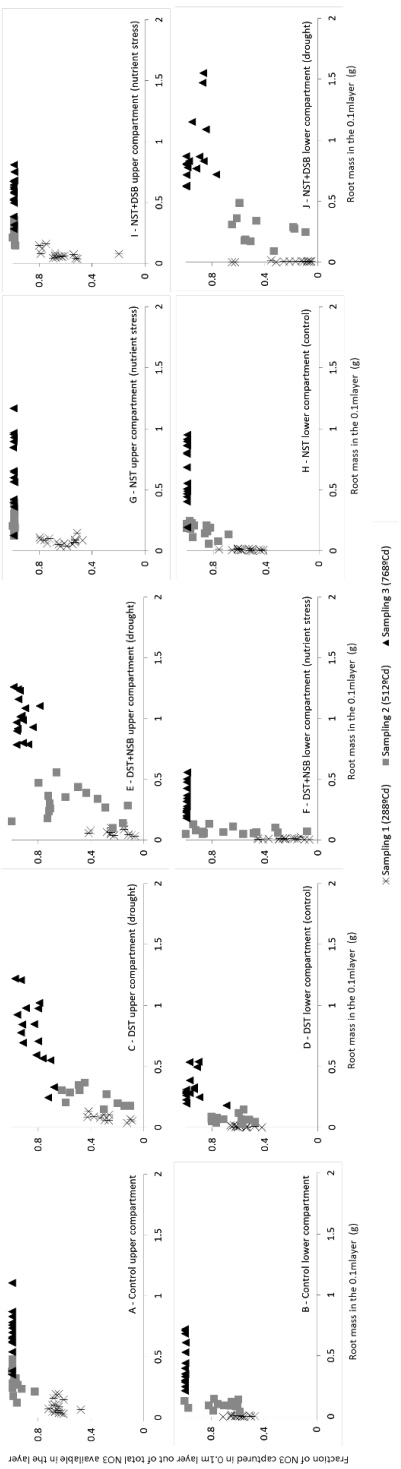

Figure 4.2 Relationship between the root mass in a 0.1 m layer and the fraction of NO_3 captured in that layer (out of total NO_3 available in that layer) at each sampling date (Sampling 1 (288 ºCd), Sampling 2 (512 ºCd) and Sampling 3 (768 ºCd)) for each treatment and compartment (A and B: control treatment upper and lower compartments, respectively; C and D: 'DST' treatment (drought stress applied in the upper compartment) upper and lower compartments, respectively; E and F: 'DST+NSB' treatment (drought stress applied in the upper compartment combined with nitrogen stress applied in the lower compartment) upper and lower compartments, respectively; G and H: 'NST' (nitrogen stress applied in the upper compartment) upper and lower compartments, respectively; I and J, 'NST+DSB' (nitrogen stress applied in the upper compartment combined with drought stress applied in the lower compartment) upper and lower compartments, respectively). Each point represents a value for one cultivar ('Pronto' or 'Matilda'), one 0.1 m layer and one replicate of the pot experiment.

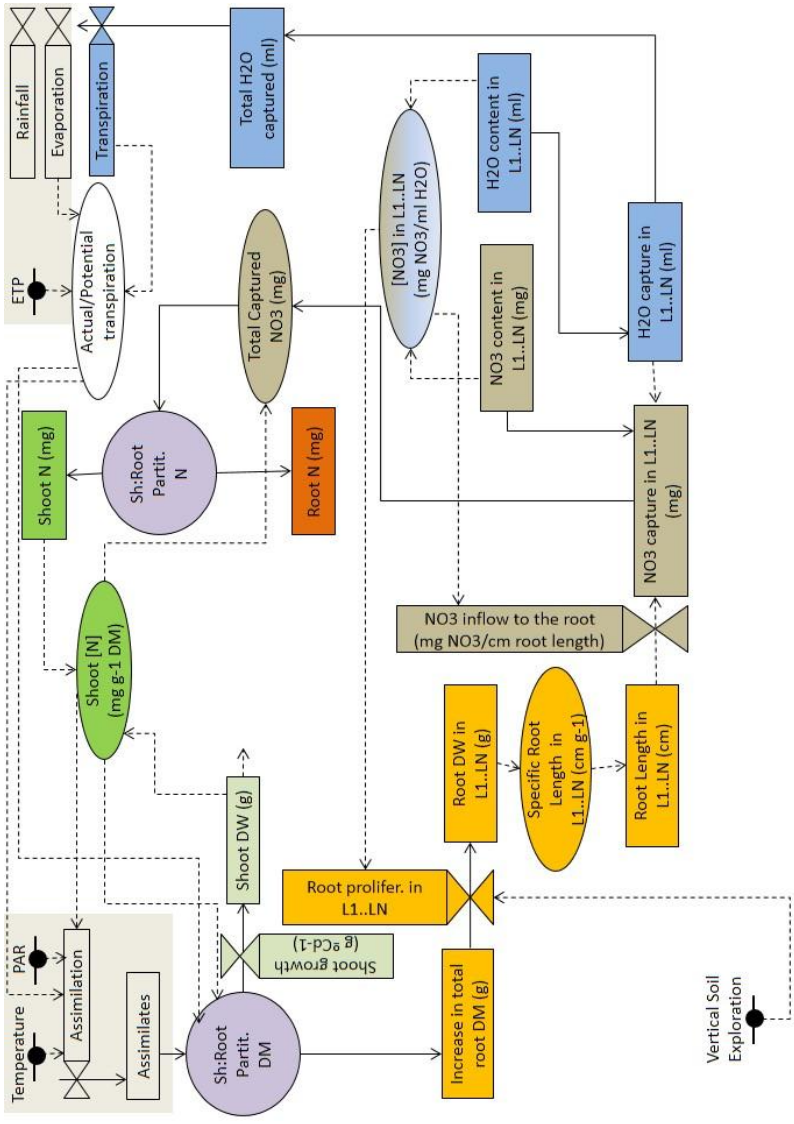

Figure 4.3 Model concept flow chart (Sh: shoot; Partit.: Partitioning; DM: Dry Matter; L1… LN: soil layer L1 to soil layer LN); PAR: Photosynthetically Active Radiation; N: nitrogen; ETP: evapotranspiration. Continuous lines indicate a flow of material; Dotted lines indicate a flow of information.

Water capture mechanisms are purely physical and only partially impact nitrate capture processes

Environmental conditions influence transpiration which determines the overall water capture from the soil; combined with the moisture content within a layer, transpiration indirectly affects the amount of water to be captured in a given soil layer. As shown previously, in optimal conditions additional root growth does not lead to additional nitrate capture, thus in the model concept, nitrate capture in a certain layer is only impacted by the moisture content of that layer and the amount of nitrate available in that layer.

The overall amount of N captured below-ground is a key element by influencing the nutritional status of the plant and determining spatial nitrate capture

Overall nitrate captured in all layers is then allocated to the shoot (impacting shoot [N]) or to the roots. The shoot [N] then regulates the amount of N to be captured below-ground as a feedback loop as the nutritional status of the plants determines the quantity of resources required to sustain the shoot growth rate. The total amount of nitrogen captured below-ground may also affect the amount of nitrogen captured in a specific layer as if the whole requirement is not met by resource capture in certain layers, it may increase the capture in other layers as a compensation mechanism.

4.3.3 Assessing genetic variation in physiological processes determining resource capture and use

Variation in physiological mechanisms involved in resource capture and use efficiency

Table 4.3 summarizes the results for the above-ground and below-ground measurements performed on the 148 cultivars at intermediate and final harvest during the four field trials.

Under optimal growing conditions (Trial 2, 2010) the highest dry matter production and highest Nitrogen Use Efficiency (NUE) at final harvest were achieved. Highest levels of nitrate left in each soil layer and over the whole soil profile at final harvest were also recorded during this trial. Significant genetic variation was found in fresh and dry

yields, plant H_2O and plant [H_2O] as well as in the amount of nitrate left in the soil layers and over the whole profile at final harvest. No genetic variation was found in the NUE in this trial.

Under dry conditions (Trial 1, 2010 and Trial 1, 2011), genetic variation was found in all shoot measurements at final harvest, except for fresh yield in Trial 1, 2010. No significant genetic variation was found in soil moisture or nitrate measurements at final harvest for Trial 1, 2010, with relatively mild temperatures, whereas significant genetic variation was found in moisture content in each soil layer and over the whole soil profile at final harvest in Trial 1, 2011 under much colder temperatures.

Under wet conditions (Trial 2, 2011) significant genetic variation was found in shoot measurements at final harvest; such significant genetic variations were also found in the moisture content in the top soil layer (0.0-0.1 m layer) at final harvest, and in soil nitrate measurements in the layers 0.1-0.2, 0.2-0.3 and 0.3-0.4 m at final harvest.

Partitioning the total variance into variance components

The partitioning (%) of the total variance recorded for each trait into different variance components is summarized in Table 4.4. For the below-ground measurements (soil moisture content in each 0.1 m layer of the 0.4 m soil profile and the volume of water left over the whole 0.4 m soil profile and [NO_3] in each 0.1 m layer of the 0.4 m soil profile and total NO_3 of the whole 0.4 m soil profile) the moment of sampling had the largest contribution to the total variance observed, with 45 to 89% of the total observed variance in below-ground traits accounted for by the Y(year) × T(trial) × S(sampling) effect compared to Y × T. This confirms the results in Table 4.3 showing that differences found in below-ground measurements were much larger between samplings within trials than between trials within a sampling date. For all water measurements, the contribution of the main genotypic effect to the variance was null; the effect of G × Y × T accounted for 1% of the total variance recorded for both [H_2O] and [NO_3] measured in the 0.2-0.3 m and the 0.3-0.4 m layers. Two percent (2%) of the total variance recorded in the [NO_3] measured in the 0.0-0.1 m layer was due to genotypic effect only (1%) or to G × Y (1%). A small proportion of the total variance within the total amount

of NO_3 left over the whole 0.4 m soil profile was attributed to the genotypic effect alone (1%) or to the G × Y × T interaction (1%).

The largest proportion of the total variance recorded for shoot measurements was attributed to the effect of the growing conditions within a single environment (i.e. Y × T) with 35 to 71% explained by Y × T. The main year effect ('Y') explained 45% of the variance recorded in shoot dry weight across trials, and 56% and 34% of the total variance observed in plant [H_2O] and plant H_2O. The main genotypic effect ('G') explained 1% of the total variance recorded in shoot dry weight and plant N, 2% of plant [N] and 4% of shoot fresh weight. The largest proportion of the total variance attributed to the interactions between genotypic effect and single growing environment ('G × Y × T') was found for plant fresh weight (11%).

Table 4.4 therefore shows that the effects of sampling time and environmental conditions during growth and their interactions were causing the largest proportions of the total variance of the below-ground traits. For above-ground traits, Year ('Y') and Year × Trial interactions ('Y × T') were important variance components. Nevertheless, within trials there were significant cultivar differences that were relevant for practice (bold data in Table 4.3) and the ranges of the cultivar means were also large in many cases. Almost all above-ground variables and several below-ground variables showed significant cultivar effects within trials. However, the residual variances were large for all below-ground variables and several of the above-ground variables. Moreover, when cultivar means of the variables of one of the four trials were plotted against cultivar means of the variables in one of the other three trials then the correlations were very small and the rankings were very inconsistent, demonstrating very large genotype × environment interactions (relations not shown). This type of inconsistent genotype × environment interactions were also demonstrated by Des Marais et al. (2013) (their Fig. 4.1E). Moreover, in-depth analysis of the above-ground and below-ground data on presence of nitrogen showed that combining information on uptake by the plant and residual soil N does not provide full insight into the dynamics of nitrogen in the lettuce crop (analysis not shown). Improved phenotyping supported by modelling is needed to reduce the residual variance and to improve the expression and evaluation of cultivar differences.

Table 4.3 Mean, minimum (Min) and maximum (Max) values for soil and plant measurements at intermediate (Inter.) and final (Final) sampling for the four field trials across the population of 148 lettuce cultivars.

	2010				2011			
	Trial 1		Trial 2		Trial 1		Trial 2	
Sampling	Inter.	Final	Inter.	Final	Inter.	Final	Inter.	Final
Soil [H_2O] left in layer (v:v)								
0.0-0.1 m	0.16	0.07	0.13	**0.20***	0.15	**0.09**	0.13	**0.27**
Min-Max	0.06-0.45	0.00-0.60	0.08-0.32	0.09-0.32	0.12-0.24	0.06-0.14	0.11-0.17	0.20-0.36
0.1-0.2 m	0.20	0.08	0.16	0.18	0.17	**0.09**	0.16	0.26
Min-Max	0.06-0.31	0.00-0.29	0.13-0.26	0.10-0.30	0.14-0.26	0.07-0.19	0.12-0.17	0.22-0.30
0.2-0.3 m	0.20	0.08	0.18	0.15	0.18	**0.10**	0.17	0.24
Min-Max	0.14-0.41	0.00-0.42	0.11-0.34	0.01-0.41	0.13-0.26	0.07-0.16	0.12-0.19	0.20-0.29
0.3-0.4 m	0.19	0.10	0.18	0.15	0.15	**0.11**	0.14	0.20
Min-Max	0.12-0.32	0.01-0.38	0.12-0.29	0.09-0.32	0.08-0.24	0.08-0.16	0.10-0.17	0.16-0.28
Water left over the 0.4 m soil profile (mL)	740	318	**646**	689	652	**388**	474	**959**
Min-Max	474-1048	167-784	440-855	464-1000	637-725	335-628	407-667	694-1093
Soil [NO_3] left in layer (mg kg^{-1} soil)								
0.0-0.1 m	178	51	132	**129**	163	103	175	117
Min-Max	89-245	6-261	31-393	23-247	71-259	0-530	62-307	4-215
0.1-0.2 m	141	33	160	**115**	110	24	151	**38**
Min-Max	76-278	6-272	11-278	9-228	56-307	0-260	68-217	6-155
0.2-0.3 m	157	49	**164**	**111**	**105**	21	**154**	**50**
Min-Max	13-230	7-143	71-251	9-328	51-164	0-164	71-230	4-196
0.3-0.4 m	136	67	148	120	119	101	149	**57**
Min-Max	82-218	7-338	78-241	38-189	30-199	0-261	32-306	3-216
NO_3 left over the 0.4 m soil profile (g)	0.44	0.14	0.44	**0.34**	0.36	0.18	0.42	0.19
Min-Max	0.22-0.57	0.03-0.39	0.25-0.60	0.12-0.54	0.27-0.53	0.04-0.62	019-0.58	0.02-0.42

	At final harvest	At final harvest	At final harvest	At final harvest
Plant Fresh Weight (g)	294	**483**	**344**	**514**
Min-Max	162-539	201-685	193-467	256-785
Plant Dry Weight (g)	**28.8**	**46.8**	**22.5**	**20.7**
Min-Max	18.3-51.2	22.0-74.5	16.2-28.2	10.7-42.6
Plant [H_2O] (g H_2O g^{-1} DM)	**9.3**	**9.5**	**14.3**	**24.0**
Min-Max	5.1-16.7	4.3-17.5	9.6-19.8	13.0-33.2
Plant H_2O (g per plant)	265	**436**	**322**	**493**
Min-Max	185-456	186-601	236-430	259-710
Plant [N] (g N kg^{-1} DM)	**24.2**	22.4	**23.8**	**37.0**
Min-Max	7.8-32.2	6.2-36.8	16.7-32.1	28.4-46.8
Plant N (g per plant)	**0.69**	1.03	**0.53**	**0.76**
Min-Max	0.41-1.48	0.29-1.89	0.36-0.80	0.40-1.59
Plant NUE (g DM g^{-1} N in head)	**41.9**	49.4	**42.5**	**27.2**
Min-Max	31.0-56.1	27.2-161.7	31.2-59.9	21.4-35.2

*For values in bold, significant genetic variation was found at $p \leq 0.05$.

Table 4.4 Partitioning of variance components (as % of the total variance) for below- and above-ground variables between Genotype ('G'), Year ('Y': 2010 or 2011), Trial ('T': Trial 1 or 2 within a year), Sampling ('S': Intermediate or Final Sampling within a trial), the interactions between components ('G × Y', 'Y × T', 'G × Y × T', 'Y × T × S' and 'G × Y × T × S'), and the residual error (Res.).

	G	**Y**	**G × Y**	**Y × T**	**G × Y × T**	**Y × T × S**	**G × Y × T × S**	**Res.**	**Total**
Soil [H_2O] left in layer (v:v)									
0.0-0.1 m	0.0	0.0	0.0	0.0	0.0	83.9	0.0	16.1	100
0.1-0.2 m	0.0	0.0	0.0	0.0	0.3	89.4	0.0	10.3	100
0.2-0.3 m	0.0	0.0	0.0	0.0	0.7	81.5	0.0	17.8	100
0.3-0.4 m	0.2	0.0	0.1	0.0	1.0	62.5	0.0	36.3	100
Water left over the									
0.4 m soil profile (mL)	0.0	0.0	0.0	0.0	0.0	79.9	0.0	20.1	100
Soil [NO_3] left in layer (mg kg^{-1} soil)									
0.0-0.1 m	0.9	0.0	0.6	0.0	0.1	51.1	0.0	47.3	100
0.1-0.2 m	0.4	0.0	0.4	0.0	1.1	76.4	0.0	21.7	100
0.2-0.3 m	0.4	0.0	0.4	0.0	1.2	78.9	0.0	19.2	100
0.3-0.4 m	0.0	0.0	0.0	0.0	0.0	45.0	0.0	55.0	100
NO_3 left over the									
0.4 m soil profile (g)	0.6	0.0	0.2	0.0	1.0	73.8	0.0	24.3	100
Plant Fresh Weight (g)	4.3	0.0	0.0	62.5	10.6	n.a.*	n.a.	22.6	100
Plant Dry Weight (g)	1.3	45.2	0.1	40.5	2.4	n.a.	n.a.	10.5	100
Plant [H_2O]									
(g H_2O g^{-1} DM)	0.2	56.5	0.0	36.3	0.8	n.a.	n.a.	6.3	100
Plant H_2O (g per plant)	0.0	34.3	1.9	53.2	0.0	n.a.	n.a.	10.6	100
Plant [N]									
(g N kg^{-1} DM)	1.5	5.6	0.0	70.6	0.0	n.a.	n.a.	22.2	100
Plant N (g per plant)	1.4	3.2	0.0	55.4	0.1	n.a.	n.a.	39.9	100
Plant NUE									
(g DM g^{-1} N in head)	0.0	10.3	0.0	34.7	0.0	n.a.	n.a.	55.0	100

*Not applicable: as shoot measurements were only made at final harvest, the sampling term ('S') was removed from the model.

4.3.4 Implications of phenotyping results for model development

A model specifically targeting breeding for resource capture under limiting environment

The results of the field experiments showed that under optimal growing conditions (Trial 2, 2010) nitrogen use efficiency above-ground does not seem to be a trait of interest for improvement, as no genetic variation was found in plant [N] (in g N kg^{-1} DM), plant N (in g per plant) or plant NUE (in g DM per g N) (Table 4.3). In contrast, below-ground traits displayed a higher level of genetic variation and higher repeatability values in limiting growing conditions such as in Trial 1, 2011; this suggests that a mild level of drought or nitrogen stress during growth is conducive to the expression of diverse coping strategies and consequently leads to a broader range of

variation in such strategies. On the other hand, harsh growing conditions like in Trial 1, 2010 do not seem suitable as a breeding environment as they suppressed potential genetic variation in resource capture and growth responses. Being able to simulate different growing conditions and their effect on the different traits would thus be useful in breeding programmes targeting specifically organic growing conditions where crops are often subjects to mild and temporary shortage of resources during growth.

Using a model approach to cope with Genotype × Environment interactions

The experimental results obtained in the field trials highlighted the strongly inconsistent cultivar effects across trials (both within and between years) affecting the expression of the various traits. The physiological mechanisms identified in the pot experiment and their function as integrated in the model design could hardly be retrieved in the field trials results. Especially the combination of the influence of the genetic variation and the impact of the growing conditions made the results of the measurements on moisture and nitrate content over the soil profile very complex to analyse and to understand. As shown in Table 4.4, the contribution of the genotypic effect on the variance in measurements made on below-ground traits was very limited compared to the impact of the growing conditions, highlighting the inconsistent cultivar differences across trials affecting the expression of the traits measured in these trials – which would make them very difficult to breed for. The measurements made for below-ground traits during the field experiments are hardly possible to integrate as such in a breeding programme, partly because of the enormous amount of labour requirement and partly because of such large residual variances and inconsistent cultivar effects. However, such large datasets provide an excellent basis to build and test the model. Moreover, a model accounting for inconsistent cultivar behaviour across environments would be a useful tool in a breeding programme as it would point out which traits are of interest for a given breeding environment.

Greater details in the interactions between soil resource availability, resource capture and root growth and the genetic variation thereof are needed as a step forward building the model

This being said, the traits involved in resource and use efficiency measured in this study nevertheless displayed large and significant genetic variations within trials; once their dynamics over time and space will be better understood and dissected in more stable variables, they will present an interesting potential for breeding purposes. In particular, the pot trial results shed light on the possible effect of localized change in nitrate concentration and/or moisture content on root growth. The results seem to confirm what was observed previously by Drew et al. (1973) who found that N-rich patches increases lateral root growth in barley; these findings also seems to be in line with the conclusions of Chapman et al. (2011), who found that in *Arabidopsis thaliana*, while higher nitrate concentration increases basal root growth, more water supply increases primary root growth. Overall, the different roles of localized nitrate concentration, and moisture content on root growth should therefore be studied in more detail to enable the model to take into account the interactive effects of these two resources on root growth in space and time.

Additionally, the experiments carried out in this study demonstrated that in lettuce, additional root growth does not necessarily lead to a higher amount of resource being captured in a non-limiting environment (Figure 4.2). This is in contradiction with the study by King et al. (2003): their model was based on an exponential relationship between root length density within the soil profile and resource capture. This relationship, however, seems more in accordance with the mechanisms triggered when lettuce roots experience a dry environment. It might also apply to a nutrient that is less mobile in moist soil such as phosphorus. More research is needed to understand exactly the relationships between root growth and the amount of resource captured over time and space in lettuce.

4.3.5 Implications of phenotyping for breeding: What to breed for and in which selection environment?

The findings of this study underline the importance of breeding for below-ground traits in a growth-limiting environment. The lower levels of genetic variation and repeatability in the traits involved in resource capture and use efficiency found in the trial carried out under optimal conditions (Trial 2, 2010; Table 4.3) show that under optimal conditions, below-ground traits are not crucial for shoot performance. As all

resources are available for uptake, no changes in the plant morphological or physiological processes are required to maintain its growth rate. In this system, both plastic (highly adaptable to their environment) and non-plastic (inert to changes in their environment) plants can perform. Therefore, if genetic variation in yield is observed, it might purely be caused by head morphological characteristics and the total amount of resource the plant is able to capture in the soil given its morphological features. In an optimal environment, varieties with larger overall biomass above- and below-ground are more likely to display higher yields than a variety with a lower overall biomass.
In a system in which resources are limiting, results highlighted that not only improved morphological features are necessary to capture the resource (e.g., a larger root system leads to an improved nitrate capture) but also implicitly that plasticity, as the manner a plant adapts to its environment in a timely fashion, in the processes involved in resource capture and use efficiency seems crucial. This concept was already mentioned by Hodge (2004). Therefore, breeding for resource capture and use efficiency should be done in a mildly limiting environment to trigger the expression of genetic variation; moreover more efforts should be put into understanding the dynamics of the responses in root growth, resource capture and use efficiency in time. As lettuce is a short cycle crop, new cultivars require a high level of plasticity in adaptation to their environment, especially to adapt to organic and low-input environments.

4.4 Concluding remarks

This study highlighted the following points:

- Root growth in a soil profile with localized resource shortage depends on the resource that is in short supply: root growth in relation to localized nitrate concentration and moisture content should be studied in more detail.
- Resource capture may be improved by increased root growth in a limiting environment only; selection for root traits and resource use efficiency only makes sense in such a limiting environment.
- There is considerable genetic variation in resource capture.

- The interaction between processes in the upper rooted soil layer and the lower rooted soil layer under conditions in which resources are not abundant and not equally distributed should be further investigated.
- Incorporating the time dimension is an important step to identify cultivars which are more plastic in root development and are capable of responding quickly to changes in their environment by adapting their physiological mechanisms and morphological and architectural characteristics.

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Chapter 5

Genetic control of resource capture and use efficiency of lettuce (*Lactuca sativa* L.)

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Kerbiriou PJ, Maliepaard CA, Stomph TJ, Koper M, Froissard D, Lammerts van Bueren ET, Struik PC (2014) Genetic control of resource capture and use efficiency of lettuce (*Lactuca sativa* L.)

Abstract

Robustness in lettuce, defined as the ability to produce stable yields across a wide range of environments, may be associated with below-ground traits such as water and nitrate capture. We assessed the genetic variation for such traits and shoot performance in lettuce across four environments (2 years × 2 sites) at two sampling dates, using a population of 142 cultivars. We used these results to carry out an association mapping study based on 1170 Single Nucleotide Polymorphism markers (SNPs). Significant marker-trait associations were detected across trials for below-ground and shoot traits, in number and position varying with trial, highlighting the importance of the growing environment on the expression of the traits measured. The difficulty of identifying general patterns in the expression of the QTL calls for a more in-depth analysis of the physiological mechanisms at root level allowing sustained shoot growth.

Keywords: Lettuce; Resource acquisition; Association mapping; Quantitative Trait Loci; Soil sampling; Nitrogen use efficiency

5.1 Introduction

Agronomic research has contributed to the design of lettuce cropping systems that maximise yields and optimise quality by supplying abundant water and nutrients, avoiding stress conditions (Gallardo et al. 1996a,b; Broadley et al. 2000; Frantz et al. 2004). In lettuce, drought induced by a shortage in water supply, even temporary, significantly reduces yields, as drought limits shoot growth rate (Biddington and Dearman 1985; Kerbiriou et al. 2013a). With costs of fossil fuel-based inputs forecasted to increase steadily in the future (Mou 2011), the environmental and economic sustainability of such intensive systems is becoming more and more questionable, calling for the design of more resilient systems.

Defined as the adaptive capacity to achieve sustainability in a dynamic fashion (Milestad and Darnhofer 2003), resilience is an important trait of organic farming systems. As organic systems aim at optimising the production system more than the individual crop, they are considered more resilient than conventional systems which emphasise the productivity of a single crop based on high levels of inputs (Lammerts van Bueren et al. 2011). However, the use of organic manure instead of mineral fertiliser to improve long term soil fertility combined with smaller amounts of irrigation in organic systems, may lead to irregular supply of nutrients and water compromising the certainty of high yields: as soil temperature and moisture conditions affect mineralisation of organic matter, crop growth may be more variable in organic systems than in conventional systems which are able to provide the plants with a continuous supply of nutrients available for uptake, though, at the expense of potentially large losses to the environment.

Not only improved cultural practices and crop management, but also breeding for robustness – allowing crops to maintain growth despite variable and irregular growing conditions during cropping (Kitano 2007) – can contribute to the sustainability of more demanding (low input, organic) horticultural systems (Lammerts van Bueren et al. 2002; Wolfe et al. 2008). For instance, new cultivars with more efficient resource uptake and use efficiency may display yield stability under low input or organic farming systems where resource availability is more irregular. Therefore traits relevant to

efficient uptake and use of resources and the possible genetic factors influencing these traits need to be identified.

The traits and the genetics of these traits did not receive much attention in recent breeding programmes of lettuce, a species with nine chromosome pairs. Contemporary approaches have been focusing on breeding for stress tolerance based on head characteristics. For instance, Uwimana et al. (2012) found 17 QTL associated with vigour in a cultivated (*L. sativa* L.) × wild lettuce (*L. serriola* L.) population subjected to drought, salinity and nutrient deficiency. Jenni et al. (2013) found 36 QTL significantly associated with eight traits linked to heat-stress related physiological disorders in lettuce in recombinant inbred lines derived from an intra-specific cross between two commercial lettuce cultivars.

In lettuce, research on the role of root traits in resource acquisition has been rather limited. As lettuce breeding has been taking place under optimal growth conditions in conventional systems, breeders could afford to select types with a small root systems and a high shoot: root ratio, thus increasing harvestable yield (Johnson et al. 2000). Consequently, the root system of modern lettuce varieties is shallow, mainly present in the top 0.2 m of the soil profile where resources are abundant and directly available for uptake in conventional systems (Gallardo et al. 1996b). This morphological feature may affect harvestable yields when these top layers dry out, as no roots are present in the deeper layers of the soil profile where water is available for capture (Jackson 1995).

One way to improve resource capture and use efficiency and consequently the robustness of new lettuce cultivars may thus be to select for genotypes with a longer, more developed root system able to forage water and nutrients in the lower layers of the soil and compensate for the unavailability of resources in the top layers during a period of drought. With this idea, Johnson et al. (2000) tested whether deeper root foraging and water capture in lower layers of the soil profile was significantly associated with genetic markers in directly sown cultivated (*L. sativa* L.) × wild lettuce (*L. serriola* L.) $F_{2:3}$ families. Thirteen QTL, each accounting for 28-83% of the phenotypic variation in root traits, were identified, and they showed that the loci for taproot length co-localised with the ability to extract water from deeper soil layers.

However, assessing the genetic diversity of root systems with the objective to breed for improved root system architecture, is very intensive and labour-consuming, especially under field conditions where roots have to be sampled, washed, manually cleaned to remove organic litter and scanned. Instead, it might be easier to take soil samples to measure resource capture, and by a modelling approach, predict root characteristics – based on the assumption that root characteristics and resource capture are strongly correlated within relevant ranges, as shown by King et al. (2003) in barley and surmising that nitrogen accumulation in the heads is correlated with resources removed from the soil.

Chapter 4 (Kerbiriou et al., 2014) showed that in lettuce the relationship between root mass and nitrate capture does not follow the relationship found by King et al. in barley (2003), where the non-captured resource logarithmically declines with an increase in the amount of roots or with the root length density. Although nitrate capture in lettuce is generally fairly correlated to root mass or root length density when field conditions are conducive to growth (Kerbiriou et al. 2013a, Chapter 3), in lettuce localised root growth is related to specific, localised resource availability as demonstrated by Kerbiriou et al. (2013b, Chapter 2) in a pot trial. In case localised nitrate shortage was applied, root growth was more abundant in N rich soil layers – as previously noted by Hodge (2004) in grass species under various conditions – whereas when localised drought was applied, root growth occurred in the dry compartment (as opposed to the moist compartment). These findings highlighted that the relationship between root growth and resource capture in lettuce is complicated, and requires a novel modelling approach before resource capture can be related to root traits – as discussed in Chapter 4 (Kerbiriou et al., 2014).

This Chapter 4 also revealed that large genetic variation can be found in the temporal and spatial dynamics of resource capture below-ground and use of these resources above-ground. The patterns of nitrate and water capture in 0.1 m soil layers over a 0.4 m soil profile in a population of 148 lettuce cultivars grown in four environments proved to be highly diverse and complex, supporting the idea that it would be possible but difficult to breed for traits related to below-ground performance.

While the mechanisms involved in resource capture and use were analysed in Chapter 4, the current paper addresses the genetic control of such traits, in other words explores the association between the phenotypic traits involved in resource capture and use efficiency, and genotyping information provided by Single Nucleotide Polymorphism (SNP) markers. With this objective, a population of 148 lettuce cultivars was planted during two seasons (spring and summer) in two different locations under organic cropping conditions, and nitrate and water capture below-ground and in the shoots were assessed during growth and when the plants reached a harvestable size. Simultaneously, 1170 SNP markers were scored for each cultivar using the KASP™ technology (LGC Genomics, Hents, UK). The statistical significance of the association between the measured traits and the markers was tested with the aim to find QTL associated with nitrate and water capture and use efficiency, and to understand their interaction with the growing environment. A complete set of reliable data was obtained for 142 cultivars.

5.2 Materials and Methods

5.2.1 Cultivar choice

Two-hundred-fifty lettuce accessions, commercially available in the period between 1960 and 2008 were grown under field conditions in 2008 and were evaluated for a broad range of crop growth parameters. Out of these 250 accessions, 148 butterhead types suitable for field cultivation under either spring or summer conditions, or both, were selected for this study. Criteria for selection included diversity in head characteristics (large vs. compact heads, colour, leaf shape, leaf texture etc.), commercial origin (seed company), and country and date of release. Criteria for selection did not include traits related to root characteristics, but we surmised that cultivars released before 1970 had larger root systems and lower harvest indices. The origin of the selected varieties is illustrated in Figure 5.1. In the selected population, 27 cultivars were released before 1970, 24 cultivars were released between 1970 and 1990, and 95 cultivars were released after 1990; the time of release of two cultivars was unknown. Eight cultivars were known to be grown by amateur gardeners, and two

cultivars came from breeding programmes targeting specifically organic farming systems.

5.2.2 Transplants raising and transplanting

Seeds used originated from randomly selected plants from the screening trial in 2008. Prior to transplanting, seeds were sown in 4 cm × 4 cm × 4 cm organic peat blocks (Jongerius, Houten, the Netherlands) after breaking seed dormancy by exposure to 4 ºC for 24 h. Transplants were raised in a greenhouse with a day temperature of 20 ºC and a night temperature of 15 ºC.

Transplanting was done when the transplants had 5-7 leaves and few roots started to emerge out of the peat block. In the field, plant arrangement was 0.3 m × 0.3 m.

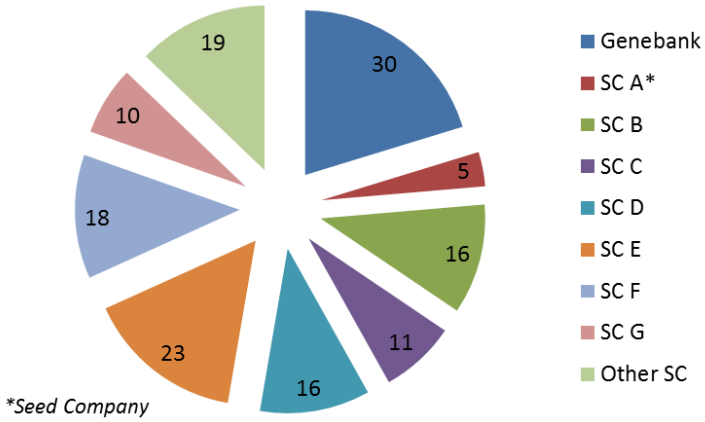

Figure 5.1 Cultivar partitioning per breeding company within the population. Cultivars owned by a breeding company which was represented less than 3 times in the population fell under the 'Other Seed Companies' category.

5.2.3 Experimental design

Four field trials were performed: two in Wageningen (51.97° N, 5.67° E, The Netherlands), in spring and summer 2010, and two in Voorst (52.23° N, 6.08° E, The Netherlands), in spring and summer 2011. Each trial included two repetitions. The experimental set up was a randomised complete block design, each block consisting of 150 plots. Two plots per block were left empty for measurements in bare soil. Not bare

plots were planted to 25 plants (5 × 5 plants) of the same cultivar (cf. Figure 5.2). Measurements were done on the nine inner plants.

5.2.4 Field management

Field sites were chosen according to their soil quality (uniform soil profile up to 0.5 m depth and adequate structure) and previous crop management. All sites had been cropped uniformly in the previous 5 years on a larger surface than the area covered by the trials, in order to avoid influence of previous crops or field management on soil characteristics. In both locations the soil was sandy, poor with a low content in organic matter (8-10%), and low water retention capability. All trial fields were certified organic and managed according to organic standards during the experiments.

For even distribution of nutrients, fertilisation was provided by applying 100 kg/ha nitrogen, from seaweed pellets (9% N, 3% P, 3% K + 3% MgO, EcoFertiel, EcoStyle, Appelscha, The Netherlands) on the day before transplanting, instead of using compost or manure. Weeding was done manually every week. Irrigation was not applied.

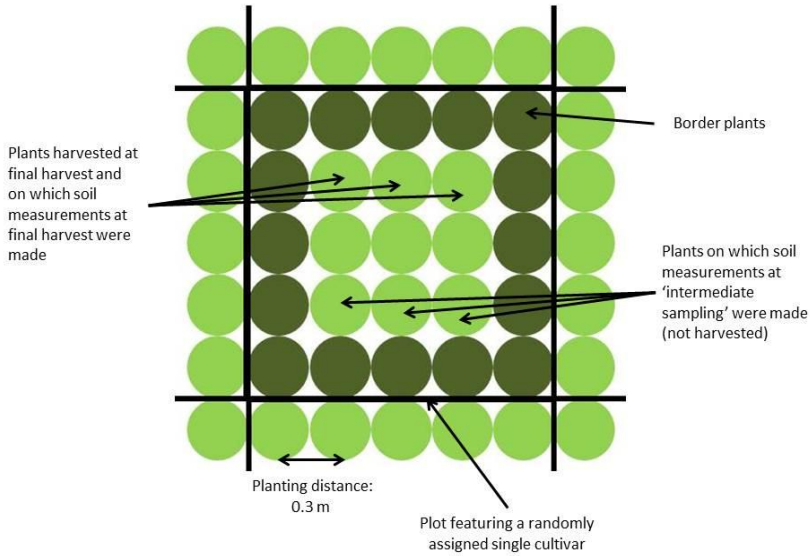

Figure 5.2 Sampling scheme for a plot featuring a single cultivar.

5.2.5 Field conditions

For each trial, weather data (air temperature, radiation, rainfall) were recorded daily (Voorst) or hourly (Wageningen) at the nearest weather station (for the Wageningen trials, data were collected from http://www.met.wau.nl/ and for the Voorst trials, data were collected from the on-farm weather station). Soil temperatures were measured in 4 horizons (0.0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4 m) using a data logger. Cumulated degree-days (based on air temperatures, calculations see below), as well as cumulated rainfall at each sampling date for each trial are shown in Table 5.1. Details of daily temperature fluctuations and daily rainfall events are shown in Figure 5.3.

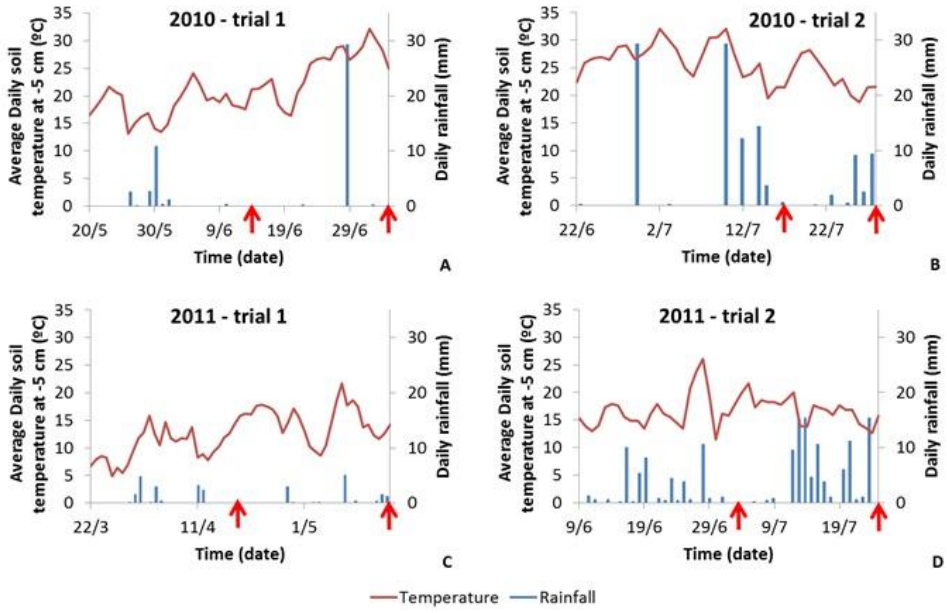

Figure 5.3 Average daily temperature recorded at 5 cm below ground and average daily rainfall for the Wageningen trials (A, B) and for the Voorst trials (C, D). Arrows indicate the time at which intermediate sampling and final harvest occurred (cf. Materials and Methods).

Table 5.1 Transplanting, intermediate sampling and final harvest dates, and weather conditions during the four experiments.

Year	2010				2011			
Location	Wageningen				Voorst			
	Trial 1		Trial 2		Trial 1		Trial 2	
Planting date	20-05-2010		22-06-2010		22-03-2011		09-06-2011	
	Intermediate sampling	Final harvest	Intermediate sampling	Final harvest	Intermediate sampling	Final harvest	Intermediate sampling	Final harvest
Sampling date	14-06-2010	05-07-2010	19-07-2010	28-07-2010	19-04-2011	17-05-2011	05-07-2011	25-07-2011
Cumulative rainfall (mm)	18	48	90	104	16	27	49	145
CDD* (°Cd)	357	793	607	782	174	481	329	590

*Cumulated Degree-Days (using 4 °C as base temperature).

5.2.6 Phenotyping

Calculation of thermal time

Cumulated degree days at each sampling date were calculated as the sum, between the date of transplanting and the sampling date, of the degrees above 4 ºC (base temperature for lettuce; Kristensen et al., 1985), based on average daily temperature:

$$CDD_{sampling\ x} = \sum_{day\ 0}^{sampling\ date\ x} \left[\frac{(T_{max} + T_{min})}{2} - T_{base} \right]$$

where T_{max} and T_{min} correspond respectively to the maximum and to the minimum temperatures recorded on a certain day and with $T_{min} > T_{base}$.

Soil measurements

Soil samples were taken every 0.1 m over a depth of 0.4 m outside of the peat block, using a 0.06 m diameter and 0.40 m long auger, during growth ('intermediate sampling') and at final harvest ('final sampling'). For three plants per plot, soil samples taken in each soil layer were pooled to account for plant-to-plant variation.

Volumetric soil moisture content (% v:v) was recorded after drying at 40 °C for 48 h.

Nitrate content (soil [NO_3], assessed in ppm) in each 0.1 m soil layer was measured using an Ion Selective Electrode (ThermoFisher, Waltham, MA, USA) using the method described previously by Sibley et al. (2009) and also used in Kerbiriou et al. (2013a), cf. Chapter 3.

Shoot measurements

Shoot measurements were done only at final harvest. Fresh weight and dry weight (g per plant) were assessed based on three plants per plot at final harvest, which took place 5 to 9 weeks after transplanting depending on the trial (for sampling method, see Figure 5.2). The averages over six plants per cultivar per trial (three plants per replicate, two replicates per trial) were used in the association mapping study. Nitrogen concentration (g N per kg dry matter) in the head was measured using the Kjeldahl method, based on the ground material of three plants per cultivar and per replicate within a trial. Physiological Nitrogen Use Efficiency (NUE, g DM per g N in head) was calculated based on the head [N] as NUE = 1 / (head [N]). The average value over the two replicates within a trial was used for the association mapping study.

5.2.7 Heritability

The genotypic and residual variance components were estimated using the Residual Maximum Likelihood Estimations (REML) analysis of Genstat 15th Edition (Hempstead, UK) with the following mixed model: response = general mean + genotype + block + error. Heritability (h^2) estimates were then calculated based on the variance components as follows: $h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_e)$ with σ^2_g the estimate of the genotypic variance and σ^2_e the residual variance.

5.2.8 Genotyping

Lettuce DNA was isolated from leaf material taken when they had reached the 5th leaf stage; these plants were specifically grown in a greenhouse for the purpose of genotyping. The plants were grown from seeds originating from the same seed lot as was used for the phenotyping experiments.

Single Nucleotide Polymorphisms (SNPs) were mined from various transcriptome sequencing projects done on the leaves of two lettuce lines (proprietary markers by Enza Zaden). SNPs were identified in lettuce Expressed Sequence Tags (EST) and only the 1348 SNPs with high probability scores were conferred into KASP™ assays (LGC Genomics, Hents, UK). Six cultivars from the 148 cultivars tested in the field were discarded in the association mapping studies because of large amounts of missing values (more than 10%) for these cultivars, therefore only 142 cultivars in total were kept in the analyses. Markers of poor quality or rare alleles (less than 10% occurrence) were also removed from the analysis and at the end 1170 markers were used. SNP markers were run with the DNA of the lettuce population on a Fluidigm chip on a Biomark HD system (Fluidigm, San Francisco, USA). The SNPs were scored using an in-house software package based on base pair codes for homozygous SNPs (0101 = A, 0202 = C, 0303 = G, 0404 = T) or heterozygous SNPs (0102 = A or C, 0304 = G or T). The SNPs were then mapped on nine linkage groups, plus an additional linkage group, used for markers for which the position was unknown. The average distance between markers was 0.4 cM. A summary of genotypic information is given in Table 5.2.

Table 5.2 Summary of marker information (generated with Genstat 15th Edition (Hempstead, UK))

Chromosome	Length (cM)	Number of markers	Median distance between markers	95% percentile of distance
1	132	171	0.2	4.7
2	124	89	0.5	6.1
3	92	65	0.7	6.4
4	162	209	0.3	3.3
5	156	172	0.3	4.4
6	98	36	0.5	15.1
7	112	118	0.4	4.0
8	169	166	0.3	4.7
9	97	67	0.2	8.3
10*	76	77	1.0	1.0
Genome	1217	1170	0.4	4.6

*Used to map markers of unknown position

5.2.9 Association mapping procedure for QTL detection

Principal components analysis (Eigenanalysis)

Population structure was investigated following the approach by Price et al. (2006) and Patterson et al. (2006) using the QEIGENANALYSIS procedure in Genstat 15th Edition (Hempstead, UK) and the 1170 SNP markers set. Seventeen significant eigenvectors were obtained and used as covariates to account for population structure in the marker-trait association models.

Linkage Disequilibrium (LD) decay investigation

Marker-marker associations (LD decay) were investigated on the set of 1170 SNP markers correcting for relatedness using the significant eigenvectors as covariates in the QLDDECAY procedure in Genstat 15th Edition (Hempstead, UK). For each chromosome, pairwise LD between markers was calculated using the square of the corrected correlation coefficient, r^2 (Pritchard and Przeworski 2001). r^2 coefficients were plotted against the genetic distance between markers (in cM) to evaluate LD decay for each chromosome (Figure 5.4).

Association mapping analysis

All the mean shoot and soil measurements obtained for each cultivar in each environment were used as phenotypic data to be related to the genotypic data. Association mapping studies were carried out for each trait at each sampling date within

each environment using the QASSOCIATION procedure in Genstat 15th Edition (Hempstead, UK). Population structure was corrected based on the results of the eigenanalysis. The eigenvectors were used as covariates as random terms in the mixed-model-based marker-trait association approach, in which the QTL effects were fitted as fixed effect at the marker position. The Wald-test was used to test significance; *p-values* were derived from this test and transformed using a $-\log_{10}(p\text{-}value)$ transformation. To account for multiple testing, a number of effective tests (# tests) was calculated as the ratio of the total genome size to the average LD over the nine chromosomes, and used to calculate the threshold of significance to claim a significant QTL as: threshold = $-\log_{10}(0.05/\#\ tests)$. Because a threshold of 3.5 was more stringent than a 5% false discovery rate, this value was used to identify significant marker-trait associations throughout the analyses.

The threshold for the minor allele frequency (MAF) was set to 7% (at least 10 accessions should have the minor allele) for testing marker-trait associations.

5.3 Results

5.3.1 Phenotyping results

Figure 5.5 (below-ground traits at both sampling dates) and Figure 5.6 (shoot traits at final harvest) summarize the mean values and genetic variation in the population of the 142 cultivars used in this study. Which variables showed significant genetic variation is indicated in Table 5.3 (bold numbers for heritability). Moisture or nitrate measurements in the soil at intermediate or final sampling did not show significant variation caused by cultivar differences for Trial 1, 2010, with relatively mild temperatures and dry weather. Significant genetic variation was found in moisture content in each soil layer and over the whole soil profile at final sampling in Trial 1, 2011 with relatively low temperatures and dry weather; nitrate left in the soil did not show much genetic variation in Trial 1, 2011. Highest levels of nitrate left in each soil layer and over the whole soil profile at final harvest were recorded for Trial 2, 2010, under optimal growing conditions, with in most cases significant genetic variation. Also in Trial 2,

2011, an experiment under conducive growing conditions, several soil variables showed significant genetic variation (Figure 5.5; Table 5.3).

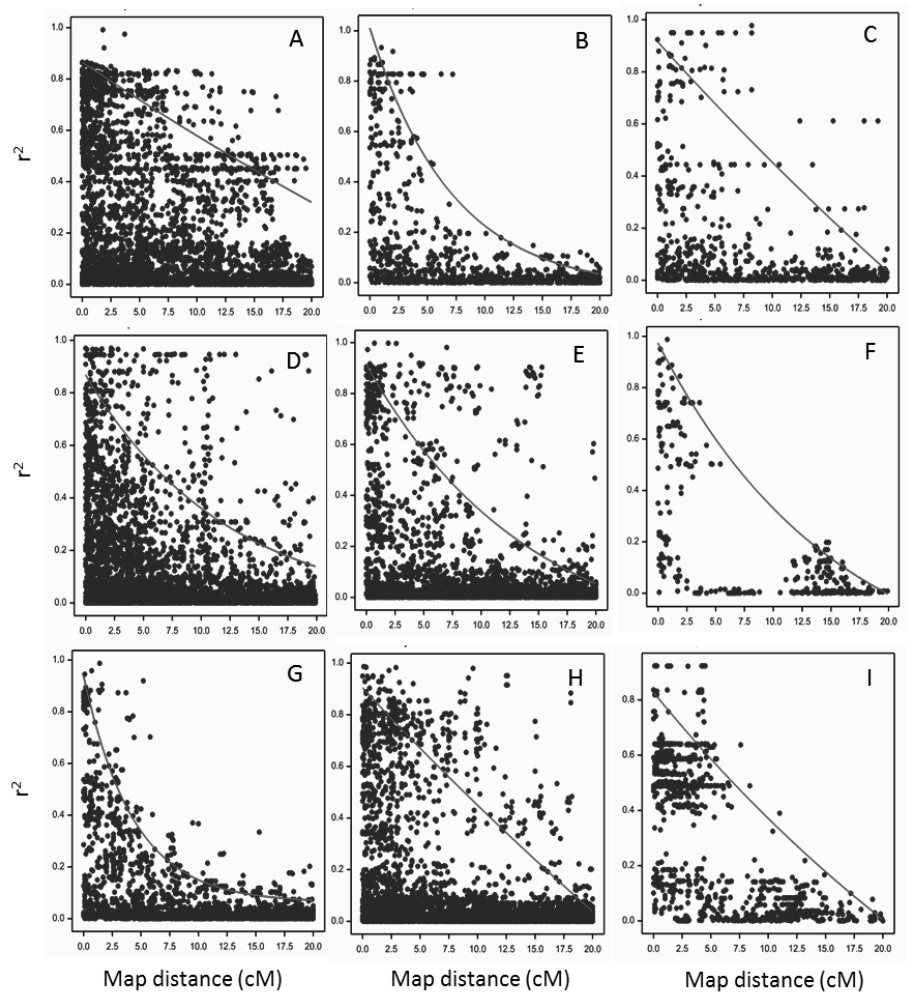

Figure 5.4 Visualisation of Linkage Disequilibrium (LD) decay as the squared coefficient of the relation between two markers (r2) plotted against the genetic distance between two markers in cM (dots) for each chromosome (A: chromosome 1; B: chromosome 2; C: chromosome 3; D: chromosome 4; E: chromosome 5; F: chromosome 6; G: chromosome 7; H: chromosome 8; I: chromosome 9). The trend line illustrates the LD decay based on the non-linear regression of the r2 on genetic distance.

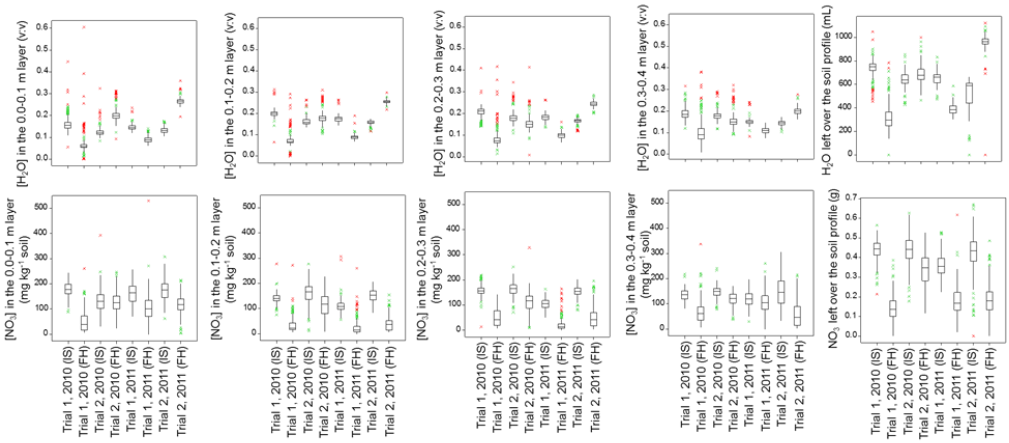

Figure 5.5 Boxplots of the below-ground traits ([NO_3] and [H_2O] in 0.0-0.1, 0.1-0.2, 0.2-0.3 and 0.3-0.4 m layers) for the population of 142 lettuce cultivars in each trial and at each sampling date (IS = Intermediate Sampling; FH = Final Harvest).

Under optimal growing conditions (Trial 2, 2010) the highest dry matter production and highest Nitrogen Use Efficiency (NUE) at final harvest were achieved (Figure 5.6). Significant genetic variation was found in fresh and dry yields. No significant genetic variation was found in plant nitrogen or NUE in this trial. Under dry conditions (Trial 1, 2010 and Trial 1, 2011), genetic variation was found in all shoot measurements at final harvest, except for fresh yield in Trial 1, 2010. Trial 2, 2011 had the highest values for plant nitrogen, with relatively small, but significant genetic variation (Figure 5.6; Table 5.3).

5.3.2 Heritability of the traits

Per trial, the heritability estimates were low for the soil moisture content measurements at each layer and over the whole soil profile, except for the measurements made at final harvest in Trial 1, 2011 (moderately dry conditions), where estimates ranged from 14 to 38% (Table 5.3).

The heritability in the [NO_3] traits was the largest at final harvest in Trial 2, 2010 (optimal growing conditions) and Trial 2, 2011 (wet conditions) with values ranging from 0 to 25% in the layers 0.1-0.4 m of the soil (Table 5.3).

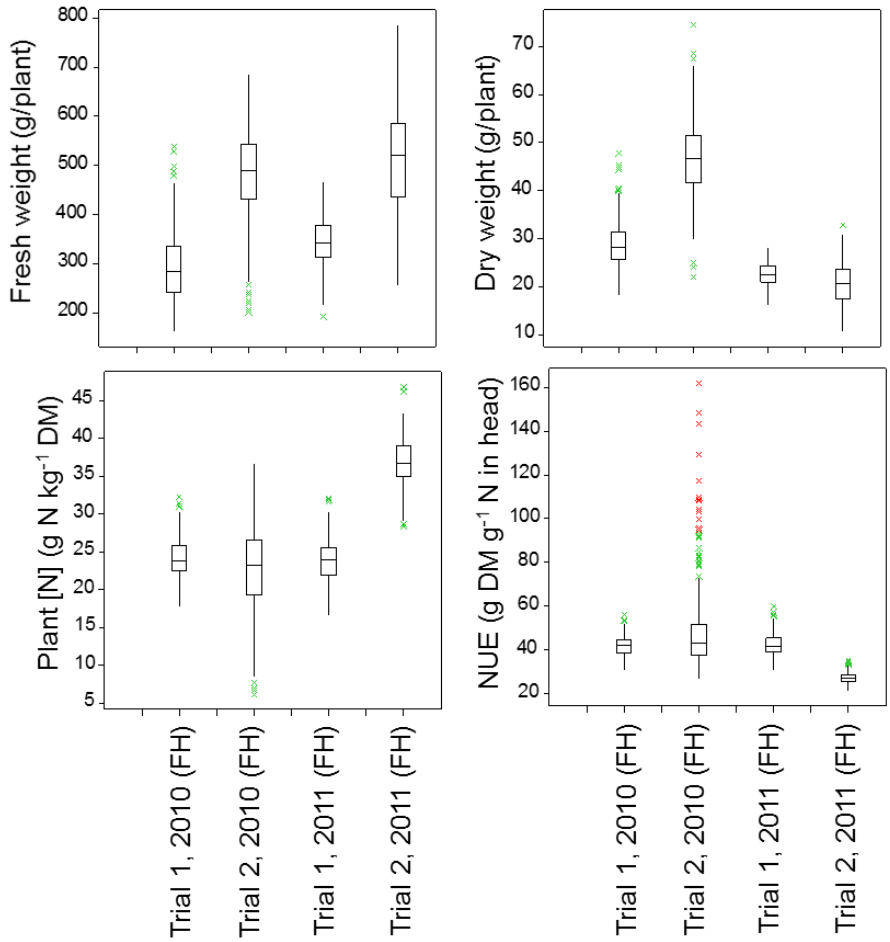

Figure 5.6 Boxplot of the shoot traits for the population of 142 lettuce cultivars in for the population of 142 lettuce cultivars in each trial (FH = Final Harvest).

Shoot traits (plant fresh and dry weight, plant [N] and plant NUE) were generally the traits for which the heritability was the largest, with values up to 55% (Table 5.3). The highest heritabilities for shoot traits were obtained in the trials in Voorst 2011, with values ranging from 17% to 55%, compared to the trials carried out in Wageningen in 2010 where values ranged from 2 to 48%.

Table 5.3. Heritability (%) of soil and plant measurements at intermediate (Inter.) and final (Final) sampling for the four trials across the population of lettuce.

	2010				2011			
	Trial 1		Trial 2		Trial 1		Trial 2	
Sampling	Inter.	Final	Inter.	Final	Inter.	Final	Inter.	Final
Soil [H_2O] left in layer (v:v)								
0.0-0.1 m	9	0	9	**21**	0	**14**	0	**15**
0.1-0.2 m	0	1	0	0	0	**25**	7	0
0.2-0.3 m	0	7	6	9	0	**38**	4	0
0.3-0.4 m	0	2	3	0	2	**16**	0	0
Water left over the 0.4 m soil profile (mL)								
	0	9	**18**	13	0	**30**	2	**15**
Soil [NO_3] left in layer (mg kg^{-1} soil)								
0.0-0.1 m	0	0	5	**25**	0	3	0	0
0.1-0.2 m	0	0	1	**17**	0	0	0	**15**
0.2-0.3 m	0	0	**14**	**16**	**13**	0	**21**	**16**
0.3-0.4 m	0	2	9	7	0	0	9	**17**
NO_3 left over the 0.4 m soil profile (g)								
	0	0	5	**23**	1	4	0	9
Plant Fresh Weight (g)		8		**48**		**38**		**55**
Plant Dry Weight (g)		**14**		**27**		**52**		**48**
Plant [N] (g N kg^{-1} DM)		**43**		5		**46**		**17**
Plant NUE (g DM g^{-1} N in head)		**39**		2		**43**		**18**

For values in bold the genetic variation as illustrated in the box plots of Figures 5.5 and 5.6 was statistically significant at $p \leq 0.05$.

5.3.3 LD decay analysis

Pairwise LD showed to decrease rapidly with genetic distance on all chromosomes except chromosome 1 (Figure 5.4). For chromosomes 1, 3, 4, 5, and 8 (Figure 5.4A, 4C, 4D, 4E and 4H, respectively), regions of high LD were mixed with regions of low LD. Basal LD, defined as the critical value of r^2 beyond which LD was assumed to be due to genetic linkage, was estimated to be 0.2 over the whole genome. For each chromosome, intra-chromosomal LD was calculated as the intersection of the LD trend line with the basal r^2 (Figure 5.4). Intra-chromosomal LD was found to decay between 8 and 17 cM for individual chromosomes (except for chromosome 1 where it was at about 35 cM) and average LD decay over the whole genome was estimated at 15 cM.

5.3.4 Marker-trait associations

Many significant QTL were found in the association mapping study, especially for the traits measured at final harvest. Most of the QTL found were located on chromosomes

Table 5.4. Significant marker-trait associations ((–Log10(P) >3.5) for overall NO3 left over the soil profile (g) at final sampling, and their position (cM) on the lettuce chromosome (Chr.) identified in each environment (Year × Trial combination) with –Log10(P) score, allele frequency (Allele fq.), allele effects and absolute value of the standard error (SE).

Year	Trial	Chr.	Marker	cM	$-Log_{10}(P)$	Allele fq. (%)	Allele effect	SE
2011	1	4	LSM00408	79.3	3.74	88.0	0.024	0.006
2011	1	4	LSM00032	80.1	3.72	86.4	0.024	0.006
2011	1	4	LSM01321	83.2	4.52	89.4	0.028	0.007
2011	2	4	LSM00408	79.3	4.19	88.0	0.034	0.008
2011	2	4	LSM00496	80.5	4.37	87.1	0.031	0.008
2010	2	5	LSM00319	92.3	4.02	92.9	0.007	0.000
2010	2	7	LSM00610	43.6	5.52	92.2	0.055	0.012

4, 5, 7 and 9, while only very few significant associations were found on chromosomes 1, 2, 3, 6, and 8 (Table 5.4, 5.5 and 5.6).

Below-ground traits

NO_3 left both over the full soil profile and in each soil layer showed the highest counts of significant marker-trait associations across environments (cf. Table 5.4 and 5.5); there significant associations were consistent across trials and over the different layers of the soil profile. Contrastingly, significant marker-trait associations for water left over the soil profile were found only in Trial 2, 2011 (on chromosome 4 at 88.6 cM; on chromosome 5 at 92.3 cM; on chromosome 9 at 53.8 and 58.0 cM) and in Trial 2, 2010 (on chromosome 1 at 68.3 cM and on chromosome 7 on 43.6 cM).

As shown in Table 5.4, significant marker-trait associations for NO_3 left over the full soil profile were found on chromosome 4, 5, and 7 and mostly at final harvest. The frequency of the major allele for these markers were high over the population, with frequencies ranging from 92.2 to 86.4%. The effects of these QTL were intermediate, with approx. 15% difference in overall NO_3 content over the whole soil profile between the two parts of the population bearing the different alleles.

The same was true for the marker-trait associations tested for the [NO_3] in the different soil layers (cf. Table 5.5). The frequency of the major allele for these markers was also high among the population with values above 65%. The effect of the QTL located in the region around 80 cM on chromosome 7 was intermediate to high, with in Trial 2, 2010

11% difference and about 40% difference in Trial 2, 2011 between the cultivars bearing one allele and the cultivars bearing the other allele. The effect of the QTL located between 50 and 60 cM on chromosome 9 was also moderate with about 30% in [NO_3] in the considered layers between the part of the population bearing one allele compared to the part of the population bearing the other allele. Only the significant QTL detected at final harvest are displayed in Table 5.5.

Significant marker-trait associations were identified for moisture content in specific layers only in Trial 2, 2011 at final harvest for layer 0.1-0.2 m on chromosome 7 (69.8 cM) and chromosome 9 (52 cM), for layer 0.2-0.3 m on chromosome 9 (53.7 and 57.7 cM) and for layer 0.3-0.4 m on chromosome 7 (97.2 cM).

Because of the large number of QTL detected on chromosome 9, we had a closer look on this region, and we identified a group of 11 cultivars bearing a different allele than the rest of the population for the detected markers and traits. This cluster was composed of 4 cultivars released before 1970, 3 cultivars released between 1970 and 1990 and 5 cultivars released after 1990. They came from a gene bank (5), from a single seed company (3), or from diverse seed companies (3). The ANOVA based on this grouping showed that this cluster left significantly more H_2O and NO_3 (*p-value* ≤ 0.05) in the deeper soil layers than the rest of the group (Figure 5.7). The cultivars in this group also had significantly lower fresh and dry yields.

Shoot traits

Several significant QTL were detected for the shoot traits, mainly for the shoot fresh and dry weights. For Trial 2, 2011 only few significant QTL were found for shoot traits (Table 5.6). Significant QTL associated with plant fresh weight were detected in Trial 1, 2010 and Trial 2, 2010, for both trials located on chromosome 5 (at 56.0 and 92.3 cM, respectively) and on chromosome 7 at 43.6 cM (Trial 2, 2010) (Table 5.6). Significant QTL associated with plant dry weight were found on chromosome 3 for Trial 1, 2011 and Trial 2, 2011 at 69 cM approx., as well as on chromosome 4 at 43 cM (Trial 2, 2010), chromosome 5 at 56.0 cM (Trial 1, 2010), chromosome 6 at 61.9 cM (Trial 2, 2011), chromosome 8 at 92 cM (Trial 1, 2010) and 68.1 cM (Trial 1, 2011) and on chromosome 9 at 52.0 cM (Trial 1, 2010) (Table 5.6). A significant QTL associated

with NUE was found only in one trial and one chromosome (chromosome 7 at 67.0 cM in Trial 1, 2011) (Table 5.6).

Table 5.5 Significant marker-trait associations ((–Log10(P) > 3.5) for [NO_3] left in a layer (ppm) at final sampling and their position (cM) on the lettuce chromosome (Chr.) identified in each environment (Year × Trial combination) with –Log10(P) score, allele frequency (Allele fq. in %), allele effects (ppm) and absolute value of the standard error (SE).

Year	Trial	Layer	Chr.	Marker	cM	$-\text{Log}_{10}(P)$	Allele fq. (%)	Allele effect	SE
2010	2	0.3-0.4 m	4	LSM00409	42.4	3.52	84.6	7.1	2.0
2010	2	0.2-0.3 m	4	LSM00408	79.3	3.75	88.0	13.4	3.6
2010	2	0.2-0.3 m	4	LSM00496	80.5	4.63	87.1	14.6	3.5
2010	2	0.2-0.3 m	4	LSM00344	84.8	3.91	86.4	13.1	3.4
2011	2	0.2-0.3 m	4	LSM00644	79.1	4.91	86.5	19.0	4.4
2011	2	0.2-0.3 m	4	LSM00408	79.3	8.42	88.0	24.3	4.1
2011	2	0.2-0.3 m	4	LSM00032	80.1	5.18	86.4	19.4	4.3
2011	2	0.2-0.3 m	4	LSM00106	80.2	3.56	85.2	15.7	4.3
2011	2	0.2-0.3 m	4	LSM00496	80.5	4.31	87.1	17.5	4.3
2011	2	0.3-0.4 m	4	LSM00496	80.5	10.8	87.1	29.0	4.3
2011	2	0.2-0.3 m	4	LSM01560	81.3	5.27	86.5	19.3	4.3
2011	2	0.2-0.3 m	4	LSM01439	81.5	4.40	86.4	17.7	4.3
2011	2	0.3-0.4 m	4	LSM00233	81.5	5.10	84.8	21.5	4.8
2011	2	0.2-0.3 m	4	LSM00434	82.3	4.62	87.2	18.7	4.4
2011	2	0.2-0.3 m	4	LSM01612	82.3	4.43	85.7	17.7	4.3
2011	2	0.3-0.4 m	4	LSM00085	84.8	4.50	85.1	20.5	4.9
2011	2	0.3-0.4 m	4	LSM00344	84.8	6.34	86.4	25.0	4.9
2010	2	0.1-0.2 m	5	LSM00319	92.3	6.31	92.9	30.5	6.1
2010	2	0.0-0.1 m	7	LSM00610	43.6	12.8	92.2	31.1	4.2
2010	2	0.1-0.2 m	7	LSM00610	43.6	5.84	92.2	31.3	6.5
2010	2	0.3-0.4 m	7	LSM01598	94.1	3.68	69.1	5.7	1.5
2010	2	0.3-0.4 m	7	LSM01060	94.2	3.51	70.2	5.5	1.5
2010	2	0.3-0.4 m	7	LSM01558	94.2	3.88	69.3	5.7	1.5
2010	2	0.3-0.4 m	7	LSM00539	94.9	3.71	69.1	5.6	1.5
2010	2	0.3-0.4 m	7	LSM01772	97.2	3.89	67.9	5.7	1.5
2011	2	0.1-0.2 m	7	LSM00610	43.6	4.25	92.2	15.8	3.9
2011	2	0.2-0.3 m	9	LSM00075	53.4	5.98	88.7	17.7	3.6
2011	2	0.3-0.4 m	9	LSM00075	53.4	4.22	88.7	18.3	4.6
2011	2	0.2-0.3 m	9	LSM00690	53.5	5.68	92.3	19.2	4.0
2011	2	0.3-0.4 m	9	LSM00690	53.5	3.54	92.3	18.6	5.1

Table 5.5 Significant marker-trait associations ((–Log10(P) > 3.5) for [NO_3] left in a layer (ppm) at final sampling and their position (cM) on the lettuce chromosome (Chr.) identified in each environment (Year × Trial combination) with –Log10(P) score, allele frequency (Allele fq. in %), allele effects (ppm) and absolute value of the standard error (SE). (Continued)

Year	Trial	Layer	Chr.	Marker	cM	$-\text{Log}_{10}$(P)	Allele fq. (%)	Allele effect	SE
2011	2	0.2-0.3 m	9	LSM00232	53.7	7.62	90.8	20.7	3.7
2011	2	0.2-0.3 m	9	LSM00701	53.7	5.68	92.3	19.2	4.0
2011	2	0.3-0.4 m	9	LSM00232	53.7	4.19	90.8	19.1	4.8
2011	2	0.3-0.4 m	9	LSM00701	53.7	3.54	92.3	18.6	5.1
2011	2	0.2-0.3 m	9	LSM00123	53.8	6.94	91.5	20.5	3.9
2011	2	0.2-0.3 m	9	LSM00256	53.8	5.68	92.3	19.2	4.0
2011	2	0.3-0.4 m	9	LSM00123	53.8	4.23	91.5	19.8	4.9
2011	2	0.3-0.4 m	9	LSM00256	53.8	3.54	92.3	18.6	5.1
2011	2	0.2-0.3 m	9	LSM00443	54.3	7.15	91.3	20.8	3.9
2011	2	0.2-0.3 m	9	LSM00605	55.0	5.63	92.1	19.2	4.1
2011	2	0.2-0.3 m	9	LSM01377	57.7	6.07	91.4	19.0	3.9
2011	2	0.3-0.4 m	9	LSM01377	57.7	3.52	91.4	17.7	5.1
2011	2	0.2-0.3 m	9	LSM01150	57.8	5.82	88.4	17.3	3.6
2011	2	0.3-0.4 m	9	LSM01150	57.8	3.73	88.4	17.0	4.9
2011	2	0.2-0.3 m	9	LSM01604	58.0	5.68	92.3	19.2	4.0
2011	2	0.3-0.4 m	9	LSM01604	58.0	3.54	92.3	18.6	5.1
2011	2	0.2-0.3 m	9	LSM01220	58.7	6.96	89.0	18.1	3.4

Table 5.6 Significant marker-trait associations ((–Log10(P) > 3.5) for the shoot traits fresh weight (FW; g per head), dry weight (DW; g per head), and nitrogen use efficiency (NUE g dry matter per g nitrogen taken up) at final harvest, and their position (cM) on the lettuce chromosome (Chr.) identified in each environment (Year × Trial combination) with –Log10(P) score, allele frequency (Allele fq.), allele effects and absolute value of the standard error (SE).

Year	Trial	Trait	Chr.	Marker	cM	$-\text{Log}_{10}$ (P)	Allele fq. (%)	Allele effect	SE
2011	2	FW	2	LSM00500	60.7	7.25	25.0	42.5	7.82
2011	2	DW	2	LSM00500	60.7	6.82	25.0	1.69	0.32
2011	1	DW	2	LSM01045	67.6	3.78	28.6	0.66	0.17
2011	1	DW	3	LSM01342	69.4	4.99	38.0	-0.74	0.17
2011	2	DW	3	LSM01342	69.4	3.69	38.0	-1.09	0.29
2010	2	DW	4	LSM00604	43.0	4.48	34.3	2.44	0.59
2011	1	DW	4	LSM01595	72.7	3.85	17.1	-0.96	0.25
2010	1	DW	5	LSM00513	56.0	3.67	7.1	2.18	0.59
2010	1	FW	5	LSM01378	56.1	3.78	7.7	27.1	7.18
2010	2	FW	5	LSM00648	92.5	4.27	8.5	-51.5	12.75
2011	1	DW	6	LSM00165	61.9	3.83	17.5	0.77	0.20
2010	2	FW	7	LSM00610	43.6	11.59	7.8	-10.7	15.31
2011	1	NUE	7	LSM00730	67.0	4.97	23.4	1.64	0.37
2011	1	DW	7	LSM00928	68.1	4.10	31.2	0.66	0.17
2010	1	DW	8	LSM01651	92.0	5.81	8.9	2.53	0.53
2010	2	DW	9	LSM00519	52.0	3.72	10.6	-1.71	0.46

5.3.5 Comparisons across trials and across genome

QTL across trials:

The QTL detected for the below-ground traits showed reasonable consistency across trials: for instance on chromosome 7, the region around 43.6 cM was significantly associated with [NO_3] in a 0.10 m soil layer at final harvest in Trial 2, 2010 (0.0-0.1 m; 0.1-0.2 m; 0.3-0.4 m) and in Trial 2, 2011 (0.1-0.2 m). On chromosome 8, the region around 100 cM was significantly associated with NO_3 content over the whole soil profile in Trial 2, 2010 (intermediate sampling) and Trial 1, 2011 (final sampling).

Contrastingly, the QTL detected for the shoot traits did not show consistency across trials for these traits: if a QTL was detected for one shoot trait in one trial, it was not found for the same trait in another trial – with the exception of a region around 68 cM on chromosome 3, which was significantly associated with dry weight at final harvest in Trials 1 and 2, 2011.

QTL for multiple traits:

The same QTL were often detected for multiple traits across trials. For instance, the region around 50 cM on chromosome 5 was associated with fresh and dry weight in Trial 1, 2010. On this same chromosome, the region around 90 cM was significantly associated with fresh weight and NO_3 left over the whole soil profile in Trial 2, 2010 (final harvest), and with water left over the whole soil profile in Trial 2, 2011 (final harvest). The region around 45 cM on chromosome 4 was significantly associated with dry weight, and with the [NO_3] in layers 0.1-0.2 and 0.3-0.4 in Trial 2, 2010 (final harvest). On chromosome 7, the region around 43.6 cM was very significantly associated with shoot fresh weight in Trial 2, 2010 and NO_3 left over the soil profile in the same trial. On chromosome 9, the region between 50 and 60 cM was significantly associated with the dry weight at final harvest (marker at 52 cM) and diverse below-ground traits in Trial 2, 2011 at final harvest ([NO_3] in layer 0.2-0.3 m and 0.3-0.4 m, overall water left over the whole soil profile, and moisture content in 0.1-0.2 m and 0.2-0.3 m layers of the soil profile): the same marker was associated with several traits.

5.4 Discussion

5.4.1 Evaluation of the soil nitrate measurements method

The nitrate measurements in each soil layer were made following the method described previously by Sibley et al. (2009) and used in Chapter 2 in pot experiments. Using an ion-selective electrode enables quick and reliable nitrate measurements in the soil solution and allows the analysis of an important number of samples within a reasonable period of time and at low cost. Most studies dealing with nitrate capture at the root level use ^{15}N labelling (e.g. Robinson et al. 2001; Popay and Crush 2010; Yang et al. 2013; Yang et al. 2014), quantify root N (e.g. Ehdaie et al. 2010) or use molecular tools to quantify NO_3^- concentrations in roots (e.g. Sorgona et al. 2011; Wang and Chen 2012). However, these methods can become expensive and time consuming when the objective is to quantify nitrate capture over a population of individuals. Although the range of values obtained with the electrode was sometimes large (cf. Chapter 4), the values found within a sampling date were consistent across trials. The potential of this method for nitrate uptake quantification seems promising as a relatively high throughput technique for breeding programmes targeting improved resource capture below-ground.

5.4.2 Timing matters

This study demonstrated that genetic control over resource capture below-ground exists, but is difficult to comprehend at early growth stages. Heritability values found at intermediate sampling for the below-ground traits were very low, if not null (Table 5.3) and therefore QTL were not detected for these traits at early sampling date. Genetic variation in below-ground measurements may have been so low at early sampling because transplanted seedlings were used in this study, as opposed to direct sowing used in other studies (e.g. Johnson et al. 2000). Using transplants (a common cultivation practice in European lettuce production systems) damages the root system at transplanting and therefore may affect resource capture at early stages (Biddington and Dearman 1985). Potentially, impaired resource capture during transplant establishment in the field may have created a residual variance due to soil conditions larger than the

genotypic variance, consequently considerably lowering heritability values. On the other hand, while this was not detected in this study, Chapters 2 and 3 showed that genetic variation exists in the way lettuce recovers from transplanting stress; such genetic variation was observed in resource capture and shoot traits observed at final harvest.

5.4.3 Relevance of the QTL detected

In the trials performed in this study, the variance in the dataset generated by the field conditions was so high in some cases (e.g. for the traits related to water capture) that barely any genetic variance and consequently no QTL were detected for these traits. Although heritability values were higher for the shoot traits, the significant QTL detected for these traits were relatively less consistent and less numerous than the significant marker-trait associations detected for the below-ground traits. One reason for this discrepancy might be that, as shown in Chapter 4, the range of measurements obtained for the shoot traits were high, with for instance values ranging from 18.3 to 51.2 g dry matter per plant in dry conditions (Trial 1, 2010) or from 10.7 to 42.6 g dry matter per plant in wet conditions (Trial 2, 2011). The fact that the significant marker-trait associations were less consistent than expected for the shoot traits may be an artefact of the high level of G × E interactions in these trials, as was also experienced by Hartman et al. (2014) who found numerous non-overlapping QTL among experiments correlating with stress components. Furthermore, not only the level of G × E interactions was very high, but also the physiological mechanisms regulating shoot and root growth seem to have been largely impacted by the field conditions, i.e. mechanisms regulating resource capture and use efficiency seemed specific to each field condition, making the results very difficult to generalise and extend to overall interpretations. This can be illustrated by correlation analyses carried out between shoot and root traits based on phenotypic measurements (results not shown). For instance, heavy rainfall affected Trial 2, 2011 towards the end of the experiment – just before final harvest (cf. Figure 5.3). This caused the nitrate in the top layers of the soil profile (0.0-0.2 m) to leach towards the lower layers of the soil profile (0.2-0.4 m); the $[NO_3]$ in the lower layers of the soil profile was thus larger than the $[NO_3]$ in the upper layers of

the soil profile. This phenomenon might have impacted resource foraging for the plants, as in this trial, the shoot dry- and fresh weights are highly and significantly negatively correlated with the [NO_3] in the lower layers of the soil profile (0.2-0.4 m). As shown by Hodge (2004), Gallardo et al. (1996) and Kerbiriou et al. (2013b, Chapter 2) localised root elongation happens in N-rich zones – in contrast to neighbouring N-poor zones. One can thus hypothesise that during this trial, efficient N-foraging in these layers significantly contributed to shoot field performance. Such active N-foraging may have been genetically controlled as numerous QTL were expressed on chromosome 9 around 52 cM for the below-ground traits ([NO_3] and [H_2O] in the lower layers of the soil profile) in this very specific environment (cf. Table 5.5). As shown in Figure 5.7, the group bearing a different allele for this marker than the rest of the population seem not to have been able to capture as much nitrate in the lower layers of the soil profile, which significantly impacted shoot growth.

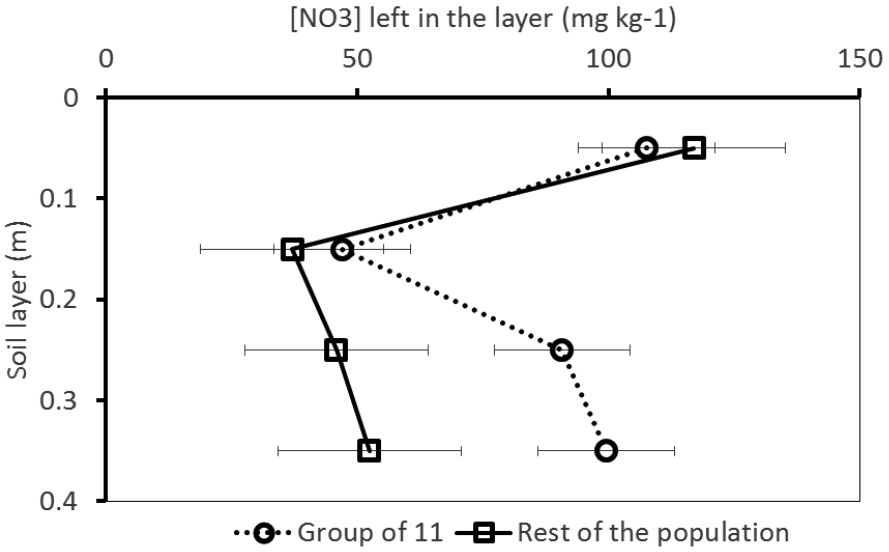

Figure 5.7 [NO_3] pattern over the soil profile for the group of 11 cultivars bearing a different allele for the significant markers identified on chromosome 9 compared to the rest of the population (Trial 2, 2011, final harvest).

In contrast, the mechanisms regulating shoot growth in Trial 2, 2010, seem different, as the correlation analysis shows that dry weight (and fresh weight to a lesser extent) were significantly negatively correlated with the [NO_3] concentration in the different layers of the soil profile. One can imagine that in relatively warm and optimal conditions with regular rainfall which replenished the soil profile at regular intervals (cf. Figure 5.3), the ability of the genotypes to display good field performance may mainly have been linked to their ability to extract nitrate from the soil profile – assuming that genotypes with a larger root systems (not investigated in this study) allowing them to capture a larger amount of nitrate, would perform better than cultivars with a smaller root system. This mechanism may have been genetically controlled as interestingly, neighbouring regions on chromosome 4 were significantly associated with shoot and below-ground traits: shoot dry weight and [NO_3] in the 0.3-0.4 layer of the soil profile were significantly associated with a marker around 40 cM on the one hand, and markers in a region between 70 and 80 cM were associated with [NO_3] in the 0.2-0.3 m layer of the soil (Table 5.5).

Overall, several regions showed to be significantly associated with below-ground traits, e.g. the region around 80 cM on chromosome 4, the region around 90 cM on chromosome 7, and the region between 50 and 60 cM on chromosome 9. The exact same regions were not identified before, although there seems to be some overlap with some regions previously identified by Uwimana et al. (2012). For instance, in our study the region between 50 and 60 cM on chromosome 9 was significantly associated with [NO_3] of the lower layers of the soil profile in Trial 2, 2011; in Uwimana et al. (2012) a neighbouring region on this chromosome was significantly associated with relative moisture content of the soil. However, the lower heritability of the data for the below-ground traits, soil moisture for instance, prevented finding QTL for these traits. In this study, the data for soil moisture content were not corrected for water movement caused by rainfall across the soil profile; also, the data for nitrate content in the soil layers did not take into account soil moisture content data. Fitting the experimental data into a model accounting for these movements (such as 'tipping-bucket' models; Guswa et al. 2002) would improve the fitness of the data and may therefore allow better correlation with genotypic data.

5.4.4 Recommendations for future research

Provided the observations above, the genotypic data used in this study may be further transformed to gain a more accurate understanding of the G × E, and exploited more in-depth to get a better insight into the mechanisms explaining the results. As this study was based on the assumption that root characteristics are strongly correlated with resource capture, it would be interesting to assess the root system architecture of the cultivars used in this association panel. It would also be interesting to investigate further how the regions identified in this study relate to each other, and how they interact with the environment, by for instance designing experiments where different stresses are applied (such as in Chapter 2). It is possible that regions located on different chromosomes are simultaneously or differentially expressed in contrasting environments.

Although these traits can be more easily measured in greenhouse experiments, such greenhouse experiments may not always reflect the reality of the field conditions. This was illustrated by a study by Hartman et al. (2012) who found different QTL patterns for fitness-related traits in lettuce (measured on shoots) in trials carried out in the field compared to greenhouse conditions.

5.4.5 Implication for lettuce breeding

Most of the recent literature investigating the potential of marker use in lettuce breeding has been focusing on cultivated × wild lettuce crosses (Johnson et al. 2000; Kuang et al. 2008; Jeuken et al. 2008; Uwimana et al. 2012; Hartman et al. 2013a, b; Hartman et al. 2014), on intra-specific crosses (Waycott et al. 1999) or recombinant inbred lines (Hayashi et al. 2012). Cultivated and wild lettuce are very different species morphologically, not only for shoot traits, but also for root traits (Uwimana et al. 2012); for instance wild lettuce develops a strong tap root which allows it to forage resources in deep soil layers, while transplanted cultivated lettuce cultivars have a small root system mostly located in the top soil layers (0.0-0.3 m) (Johnson et al. 2000). Although introgressing genes from wild species into cultivated species seems a promising approach, particularly for root traits in lettuce, this a long term strategy which requires a

better understanding of the interaction patterns existing between genes located on different chromosomes. Bi-parental QTL mapping studies also tend to produce longer linkage blocks, where association panels allow a more precise localisation of the regions of interest as it is based on the recombination events which occurred during the breeding history (Long et al. 2013). In this view the information provided by this study may be used immediately for breeding purposes. Indeed breeders could design new trials including diverse soil treatments (localised drought or nitrate limitation) in order to investigate if they could retrieve the QTL found in this study and how they are expressed in controlled conditions.

However, the high frequency of the alleles shown in Tables 5.4 and 5.5 for instance suggests that the genetic basis of the population chosen for the association panel may have been rather narrow. Lettuce has been bred intensively since the industrialisation of the horticultural sector in the 1970s which may have reduced the genetic diversity in the commercial varieties currently available. Although population structure is visible between types (e.g. stem lettuce compared to leaf types), genetic variation within types – such as butterhead in this study – may be rather narrow. In this study, the two most different genotypes still shared about 55% of the alleles, which is a relatively high proportion. Molecular tools may therefore be useful to re-introduce genetic diversity in lettuce without the lengthy efforts of classical breeding techniques.

Furthermore, the development of more affordable and faster molecular techniques will soon allow systematic genotyping as a molecular-assisted breeding tool and might replace current techniques using genotypic markers. Indeed, sequencing the whole genome allows a more precise localisation of genomic regions of interest and thus the identification of potential candidate genes regulating the expression of the trait of interest. In contrast, marker technologies can only point out potential regions of interest but do not bring much information in regards to the expression of the trait. For breeding for complex traits though, the bottleneck remains in the phenotyping. As pointed out by Johnson et al. (2000) below-ground traits are extremely difficult to evaluate and more efforts are needed to understand and quantify resource capture and use efficiency before meaningful molecular tools can be developed to breed for these traits.

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Chapter 6
General discussion

Preamble

In Chapter 1 of this thesis it was argued that organic lettuce production could be improved by using more robust cultivars with improved below-ground traits. Below-ground traits such as root system architecture and the dynamics of capture of nitrate and water in space and time were analysed to increase our understanding of the physiological mechanisms regulating such traits (Chapter 2). The contribution of root traits to field performance of lettuce under organic conditions and the genetic variation therein (Chapter 3) were also studied. Based on these results a concept for a new eco-physiological model as a tool for breeding for robustness in lettuce under organic conditions (Chapter 4) was developed. Chapter 5 investigated the potential of breeding for below-ground traits by identifying quantitative trait loci for below-ground and above-ground traits.

This Chapter 6 broadens the discussion in the preceding chapters evaluating the achievements realized in this thesis and analysing issues related to breeding for robustness in lettuce. The following specific aspects are discussed:

(1) Root traits and resource acquisition mechanisms in lettuce: unravelling complexity through technical innovations. Chapters 2 and 3 showed that it is difficult to phenotype accurately root traits and resource capture traits. More effort is needed in research and technology to develop new *in-situ* tools enabling easy and reliable root measurements.

(2) Plasticity in below-ground traits – and not below-ground traits *per se* – may actually be the most relevant trait conferring robustness in lettuce. Plasticity is defined as the ability for a genotype to display diverse phenotypes in order to overcome environmental stress (Des Marais et al., 2013). Large plasticity may improve adaptability to diverse ranges of environments and consequently robustness.

(3) Stimulating the dialogue between physiologists and breeders is the way to move the horticultural industry forward. This thesis illustrates that the dialogue between physiologists and breeders is necessary to comprehend complex traits such as resource use efficiency.

6.1 Introduction

In lettuce, and more specifically low-input- or organically-grown lettuce, cultivars with improved robustness and more stable yields would benefit the industry, as growers would be able to consistently supply retailers with larger quantities of high quality produce. They are not yet able to realize this as they are currently relying on cultivars requiring high levels of inputs and sensitive to the more heterogeneous growing conditions typical of organic farming systems. It was hypothesized that such sensitivity may be due to the fact that the commercial varieties available nowadays have been selected under high input and conventional conditions, which did not favour the expression of – and consequently the selection for – traits contributing to robustness, such as a specific root system architecture allowing resource capture in deeper layers and dynamic resource acquisition below-ground (Johnson et al., 2000).

The impact of below-ground traits on field performance of cultivated crops has received more and more attention in the last 10 years (Postma et al., 2009). Including such traits into a breeding strategy is being increasingly investigated, with the aim of providing growers with varieties that have both the robustness and plasticity to produce stable yields under a wide range of environmental conditions. However, in lettuce, being a short cycle, vegetative crop, not much is known about the physiological mechanisms controlling resource capture below-ground, and the contribution of root phenes to overall field performance. Moreover, the question about how to take these traits into account in lettuce breeding has remained unanswered.

Therefore, the main objective of this thesis was to develop a breeding strategy to increase abiotic stress tolerance in lettuce, based on the below-ground traits that could confer plasticity and robustness. To achieve this objective, the physiological and morphological mechanisms regulating resource capture and resource use efficiency at the root and shoot level were investigated in the context of both temporary and localized resource shortage (Chapter 2). Moreover, the contribution of root traits, as well as the genetic variation therein, to (variation in) resource capture and field performance were examined (Chapter 3). In addition, the genetic variation in these mechanisms was assessed and used as a basis to develop a modelling concept to assist breeders in

breeding for robustness (Chapter 4). Finally, the genetic control of resource capture below-ground in time and space was evaluated, the influence of the environment on the regulation of the expression of the traits reviewed and the consequences for above-ground crop performance (in terms of resource accumulation and production) analysed (Chapter 5).

In this General discussion, the main findings of the four component studies are presented and the following propositions are discussed:

1- Root traits and resource acquisition mechanisms in lettuce: unravelling complexity through technical innovations.
2- Plasticity in belowground traits – and not below-ground traits *per se* – may actually be the most relevant trait conferring robustness in lettuce.
3- Stimulating the dialogue between physiologists and breeders is the way to move the horticultural industry forward.

6.2 Overview of the main findings

The shoot:root dialogue as a feed-forward mechanism for robustness

In lettuce the nutritional status of the plant determines the type of response to temporary resource shortage (Chapter 2). To maintain the initial shoot growth rate during mild and temporary drought, lettuce increased the rate of nitrate inflow to the roots when drought was applied at an early stage; this mechanism is less costly for the plant than producing and maintaining new roots, and might be activated when resources are not yet strongly limiting shoot growth. To maintain shoot growth during a later stage – when resources were becoming increasingly limiting for shoot growth – root elongation was stimulated. These contrasting mechanisms highlight different strategies regulating resource allocation to the shoot and to the root based on the shoot status, not necessarily in line with the widely accepted concept of the functional equilibrium (Brouwer, 1962) found elsewhere in the literature (e.g. Forde and Lorenzo, 2001).

Furthermore, when root elongation was triggered by resource limitation, the zone in which it was triggered was determined by the type of resource being limiting in that zone. When roots encountered a dry zone in a soil profile, they tended to proliferate in

that zone, whereas simultaneously no increase in root mass was detected in the moist zone of the same soil profile. The contrary happened when roots encountered an N-poor zone in a soil profile: the plant tended to enhance root growth in the N-rich zone, as opposed to the N-poor zone. These results may have actually been related to the same mechanism: root growth may have been triggered to the nitrate concentration in the soil solution, the concentration of nitrate in the soil solution may have increased in a drying soil. In these trials, nitrate concentration was not measured as such and this hypothesis could not be verified.

Besides root traits related to resource acquisition below-ground, resource use efficiency above ground significantly contributes to robustness in field conditions

Different transplant sizes can be used as proxies for different root:shoot ratios at transplanting. Investigating different root:shoot ratios at transplanting gave us information about the importance of root traits for field performance in lettuce (Chapter 3). Changes in initial root:shoot ratios did not impair further field performance and final yield in lettuce, unless the initial root:shoot ratio was very small (under-developed transplant size). The elements enabling lettuce to overcome an unbalanced root:shoot ratio so quickly could be found below-ground, in the efficiency of resource acquisition by roots. In field conditions, cultivars with a larger root system exploring deeper layers of the soil generally displayed better field performance and yield stability across environments. However, if additional root proliferation generally correlated with improved resource capture below-ground in conditions conducive to growth, that relationship was not clear when field conditions were sub-optimal. Different mechanisms may then be triggered such as an increased nitrate inflow into the roots – as highlighted previously – or an improved resource use efficiency. On the other hand, the ability to transform the resource acquired below-ground into shoot mass may also confer yield stability and thus robustness at a relatively low cost for the plant. These findings highlight that genetic variation in root:shoot assimilates partitioning exists between genotypes and that breeding for such traits may be possible.

Because it can tackle Genotype × Environment interactions and account for genetic variation, modelling is a necessary step towards breeding for robustness

Creating more robust cultivars of lettuce which can perform well in a wide range of environments based on improved resource acquisition and use efficiency requires a better understanding of the physiology behind these traits and of the genetic variation therein (Chapter 4). In that regards, modelling can significantly improve our understanding of the mechanisms below-ground, their contribution to field performance, and how the communication within the plant (the shoot-to-root dialogue) and organization (the implementation of the outcome of that dialogue) are impacted by the environment. Experimental findings underline the high level of Genotype × Environment interactions in the mechanisms regulating resource capture and use efficiency masking both the direct genotypic effects, and which should be accounted for in a model concept. Such a model concept would then allow for a more effective analysis of the genotypic effects and the Genotype × Environment interactions. Based on this concept, the model to be developed could help breeders to identify traits of interest when selecting robust genotypes in a given environment, and conversely, identify the selection environment in which the trait conferring robustness would be best expressed. For instance, the model could help the breeder choosing which trait(s) could confer adequate field performance when heavy rainfalls during cropping make the nitrate to leach to lower layers of the soil profile and create N-rich and N-poor patches within the soil profile. Conversely, it could predict in which environment a genotype displaying a lower root:shoot ratio together with a higher NUE would perform best. To improve the effectiveness of this model, further elaboration will be needed on accurate modelling of the water and nitrate flows over the soil profile as well as accurate determination of the range of the input parameters, based on observed genetic variation.

The mechanisms of resource capture below-ground, rather than the traits per se, are impacted more by the environment than by the genetic background

In the context of increasing interest in molecular tools which can make the breeding progress more effective, assessing and evaluating the genetic control of resource acquisition and resource efficiency is necessary (Chapter 5). The association between

the traits related to resource capture below-ground in space and time proved to be highly impacted by the environmental conditions. This shows that a complex combination of external factors, such as weather conditions, the nutritional status of the plant, and the availability of localized resources below-ground, significantly regulate the expression of the genetic background. In this view, the high impact of the environment on the physiological mechanisms regulating resource capture and resource use make the identification of specific genomic regions correlated with favourable alleles very difficult; indeed, the expression of favourable traits identified in a specific environment may impair the expression of other traits which may be useful in another environment. Moreover, observed phenotypic effects may be controlled by numerous interacting quantitative trait loci, of which the individual expression greatly varies with the environment. However, such findings may also be due to the narrow genetic basis of most lettuce cultivars commercially available nowadays; this narrow base reduces the genetic diversity for traits related to resource capture and resource use as most of the selection has been done under high input systems. This forces us to look for small differences in trait expression which often can be masked by the "noise" in the data created by the environmental conditions.

The main findings of the four component studies highlight the complexity of breeding for below-ground traits as contributors to plasticity and robustness. In the light of these findings, the following paragraphs will discuss three aspects:

1- The complexity of the relationships between root traits and resource acquisition mechanisms can mainly be unravelled through technical innovations.
2- Plasticity in belowground traits – and not below-ground traits *per se* – may actually be the most relevant trait conferring robustness in lettuce.
3- Stimulating the dialogue between physiologists and breeders is the way to move the horticultural industry forward.

6.3 Discussion of the main findings

The complexity of the relationships between root traits and resource acquisition mechanisms can mainly be unravelled through technical innovations

In this thesis, the relationships measured between root mass and resource capture proved to be inconsistent in regards to the type of resource considered, and difficult to unravel in great detail under field conditions.

On the one hand, Chapter 2 and Chapter 4 showed that – in an environment that does not show resource limitations – nitrate capture was only correlated to root mass when the root mass in a specific soil horizon was low (up to approx. 0.16 g DM L^{-1} soil). Figure 4.2 in Chapter 4 illustrates that when roots grew in a N-poor environment, this relationship existed only for an even smaller range of root mass (i.e. up to approx. 0.07 g DM L^{-1} soil in the considered soil horizon). The roots continued to grow in the N-poor compartment even once all the nitrate was captured in the considered layer, so the roots continued to grow but were not capturing nitrate anymore. In contrast, when roots grew in a dry zone, the relationship between the amount of nitrate captured and the root mass present in the considered zone persisted beyond 0.5 g of root dry matter in the layer (up to 1.2 g root dry matter in the layer), meaning that in the case of dry soil, additional root length helps capturing more nitrate. These results highlight that more roots does not necessarily mean better resource capture – at least not in a pot experiment. The question why roots continued to grow in the zone where nitrate was fully depleted remains unanswered. As other nutrients were not quantified in this study, we can hypothesize that the roots may have been foraging for another resource (e.g. phosphorus or micro nutrients such as calcium or boron) once nitrate was fully depleted in the layer.

On the other hand, Chapter 3 shows that the range of estimations for the root mass values in field conditions (up to 0.5 g for the overall root mass over the 0.40 m soil profile, cf. Table 3.5) was much smaller under field conditions than in the pot trials. These results showed that under field conditions, the root system was much smaller, compared to the root systems of plants grown under controlled conditions. This may have been due to the sampling error on the one hand, but also to the soil characteristics (organic matter) and soil conditions (soil temperature) on the other hand. Indeed, the method for root sampling in Chapter 2 under controlled conditions and in Chapter 3

under field conditions (cf. Material and Methods sections in these chapters) may have missed the finer roots which were present in more important quantities under the field conditions, due to the coarser textured and more compacted soil in the field.

Furthermore, as shown in Chapter 3, the root measurements only partially correlated with the nitrate measurements in the field conditions; besides, the nitrate measurements carried out for the population of commercial lettuce cultivars (Chapter 5) showed high variability and very wide ranges (up to a threefold in some environments) for resource capture.

Resource capture measurements in the soil were simple and straightforward, using a volumetric method based on fresh and dry weight difference in the soil for the soil moisture, and using an ion-specific electrode for the nitrate content. The data obtained by these methods were not corrected for the water movement over the soil profile. Carrying out such a correction might have improved the precision of the data. Indeed, in the sandy soils where the trials were carried out, the porous structure of the soil allowed for ample vertical water movements where no roots were present. Accounting of the presence of roots which increases the water retention capacity of the soil, and quantifying the effect of rainfalls (intensity, quantity, frequency) would improve our understanding of the water movement and consequently the water capture patterns in space and time.

These observations can be summarized as follows: the difficulties experienced in identifying the relationships between the root traits and the resource capture were due to a discrepancy in the pot vs. field root data which make the greenhouse findings difficult to extrapolate; the resource capture measurements in the field were not corrected for water movement over the soil profile, loosing accuracy and therefore impairing the potential relationships. This calls for further improvements in root and resource capture phenotyping in future research. As the investigation of below-ground traits has been gaining more and more popularity recently, many research groups around the world are looking for new methods to accurately phenotype root traits and quantifying resource capture. The use of soil cores (as in our study) or shovelomics (digging out the whole root system of a plant to measure different traits, cf. Penn State root lab; Trachsel et al. 2011) for field measurement require a lot of labour and are difficult methods to use for

population screening. They simply might be too inaccurate and too laborious. Other in situ methods have been developed, such as the rhizotron (analysis of the pictures of roots on the walls of a glass pipe buried in the field, Majdi, 1996; Johnson et al., 2001; Smit and Groenwold, 2005) or measuring the force required to pull a root clump out of the soil as a proxy for root mass (root pulling resistance, Lebreton et al., 1995; Sanguineti et al., 1998; Landi et al., 2002).

In controlled conditions the use of x-ray tomography (Bauerle and Centinari, 2014; Kuka et al., 2013; Zappala et al., 2013) allows the discovery of root traits in relation to water capture in a non-destructive way. Recently Schultz et al. (2007) developed a system to visualize the root system in 3D via Magnetic Resonance Imaging with promising outcomes. However, these methods do not seem yet applicable to the field conditions, partly due to the cost of operation and because of the special conditions in which measurements have to be taken. Moreover, as mentioned previously, the size of the root system may vary considerably between the field- and the greenhouse situation, making the use of certain techniques used for controlled conditions difficult to extrapolate to outdoor conditions. This limits the methods available for the root researcher to the in-situ systems mentioned before which are more laborious and less easy to use for large population screening.

However, more combined efforts from the horticultural, the technological and the research sector is needed to bring new technologies to the field to ensure reliable in situ measurements of root traits in space and time. Most of the methods cited above can be used for only a few time points and might not provide adequate information on the dynamics of the root growth and resource capture. The root researchers are in need of innovative and reliable methods that can translate the continuous changes happening at the root system in time and space, as such changes can explain the various degrees of a plant's adaptability to its environment. For instance non-destructive continuous monitoring of nitrate and water capture over time would be very useful in understanding the evolution of nitrate concentration in time and space; analysing the associated root growth would provide valuable information to include in the model concept developed in Chapter 4.

Plasticity in below-ground traits – and not below-ground traits per se – may actually be the most relevant trait conferring robustness in lettuce

The way a plant adapts to its environment depends on its level of plasticity, defined as "the ability for a single genotype to sense, respond to, and survive a variety of abiotic stresses (Des Marais et al., 2013). Plasticity can then be expressed as the ability for a genotype to display multiple phenotypes in response to the environment. It seems that variations in the phenotypic plasticity are often the greatest among species and within traits classes (phenological vs. nutrient accumulation) (Des Marais et al., 2013).

Chapter 4 showed that there is a large variation in resource capture below-ground in lettuce within a sampling date and that there is a high level of quantitative trait locus (QTL) × Environment interaction in below-ground traits as QTL numbers and chromosomal locations were subject to changes across environments (cf. also Chapter 5). Among the population tested in this Chapter, only one cultivar displayed consistent good field performance across all environments. Among the rest of the population it was impossible to identify consistent patterns across environments for the shoot and the root traits. This could be perfectly illustrated the figure 1E in the paper by Des Marais et al. (2013), where the trait measured over the population in two environments does not change linearly with the environment. This type of Genotype × Environment interaction actually underlines the diverse levels of plasticity existing in the population for the observed trait (below-ground traits in our case). Because the observed trait changes so unpredictably with the environment, it makes them difficult to breed for. This then questions whether it would actually not be more fruitful to investigate the plasticity in below-ground traits as a selection criterion when breeding for robustness, instead of breeding for below-ground traits *per se*. This would imply identifying more or less plastic genotypes and determine how the environment stimulates plastic responses.

In literature there is wide body of evidence that plasticity in root traits confers tolerance to resource limitation (Mou et al., 2013; Grossman and Rice, 2012; Useche and Shipley, 2010). The contribution of plasticity in root traits to good field performance was shown in rice under drought by Tran et al. (2014) and Kano-Nakata et al. (2011) and in bread wheat by Ehdaie et al. (2012). The dataset in Chapter 5 proved that in lettuce, the

contribution of root traits to shoot performance was strongly influenced by the environment. As shown in Figure 6.1 (based on the data obtained in Chapter 5), the correlation between root traits and shoot trait varied considerably across environments, making the identification of favourable below-ground traits quite difficult. This calls for a better understanding of the shoot:root communication and the impact of the environment thereon. As shown by this study, there is a need to create synergy among physiologists and breeders given what they could achieve working together.

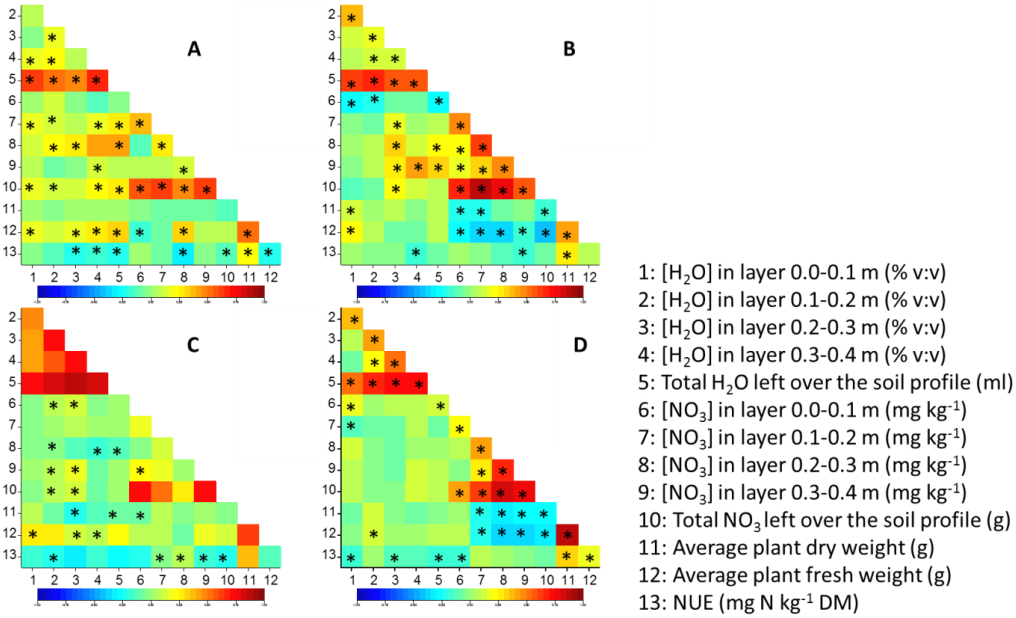

Figure 6.1 Correlations between the shoot and root traits in the four environments at final harvest (A: Trial 1, 2010; B: Trial 2, 2010; C: Trial 1, 2011; TD: Trial 2, 2011). Stars (*) indicate a significant correlation at $\alpha = 0.05$. Colours indicate the value of the correlation from blue to red indicating a correlation increasing from -1.00 to +1.00.

Stimulating the dialogue between physiologists and breeders is the way to move the horticultural industry forward

The necessity to bridge the gap between phenotypes and genotypes has already been emphasized some years ago (e.g. Yin & Struik, 2008). The research carried out in this study highlights that without a good understanding of the physiological mechanisms controlling resource capture below-ground and its use above-ground, it is very difficult to identify the traits to breed for when breeding for robustness, and to analyse the effects of Genotype × Environment interactions. Building a model-based breeding approach requires close collaboration between both disciplines allowing teams to learn from each other.

In practice, the industry needs both types of specialists. On the one hand physiologists are able to explain the plant developmental patterns but they often use only a few genotypes to calibrate their model parameters. On the other hand, breeders, and particularly molecular specialists, focus a lot on the genetics of the crop and tend to overlook the (whole-plant) physiological mechanisms explaining variations in yield or related shoot traits. Such a multidisciplinary team is fruitful for complex traits such as exploring root development in relation to nutrient use efficiency. Most successful seed companies nowadays have united scientists of diverse disciplines to ensure that all the aspects of a successful variety development are covered. For instance, within a breeding team in the horticultural sector, pre-breeders, genomic breeders, (practical) breeders, and crop researchers work together on a daily basis and exchange information related to the phenotypic and the genotypic data. However, the breeding effort nowadays is barely supported by modelling. Indeed, modelling – especially when investigating complex traits – requires long term investment in research capability and does not yield immediate results as it needs a lot of research to become more robust and make accurate predictions. For instance seed companies nowadays struggle in onion breeding as this crop is highly influenced by day-length. In this case, the use of modelling could be an excellent tool to get a better understanding of the crop and improve the breeding efficiency. The full potential of the use of modelling in breeding can also be seen in perennial crops. For instance modelling can help to understand the influence of the environment and the genetic variation in yield components of rice, wheat, maize, potato

as well as fruit crops such as blueberries, apples, strawberries etc. – crop which are grown globally and require a good understanding of the influence of external factors such as the day length, temperature accumulation, abiotic stress on vegetative and generative growth. Furthermore, other complex traits which are nowadays the focus of breeding such as fruit quality, shelf life, seasonality could be considerably better understood and consequently more efficiently bred for with a modelling approach. Soon, the next generation of models will enable the integration of genomic data (QTL-based Eco-Physiological models) and will help breeding tremendously in providing breeders with a better understanding of the QTL × Environment interactions. In this view, the model approach will help to understand how interesting genomic regions are expressed in given environments and which regions should be taken into account by the breeder when breeding for a specific trait in a specific environment.

6.4 Outlook on future research

The dataset generated by the component studies contained in this thesis is very large and rich and would require further analyses to be fully understood. Using the genotypic data generated in Chapter 5, more trials would be required to comprehend the complexity of the genetic control of below-ground mechanisms, under limiting conditions. Moreover, this study only focused on nitrate capture which moves with water, but what would happen if we would look at phosphorus capture and use efficiency? It would be interesting to compare the genomic regions identified in Chapter 5 for nitrate capture with data obtained for phosphorus capture. Furthermore, the influence of nutrient limitation on product quality was not analysed in this study. Investigating the genetic variation in the consequences of nutrient limitation or drought on shelf life and head colour for instance would provide excellent information from a breeding perspective.

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Summary

Growers are not yet capable of providing supermarkets with a constant supply of high quality organic lettuce among others because they are relying on cultivars requiring high levels of inputs and sensitive to the fluctuating resource availability typical of organic or low-input faming systems. Modern varieties of lettuce have been bred in conventional and high-input systems, which may not have favoured the expression of – and consequently the selection for – traits conferring the ability to sustain mild abiotic stresses during growth, such as root traits. The contribution of such traits to the performance of crops is being increasingly investigated with the objective of providing growers with cultivars of improved robustness and plasticity enabling them to produce stable yields over a wide range of environmental conditions. In lettuce, a short cycle and vegetative crop, the physiological mechanisms controlling resource capture and the contribution of root phenes to overall field performance have not been well studied, and the possibility to take these traits into account in lettuce breeding have not yet been investigated. Therefore, the main objective of this thesis was to contribute towards a breeding strategy to increase abiotic stress tolerance in lettuce, based on the below-ground traits that could confer plasticity and robustness. To achieve this objective, the physiological mechanisms regulating resource capture and resource use efficiency at the root and shoot level were investigated in the context of both temporary and localized resource shortage (Chapter 2). Moreover, the contribution of root traits, as well as the genetic variation therein, to (variation in) resource capture and field performance were examined (Chapter 3). In addition, the genetic variation in these mechanisms was assessed and used as a basis to develop a modelling concept to assist breeders in breeding for robustness (Chapter 4). Finally, the genetic control of resource capture below-ground in time and space was evaluated, the influence of the environment on the regulation of the expression of the traits reviewed and the consequences for above-ground crop performance (in terms of resource accumulation and production) analysed (Chapter 5).

In Chapter 2, it was found that in lettuce, the nutritional status of the plant determines the type of response to temporary resource shortage. To maintain the initial shoot

growth rate during mild and temporary drought, lettuce increased the rate of nitrate inflow into the roots when drought was applied at an early stage; this mechanism may be less costly for the plant than producing and maintaining new roots, and might be activated when resources are not yet strongly limiting shoot growth. To maintain shoot growth during a later stage – when resources were becoming increasingly limiting for shoot growth – root elongation was stimulated. These contrasting mechanisms highlight different strategies regulating resource allocation to the shoot and to the root based on the shoot status, not necessarily in line with the widely accepted concept of functional equilibrium. Furthermore, when root elongation was triggered by resource limitation, the zone in which it was triggered was determined by the type of resource being limiting in that zone. When roots encountered a dry zone in a soil profile, they tended to proliferate in that zone, whereas simultaneously no increase in root mass was detected in the moist zone of the same soil profile. The contrary happened when roots encountered an N-poor zone in a soil profile: the plant tended to enhance root growth in the N-rich zone, as opposed to the N-poor zone. These results may have actually been related to the same mechanism: root growth may have been triggered by the nitrate concentration in the soil solution, the concentration of nitrate in the soil solution may have increased in a drying soil. In these trials, nitrate concentration was not measured as such and this hypothesis could not be verified.

In Chapter 3, different transplant sizes were used as proxies for different root:shoot ratios at transplanting. Investigating different root:shoot ratios at transplanting provided information about the importance of root traits for field performance in lettuce. Changes in initial root:shoot ratios did not impair further field performance and final yield in lettuce, unless the initial root:shoot ratio was very small (under-developed transplant size). The characteristics enabling lettuce to overcome an unbalanced root:shoot ratio so quickly could be found below-ground, in the efficiency of resource acquisition by roots. In field conditions, cultivars with a larger root system exploring deeper soil layers generally displayed better field performance and yield stability across environments. However, if additional root proliferation generally correlated with improved resource capture below-ground in conditions conducive to growth, that relationship was not clear when field conditions were sub-optimal. Different mechanisms may then be triggered

such as an increased nitrate inflow into the roots – as highlighted previously – or improved resource use efficiency. On the other hand, the ability to transform the resource acquired below-ground into shoot mass may also confer yield stability and thus robustness at a relatively low cost for the plant. These findings highlight that genetic variation in root:shoot assimilates partitioning exists among genotypes and that breeding for such traits may be possible.

Chapter 4 emphasizes that creating more robust cultivars of lettuce which can perform well in a wide range of environments based on improved resource acquisition and use efficiency requires a better understanding of the physiology behind these traits and of the genetic variation therein. In that regards, modelling can significantly improve our understanding of the mechanisms below-ground, their contribution to field performance, and how the communication within the plant (the shoot-to-root cross talk) and organization (the implementation of the outcome of that cross talk) are impacted by the environment. Experimental findings underline the high level of Genotype × Environment interactions in the mechanisms regulating resource capture and use efficiency masking the direct genotypic effects and stressing the need for an advanced analytical tool. Therefore a model concept has been proposed allowing for a more effective analysis of the genotypic effects and the Genotype × Environment interactions. Based on this concept, the model to be developed could help breeders identify traits of interest when selecting robust genotypes in a given environment, and conversely, identify the selection environment in which the trait conferring robustness would be expressed best. Further elaboration of the model will be needed, especially relating to accurate modelling of water and nitrate flows over the soil profile and accurate evaluation of the range of model input parameters, based on observed genetic variation.

Chapter 5 highlighted that in the context of increasing interest in molecular tools which can make the breeding progress more effective, assessing and evaluating the genetic control of resource acquisition and resource efficiency is necessary. The association between the traits related to resource capture below-ground in space and time proved to be highly impacted by environmental conditions. This shows that a complex combination of external factors, such as weather conditions, the nutritional status of the

plant, and the availability of localized resources below-ground, influence the expression of the genetic variation. In view of the high impact of environmental factors on physiological mechanisms regulating resource capture and resource use, identifying specific genomic regions correlated with favourable alleles is very difficult; indeed, the expression of favourable traits identified in a specific environment may impair the expression of other traits which may be useful in another environment. Moreover, observed phenotypic effects may be controlled by numerous interacting quantitative trait loci, of which the individual expression greatly varies with environment. However, such findings may also be due to the narrow genetic basis of most lettuce cultivars commercially available nowadays; this narrow base reduces the genetic diversity for traits related to resource capture and resource use as most of the selection has been done under high input systems. This forces us to look for small differences in trait expression which often can be masked by the "noise" in the data created by the environmental conditions.

In the general discussion, the proposition that the complexity of the relationships between root traits and resource capture can mainly be unravelled through technical innovations is debated. Indeed, in the different chapters, the difficulties experienced in identifying the relationships between root traits and resource capture were partly due to a discrepancy between pot trial and field trial data and to the fact that resource capture measurements in the field were not corrected for water movement over the soil profile. These observations call for further improvements in root and resource capture phenotyping in future research.

While literature shows that plasticity in root traits can confer tolerance to resource limitation, this thesis suggests that the plasticity in belowground traits – and not below-ground traits *per se* – may actually be the most relevant trait conferring robustness in lettuce. Chapter 4 showed that there is a high level of quantitative trait locus (QTL) × environment interaction in below-ground traits as QTL numbers and chromosomal locations were subject to changes across environments (cf. also Chapter 5). Because the observed trait changes so unpredictably with the environment, breeding progress is small; actually it might be more fruitful to select for plasticity in below-ground traits when breeding for robustness than for below-ground traits *per se*.

Samenvatting

Telers zijn nog niet in staat om supermarkten continu te bevoorraden met hoogwaardige biologische sla. Zij vertrouwen immers nog steeds op rassen die veel *inputs* behoeven en gevoelig zijn voor fluctuaties in beschikbare hoeveelheden water en nutriënten kenmerkend voor biologische en *low-input* bedrijfssystemen. Moderne slarassen zijn geselecteerd onder conventionele, *high input* condities. Daarin komen eigenschappen gerelateerd aan tolerantie voor milde vormen van abiotische stress gedurende de groei niet tot expressie en is de selectievoortgang ten aanzien van zulke eigenschappen, bijvoorbeeld wortelkarakteristieken, dus gering. De bijdrage van deze eigenschappen aan de prestaties van de gewassen wordt steeds meer onderzocht teneinde telers van robuuste en plastische rassen te voorzien, die in staat zijn onder diverse omstandigheden een constante opbrengst te leveren. Sla is een vegetatief gewas met een korte groeicyclus. Fysiologische mechanismen van het vangen van water en nutriënten en de bijdrage van wortels aan het opbrengend vermogen in het veld zijn niet goed bestudeerd; ook de mogelijkheid om met dergelijke eigenschappen rekening te houden in de slaveredeling zijn nog niet onderzocht. Daarom was de belangrijkste doelstelling van dit proefschrift het ontwikkelen van een strategie om te veredelen op tolerantie voor abiotische stress in sla, op basis van ondergrondse eigenschappen die plasticiteit en robuustheid kunnen verschaffen. Om deze doelstelling te bereiken, werden de fysiologische mechanismen onderzocht die het vangen en benutten van hulpbronnen reguleren op wortel- en spruitniveau. Daarbij werd specifiek gekeken naar tijdelijke en lokale schaarste van hulpbronnen (Hoofdstuk 2). Bovendien werd de bijdrage van wortelkenmerken, alsmede de genetische variatie daarin, aan (variatie in) het vermogen om hulpbronnen te vangen en opbrengst te leveren onderzocht (Hoofdstuk 3). Daarnaast werd de genetische variatie in deze mechanismen vastgesteld en benut als basis voor een modelconcept om veredelaars te ondersteunen in hun veredelingsactiviteiten gericht op robuustheid (Hoofdstuk 4). Ten slotte werd de genetische aansturing van ondergronds vangen van hulpbronnen in tijd en ruimte geëvalueerd, de invloed van de omgeving op de regulatie van de expressie van de

eigenschappen beoordeeld en werden de gevolgen voor het vastleggen van hulpbronnen bovengronds en het opbrengend vermogen van het gewas geanalyseerd (Hoofdstuk 5).
In Hoofdstuk 2 bleek dat de voedingstoestand van sla het type reactie op tijdelijke schaarste van hulpbronnen bepaalt. Om de initiële bovengrondse groeisnelheid tijdens milde, tijdelijke droogte te handhaven, verhoogde sla bij droogte in een vroeg stadium de snelheid van nitraatinstroom in de wortels; dit mechanisme is wellicht goedkoper voor de plant dan het produceren en onderhouden van nieuwe wortels, en kan worden geactiveerd als de schaarste aan hulpbronnen nog niet leidt tot een sterke beperking van de groei. Om de spruitgroei tijdens een later stadium - wanneer de hulpbronnen in toenemende mate beperkend worden voor de spruitgroei – te bestendigen, werd de lengtegroei van de wortels gestimuleerd. Deze verschillende mechanismen geven aan dat er verschillende strategieën zijn voor de verdeling van hulpbronnen over spruit en wortel op basis van de toestand van de spruit, niet *per se* overeenkomend met het algemeen aanvaarde concept van het functionele evenwicht. Bovendien, wanneer wortellengtegroei door beperking in hulpbronnen werd *getriggerd*, werd de zone waarin deze *trigger* plaatsvond, bepaald door de aard van de in die zone beperkende hulpbron. Wanneer wortels op een droge zone in een bodemprofiel stuitten, hadden ze de neiging om zich in die zone sterk uit te breiden, terwijl tegelijkertijd geen toename van de wortelmassa werd waargenomen in de vochtige zone van hetzelfde bodemprofiel. Het tegenovergestelde gebeurde wanneer wortels op een stikstofarme zone van een bodemprofiel stuitten: de plant had de neiging om de wortelgroei in de N-rijke zone te versterken en in de N-arme zone te matigen. Deze resultaten kunnen in feite veroorzaakt worden door hetzelfde mechanisme: wortelgroei kan worden geactiveerd door de nitraatconcentratie in het bodemvocht en de nitraatconcentratie in het bodemvocht kan zijn toegenomen in droge grond. In deze experimenten was de nitraatconcentratie zelf niet gemeten en derhalve kon deze hypothese niet worden geverifieerd.
In Hoofdstuk 3 werden slaplantjes van verschillende grootte gebruikt om bij het verspenen verschillende spruit-wortelverhoudingen te creëren. Het onderzoek naar de effecten van verschillende spruit-wortelverhoudingen bij het verspenen leverde informatie omtrent het belang van worteleigenschappen voor het opbrengend vermogen

van sla in het veld. Veranderingen in de oorspronkelijke spruit-wortelverhoudingen tastten het opbrengend vermogen in het veld, en daarmee de uiteindelijke opbrengst, niet aan, tenzij de initiële spruit-wortelverhouding erg klein was (bij het verspenen van te kleine slaplantjes). De kenmerken die sla in staat stellen om een onevenwichtige spruit-wortel verhouding zo snel te boven te komen, bleken zich ondergronds te bevinden en wel in de efficiëntie van het vangen van hulpbronnen door wortels. Onder veldomstandigheden toonden rassen met een groter wortelstelsel, en dus in staat om ook diepere bodemlagen te exploreren, in het algemeen een beter opbrengend vermogen en een groter opbrengststabiliteit onder verschillende condities. Echter, in die gevallen waarin extra wortelgroei in het algemeen gecorreleerd was met betere opname van hulpbronnen ondergronds in voor groei gunstige omstandigheden, was dat verband niet duidelijk wanneer de veldomstandigheden niet optimaal waren. Verschillende mechanismen kunnen dan worden geactiveerd, zoals een verhoogde nitraatinstroom in de wortels - zoals eerder vermeld - of betere efficiëntie in het gebruik van hulpbronnen. Anderzijds kan ook het vermogen om de ondergronds verkregen hulpbron om te vormen in spruitmassa opbrengststabiliteit en daarmee robuustheid verlenen tegen relatief lage kosten voor de plant. Deze bevindingen benadrukken dat er genetische variatie in verdeling van assimilaten over wortel en spruit bestaat en dat veredelen op deze eigenschappen mogelijk kan zijn.

In Hoofdstuk 4 wordt benadrukt dat het creëren van meer robuuste slarassen die goed kunnen presteren onder een breed scala van condities op basis van een verbeterd vermogen om hulpbronnen te vangen en efficiënt te benutten, een beter begrip vereist van de fysiologie achter deze eigenschappen en van de genetische variatie daarin. Hierbij kan modelleren een aanzienlijke verbetering leveren van ons begrip van de ondergrondse mechanismen, hun bijdrage aan het opbrengend vermogen in het veld, en hoe de communicatie binnen de plant (de cross talk tussen spruit en wortel) en de organisatie (de implementatie van de resultaten van die cross talk) worden beïnvloed door de omgeving. Experimentele bevindingen onderstrepen dat de genotype x standplaats interacties sterk zijn voor de mechanismen die het vangen van de hulpbronnen en de efficiëntie van hun gebruik reguleren. Daarmee worden de directe genotypische effecten gemaskeerd, hetgeen eens te meer laat zien dat een geavanceerd

analyse-instrument nodig is. Daarom is een modelconcept voorgesteld dat een effectievere analyse van de genotypische effecten en de genotype x standplaats interacties mogelijk maakt. Op basis van dit concept kan het nog te ontwikkelen model veredelaars helpen bij het identificeren van belangwekkende eigenschappen bij de selectie van robuuste genotypen in een bepaalde omgeving. En omgekeerd kan het selectiemilieu worden geïdentificeerd, waarin de eigenschap die leidt tot robuustheid het best tot expressie komt. Nadere uitwerking van het model zal nodig zijn, vooral met betrekking tot het nauwkeurige modelleren van bewegingen van water en nitraat over het bodemprofiel en nauwkeurige toetsing van de reeks model-inputparameters, gebaseerd op waargenomen genetische variatie.

Hoofdstuk 5 benadrukt dat in het kader van de toenemende belangstelling voor moleculaire technieken, die de vooruitgang in de veredeling effectiever kunnen maken, het vaststellen en evalueren van de genetische sturing van het vangen en efficiënt benutten van hulpbronnen noodzakelijk is. De associatie tussen de kenmerken met betrekking tot opname van ondergrondse hulpbronnen in ruimte en tijd bleek sterk te worden beïnvloed door omgevingsfactoren. Dit toont aan dat er een complexe combinatie bestaat van externe factoren, zoals weersomstandigheden, de voedingstoestand van de plant, en de beschikbaarheid van plaatsgebonden ondergrondse hulpbronnen, die van invloed is op de expressie van de genetische variatie. Gezien de sterke invloed van milieufactoren op de fysiologische mechanismen die het vastleggen en gebruiken van hulpbronnen reguleren, is het identificeren van specifieke regio's op het genoom waar zich gunstige allelen bevinden, heel moeilijk; inderdaad kan de expressie van gunstige eigenschappen in een specifieke omgeving de expressie van andere eigenschappen, die nuttig zijn in een andere omgeving, beïnvloeden. Bovendien kunnen waargenomen fenotypische effecten worden bestuurd door tal van interacterende *quantitative trait loci*, waarvan de individuele expressie sterk varieert met de omgeving. Dergelijke bevindingen kunnen echter ook veroorzaakt worden door de smalle genetische basis van de meest tegenwoordig commercieel verkrijgbare slarassen; deze smalle basis vermindert de genetische diversiteit van kenmerken die gerelateerd zijn aan het vastleggen en gebruiken gebruik van hulpbronnen, aangezien de selectie vooral uitgevoerd wordt onder *high input*. Dit

dwingt ons om te zoeken naar kleine verschillen in expressie van kenmerken die vaak kan worden gemaskeerd door de "ruis" in de gegevens veroorzaakt door milieuomstandigheden.

In de algemene discussie wordt de stellingname dat de complexiteit van de relaties tussen de worteleigenschappen en het vangen van hulpbronnen voornamelijk kan worden ontrafeld door middel van technische innovaties bediscussieerd. Inderdaad werden in de verschillende hoofdstukken de problemen bij het identificeren van de relatie tussen wortelkenmerken en vangen van hulpbronnen deels veroorzaakt door een discrepantie tussen potproeven en veldproeven en door het feit dat de metingen aan het vangen van hulpbronnen in het veld niet konden worden gecorrigeerd voor waterbeweging in het bodemprofiel. Het is daarom noodzakelijk om in vervolgonderzoek aandacht te besteden aan betere technieken voor het fenotyperen van wortelsystemen en opname van water en nutriënten.

Uit de literatuur blijkt dat plasticiteit in worteleigenschappen kan bijdragen tot tolerantie voor een beperkte beschikbaarheid van water en nutriënten. Dit proefschrift suggereert dat plasticiteit in ondergrondse eigenschappen - en niet ondergrondse eigenschappen *per se* – in feite de meest relevante eigenschap is die robuustheid van sla bepaalt. Uit Hoofdstuk 4 bleek dat er een sterke *quantitative trait locus* (QTL) × milieu interactie bestaat in ondergrondse kenmerken. Immers de aantallen QTLs en posities van deze QTLs op het genoom vertoonden verschillen als gevolg van verschillen in omgevingsfactoren (zie ook Hoofdstuk 5). Omdat de waargenomen eigenschap zo onvoorspelbaar verandert met de omgeving, is de veredelingsvoortgang klein; eigenlijk is het wellicht vruchtbaarder om te kiezen voor plasticiteit in ondergrondse kenmerken bij het veredelen op robuustheid dan voor ondergrondse eigenschappen *per se*.

About the author

Pauline J. Kerbiriou was born in France, where she obtained a Master's degree in horticultural engineering from the National Institute for Life, Food, Horticultural Sciences and Landscaping (AgroCampus Ouest, Angers, France) in 2007. She also completed a Master's degree in Organic Agriculture at the University of Wageningen (the Netherlands). Pauline's first interest in plant breeding dates back from 2005, when she worked with the Citrus breeding program developed at the CIRAD (International Centre for Agricultural Research for Development) in Corsica (France), evaluating diverse methods used to obtain tangerine triploid hybrids. This led her to move to Florida (USA) in 2007 to work on a project aiming at breeding tangerine for improved organoleptic quality at the Citrus Research and Education Centre with Dr. Fred Gmitter and Dr. Anne Plotto. In 2009, Pauline came back to the University of Wageningen to work on her PhD project in lettuce breeding with Dr. Edith Lammerts van Bueren and Dr. Paul Struik. In 2013 she moved to the UK where she is now raspberry, blackberry and blueberry variety development manager for Driscoll's in Europe.

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 of literature (6 ECTS)

- Resource capture in lettuce: breeding and modelling (2009)

Post-graduate courses (2.7 ECTS)

- Association mapping; Biometris (2012)
- Mixed model based QTL mapping in Genstat; Biometris (2012)
- Root ecology: drivers of foraging and interactions in a spatial context; PE&RC-KU SCIENCE-NUE Crops (2012)

Deficiency, refresh, brush-up courses (3 ECTS)

- Modern statistics for the Life Sciences (2009)

Competence strengthening / skills courses (5.6 ECTS)

- PhD Competence assessment; Wageningen Graduate Schools (2009)
- Techniques for writing and presenting scientific papers; Wageningen Graduate Schools (2009)
- Interpersonal communication for PhD students; Wageningen Graduate Schools (2010)
- Effective behaviour in professional surroundings; Wageningen Graduate Schools (2011)
- Mobilizing your scientific network; Wageningen Graduate Schools (2011)
- Career orientation; Wageningen Graduate Schools (2012)
- Writing grant proposals; Wageningen Graduate Schools (2013)

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

- PE&RC Introduction weekend (2009)
- PE&RC Day (2012)

Discussion groups / local seminars / other scientific meetings (5 ECTS)

- Abiotic stress discussion group; Plant breeding (2009-2012)
- Soil-Plant interactions discussion group (2011)

International symposia, workshops and conferences (5.1 ECTS)

- TTI networking event; poster presentation; Utrecht, the Netherlands (2011)
- EUCARPIA 2nd Conference of the Section Organic and Low-Input Agriculture on Breeding for resilience: a strategy for organic and low-input farming systems; poster presentation; Paris, France (2011)
- EUCARPIA 3rd Conference of the Section Organic and Low-Input Agriculture on Breeding for Nutrient Efficiency; oral presentation; Goettingen, Germany (2013)

Lecturing / supervision of practical's / tutorials (1.8 ECTS)

- Organic plant breeding (2009-2012)
- Research methods in crop physiology (2011-2012)

Supervision of MSc students (9 ECTS)

- Root and shoot growth of lettuce under temporary drought stress
- Root and shoot growth and resource capture of lettuce under spatially limiting resources
- Resource capture of a population of lettuce in field conditions