Institut für Bio- und Geowissenschaften- Pflanzenwissenschaften
(IBG-2), Forschungszentrum Jülich

Role of root plasticity in response to soil compaction in sorghum

Dissertation

zur Erlangung des Grades

Doktor der Agrarwissenschaften (Dr. agr.)

der
Landwirtschaftlichen Fakultät

der
Rheinischen Friedrich-Wilhelms-Universität Bonn

von

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Bonn 2022

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Tag der mündlichen Prüfung: 01.07.2021

Angefertigt mit Genehmigung der Landwirtschaftlichen Fakultät der Universität Bonn

To my Mother

ABSTRACT

Compacted soils limit crop production and affect millions of hectares of agricultural land globally. Plants vary in tolerance to soil compaction and roots express various plastic responses. Unfortunately, the importance of those responses for crop yield and the implication in breeding are practically unknown. Accordingly, to test whether the plasticity of the root system architecture is associated with the tolerance to soil compaction, we reviewed the various root responses reported in the literature and describe the consequences of soil compaction on the rooting environment. Additionally, we carried out a set of experiments to study the phenotypic diversity of shoot and root plasticity in a collection of sorghum genotypes. For that, plants were grown in a greenhouse for three to six weeks in homo- and heterogeneous soil density gradients (from 1.3 to 1.8 g cm^{-3}). Finally, a simulation-based research was conducted to study the plants functional consequence of phenotypic response to soil compaction. As a conclusion, sorghum genotypes can vary significantly in terms of their response to soil compaction. Tolerant lines are in general smaller sized genotypes which exhibit plasticity to soil compaction for fine roots only. Additionally, this tolerance is associated with the ability to compensate the limited function of an impeded portion of their root system, by growing less roots in those layers where the strength is high and growing more roots in looser zones. Although these responses are complex, root plasticity can be targeted in breeding to increase the crop yield under specific conditions such as low-input agronomic systems.

Keywords:
Root architecture; phenotypic variation; allometry; genotype-by-environment interaction; soil bulk density.

KURZFASSUNG

Verdichtete Böden begrenzen die Pflanzenproduktion und betreffen weltweit Millionen von Hektaren landwirtschaftlicher Nutzfläche. Pflanzen variieren in ihrer Toleranz gegenüber Bodenverdichtung und Wurzeln drücken dieses in verschiedenen plastischen Reaktionen aus. Die Bedeutung dieser plastischen Reaktionen auf den Ernteertrag ist bisher praktisch unbekannt. Um zu testen, ob die Plastizität der Wurzelsystemarchitektur mit der Toleranz gegenüber Bodenverdichtung zusammenhängt, verfolgt diese Arbeit zwei Ziele: Erstens die Evaluierung der vorhandenen Literatur. Zweitens wurden eine Reihe von Experimenten durchgeführt, um die phänotypische Vielfalt der Spross- und Wurzelplastizität von diversen Sorghum-Genotypen zu untersuchen. Die Pflanzen wurden drei bis sechs Wochen in einem Gewächshaus in homo- und heterogenen Bodendichtegradienten (von 1,3 bis 1,8 g cm^{-3}) angezogen, und die Reaktionen der Wurzelarchitektur auf die Gradienten gemessen. Schließlich wurde eine simulationsbasierte Studie durchgeführt, um die funktionellen Konsequenzen der phänotypischen Reaktion auf die Bodenverdichtung der Pflanzen zu evaluieren. Ich schließe aus den Ergebnissen, dass Sorghum-Genotypen hinsichtlich ihrer phänotypischen Reaktion auf Bodenverdichtung erheblich variieren können. Tolerante Linien sind im Allgemeinen Genotypen kleinerer Größe, und dass nur feine Wurzeln eine Plastizität gegenüber der Bodenverdichtung aufweisen. Zusätzlich ist diese Toleranz mit der Fähigkeit verbunden, die eingeschränkte Funktion eines betroffenen Teils ihres Wurzelsystems zu kompensieren, indem weniger Wurzeln in den Erdschichten mit hoher Festigkeit und mehr Wurzeln in lockeren Zonen wachsen. Obwohl diese Reaktionen komplex sind, kann die Wurzelplastizität bei der Züchtung gezielt eingesetzt werden, um den Ernteertrag unter bestimmten Bedingungen zu erhöhen (z.B. agronomischen Systemen mit geringem Input).

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LIST OF ABBREVIATIONS

$\bar{x}_0$: phenotypic mean in loose soil

$\bar{x}_1$: phenotypic mean in compacted soil

F_0: organic fraction of soil mass

k_{sat}: soil volumetric water content at saturation

θ_{sat}: soil volumetric content at saturation

[N]: Nitrogen concentration

3D: three dimensional

ABA: abscisic acid

ANCOVA: Analysis of Covariance

ANOVA: Analysis of Variance

Ca: Calcium

CV: coefficient of variation

DAG: days after germination

Db: soil bulk density

DM: dry mass

EC: soil electrical conductivity

Fig.: Figure

G×T: Genotype by treatment interaction

K: Potassium

K+: Potassium ion

K_2O: Potassium oxide

L1, … L6: Level of PVC cylinders indicated from the top to the bottom

Mg: Magnesium

MRI: Magnetic Resonance Imaging

N: Nitrogen

n: sample size

n.s.: not significant ($P > 0.05$)

Na+: Sodium ion

O_2: Oxygen

OC: percentage of organic carbon in soil mass

P: Phosphorus

P: *P*-value

P_2O_5: phosphate

PC1, … PC5: First, … fifth principal component

PCA: Principal Component Analysis

PVC: polyvinyl chloride

Q: soil penetration resistance

r: correlation coefficient

R/S: shoot ratios

R^2: coefficient of determination

RCA: root cortical aerenchyma

RGA: root growth angle

RSA: root system architecture

S/C: ratio of sand to clay content

SEM: standard error of the mean

SLA: specific leaf area

SRL: specific root length

SWCC: soil-water characteristic curve

Wm: gravimetric water content (wet-mass basis)

α: significance level

β: false negative rate

η^2: variance explained by treatment factor (see Cohen 1988 and 1992)

Ψ: water potential

GENERAL INTRODUCTION

Roots are the underground component of a plant that have as functions, the soil resources (water and nutrients) uptake, anchorage, storage reserves and, in some plants, serve as organs of reproduction (Weaver, 1926; Fitter, 1987). Individually, a root can be seen as a cylindrical axis with laterals; whereas, as a whole, the **plant root system** is composed of these individual axes or roots (Hackett, 1968; Gregory et al., 1978). Monocotyledonous and dicotyledonous crops form a complex root system comprising embryonic and post-embryonic roots which have their own distinct morphological and anatomical features (Weaver, 1926; Zobel, 1991; Rich and Watt, 2013). One of the most remarkable features of roots is the **root system architecture** (RSA). RSA describes the spatial arrangement of root components within the soil and is generated from the activity of growing root tips and the formation of lateral roots (Lynch, 1995; Smith and De Smet, 2012; Rogers and Benfey, 2015; Correa et al., 2019). RSA is quite an important factor which has a crucial role in plant's exploration of the soil to forage for water and nutrients (e.g. Lynch, 1995).

Phenotypic plasticity is the property of a given genotype that results in the expression of different phenotypes under different environmental conditions (Bradshaw 1965; Sultan 1987; Via et al. 1995; Pigliucci 2001; Palmer et al. 2012). Despite heterogeneous soil environments, plants are able to adjust and compensate to such variation in soil conditions through their phenotypic plasticity (Crossett et al. 1975; Goss 1977; Shani et al. 1993; Via et al. 1995; Forde 2002; Waisel and Eshel 2002; Bingham and Bengough 2003; Ho et al. 2005; Lynch and Ho 2005; Rubio and Lynch 2007; Nicotra and Davidson 2010; Palmer et al. 2012; El-Soda et al. 2014; Pfeifer et al 2014; Dara et al. 2015; Wang et al. 2016). Traditionally, the expression of phenotypic plasticity in RSA traits of different crops can be found in many works, although few experiments have addressed the phenomenon directly. According to literature, a number of different RSA can be formed depending on textural, oxygen, water and nutrient conditions of soils or other soil properties (Fitter 1986; Fitter and Stickland 1991; Lynch 1995; Glimskär 2000). These responses includes changes in root angles (Tracy et al. 2012; Uga et al. 2015) and lateral root proliferation (Drew et al. 1973; Drew 1975), root to shoot ratio (Poorter and Nagel 2000; Lynch and Ho 2005; Witzel et al. 2009), root diameter (Materechera et al. 1992; Popova et al. 2016), root hair length and density (Bates and Lynch 2001), cortical aerenchyma (Armstrong, 1979; Drew et al., 2000; Iijima et al. 2016), etc. Those responses

have been not only associated with adaptive plasticity but also with compensatory growths (Crossett et al. 1975; Goss 1977; Shani et al. 1993; Rubio and Lynch 2007; Pfeifer et al. 2014; Dara et al. 2015; Wang et al. 2016). RSA plasticity expressed through complementary or compensatory growth of distinct root system components and/or portions may be important means to optimize acquisition of multiple soil resources particularly when they are unevenly distributed in the soil profile.

Soils not often supply the most favorable conditions for crop growth. Yield imitations caused by inadequate rooting mainly occur when soil conditions such as compaction or root damage by soil-borne pathogens prevent the plants from accessing the potentially available soil resources (e.g. water and nutrients) (Hoad et al., 2001). These soil constraints may become more accentuated in degraded soils. Soil degradation is the loss of actual or potential productivity or utility due both to natural or anthropogenic causes that implies changes in soil properties and processes that have negative effects on sustainable crop production (Lal et al., 1989; Lal, 1997). This produces losses of soil structure, crusting, compaction, erosion, low levels of oxygen (hypoxia or anoxia), acidification, nutrient leaching, salinity, reduction in cation exchange capacity, organic matter reduction and decline in soil biodiversity (Lal, 1997).

One of the most important soil degradation processes is soil compaction. Soil compaction affects nearly all the bio-physicochemical properties of soil (Håkansson et al., 1988). In agricultural soils, the main factors responsible for compaction are excessive traffic, the use of farm equipment that exceeds the bearing capacity of soil, and tillage at unsuitable soil water contents, in particular wet soils (Barken et al., 1987; Håkansson et al., 1988; Lipiec and Stępniewski, 1995; Bengough et al., 2011; Casanova et al., 2013). Compaction may be very persistent, and in the subsoil even permanent (Håkansson et al., 1988). Soil compaction results in an increase in bulk density, a decrease in soil porosity or a change in the proportion of pores with water and air (mainly loss of coarse pores), and an increase in mechanical resistance or strength hindering root growth and development due to low levels of oxygen (hypoxia or anoxia), reduced water and nutrients supply, and mechanical impedance (Håkansson et al., 1988; Lal, 1997; Bengough et al., 2011; Hoad et al., 2001; Casanova et al,. 2013). High bulk densities reduces biomass production and nutrient uptake resulting in lower yields (Arvidsson 1999). The yield losses due to soil compaction have been estimated at 20 and 75% in various crops in different environments and soil conditions (Correa et al., 2019).

Highly compacted soils affect negatively root growth through several indirect

mechanisms (Passioura, 2002). This is because of the uptake of water and nutrients may become limiting due to changes in soil hydraulic, aeration, and diffusive properties alter their availability, as well as direct compaction effects on root growth and development increases soil resistant to root penetration (Håkansson et al., 1988; Lipiec and Stępniewski, 1995; Passioura, 2002). Several phenotypic responses of roots exposed to high soil bulk densities have been documented in several works. For example, decreased total length and depth of root system (total sum of root length) (Eavis, 1972; Goss 1977; Hoad et al., 2001; Bingham et al., 2010; Rich and Watt, 2013; Popova et al., 2016); lower root dry mass (Grzesiak et al., 2002; Pfeifer et al., 2014); increased root axis diameters (Eavis, 1972; Materechera et al., 1992; Hanbury and Atwell, 2005; Tracy et al., 2012; Pfeifer et al., 2014; Popova et al., 2016); less horizontally oriented lateral roots (shallower growth angles) (Dexter and Hewitt, 1978); increased root system tortuosity (ratio of root length to the shortest distance between two arbitrary points in the root such root origin and tip) (Tracy et al., 2012; Popova et al., 2016); etc.

According to these antecedents, soil strength affects root growth and development through several indirect mechanisms. Additionally, plants react to these constraints showing different RSA phenotypic changes. Even though phenotypic plasticity may have a positive adaptive value in many circumstances, few works have addressed the phenomenon directly especially in RSA studies. Additionally, it is not clear whether the degree of plasticity may vary depending of plant growth and/or development. Furthermore, the different root system components may not have the same response to soil environment. This implies that plant root system may adjust their growth and development to such changing constrains through the differential phenotypic plasticity of its components. These responses may be mainly manifested by within-plant plasticity. Phenotypic plasticity may be an active solution to the problem of adaptation to heterogeneous environments. However, little is known about the role of plasticity on the phenotypic responses to soil compaction.

This thesis is aimed to study the RSA plasticity as a response to soil compaction under a plant breeding context. As a general hypothesis, we test whether a high plasticity of the root system architecture is associated with a greater tolerance to soil compaction in terms of biomass, nutrient acquisition and water absorption. For this purpose, a theoretical framework was proposed and a series of experiment- and simulation-based studies were carried out. To answer this, the thesis is divided into six chapters as follows:

Chapter 1 - SOIL COMPACTION AND THE ARCHITECTURAL PLASTICITY OF ROOT SYSTEMS: This chapter is a comprehensive review of plant plasticity and soil compaction to explore to what extent RSA plasticity might be useful for breeding. Subsequently, a plastic ideotype was proposed for soil compaction tolerance. This review was the theoretical framework to study root plasticity of this thesis. This chapter was published in Journal of Experimental Botany, Volume 70, Issue 21, 1 Nov. 2019, Pages 6019–6034, https://doi.org/10.1093/jxb/erz383.

Chapter 2 - MATERIALS AND METHODS: This chapter describes in detail the materials and methods that support chapters **Chapter 3** to **6**.

Chapter 3 - PRELIMINARY EXPERIMENTS: Results of a set the experiments which were carried out to calculate the minimum number of replicates needed to detect a significant effect of soil compaction on plant phenotype.

Chapter 4 - PHENOTYPIC RESPONSE TO SOIL COMPACTION VARIES AMONG SORGHUM GENOTYPES BUT CORRELATES WITH PLANT SIZE: The aim of this chapter is to study whether the genotypic diversity in the degree of responses to soil compaction is more dependent on true plasticity than on plant size. Part of the results of this chapter has been published in Plant and Soil, Volume 472, 5 Jan. 2022, Pages 59–76, https://doi.org/10.1007/s11104-021-05160-z.

Chapter 5 - WITHIN-ROOT SYSTEM PLASTICITY AS A RESPONSE TO SOIL COMPACTION IN SORGHUM: In this chapter, we tested whether plants are able to compensate the effect of very compacted layers with a higher root proliferation (e.g. a higher root length) where the best condition are found (e.g. looser and more superficial soil layers).

Chapter 6 - FUNCTIONAL AND STRUCTURAL CONSEQUENCES OF PHENOTYPIC RESPONSE TO SOIL COMPACTION IN SORGHUM: A simulation-based research was carried out to study the consequences of RSA plasticity in response to soil compaction on plant performance. With this, we tested if those phenotypes with a higher RSA plasticity also express a higher nutrient and/or water uptake per unit of root length than those phenotypes with higher RSA plasticity.

1 - SOIL COMPACTION AND THE PLASTICITY OF ROOT SYSTEMS

Introduction

Plant root functions are: soil water and nutrient uptake, anchorage, reserve storage, vegetative propagation (e.g. Weaver, 1926; Fitter, 1987), and root to shoot signaling (Shabala et al., 2015). Root system architecture (RSA) describes the spatial arrangement of root components within the soil [i.e. the spatial arrangement of nodal, lateral (first-, second-, third-order, etc.), primary roots, etc.]. RSA determines the plant's exploration of the soil to forage for water and nutrients (e.g. Lynch, 1995, 2007b). RSA results from three processes: extending root tips, formation of lateral roots, and tropisms or curvatures (e.g. Lynch, 1995; Smith and De Smet, 2012; Rogers and Benfey, 2015). These processes respond dynamically to soil bio-physico-chemical properties that vary in time and space, and therefore the resulting RSA phenotype arises from both the plant genetics and the soil conditions. This responsiveness of RSA to soil conditions can be termed 'RSA plasticity'.

An individual organism cannot be considered outside the context of its environment (Bradshaw, 1965), and the actual phenotype of a particular genotype depends on the particular environment that it experiences (Via et al., 1995). In a broad sense, phenotypic plasticity is the property of a given genotype to express different phenotypes under different environmental conditions (Bradshaw, 1965; Sultan, 1987; Via et al., 1995; Pigliucci, 2001; Palmer et al., 2012). Phenotypic plasticity is thought to enable plants to cope with or even take advantage of environmental heterogeneity (Crossett et al., 1975; Via et al., 1995; Forde, 2002; El-Soda et al., 2014). Although plasticity can provide an increased environmental tolerance in many circumstances (Bradshaw, 1965; Via et al., 1995; Palmer et al., 2012; Des Marais et al., 2013), this is not always the case. If plasticity is expressed, it may not have any appreciable, beneficial effect, and it may even be counterproductive (see below).

Here we review the literature to establish the importance of RSA plasticity in the context of soil strength. Soil strength is a major cause of inadequate rooting. It affects nearly all soil bio-physico-chemical properties (Håkansson et al., 1988) such as soil

porosity, water conductivity, and nutrient availability, and millions of hectares of agricultural lands are affected globally (Oldeman et al., 1991). While the majority of affected lands are located in Europe, Africa, and Asia, some areas of the Americas are also prone to compaction (Soane and van Ouwerkerk, 1994). Yield losses by compaction have been estimated to be ~20% (Arvidsson, 1999) and 25% (Barken et al., 1987). Higher estimates (~50–75%) occur when the soil is affected by another constraint such as drought (Hoque and Kobata, 2000). In **Fig. 1.1**, we compiled an overview of the negative effect of soil compaction on yield in several crops, soils, and countries. The lower yields result from reduced uptake of water and nutrients, and lower biomass, which in turn are consequences of soil mechanical impedance on root growth and development (Håkansson et al., 1988; Lipiec and Stępniewski, 1995; Stirzaker et al., 1996; Passioura, 2002). In this chapter, we do not discuss the effect of soil compaction on the soil microbiota and their interactions with the roots and surrounding rhizosphere because it is a complex topic. Nevertheless, mechanical impedance can increase the accumulation of microorganisms on roots, making the plant more prone to infection and disease (Watt et al., 2003).

The role of RSA plasticity in providing tolerance to soil compaction is poorly understood. Few studies have addressed RSA plasticity directly and, additionally, it is challenging to distinguish adaptive mechanisms from ontological processes. To investigate the possible role of RSA plasticity in responses to soil compaction, we first discuss plasticity and how phenotypic plasticity may confer tolerance to diverse environments. Secondly, we describe the consequences of soil compaction on the rooting environment and extensively review the various root responses to soil compaction reported in the literature, such as shortened root length, increased root diameter, and fewer lateral roots. Thirdly, we propose some of those responses as plastic adaptations. Finally, we discuss to what extent these plasticity responses might have utility in agricultural production and breeding.

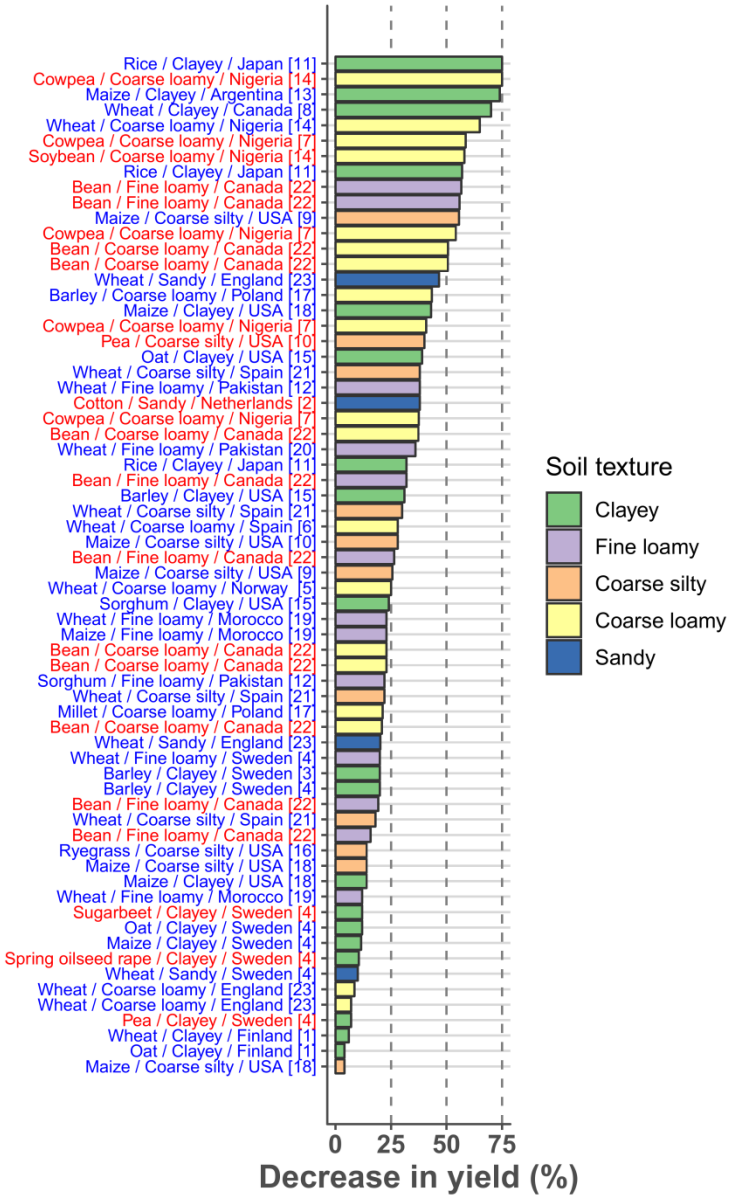


Fig. 1.1 - Yield penalties caused by compaction in different crops, soils, and countries. Labels on they-axis are different studies on soil compaction where yield was registered. If the crop is a dicot or a monocot plant, the label is red or blue, respectively. The labels are indicated as follows: Crop/Soil textural class/Country [reference]. The specific reference list for this plot can be found in **Supplementary Table S1**

This chapter expands the focus of previous reviews, conducted by Unger and Kaspar (1994), Bengough et al. (2011), Jin et al. (2013), Gao et al. (2016a), and others, by examining specifically different aspects of the RSA and highlighting the link between soil compaction and RSA plasticity not only from a theoretical point of view but also discussing their practical consequences in breeding. Our goal was not to focus on the

mechanical aspects of soil compaction, which have been extensively reviewed by Unger and Kaspar (1994), Jin et al. (2013), and Gao et al. (2016a, b). Additionally, we did not cover those soil management practices to alleviate the problems associated with soil compaction (for reviews, see Unger and Kaspar, 1994; Batey, 2009). Instead, we describe the consequences of soil compaction on the rooting environment and review the various root responses reported in the literature. Finally, we discuss to what extent these responses might be useful for breeding, and which one of them enhances the root exploration capabilities in tolerant genotypes. With this chapter, we demonstrate that RSA plasticity is key to understanding the effects of soil compaction on plant performance.

Defining phenotypic plasticity of root system architecture

RSA plasticity is the reorganization of the RSA in response to one or several exogenous disturbances that affect the RSA by influencing the extension of root tips, the formation of lateral roots, or root tropisms. The evolutionary concept of fitness is complex and its definition is outside the scope of this review chapter (see Orr, 2009). However, in plant breeding, yield integrates the 'agricultural fitness' indicators (Nicotra and Davidson, 2010). Thus, 'soil compaction tolerance' may be defined as the ability of a genotype to have stable yield or biomass production across locations varying in soil compaction.

A plastic response would be labeled as adaptive as long as it is positively correlated with some fitness components, such as the number of seeds and fruits per plant, germination and fruit set rate, and offspring survival(for more fitness components, see Primack and Kang 1989;Younginger et al., 2017). For example, plant size has been used as an estimator for plant fitness. In general, within a species, larger plants have greater fitness since they produce more seeds, leading to a greater likelihood of leaving viable offspring (Younginger et al., 2017). Below we will discuss several specific examples of adaptive plasticity in the context of soil compaction. **Fig.1.2** shows that the phenotype of a trait can be divided into two main components: the constitutive and the facultative phenotype (apparent plasticity and apparent plasticity). These two components can also be divided in turn into other subcomponents, such as genotypic and environmental effects. We expressed the relationships among those subcomponents as a linear model for reasons of simplicity. Note that many possible models may exist, such as second- and third-order non-linear relationships, models with only one subcomponent; and/or with no constitutive phenotype at all. Accordingly, if a plastic response has a genetic component, then it is a manifestation of the Genotype×Environment interaction. When this plastic response is

positively associated with fitness, it should be labeled as adaptive.

Tolerance and adaptive plasticity

Theoretically, plasticity may provide environmental tolerance especially in heterogeneous environments. If tolerance is defined as the ability to maintain fitness while facing environmental stress, it is necessary to define 'plant stresses' as well. Lichtenthaler (1996) defines plant stress as any unfavorable condition, or substance that affects the plant metabolism, growth, or development. In crop production, the stress is any condition that decreases yield (Wallace, 1986). Thus, when yield reductions are minimal, the genotype might be considered tolerant or resistant (Negin and Moshelion, 2016). Plants respond phenotypically to stress. The initial result of stress is strain and has been defined as the phenotypic expression of stress before damage occurs (Lichtenthaler, 1996; Blum, 2016). Therefore, by definition, strain includes both morphological (structural) changes and physiological responses (Blum, 2016). The term 'strain' is rarely used and is usually replaced by stress responses (Lichtenthaler, 1996). Thus, strain, unlike stress, can be phenotyped (Blum, 2016). For instance, the primary strain under drought stress is water loss from cells (Blum, 2016). Biological systems have developed adaptive mechanisms to cope with stress (Kranner et al., 2010; Blum, 2016). It may be difficult to distinguish between adaptive responses and damage, especially as adaptive responses have costs and limits (see below). In some cases, however, plants may recover from stress and reverse the damage. Such recovery may be considered adaptive and is sometimes referred to as an 'elastic response' (Kranner et al., 2010; Blum, 2016). When elastic responses allow the plant to return to a reference or pre-stress state, the genotype may be labeled as 'resilient' (Grimm and Wissel, 1997; Negin and Moshelion, 2016). For example, a resilient plant decreases its stomatal conductance as a response to drought stress, but it is able to return to its previous stomatal conductance levels after the stress ceases (Negin and Moshelion, 2016). Thus, plasticity encompasses strain, damage, and adaptive responses (**Fig. 1.2**), and that these adaptive responses can cause a genotype to be tolerant, resistant, or resilient.

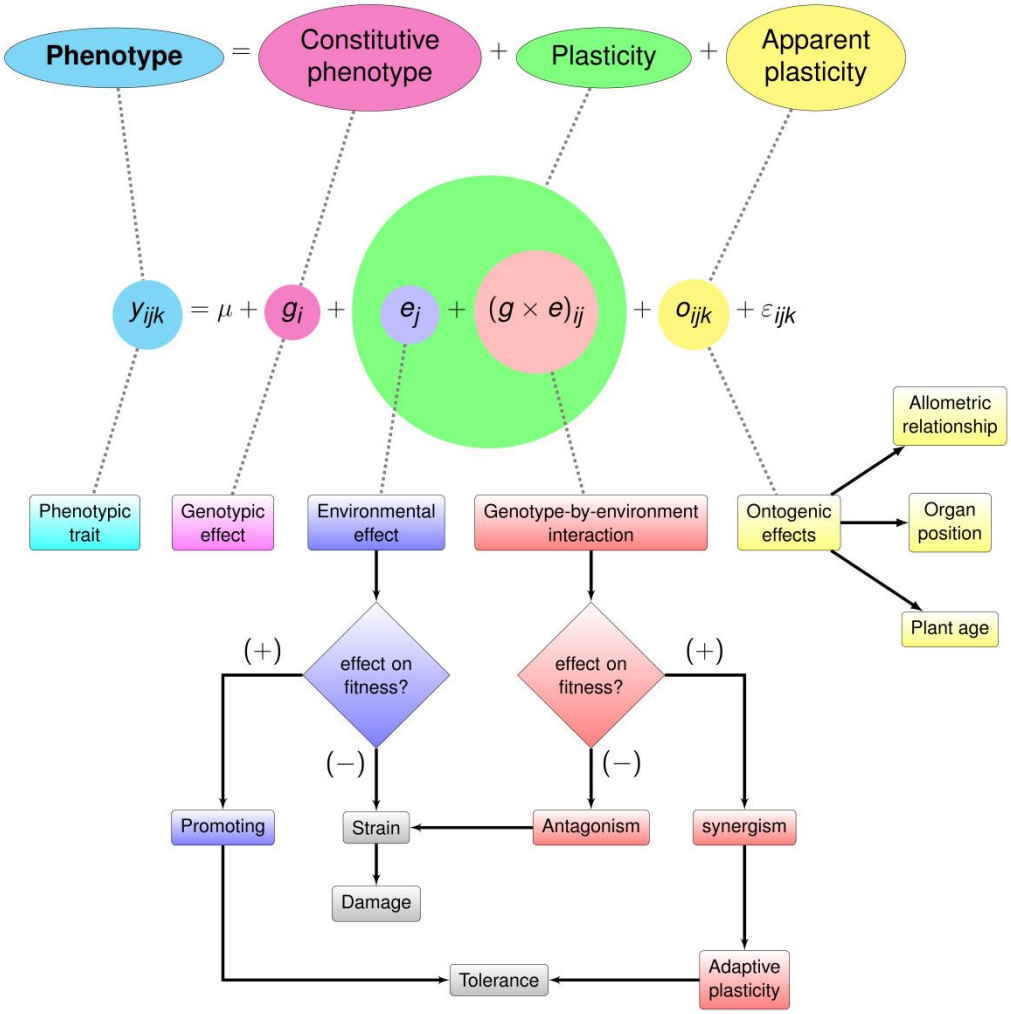


Fig. 1.2 - Model for plant phenotypic plasticity. At the population level, we can split a phenotypic trait into three components: (i) the constitutive phenotype; (ii) plasticity; and (iii) apparent plasticity. The relationships among those components for simplicity may be expressed as a basic linear model: where y_{ijk} is the phenotype measured for the trait y on the plant k of the genotype i under the environment j; μ corresponds to the overall mean; g_i is the effect of genotype i representing the effect of each genotype or genotypic effect on trait y (constitutive phenotype); e_j is the effect of environment j; $(g \times e)_{ij}$ is the interaction between genotype i and environment j (i.e. not all genotypes have the same degree of response to e_j); and ε_{ijk} is the residual error. Additionally, an additive ontogenic effect is assumed, o_{ijk}, as a covariable. For instance, the phenotype of a flower may depend on the position and developmental stage of its node along the shoot. Thus, the plasticity is given by e_j and $(g \times e)_{ij}$. In addition, the effects of plasticity on performance can be both negative and positive, leading to damage or tolerance, respectively.

Costs and trade-offs of phenotypic plasticity

As mentioned above, phenotypic plasticity may have positive or negative consequences on plant performance. Additionally there may be negative interactions among root traits, such as trade-offs within a single environment, or across different environments. A plastic response may be adaptive in one environment, but detrimental in another (Lynch, 2007a). This is particularly evident when resources are stratified in the soil profile (Ho et al., 2005; Lynch and Ho, 2005).

The type of RSA expressed is controlled by the genetic background of a particular plant and the available resources and environmental condition (Fitz Gerald et al., 2006). Since the resource costs associated with production or maintenance associated with soil exploration (metabolic costs) by root systems have been shown to be relatively high, sometimes exceeding 50% of daily photosynthesis (Nielsen et al., 1998, 2001; Lambers et al., 2002), breeding for genotypes having an increased allocation of resources to roots may carry negative consequences for yield, especially in resource-poor environments. The metabolic costs of enhanced root growth should be subsidized by resources which might be used for yield instead (Ho et al., 2005; Lynch and Ho, 2005; Lynch, 2007b; Mi et al., 2010; Lynch, 2013). Thus, traits that enhance the effectiveness or efficiency of roots in acquiring soil resources would be better selection targets than root size per se (Lynch, 2007b).

The carbon costs associated with any plastic root response are assumed as long as there are greater returns in terms of soil resources for the carbon investment (Eissenstat, 1992). For instance, fine root proliferation may be costly in terms of carbon, oxygen, and nitrogen since those roots have high respiration rates, a relatively short lifespan, rapid turnover, and quick decomposition (Eissenstat and Yanai, 1997; Jackson et al., 1997; Pregitzer et al., 1998). The low availability of nitrogen and oxygen in compacted soil (Håkansson et al., 1988; Passioura, 2002; Tubeileh et al., 2003; Bengough et al., 2011) would hinder the production of fine roots. To test these, specific studies on the carbon economy under soil compaction conditions are needed.

True adaptive versus apparent plasticity

As we previously discussed, plasticity responses encompass strain, damage, and adaptive responses. The distinction between these types of plasticity from stress and/or ontological effects may be challenging. Changes in biomass allocation may also result from

‘ontogenetic drift’ (Evans, 1972) since biomass allocation usually changes as a function of plant size or total biomass during growth and development.

In general, edaphic stress causes whole-plant growth to be reduced while growth of roots is favored over that of shoots (e.g. increased resource allocation to the root system). For example, increased root to shoot ratios (R/S) have been found to be associated with nutrient deficiencies (Poorter and Nagel, 2000; Lynch and Ho, 2005; Walk et al., 2006) and drought (Huang and Fry, 1998; Verslues et al., 2006). In the case of compaction, both increases and decreases in R/S have been observed, and we will discuss the various explanations.

These changes are often explained using theories such as the ‘functional equilibrium theory’ (Poorter and Nagel, 2000), which states that plants shift their allocation of biomass towards shoots or roots, depending on the availability of above- or below-ground resources, respectively. This is an important limiting factor for plant growth, prioritizing and optimizing the acquisition of resources in a manner that maximizes plant growth (Poorter and Nagel, 2000; Reich, 2002). For example, an increase in the R/S in response to a reduced availability of nutrients, such as nitrogen, occurs as long as the availability of assimilates is not limiting (Ericsson, 1995). Under these conditions, carbon may have little value relative to the value of the most limiting resource, and large amounts of carbon may be allocated to acquire the most limiting resource (Eissenstat, 1992). Thus, these plastic responses could be clearly indicated as adaptive (Poorter and Nagel, 2000). However, smaller or younger plants generally have a greater R/S, and thus at least part of the observed plasticity might be explained by ontogeny if it is assumed that the stressed plants are simply ‘behind schedule’. For instance, under stressful conditions, plant size may be reduced and show changes in R/S. However, for each plant size there seems to be a ‘pre-defined’ R/S independent of the environmental conditions, and the observed R/S may merely be a result of the reduction in plant size and not an active response to cope with this stress. Thus, the changes in biomass allocation may also result from ‘ontogenetic drift’ (Evans, 1972) since biomass allocation usually changes as a function of plant size or biomass during growth and development. In general, during the vegetative growth phase of most herbaceous plants, seedlings have the highest R/S values, which decline over time as plants grow and develop (McConnaughay and Coleman, 1999). These changes in allocation may result from environmental gradients (true plasticity), ontogenetic drift (apparent plasticity), or both (McConnaughay and Coleman, 1999; Poorter and Nagel, 2000; Reich, 2002; Geng et al.,

2007; Xie et al., 2012). Therefore, to understand plasticity in biomass allocation, it is necessary to distinguish these sources of variation (Xie et al., 2012). For that, the log–log relationship (e.g. log–log plots to describe the growth of one plant component or organ in relation to the growth of another component) during different developmental stages has been used (Poorter and Nagel, 2000; Reich, 2002). This growth covariation among plant components may be referred to as allometric trajectory (Alfoncillo et al., 2016). According to that, two treatments have a different allometric trajectory between root and shoot if they have different slopes in the log–log model of root versus shoot biomass (Reich, 2002). Otherwise, the differences in terms of R/S are given by differences in size or age (apparent plasticity). This allows the experimental distinction between true plasticity and apparent plasticity. The latter not only is key for the theoretical interpretation of the data but also has practical consequences (**Fig. 1.2**). Without this distinction, an involuntary selection could be made in favor of genotypes that present juvenile traits such as a low rate of development and/or growth if genotypes with a greater allocation to the roots are selected in a breeding program.

In summary, plasticity might encompass strain, damage, and adaptive responses (**Fig. 1.2**). As long as an adaptive response has a clear genetic basis, it will be useful for breeding. However, it is not always possible to differentiate between adaptive and non-adaptive responses. Additionally, these responses may be restricted by costs and limits. In this chapter, we will focus on RSA plasticity in response to an agronomically important stress—soil compaction—and ask to what extent the observed responses might be termed as strain, damage, or ontological (apparent) or true adaptive responses (**Fig. 1.2**). Before reviewing the various reported phenotypic responses to soil strength, we will discuss in what ways soil strength may cause strain in plants.

Soil compaction and strength

Soil compaction is a process by which the soil particles are pressed together, decreasing the space between them when external forces are applied (Soil Science Society of America, 2008). Almost all soil properties are affected by compaction (**Fig. 1.3**) which interact with each other producing complex temporal and spatial patterns of resistance to penetration (Håkansson et al., 1988; Zobel, 1992). Compaction results in an increase in bulk density, a decrease in soil porosity or a change in the proportion of pores with water and air (mainly loss of large pores), and an increase in mechanical resistance or strength. The resulting low levels of oxygen (hypoxia or anoxia), reduced water and nutrient

supply, and mechanical impedance cause reductions in root growth and development (Håkansson et al., 1988; Lal, 1997; Bengough et al., 2011; Hoad et al., 2001; Casanova et al., 2013).

Soil compaction can occur in both top and subsoil. At the top, it may cause the formation of a crust which seals the soil surface. More often subsurface compaction, namely the formation of a dense soil layer some distance below the soil surface, is intended when authors write about 'soil compaction' (Nortjé et al., 2012). In agricultural soils, the main factors responsible for compaction are excessive traffic, the use of farm equipment that exceeds the bearing capacity of soil, and tillage at unsuitable soil water contents, in particular wet soils (Barken et al., 1987; Håkansson et al., 1988; Lipiec and Stępniewski, 1995; Bengough et al., 2011; Casanova et al., 2013).

Soil compaction is often described by measurements such as bulk density and penetrometer resistance (Passioura, 2002). Bulk density is the weight of dry soil divided by the total soil volume, and its commonly used units are g cm^{-3}. Penetrometer (or penetration) resistance has been used to provide a relative measure of the resistance offered by soil to the penetration of roots or soil strength (van Huysteen, 1983; Nortjé et al., 2012; Gao et al., 2016a, b; Kolb et al., 2017). It has been shown to be a good predictor of the ability of roots to penetrate soil (Bengough and Mullins, 1990; Jin et al., 2013; Gao et al., 2016b).

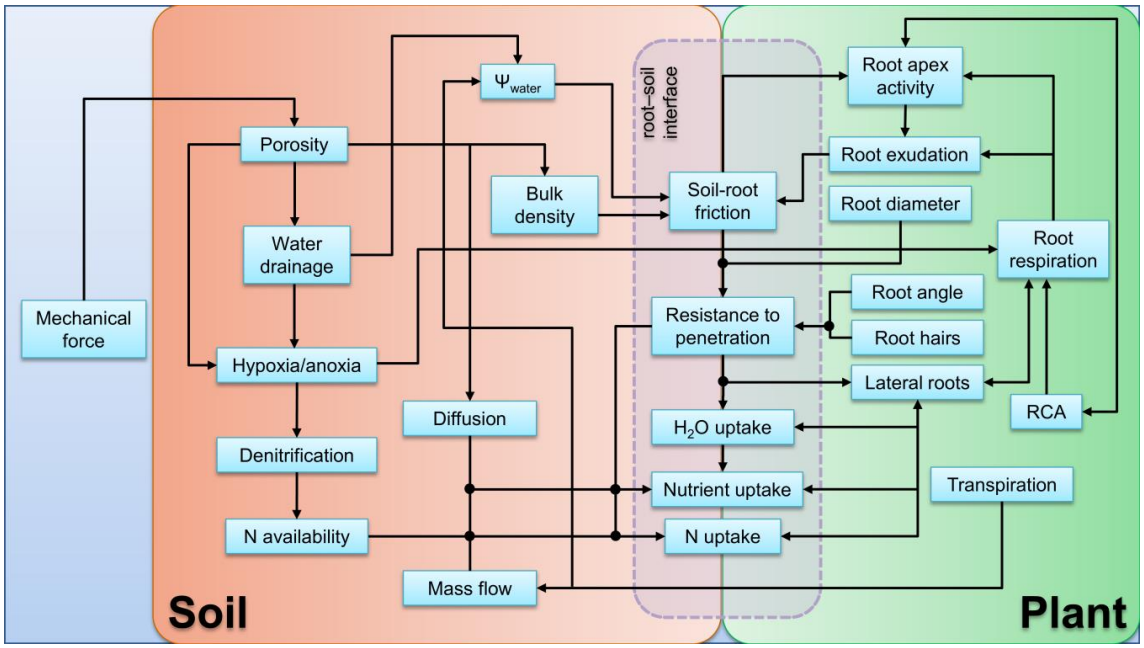


Fig. 1.3 - Interactions among soil properties and root function and structure under soil compaction conditions. Ψ_{water}, water potential; RCA, root cortical aerenchyma. The arrow (→) indicates the influence of one property on another whose interaction can be of synergistic or antagonistic nature (explained in the main text); a two-way arrow (↔) indicates a reciprocal influence between two properties; a black bullet (•) indicates converging influence between two or more properties on the following property; if two or more arrows have a point of intersection without a bullet, no direct interaction between them is indicated.

Soil properties affecting compaction

Many soil properties affect how easily the soil gets compacted and how resistive the soil is to root penetration. For instance, the soil density level at which root growth and penetration begin to be reduced depends on the soil texture (Jones, 1983; Pierce et al., 1983; Unger and Kaspar, 1994). For example, soils with high clay content are thought to be most inhibitory when compacted (Atwell, 1993). Even though there is a strong negative correlation between percentage clay and soil bulk density (Jones, 1983), clayey soils have higher soil strength than soils with a lower clay proportion at the same density values. For instance, root growth ceases in clayey and sandy soils at 1.47 g cm cm^{-3} and 1.85 g cm^{-3}, respectively (Jones, 1983; Pierce et al., 1983; Jin et al., 2017). At the same bulk density, clayey soils have a larger contact area between soil particles per soil volume than sandy soils, which in turn would increase the soil strength (Mathers et al., 1966).

A decrease of soil organic matter leads to a loss of structural stability, causing soils to be more susceptible to compaction (Casanova et al., 2013) and to increase the soil mechanical resistance under different ranges of water potentials (To and Kay, 2005). This is because increasing levels of soil organic matter has been associated with an improved aggregation, decreased dispersible clay content, decreased soil bulk density, increased number of failure zones, reduced strength, and increased ease of formation of micro-cracks (Kay, 1990). Soil organic matter is thereby a key contributor to the formation of the soil pore structure, and it greatly affects the diffusion behavior of soil gases such as O_2 (Hamamoto et al., 2012). However, when soil bulk density is held constant, soil mechanical resistance increases as the organic matter content increases, especially when soil is dryer. Under these conditions, increased cementation within substrate micro-aggregates may occur (for a graphical illustration, see To and Kay, 2005).

Physically, soil strength increases with decreasing soil water content (Gerard, 1965; Mathers et al., 1966; Whalley et al., 2005; Bengough et al., 2011). Thus, root growth in drying soil is generally limited by a combination of increased resistance to root penetration and water shortage (Bengough et al., 2011; Kolb et al., 2017). It should be noted that this is not always the case. For example, vermiculite shows a very small decrease in mechanical strength as it dries (Sharp et al., 1988).

High levels of exchangeable cations, such as K+ or Na+, can cause an increase in soil strength, especially when the soil dries out (Mathers et al., 1966; Dexter and Chan, 1991; Unger and Kaspar, 1994). Cations cause small clay particles to repulse each other, which facilitates the dispersion of the particles and eventually results in a denser packing arrangement (Dexter and Chan, 1991).

Soil properties affected by compaction

Increasing bulk density occurs at the cost of soil porosity, especially larger air-filled pores (Kolb et al., 2017). Evaluating a sandy loam soil mix at 15% moisture content, Tubeileh et al. (2003) found that air-filled porosity occupied 29% and 35% of the total volume under a soil density of 1.45 g cm^{-3} and 1.3 g cm^{-3} respectively. Such a loss in pore space decreases the water conductivity and holding capacity substantially (Douglas and Crawford, 1993; Tubeileh et al., 2003). Waterlogging may occur when a compacted layer interferes with the water drainage capacity of soil (Unger and Kaspar, 1994; Batey, 2009). Additionally, gas diffusion (m^2 s^{-1}) in soil is reduced significantly in compacted soils which quickly may lead to (locally) anaerobic conditions (Fujikawa and Miyazaki, 2005;

Hamamoto et al., 2012). Consequently, soil microbial activity may switch from aerobic mineralization to anaerobic denitrification, and thereby the nitrogen availability to the plant might be reduced significantly (Smith and Tiedje, 1979; Barken et al., 1987; Sitaula et al., 2000).

Root system plasticity in response to soil compaction and strength

RSA and other trait responses to soil compaction and strength are summarized in **Fig. 1.4**. Additionally, **Fig. 1.3** shows some relationships between some soil properties associated with soil compaction and plant responses. In this section, the main plasticity responses are described and the extent to which these responses may be adaptive is discussed.

Root length and number

The main influence of higher impedance by soil compaction is the decrease in total root length (Grzesiak et al., 2002; Bingham et al., 2010; Pfeifer et al., 2014) with a coincident increase in root diameter (Eavis, 1972; Goss, 1977; Rich and Watt, 2013; Popova et al., 2016). Roots begin to undergo a reduction of their growth with bulk density values of 1.39–1.49 g cm^{-3} and 1.69 g cm^{-3} in clay and in sandy texture soils, respectively (Pierce et al., 1983). The limiting values of soil bulk density at which root growth and penetration cease range from ~1.47–1.58 g cm^{-3} in clay texture soils (depending on the percentage of clay) to 1.85 g cm^{-3} in sandy texture soils (Pierce et al., 1983). In terms of penetrometer resistance, root elongation is typically affected in soils with values >0.8–2 MPa and may arrest root growth completely at a resistance of ~5 MPa (Passioura, 2002; Bengough et al., 2011).

Grzesiak et al.(2002), comparing the effect of bulk densities (1.33 g cm^{-3} versus 1.50 g cm^{-3} in a 1:1:3 mixture of garden soil, peat, and sand) on triticale root systems, found a decrease of seminal root length, number and length of lateral roots, and number and length of nodal roots with higher soil densities. In 14 winter wheat, decreased axial and lateral root numbers in response to soil compaction (soil column, 1.6 g cm^{-3}, 1.06 MPa) were found (Colombi and Walter, 2017). In addition, lateral root initiation is delayed under compacted soil in tomato (Tracy et al., 2012), wheat (Colombi and Walter, 2017), and triticale and soybean (Colombi and Walter, 2016).

If a plant keeps a relatively greater number of roots under compacted soil, it would supposedly have a better soil exploration than a plant with a severely affected root system with few roots. However, the root penetration and consequent growth into a compacted soil layer may also depend on how plastic the root diameter and angle are (see below).

Root diameter

Several studies have shown that root diameter is increased in compacted soil (Eavis, 1972; Materechera et al., 1992; Hanbury and Atwell, 2005; Tracy et al., 2012; Pfeifer et al., 2014; Popova et al., 2016). Increased diameter of the main roots is thought to lead tofavorable mechanical properties, such as greater axial root growth pressure, radial expansion, and potential growth rate (Eavis, 1972; Crossett et al., 1975; Materechera et al., 1992; Atwell, 1993; Whalley et al., 1995; Pagès et al., 2010; Kolb et al., 2017; Potocka and Szymanowska-Pulka, 2018). Consequently, thicker roots have a greater ability to explore hard soil (Bengough et al., 2011). Concordantly, Materechera et al. (1992), studying several dicot and monocot species (barley, fava bean, lupine, oats, pea, ryegrass, safflower, and wheat), found that a greater proportion of thicker roots is associated with a higher penetration ratio under compacted soil.

Roots must exert a growth pressure in order to displace soil particles, overcome friction, and elongate through the soil. Differences between species in their ability to penetrate compacted soil layers are not only related to differences in growth pressure, but are also due to differences in root diameter and in the tendency of roots to deflect or buckle (Clark et al., 2003). The increased diameter would allow the root to penetrate substrates with higher penetration resistance at the same root penetration pressure (Popova et al., 2016). The observed increase in the diameter of root tips and roots in compacted soil may reduce buckling and deflecting of roots as they attempt to displace soil particles during extension growth (Clark et al., 2003; Tracy et al., 2012). Otherwise, a greater tortuosity level will be found in the root system (see: ‘Root tortuosity’). Thus, a genotype that is tolerant to soil compaction is expected to have increased root diameter which would allow it to penetrate compacted layers, and explore more soil with a greater root length.

Root angle

The angle of incidence of a root at a soil layer, or simply ‘root growth angle’ (RGA, i.e. degrees from the horizontal), determines the direction of root elongation, and the volume

of soil in which roots can forage for water and nutrients. Thereby, RGA defines whether a plant will develop a shallow or deep RSA (Uga et al., 2015). The empirical model proposed by Dexter and Hewitt (1978) shows that the proportion of roots penetrating into a denser soil layer decreases as the soil strength increases; however, this relationship varies as a function of RGA. Thus, at a given level of soil strength, as RGA increases (i.e. steeper root angles and an interface perpendicular to the gradient of the gravity), the proportion of roots that can penetrate the interface also increases. For example, Ramalingam et al.(2017) found in rice genotypes that the proportion of roots with steeper angles (45°–90° from the horizontal) is highly and positively correlated with root length density (cm cm^{-3}) at a depth from 30 cm to 60 cm at both maximum tillering and maturity stages under both loose (0–0.5 MPa, on average) and compacted soil (with a maximum strength of ~1.8 MPa at 10 cm depth), and this proportion is lower under compacted soil. Additionally, the proportion of roots with steeper angles in response to compaction was genotype dependent, which suggests that this may be an adaptive trait.

When roots suddenly hit a compacted soil layer, such as a plough pan, they have three options: (i)circumvent it by deflecting themselves sideways; (ii) penetrate it in order to grow downwards through the strong soil; or (iii) stop growing (Dexter and Hewitt, 1978; Clark et al., 2003). Thus, if the root diameter and angle are not thick and steep enough to penetrate a strong soil layer, roots may be horizontally deflected when growth continues. Less steep angles in compacted soil have been found in triticale (Colombi and Walter, 2016) and lupine plants (Chen et al., 2014). This may be a strategy to compensate the limited function of an impeded taproot, due to subsoil compaction, by horizontal exploration of the top soil as long as lateral roots become stronger and longer as they grow (Chen et al., 2014), but this also may be a purely mechanical effect.

Even though these antecedents show an existing link between angles and soil strength, it is not clear yet whether those responses are an example of adaptive plasticity or passive consequences of the effect of compaction on root growth. In the case of having adaptive plasticity for RGA, a plant would produce much steeper root angles as a response to soil strength to explore deeper soils. This would be beneficial as long as the compact soil layers were thin enough to be penetrated and if deeper layers were looser and richer in soil resources. However, this response would be counterproductive in soils that at depth are even more compacted, anoxic, or cold.

Root tortuosity

Even though roots often grow through cracks, biopores, and holes in the soil, they have the tendency to nutate as they are flexible organs that follow tortuous paths through the soil, apparently seeking out the path of least resistance. Following planes of weakness between soil particles, they may reduce soil frictional resistance to root tip penetration (Bengough and Mullins, 1990). Roots may be buckled as a result of physical impedance imposed by the soil as the roots are forced to follow more convoluted pathways (Dexter and Hewitt, 1978; Clark et al., 2003). Root tortuosity can be described as the waviness of the growth pattern (Popova et al., 2016). The degree of tortuosity of a root system is dependent on both soil bulk density and soil type, as Tracy et al. (2012) and Popova et al. (2016) have demonstrated for tomato and maize plants, respectively. Both works found greater values of tortuosity for plants grown in compacted soil, with greater values in coarser textured soils. An active increased tortuosity, as an adaptive plastic response, may improve the chances to explore a larger volume of soil which in turn potentially increases acquisition of soil resources (Popova et al., 2016). However, as commented on previously for root angles, questions are still open regarding whether roots respond passively by bending physically as they face a strong layer, or whether they are able to actively guide a new orientation of growth (Clark et al., 2003; Popova et al., 2016). Furthermore, there must be a compromise between an increase in soil exploration that requires more allocation of resources and energy to the roots, and the limited availability of resources that are often found in compacted soils (Popova et al., 2016).

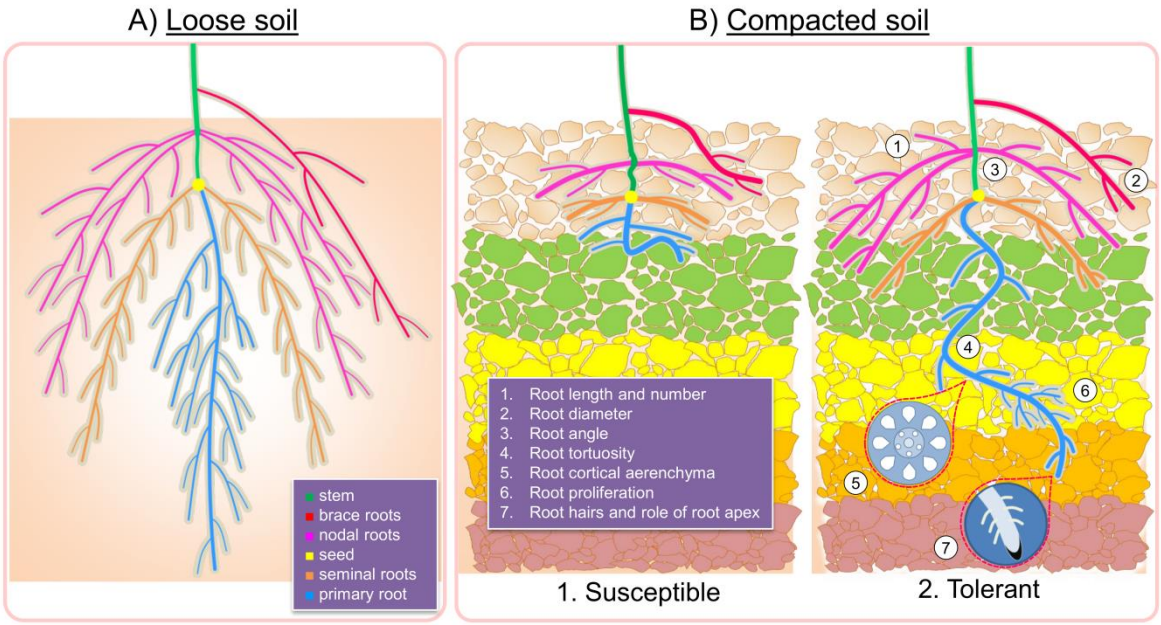


Fig. 1.4 - Generalized root phenotype for maize or sorghum in non-compacted and compacted conditions. **A**) Root system expressing its full, potential suite of phenotypes under an ideal soil condition, which is neither too hard nor too loose, but has the optimum density homogeneously distributed thoroughly the soil profile. **B**) Two root systems growing into several layers of soil with different degrees of compaction (highlighted in colors and indicated by the right arrow) that increases with depth. Root system1: if the resistance to the penetration is too high and/or the genotype is susceptible to soil strength, measurable changes in the root system are as follows: (1) reduction in root length and number, which results in a smaller root system size; (2) increased root diameter; (3) less steep root angles; and (4) deflected root growth. These changes make the plant susceptible to compaction especially under rain-fed conditions when the crop depends on water from deeper soil layers. Root system 2: the contrasting, expected responses of a tolerant plant, which include an increased root diameter and higher tortuosity. This would allow an improved exploration of soil by increasing both their penetration rate and chances to grow into those paths of least resistance (see text for details).

Root to shoot ratio

Occasionally the carbon allocation to below-ground organs is decreased, which is associated with a lower R/S. This phenomenon has also been observed as a response to soil compaction. Thus, in maize cultivated in cylindrical pots (40 cm height×15.5 cm diameter) with a soil mix as substrate but with two bulk densities (1.3 g cm^{-3} and 1.45 g

cm^{-3}), the biomass-based R/S decreased under the denser substrate condition at 42 days after planting. Also, in sugar beet, the R/S (cm mg^{-1}) decreases as bulk density increases (silt loam soil, 1.3–1.65 g cm^{-3}) (Hoffmann and Jungk, 1995). For example, Masle (1992) found that most genotypes of wheat or barley with enhanced R/S at high soil resistance (5.3–5.5 MPa) were modern lines, whereas landraces showed a decreased R/S under these soil conditions. Thus, R/S may be reduced or increased in impeded plants depending on the plant's genetic background.

As mentioned, smaller or younger plants generally are more 'rooty' (high R/S); therefore, those plants with enhanced R/S may simply lag in their development and this response may be just evidence for apparent plasticity or allometry. On the other hand, it possible to speculate that a lower R/S may help plants to reallocate more carbon to seed production, maximizing the chances for reproduction, as long as carbon fixation is not affected (Masle, 1992). It is also possible that although less carbon is spent on construction of roots, more is spent on the increased reliance on alternative nutrient acquisition strategies such as mycorrhizae or root exudation. Root exudation may not only enhance nutrient acquisition but may also play a beneficial role in penetrating compacted layers (Tubeileh et al., 2003) (see: 'Rhizosphere'). As discussed above, changes in R/S may be a function of the plant size (or development) or be truly plastic. This is also true for R/S responses to soil compaction. Thus, a correct interpretation of any change in R/S should be based on allometric analysis, which, in the best case, should be carried out in plants of different ages.

Compensatory growth

For soil compaction, Goss (1977) demonstrated that if only the apical parts of the main root axes of barley plants are exposed to compaction, the laterals freely penetrating into looser soil express a much greater length than root laterals of plants growing completely unimpeded root systems. This increased growth of laterals could mask the effect of compacted soil on the root main axis, when the total dry mass of the root system is found to be similar between unaffected and the impeded root main axes (Goss, 1977). A compensatory behavior of the whole RSA of barley plants was observed in a compaction experiment by Pfeifer et al. (2014) using vertically split rhizoboxes. These authors observed that rooting depth of roots under loose soil in a split rhizobox (compacted and loose soil) was significantly greater than rooting depth under uniform loose conditions(loose substrate in both compartments of the split system) and in all compacted

compartments. This phenomenon is accompanied by several changes in other RSA parameters in the loose compartment such as longer root length, earlier occurrence of laterals, and larger root area (the smallest polygonal area that encloses the portion of the root system observed in a rhizotron plate). Thus, the compensatory growth of laterals is analogous to that observed when the growth of part of a root system is restricted by other stresses. Compensatory growth may be a strategy of adaptive plasticity to counterbalance the limited function of an impeded portion of a root system, by growing less in those soil zones where the strength is high and growing more in looser zones.

Root hairs

Root hairs are unicellular and unbranched extensions of root epidermal cells whose principal function is to extend the root absorbing surface for water and nutrients (Evert, 2006). Various root hair traits have been shown to be important in nutrient uptake, with length and density (number of root hairs per millimeter of root length) being particularly important (Peterson and Farquhar, 1996; Bates and Lynch, 2001; Ma et al., 2001a, b). Additionally, root hairs have been associated with an improved anchoring of root to the substrate (Atwell, 1993; Müller and Schmidt, 2004; Bengough et al., 2016). Root hairs may provide anchorage due to their tensile strength (Bengough et al., 2011, 2016) and by greatly increasing the surface area in contact with the surrounding substrate (Müller and Schmidt, 2004). According to Bengough and Mullins (1990) and Bengough et al. (2011, 2016), the anchorage of the root axis may facilitate the root penetration from a looser to a denser layer. Root hairs close to the root tip may contribute to friction between the surrounding substrate and maturing tissues behind the elongation zone. They might, thereby, enable growing root axes to attach themselves firmly to the soil pore walls and penetrate further into the surrounding soil layers (Bengough et al., 2011, 2016). This may be supported by the fact that a hairless maize mutant (*rth3*-3) has been shown to have a lower penetration rate than its wild-type counterpart under soil densities between 1.0g cm^{-3} and 1.2 g cm^{-3} (Bengough et al., 2016). Similarly, Haling et al. (2013) found that barley root hair-bearing genotypes have a better root penetration into high-strength layers (1.6–1.7 g cm^{-3} versus 1.2 g cm^{-3}) than root hairless mutants. The presence of root hairs increased the proportion of roots that penetrated high-strength layers, rather than the rate of elongation through the high-strength layers. When the two genotypes (root hair-bearing versus root hairless) were grown in soils with a high and uniform compaction level, there were no significant differences in terms of total root length. Comparing the plant pulling

resistance of an Arabidopsis thaliana root hair-deficient mutant (*rhd* 2-1) with a wildtype, Bailey et al. (2002) found, in contrast, that root hairs, unlike lateral roots, do not contribute to whole-plant anchorage. Furthermore, average root hair length has been shown to decrease under higher bulk densities [i.e. 1.65 g cm^{-3} (silt loam soil)] in sugar beet (Hoffmann and Jungk, 1995). Based on these various observations, we propose that the possible benefits of an increased root hair proliferation on root penetration may be observed as long as roots are growing in loose soil conditions or when they are transitioning from looser into denser soil layers.

Rhizosphere

In addition to the tensile strength to help the anchoring of root axes by root hairs, there are also a number of chemical, physical, and biological factors in the rhizosphere such as the release of mucilages by roots and the presence of microorganism activity (associated or not with the development of the rhizosheath) that may allow the adhesion of the soil to roots and therefore the root exploration into a compacted layer of soil (Haling et al., 2013). Under compacted soil, plants may have lower R/S (see: 'Root to shoot ratio'); one consequence of this is that a hampered root system tends to accumulate much more carbon that they can use to grow which may be released into the soil (Tubeileh et al., 2003). This may help to face the soil resistance to root penetrations by facultative or modulated secretion of mucilage to reduce the friction between the root surface and soil particles (see: 'Role of the root apex').

Nutrient uptake

Lower nutrient concentrations in plants growing under compacted soil conditions have been observed. This may result not only from effects on physico-chemical soil properties which reduces their availability (e.g. anaerobic denitrification; see: 'Soil properties affected by compaction') but also from direct effects of compaction on roots. Since the total extension of the root system is reduced in compacted soil (see: 'Root length and number'), and possibly the root hair surface area as well, both the absorbing root surface and the radial access to soil resources are reduced, probably affecting nutrient uptake (Atwell, 1993; Hoffmann and Jungk, 1995; Rich and Watt, 2013).

Low yields under severely compacted soils are linked to low concentrations of nitrogen, phosphorus, and potassium in plants (Lipiec and Stępniewski, 1995; Arvidsson, 1999). For example, in a field experiment, growing wheat on a loamy soil with a

compacted soil layer (1.76 g cm^{-3}) between 10 cm and 55 cm depth and deep-tilled profile (loosened soil), Atwell (1993) found that the concentrations of N and K of shoots were reduced in plants grown in compacted soil conditions. Douglas and Crawford (1993) studied in the field (Scotland, clay loam soil) the effect of soil compaction due to wheel traffic on the biomass response of perennial ryegrass plants to the application of nitrogen. They found that there is an interaction between the N concentration applied and the degree of compaction which finally affects the plant growth: the plant biomass increases (1–5 t ha^{-1}) as the N application rate increases (0, 50, 100, and 150 kg ha^{-1}), but the degree of this increase is reduced as the soil compaction levels increase. Kuht and Reintam (2004) carried out an experiment compacting soil by riding over a field with a 17.4 t tractor. They achieved the compaction of both the plough layer and the subsoil (1.6–1.9 g cm^{-3} at the soil plough layer). They found that compaction decreased the N, P, K, and Ca contents in shoot dry matter of spring barley and spring wheat plants by almost 30% and 50% in the case of heavy soil compaction (1.9 g cm^{-3}). However, on other occasions, plants did not show any reduction in nutrient content. For instance, Masle and Passioura (1987) found that both shoot N and P concentrations are independent of soil strength (from 1.5 MPa to 5.5 MPa), a reason why the negative effect observed on shoot mass may not be mainly due to nutrient deficiencies (Masle and Passioura, 1987). Accordingly, Hoffmann and Jungk (1995) found that [P] of sugar beet shoots which were grown in pots under growth chamber conditions was not affected by bulk density (silt loam soil, 1.3–1.65 g cm^{-3}) in spite of decreased shoot dry mass, R/S (cm mg^{-1}), root hair length, and total root length. Thus, this evident loss of the absorbing surface of roots may be compensated by other mechanisms associated with increased nutrient uptake efficiency (g m^{-1} root) such as differential expression of high-affinity nutrient transporters or a higher rate of root exudation. Alternatively the nutrient demand of the plant was reduced by adapting the shoot size to the reduced root system size. We conclude that the reduced root length and soil exploration in compacted soils may limit the nutrient uptake, causing plants to have reduced nutrient concentrations in shoots. However, this has not been observed consistently, and we propose that plants, besides pre-emptively reducing shoot growth to avoid nutrient limitations, may also have compensatory mechanisms which increase the nutrient uptake per unit root length.

Root cortical aerenchyma (RCA)

RCAs are intercellular gas-filled spaces in the root cortex that form either by cell death

or by cell separation (He et al., 1996; Lynch and Brown, 2008; Postma and Lynch, 2011a, b; York et al., 2013; Lynch and Wojciechowski, 2015). Formation of aerenchyma is essential to the survival and functioning of plants subjected to waterlogging (Nishiuchi et al., 2012; Cardoso et al., 2013) because RCA contributes to the ability of plants to tolerate low-oxygen soil environments, by providing an internal aeration system for the transfer of oxygen (O_2) from the shoot to the root apical meristem (Drew et al., 2000; Nishiuchi et al., 2012; Yamauchi et al., 2013; Iijima et al., 2016). It has been proposed that the formation of RCA reduces the root metabolic cost of soil exploration by transforming living cortical tissue to air space through programmed cell death, permitting greater root growth and nutrient acquisition for a given metabolic investment (Lynch, 2007a; Lynch and Brown, 2008; Lynch and Wojciechowski, 2015). Even though soil strength stimulates the ethylene-dependent RCA formation in maize roots, its role in response to mechanical impedance stress is not clear (He et al., 1996). In addition, the RCA induction by soil compaction, found at 5, 10, and 15 cm from the root base, has been observed in triticale and to a smaller extent also in soybean (Colombi and Walter, 2016). In the first crop, the proportion of RCA depended on the root type, being higher in seminal roots than in primary and nodal roots. Due to the low levels of oxygen found in compacted soils, the mechanical induction of RCA under mechanical impedance could be potentially adaptive for root growth. However, RCA would not affect root penetration ability since it forms in mature root tissue behind the zone of active root elongation and root hair formation (Chimungu et al., 2015; Lynch and Wojciechowski, 2015).

Role of the root apex

The root apex with the root cap is thought to be an important sensory organ, sensitive to soil compaction. Goss and Russell (1980) observed the elongation rate of intact and decapped maize root apices when they faced a high density layer (made of 'ballotini'). Intact apices had an abrupt reduction in elongation rate when touching the layer, whereas decapped apices did not. In contrast to this finding, Iijima et al. (2003) found that the decapped roots of maize seedlings are significantly more sensitive than intact roots to the effect of mechanical impedance. Growing in compacted soil (sandy loam soil, 1.4 g cm^{-3}, 1.06 MPa), decapped roots had a 71% lower elongation rate and 52% thicker root diameters than those growing in loose soil (0.8 g cm^{-3}, 0.06 MPa). Intact roots had a 44% reduced elongation rate and 17% increased root diameter. Growing tomato plants in a vertically split-pot with 1.1g cm^{-3} and 1.5 g cm^{-3} of soil in each half for 30 days, Hussain

et al.(1999) found that excising roots in the compacted half increased shoot dry mass and leaf area above that of plants with intact roots located in both soil compartments. On the other hand, Rao et al.(1989) found that plants of sorghum growing in soil densities of 1.4g cm^{-3} and 1.5 g cm^{-3} have slight differences in terms of root dry mass and root length, but shoot and plant dry mass were not affected appreciably. At a soil density of 1.7 g cm^{-3}, however, both root and shoot dry mass were severely decreased. Apparently, the root system of sorghum is more sensitive to soil compaction than the shoot. Based on these observations, it possible to conclude that the shoot response to soil compaction is controlled by the root, which first senses the strength. This mediated specifically by signals produced in the root apex such as ethylene or abscisic acid (ABA) (Goss and Russell, 1980; Masle and Passioura, 1987; Atwell, 1993; Mullholland et al., 1996; Hussain et al., 1999). ABA has been indicated to have a positive role in maintaining leaf expansion under compaction (1.6–1.7 g cm^{-3}) because an ABA-deficient mutant (Az34) genotype of barley produces much smaller leaves and has a higher leaf conductance than a wild-type genotype (Steptoe) under compacted soil. These responses were correlated with lower ABA concentrations in the xylem sap in Az34 (Mulholland et al., 1996). Furthermore, tomato ABA-deficient mutants had a more reduced root volume, surface area, and lateral roots than a wild-type tomato genotype at high bulk densities (Tracy et al., 2015). This suggests that ABA mediates the impact of soil compaction not only on stomatal conductance, leaf expansion, and shoot growth, but also on RSA by improving the root capabilities to explore the soil.

The root cap is located at the apex of the root and protects the root apical meristem as the root is penetrating the surrounding soil (**Fig. 1.4**; Bengough et al., 2006; Iijima et al., 2008). The root cap may protect the apex by reducing the mechanical resistance imposed by soil. This is achieved by both sloughing of root cap cells and secretion of mucilage (Atwell, 1993; Bengough and McKenzie, 1997; Iijima et al., 2003, 2004, 2008; Bengough et al., 2006; McKenzie et al., 2013; Potocka and Szymanowska-Pulka, 2018). The lubrication is thought to occur in the zone immediately behind the root apex and in the zone of extension (Bengough and McKenzie, 1997; McKenzie et al., 2013). This results in a decrease of the coefficient of friction between the root surface and soil particles (Bengough and McKenzie, 1997; Potocka and Szymanowska-Pulka, 2018). Facultative (or plastic) cell sloughing and mucilage secretion as the root penetrates harder soil layers may be adaptive strategies to face the soil strength. However, adaptive strategies should limit the carbon costs and maximize the returns in terms of soil resources

such as carbon, nitrogen, or oxygen. In other words, the amount of carbon earned, or fixed, after the response is expressed must be, at least, greater than or equal to the amount of carbon spent on the investment of such a response (see: 'Costs, limits, and trade-offs of phenotypic plasticity' and 'Root to shoot ratio').

Breeding for plasticity?

As previously stated, soil resistance to root penetration is positively associated with soil dryness (Whalley et al., 2005). The effects of soil compaction are thereby greater in warmer and dryer climates, especially when dense layers, such as a plough pan, impede access to deeper soil water (Batey, 2009). Soil strength varies not only spatially but also temporally because of changing soil water content, which in turn is very variable (Passioura, 2002), and also due to the fact that nearly all soil physico-chemical properties, which interact and are affected by compaction, are rarely uniformly distributed. Thus, each soil may have its own spatial and temporal patterns of strength, which cannot be accurately foreseen. Therefore, the ability of plant root systems to make short-term adaptations in response to those changing environmental factors altering resource allocation to the root system (i.e. plasticity) might be of great value for breeding an 'adaptive' cultivar (O'Toole and Bland, 1987). In addition, the future impact of climate change on agriculture is known to be caused not only by changes in long-term mean climate but also by changes in both inter- and intra-seasonal variability such as changes in both frequency and intensity of rainfall events per year as well as the occurrence of extreme weather events such as heatwaves, drought, and heavy rainfall (Olesen and Bindi, 2002; Porporato et al., 2004; Gornall et al., 2010; Fishman, 2016; Gray and Brady, 2016). This is likely to have negative effects on yields especially in mid- to low-latitude areas where an increased number of water shortages and extreme weather events are expected (MacDonald et al., 1994; Olesen and Bindi, 2002). As phenotypic plasticity has been proposed to have a positive adaptive value in many circumstances (Bradshaw, 1965; Via et al., 1995; Palmer et al., 2012; Des Marais et al., 2013), providing an increased environmental tolerance (Via et al., 1995), especially in heterogeneous environments (Sultan and Spencer, 2002), phenotypic plasticity in response to climate change may be critical in maintaining the agricultural productivity in the future (Gray and Brady, 2016).

Since phenotypic plasticity has been historically recognized as a heritable feature (Bradshaw, 1965; O'Toole and Bland, 1987; Via et al., 1995) and some studies have started to reveal the genetic basis of RSA traits such as root length, thickness, volume,

distribution, and allometric ratios (Fitz Gerald et al., 2006; Uga et al., 2011, 2013, 2015), study of the genetics of RSA plasticity seems like an important next research step (e.g. Fitz Gerald et al., 2006; Sandhu et al., 2016). New phenotyping methods have been developed for root traits, such as 2D image analysis, anatomy of cross-sections, shovelomics, 3D-MRI, X-ray, tomography technology, etc., which have a great potential for breeding (for an in-depth review, see Kuijken et al., 2015; Atkinson et al., 2019). Whatever the case, for an efficient root trait-based breeding, the target trait, such as yield, should be highly correlated with some root traits with high heritability (Kuijken et al., 2015). As previously stated, root phenotype is very plastic and influenced by numerous interactions between genes and between genes and the environment (Kuijken et al., 2015). All in all, this makes the breeding for root traits quite difficult (Kuijken et al., 2015).

I propose that selection in favor of RSA plasticity may be more useful under low-input farming systems or rain-fed agricultural systems. Under those conditions, the edaphic environment is often suboptimal for root growth and development, and the root system must be able to cope with temporal and spatial variability in soil properties associated with uncertain soil water status such as the amount and frequency of precipitation during the growing season, soil temperature regime, and level of native soil fertility (O'Toole and Bland, 1987).

Accordingly, a putative tolerant genotype, an ideotype for soil compaction tolerance, should have the following plastic responses for soil compaction under rain-fed agricultural systems (**Fig. 1.4**): an increased root diameter would allow improvement in the penetration and, consequently, the exploration of the soil profile (Bengough et al., 2011). A genotype which is able to produce and keep a relatively greater number of root axes with steeper root angles as the penetration resistance increases would have a better chance to explore due to the increased root length (Dexter and Hewitt, 1978; Ramalingam et al., 2017). A high degree of tortuosity could be an indicator of a greater and active reorientation of root axis growth, which would help to find paths, if they exist, with lower mechanical impedance to penetration (Clark et al., 2003; Popova et al., 2016). In those soil patches, the resources, such as oxygen and nitrogen, may be more available than in their surroundings (see 'Soil properties affected by compaction'). Proliferation of roots into patches with more favorable soil conditions may be advantageous and a way to compensate for lost root length (Jin et al., 2017). The increased presence of root cortical aerenchyma under compaction would facilitate the oxygen transport to those zones where its supply is in high demand to support the root proliferation (Colombi and Walter, 2016).

Finally, both root hairs and the apex may have an important role to improve the penetration into compacted layers by improving the root anchoring to the soil and secreting mucilage to reduce the frictional resistance of soil (Bengough and McKenzie, 1997; McKenzie et al., 2013). It is important to emphasize that not all environments might benefit from a better penetration into deeper layers since root exploration and depth might be largely restricted by harsh conditions at depth such as waterlogged soils or high and toxic concentrations of some chemical elements. In contrast, a rapid root extension rate and deep final rooting depth may be desirable features to exploit water stored in deeper soil layers under rain-fed agricultural systems (Hamblin and Tennant, 1987; Siddique et al., 1990; Colombi et al., 2018). Therefore, tolerance (see definition above) is an environment-dependent characteristic, and the list of features that makes a plant tolerant to a specific constraint may be different for different agricultural conditions.

2 - MATERIALS AND METHODS

This chapter describes in detail the materials and methods that support chapters **Chapter 3** to **6**. The chapter is divided into the following 6 sections: In the '**Kernel phenotyping**' and '**Preliminary experiments**' sections of this chapter, we describe a low-cost phenotyping methods for seeds based on scanned images and a set small experiments. These experiments were carried out to plan and design of the experimental setup of the next chapters. Their results are presented in **Chapter 3**. The '**Screening and between-plant phenotyping**' section describes the materials and method used in **Chapter 4**. This chapter was focused on knowing whether or not the degree of responses to soil compaction is plant size dependent. For that a panel of 28 genotypes of sorghum were grown and phenotyped to study the phenotypic diversity of shoot and root plasticity. In the '**Within-root phenotyping**' section, the design of modular and vertical soil columns is described in detail. This system allowed to produce heterogeneous vertical gradients of soil density (from 1.3 to 1.8 g cm^{-3}). In **Chapter 5**, the usefulness of these gradients to test if plants are able to compensate the effect of very compacted layers is discussed. In the '**Soil models and root simulation**' section, the reconstruction *in silico* both observed phenotypes and the soil conditions is described. This simulation-based research was done to study the plants functional consequence of phenotypic response to soil compaction. These consequence are discussed in **Chapter 6**.

Kernel phenotyping

Before carrying out the preliminary experiments, a genotype was chosen from a plant material and a collection of 30 sorghum genotypes (*Sorghum bicolor* L. Moench, **Table 2.1**) with different geographical and genetic origins. This selection was based on kernel phenotype as follows: (i) phenotypic mean close to the average phenotype of the collection and (ii) low within-genotype variance, respectively.

First, the kernel mass and size of the 30 genotypes was determined. Three sets of 10 kernels per genotype were sampled to determine biomass. To determine the kernel size, 80 kernels per genotypes were sampled. To assess the kernel size automatically, an

image processing script (**Fig. 2.1**, **Supplementary Material S1**) was coded in `Python` (https://www.python.org/) based on the following modules: `numpy` (https://numpy.org/), `matplotlib` (https://matplotlib.org/), `opencv` (https://opencv.org/) and `pandas` (https://pandas.pydata.org/). Afterward, one black and white image for each genotype with the 80 kernels was scanned at 400 dpi (Epson Perfection V700 PHOTO, Seiko Epson Corporation, Japan). The following kernel size traits were measured (**Fig. 2.1**): kernel projected area, length, width and perimeter. As each kernel is delimited by a region full of pixels, the kernel projected area is the actual number of pixels in each region. The kernel length and width in pixels correspond to the major and minor axes of each region, respectively. The kernel perimeter is the number of pixels around the edge of each region. Once the image is analyzed, the data is stored in an excel file where each seed is individually identified with a number. Thus, each kernel (from 2400 = 80×30) were automatically and individually phenotyped.

To select the most representative genotype, the phenotypic mean for each trait among all the kernels was calculated (collection mean). The absolute difference (deviation) between the collection mean and the genotype mean for a particular trait was calculated. Later, the genotypes were sorted from the lowest to the highest deviation from the collection mean and ranked from 1 to 30. Finally, the average ranking among all the traits of each genotype was calculated. To select the most uniform genotype, the phenotypic variance for each genotype and for each trait was calculated and ranked. Later, the average ranking among all the traits of each genotype was calculated.

Table 2.1 - Genotypes of sorghum used in this study.

id	Name	Description	Origin
1	AJABSIDO	Post-flowering drought tolerant landrace	Sudan
2	BN223	Food-grade hybrid seed-parent	Niger
3	CE-151-262-A1	Improved, open pollinated variety	Senegal
4	CSM-63	Drought tolerant landrace	Mali
5	EL_MOTA-S241	Pre-flowering drought tolerant landrace	Niger
6	FETERITA_GISHESH	Pre-flowering drought tolerant landrace	Sudan
7	FRAMIDA	Improved, Striga-resistant variety	Burkina Faso
8	GRINKAN	Improved, open pollinated variety	Mali
9	HONEY_DRIP	Sweet-stem sorghum	USA
10	ICSV1049	Improved, Striga-resistant variety	Burkina Faso
11	KORO_KOLLO	Pre-flowering drought tolerant landrace	Sudan
12	KUYUMA	Improved, open pollinated variety	Zambia
13	MACE_DA_KUNYA	Drought tolerant landrace	Niger
14	MOTA_MARADI	Pre-flowering drought tolerant landrace	Niger
15	MR732	Elite, food-grade, hybrid pollinator-parent	Niger
16	PI609567	Post-flowering drought tolerant accession	Mali
17	SARIASO-14	Improved, Striga-resistant variety	Burkina Faso
18	SC35	Post-flowering drought tolerant accession	USA
19	SC599	Post-flowering drought tolerant accession	USA
20	SEGEOLANE	Pre-flowering drought tolerant landrace	Botswana
21	SEGUETANA	Improved, open pollinated variety	Mali
22	SEPON-82	Improved, open pollinated variety	Niger
23	SK5912-SHORT_KAURA	Improved, open pollinated variety	Nigeria
24	THEIS	Sweet-stem sorghum	USA
25	TX2752	Feed-grade hybrid seed-parent	USA
26	TX430	Feed-grade hybrid pollinator-parent	USA
27	TX436	Food-grade hybrid pollinator-parent	USA
28	TX631	Food-grade hybrid seed-parent	USA
29	TXARG1	Food-grade hybrid seed-parent	USA
30	WASSA	Improved, open pollinated variety	Mali

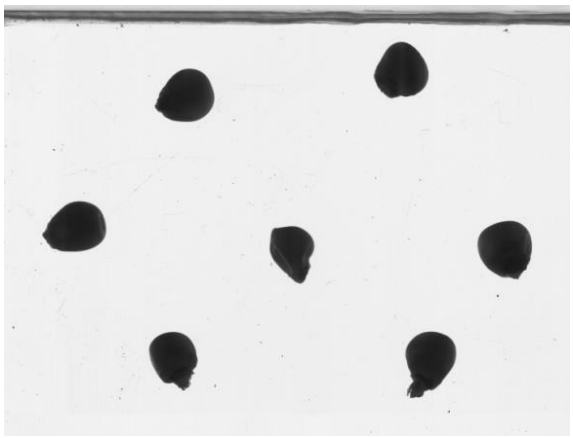
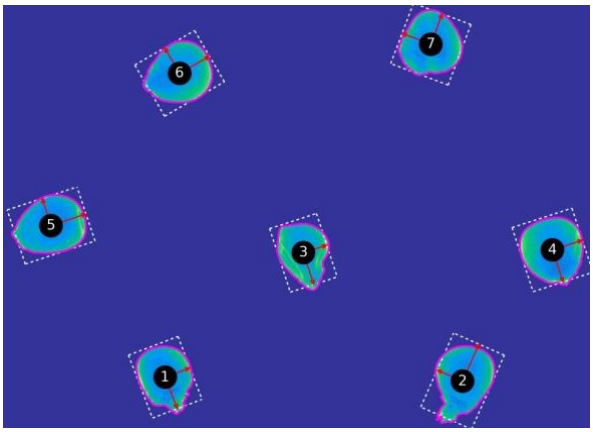
A)  B) 

Fig. 2.1 - Kernel phenotyping.A) Example of one scanned image with seven kernels. **B**) The same image with phenotyped kernels. Each kernel is identified with a number (indicated in the center of the seed, the region centroid). The kernel length and width are indicated by two red arrows emerging from the centroid to the region edge. Also, the perimeter is indicated with the edge of the region in magenta. For more details: see https://opencv.org/ and **Supplementary Material 2**.

Experiments

All the experiments were carried out in the greenhouse facilities of the Institute of Bio- and Geosciences (Plant Sciences) at the Forschungszentrum Jülich GmbH, Germany (50° 54’ 36’’N, 6° 24’ 49’’E).

Preliminary experiments

Three experiments using one genotype of sorghum under two contrasting soil densities were carried out. The genotype ‘BN223’ was chosen from 30 sorghum genotypes because it showed an average and uniform kernel phenotype (see “**Kernel phenotyping**”). Plants were grown and evaluated under greenhouse conditions in three kind of containers or pots: (i) 4 L cuboid shaped pots; (ii) 3.7 L rectangular rhizotrons; and (iii) pot trays composed of 60 small containers of 0.25 L each. Two levels of soil density were applied from 1 to 1.8 g cm^{-3}. This resulted in a penetration resistance of 0.2 and 1.8 MPa, respectively (measured with a hand penetrometer for top layers IB, Eijkelkamp, The Netherlands). A mixture 1:1 of black peat as substrate (N, 120 mg L^{-1}; P_2O_5, 20 mg L^{-1}; K_2O, 170 mg L^{-1}) and a loam field soil (10% clay, 38.6% silt, and 51.4 sand; organic

matter content 1%) was used. For pot trays, dry soil alone was used. A completely randomized design with one factor (soil density) with two levels and 6-8 replicates was proposed for all the experiments. More details about the experiments are found in **Table 2.2**.

Table 2.2 - Experimental setup of preliminary experiments.

Container	**Substrate**	**plants per container**	**number of replicates**	W_m (%)	**Soil density [strength]** ($g \cdot cm^{-3}$) [MPa]	
					Loose	**Compacted**
Pots	½soil + ½peat	1	6	30	1.0 [0.20]	1.25 [1.20]
Rhizotrons	½soil + ½peat	2	6	30	1.0 [0.20]	1.25 [1.10]
Pot trays	soil	2	8	0	1.4 [0.18]	1.80 [1.25]

Volume: container volume in liters

Substrate: mass based-substrate proportion

W_m: gravimetric water content (wet-mass basis).

Surface-sterilized seeds were previously germinated at 21°C in Petri dishes on moistened filter paper during 48 hours. Those seedlings with a healthy radicle were selected and planted into the containers. One seedling was planted in 4 L pot. For rhizotrons and pot trays were planted two seedling per container.

Plants were growing during 25 days from the Eighth of February 2017 to the sixth of March of 2017 (from seedling transplanting to harvest).Details about the environmental conditions during the experiment are found in **Table 2.3**. In all experiments, supplemental illumination was supplied to natural light during the day by mercury lamps (SON–T AGRO 400, Phillips, The Netherlands) every time that light intensity outside the greenhouse was <400 µmol m^{-2} s^{-1} for 16 hours between 06:00 and 22:00 hours local time.

Plants were watered three times per week with 100 mL of tap water for pots and rhizotrons. Pot trays were watered once a week by capillary action from the bottom to the top by putting them on a bigger tray with water until they reached an on average 30% volumetric water content.

Table 2.3 - Greenhouse air temperature and relative humidity during the experiments.

Experiments	Growing days	Air temperatures (°C)[1]		Air relative humidity (%)	
		Day	Night	Day	Night
Preliminary experiments	25	22.2 ± 0.03	18.1 ± 0.03	51.1 ± 0.09	62.1 ± 0.02
Screening experiment	25	26.2 ± 0.03	20.3 ± 0.02	47.1 ± 0.11	66.9 ± 0.01
Between-plant phenotyping	45	22.7 ± 0.01	19 ± 0.01	50.2 ± 0.05	63.6 ± 0.07
Within-root phenotyping	27	24.3 ± 0.02	18.3 ± 0.02	52.6 ± 0.06	57 ± 0.08

[1] Mean ± standard error of the mean

At harvest day, plant height and number of tillers and leaves were evaluated *in planta*. Plant height was expressed as the distance from soil surface of the pot to the apex of the uppermost fully expanded leaf. Then, the shoot was cut off from the rest of the plant at the substrate surface. Leaf area and stem projected area were measured using a LI-3100C area meter (LI-COR, Inc., Nebraska, USA). Afterwards, roots were carefully separated from the substrate and the rest of soil particle were washed away from the roots by using water. After root washing, images of scanned roots were analyzed using WinRHIZO Pro image analysis system (Regent Instruments, Inc., Quebec, Canada) to estimate total distribution of root length according to root diameter. The root length were recorded in 25 ranges of root diameter between 0 and 2.5 mm. Then, the roots, stem, leaves and shoots dry mass were obtained after drying in an oven at 65°C until constant mass. Specific leaf area and specific root length were calculated per plant as the ratio of leaf area to leaf biomass and root length to root dry mass, respectively. The complete list of traits are found in **Table 2.4**.

Table 2.4 - List of phenotypic traits recorded in the experiments and codes used.

Trait code	Phenotypic trait	Unit
$<i$	length of roots with diameters less than *i* mm	cm
$[i,\ j)$	length of roots with diameters greater than or equal to *i* and less than *j* mm	cm
$\geq i$	length of roots whose diameter is greater than or equal to *i* mm	cm
Root_length	total root length (sum of root length of all root diameter classes)	cm
Root_Diam	Average root diameter per plant	mm
Leaf_area	total leaf area per plant	cm^2
Root_DM	dry mass of roots	g
Collar_DM	dry mass of collar	g
Stem_DM	dry mass of stem	g
Leaf_DM	dry mass of leaves	g
Shoot_DM	shoot dry mass (= Leaf_DM + Stem_DM + Collar_DM)	g
SLA	specific leaf area (= Leaf_area/Leaf_DM)	$cm^2\ g^{-1}$
SRL	specific root length (= Root_length/Root_DM)	$mm\ g^{-1}$
Plant_height	plant height	cm
Leaf_number	leaf number of main stem	-
Tiller_number	number of tillers per plant	-
Root_number	number of nodal roots	-
Root/Shoot	root dry mass to shoot dry mass ratio (= Root_DM/Shoot_DM)	-
Plant_DM	plant dry mass (= Shoot_DM + Root_DM)	g

All the data analyses described in this work were conducted in the R statistical programming language (R Core Team, 2018). In order to calculate the number of replicates of further experiments, an analysis of the statistical power of the findings of this work was carried out based on the indications of Cohen (1988) and using the "pwr" package of R (Champely 2017). All the plots were drawing with the "ggplot2" package of R (Wickham 2009).

Screening and between-plant phenotyping

In **Chapter 4**, two experiments were carried out. A **screening experiment** for shoot plasticity in young plants of 28 genotypes was done. Later, based on that, a longer experiment was carried out using six of those genotypes with varying shoot plasticity to analyze the root response to soil compaction: **between-plant phenotyping** experiment.

The **screening experiment** started on July 3rd 2017 with seedling transplanting.

896 plants (28 genotypes, two soil density treatments, 8 replicates and two plants per replicate) were grown in the pot trays (see "**Preliminary experiments**"). Each container was filled with dry soil according to the following densities: 1.4 and 1.8 g cm^{-3} for loose and compacted treatments, respectively. For the compacted treatment, soil was compacted using a hand hammer and compacted until the required amount of soil would fit in the container. This resulted in a penetration resistance of 0.4 and 3.1 MPa, respectively. Like in "**Preliminary experiments**", two seedlings per genotype were transplanted together in the same and random container in each tray (two plants per container). Summing up, each tray had one soil density level and 56 plants of 28 genotypes, yielding 16 trays in total (**Fig. 2.2A**).

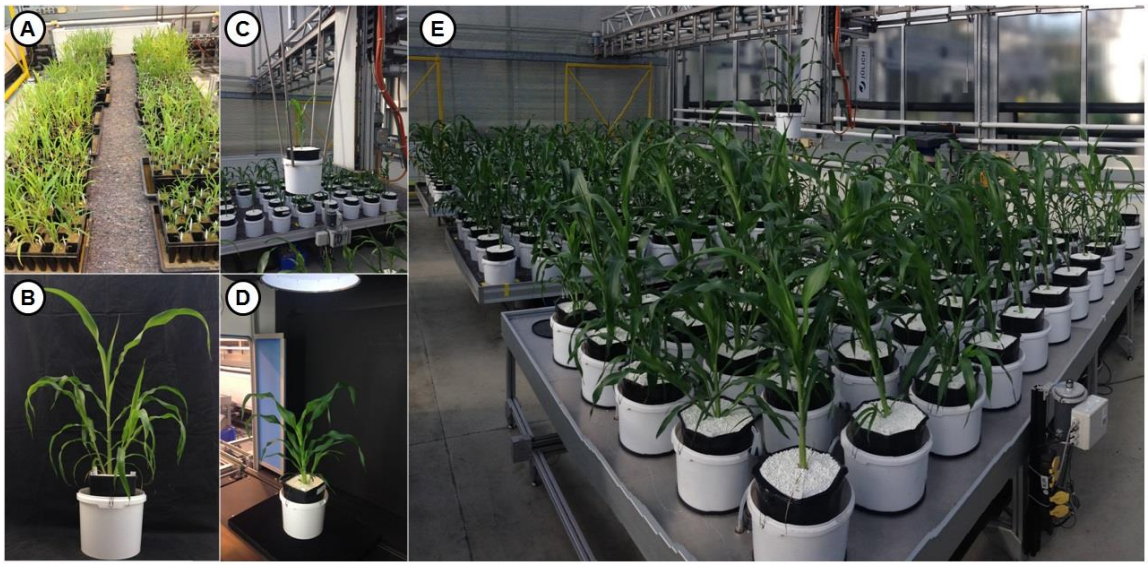


Fig. 2.2 - Screening and between-plant phenotyping experiments. A) View of screening experiment. **B**) Experimental unit of between-plant phenotyping experiment at harvest time. **C**) Crane bringing a plant to the darkroom. **D**) Plant at the fixed darkroom. **E**) Experimental view of between-plant phenotyping experiment.

Plants were growing during 25 days from the Third to the 28th of July (from seedling transplanting to harvest). Environmental conditions during the experiment are indicated in **Table 2.3**.Supplemental illumination, watering and shoot phenotyping were done as indicated in "**Preliminary experiments**".

The screening was based on shoot dry biomass to identify sensitive and susceptible genotypes. Six genotypes differing in the shoot dry mass and degree of

response to soil compaction were selected for the second experiment (**between-plant phenotyping**) in which the root growth responses in relation to the shoot responses was studied.

The **between-plant phenotyping** experiment started on September 4th 2017 with seedling transplanting. Plants were grown according to a two factorial completely randomized design with six genotypes, two soil conditions and 12 replicates (n = 144 plants). The 4 L cuboid shaped pots were filled with the same mineral field soil (see "**Preliminary experiments**") at densities of 1.4 and 1.8 g cm^{-3} (penetration resistances of 0.4 and 1.8 MPa, respectively) for loose and compacted soil, respectively. The soil was compacted with a manual bolt press (Holzmann Dop 3000, Holzmann Maschinen GmbH, Austria). Seeds of six genotypes ('HONEY_DRIP', 'KORO_KOLLO', 'SC599', 'BN223' 'TXARG1' and 'AJABSIDO') were germinated and transplanted as described in the "**Preliminary experiments**". One seedling per pot was transplanted. Plants were growing for 45 days from the 4th of September to the 18th of October (from seedling transplanting to harvest). Right before sowing, pots were irrigated from the top till 30% volumetric water content (1200 cm^3) with tap water (~7 mg L^{-1} N, 0.5 mg L^{-1} P, 2.6 mg L^{-1} K, 14 mg L^{-1} Mg; 440 mS cm^{-1}).

In order to track plant shoot development over time, from transplanting to harvest date, the shoot projected area was measured non-destructively two times per week (in total 13 times) for each plant individually. For this task, the 'ScreenHouse' automated phenotyping platform of IBG-2 was used (Nakhforoosh et al. 2016, **Fig. 2.2B-E**). This platform encompasses a mobile crane and a fixed imaging chamber. The crane transports individual pots back and forth to between the greenhouse tables and the imaging station where three cameras fixed at three different angles (0, 45 and 90º) take pictures as the pot rotates four times on its axis (90 degree rotation angles). This process yields 12 pictures per pot. Pots are measured in random order and placed in a new random position in the greenhouse. Thus, the experiment is automatically re-randomized during each measurement date. More details are found in Nakhforoosh et al. (2016).

To keep the volumetric water content at 30%, plants were watered with 100 mL of water from two times per week. Environmental conditions during the experiment are indicated in **Table 2.3**.Supplemental illumination and destructive phenotyping after harvesting were done as indicated in "**Preliminary experiments**".

To assess the effect of true and apparent plasticity on the expression of each trait, a two-way Analysis of Variance (ANOVA) and an Analysis of Covariance (ANCOVA)

for each trait were performed. In both analyses, genotype, soil compaction treatment and their interaction were used as factors. For ANCOVA, plant dry mass was added to the ANOVA model and considered as a covariable. We defined the relative importance of a factor on the phenotype of a given trait as the proportion of the total phenotypic variance explained by this factor. This proportion was calculated based on mean squares of each factor and error according to the ANOVA/ANCOVA model. Thus, plasticity effect was expressed as the sum of the relative importance of treatment and genotype-by-treatment interaction effects; and the effect of apparent plasticity as the sum of the importance on the phenotypic variation of plant dry mass and its interactions with treatment, genotype and genotype-by-treatment effects. Additionally, to analyze the allometric relationship between root and shoot biomasses, an extra ANCOVA was done considering natural logarithm of root dry mass and shoot dry mass as the dependent variable and the covariable, respectively. Before these analyses, the assumptions of normality and homoscedascity of variances of residuals were evaluated by the Shapiro-Wilks and the Levene tests, respectively. Variables that failed to meet these assumptions were transformed to natural logarithm. Significant differences among genotypes were compared by the Fisher's test (P<0.05) using the R package "agricolae" (Mendiburu, 2012). Additionally, to test how significant was the treatment within each genotype a two-sample t-test was done.

I expressed the response to soil compaction for a given trait and for each genotype asthe fold change of the logarithm base two of the ratio of the phenotypic mean in compacted ($\bar{x}_1$) and loose ($\bar{x}_0$) soil: $\log_2[\bar{x}_1/\bar{x}_0]$.This plasticity index is 0 when plants are non-plastic, and negative numbers indicate biomass is smaller under compacted conditions. For example, a value equals to -1, indicates phenotype in compacted soil was half that of loose condition.

To analyze the relationships among the traits a correlation analysis was carried out based on Pearson's correlation coefficient between traits. To identify some patterns of correlations among the response of the different traits, a hierarchical clustering of traits was performed. For this purpose, the R package "ClustOfVar" (Chavent et al., 2012) was applied on the standardized responses for each genotype (rows) and trait (columns). Then a Principal Component Analysis (PCA) was performed. Prior to the application of PCA, thelog transformation as applied to the traits, centered, and scaled the data to normalize and standardize the traits. Additionally, the effect of treatment and plant size on the variation of different principal components were examined by an ANCOVA.

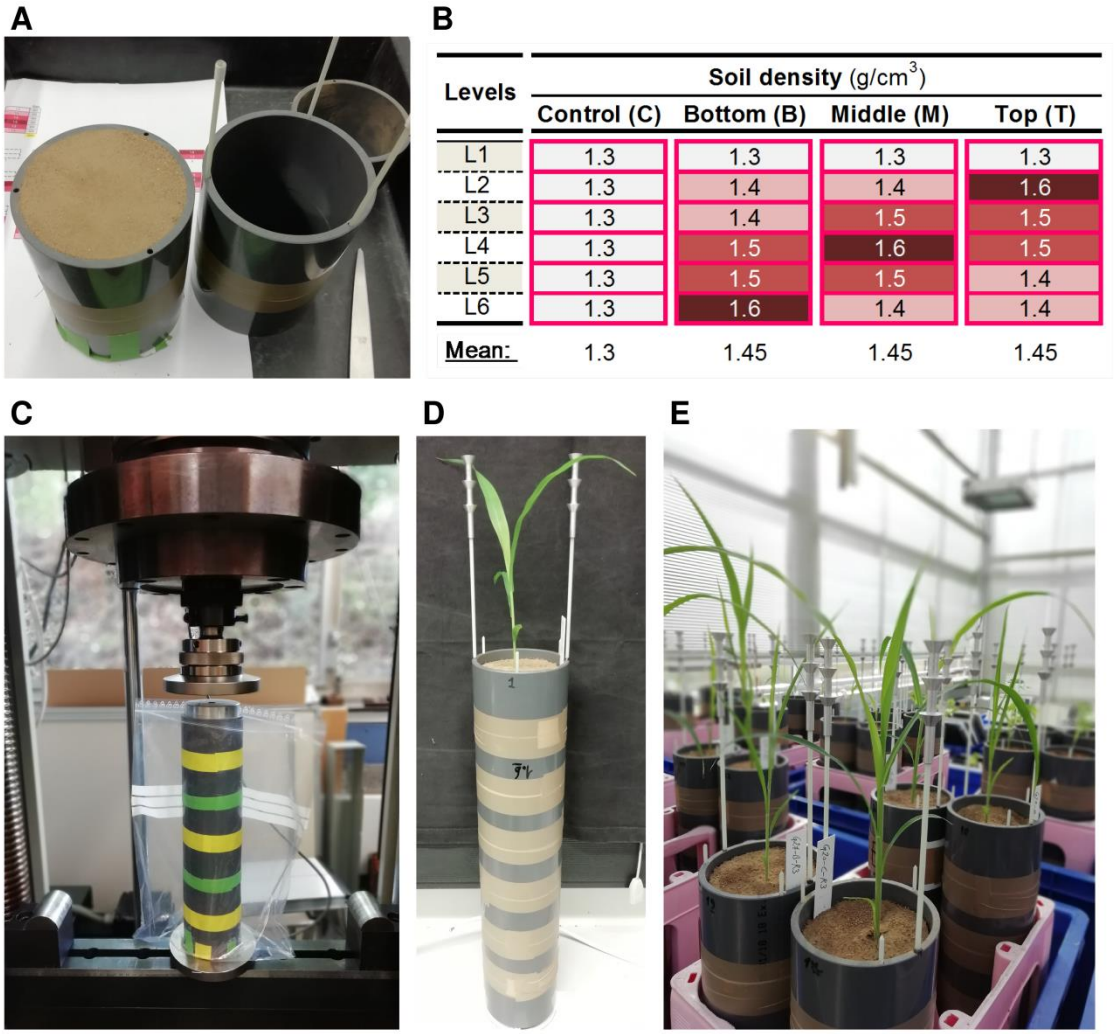
Within-root phenotyping

In **Chapter 5**, two genotypes of sorghum with different degree of response to soil compaction were chosen based on the previous experiment ("**Between-plant phenotype**"). Plants were grown under conditions of vertical and discrete gradients of soil bulk density. To generate these gradients, a system of modular and vertical soil columns was built (**Fig. 2.3**).

These genotypes express a different degree of response to soil strength: (i) 'SC599', a post-flowering drought tolerant accession from the USA with relatively small plants, low-plastic shoots and roots moderately sensitive to soil strength (10% and 35% of reduction compared to loose-soil plants, respectively). (ii) 'HONEY_DRIP', a sweet-stem genotype from the USA with relatively big sized plants (2.1 times bigger than 'SC599', in terms of plant dry mass), and sensitive shoots and highly plastic roots (38 and 57% of reduction, respectively).

The same mineral field soil described in "**Preliminary experiments**" was used as a substrate. The vertical soil columns were composed by seven interlocking and non-transparent cylinders (density levels) of the same size (**Fig. 2.3A**). The soil was compressed level by level using a universal testing machine Zwick/Roell 1495 (Ulm, Baden-Wurtemberg, Germany, **Fig. 2.3C**). Each cylinder has been designed as a custom-built circular cylinder made of polyvinyl chloride (PVC) of 8.1 cm inner diameter, 6 cm high and 4.5 mm thick wall (**Fig. 2.3A** and **Supplementary Fig. S1**). To facilitate the drainage, the columns have an empty and open cylinder at its base which supports a disk and six cylinders filled with soil. The disk is 9 cm of diameter and 5 mm thick and it was placed horizontally between the base cylinder (base) and the lowest cylinder with soil (level 6, **Supplementary Fig. S1**). This disk is made of PVC with 4 drainage holes of 5 mm of diameter. The cylinders were placed upon and connected each other by 3 fiberglass rods of 45 cm height and 3 mm of diameter. The connection between each module was covered with a packing tape to effectively prevent water leakage (**Fig. 2.3D**). Four types of treatment columns with different vertical distribution of soil density were proposed (**Fig. 2.3B**). Four levels of soil bulk density were used: 1.3; 1.4; 1.5; and 1.6 g cm^{-3}. As all the treatment columns had the same levels of soil density, they had the same average soil density and the same amount of soil. The exception was the "Control" column (loose soil and homogeneously distributed), which was only made up of cylinders at a soil density of 1.3 g cm^{-3}. The four column treatment were (**Fig. 2.3B**): (i) Control, homogeneous and loose soil; (ii) Bottom, with the density of soil increasing from the

upper to the lower cylinder; (iii) Middle, with the highest density located in the center of the column and decreasing towards the ends; and (iv) Top, with the density increasing from the base upwards. The uppermost cylinder of each column (level L1, **Fig. 2.3B**) always had the lowest density (1.3 g cm^{-3}), this was to facilitate both the seedling transplanting and their subsequent establishment during the first stages of development.



Levels	Soil density (g/cm^3)			
	Control (C)	Bottom (B)	Middle (M)	Top (T)
L1	1.3	1.3	1.3	1.3
L2	1.3	1.4	1.4	1.6
L3	1.3	1.4	1.5	1.5
L4	1.3	1.5	1.6	1.5
L5	1.3	1.5	1.5	1.4
L6	1.3	1.6	1.4	1.4
Mean:	1.3	1.45	1.45	1.45

Fig. 2.3 - Setup of within-root phenotyping experiment. A) Cylinders during the compaction process. **B**) Diagram of vertical distribution of soil densities (g cm^{-3}) for each column ("Control", "Bottom", "Middle" and "Top"). Levels (cylinders) are indicated from the top to the bottom as L1, L2, L3, L4, L5, and L6. **C**) Soil compaction process using the testing machine (hydraulic press). **D**) Experimental unit: one plant per column. **E**) View of some plant at harvest time.

The experiment started on October 15th 2018 with seed germination and plant were grown for 27 days. Seeds were germinated and transplanted as described in the "**Preliminary experiments**". A two factor randomized block design with two genotypes, four soil columns and 8 replicates was used (*n* = 64 plants). Thus, each polyethylene box containing 8 columns was considered as a statistical block (**Fig. 2.3E**). Right before sowing, pots were irrigated till 30% volumetric water content (557 cm^3) with tap water (~7 mg L^{-1} N, 0.5 mg L^{-1} P, 2.6 mg L^{-1} K, 14 mg L^{-1} Mg; 440 mS cm^{-1}). 50% of this water was added by capillary action from the bottom to the top of the column by submerging one half of the columns in water and measuring the change in weight after submergence. The other 50% was added from the top of the columns. To keep this water content, plants were weighed and watered from the top once a week. Columns were placed into open polyethylene boxes (height×length×width: 32×60×40 cm^3) and arranged according to the experimental design (**Fig. 2.3E**). Environmental conditions during the experiment are indicated in **Table 2.3**.Supplemental illumination and destructive phenotyping after harvesting were done as indicated in "**Preliminary experiments**".

At harvest time, roots of both genotypes reached the bottom of all the treatment column and destructive phenotyping of shoots were done as indicated in "**Preliminary experiments**". For root phenotyping, each cylinder were individually separated by unscrewing the rods, removing the covering tapes and carefully cutting the soil and roots with a sharp knife in the space between two modules. Then, roots were manually collected from each cylinder by carefully washing away the soil on a sieve (mesh size 600 μm) using tap water. Afterwards, the recovered roots were stored in 50% ethanol in a 50 mL in conical Falcon tube until they were scanned and analyzed using WinRHIZO. As WinRHIZO estimates the total distribution of root length according to root diameter classes. The root length was recorded in 25 ranges of root diameter between 0 and 2.5 mm for each cylinder. Afterwards, the number of diameter classes was reduced by a hierarchical clustering of variables. For this purpose, the R package "ClustOfVar" (Chavent et al. 2012) was used. Then, the root dry mass for each cylinder was obtained as indicated in "**Preliminary experiments**". The complete list of traits are found in **Table 2.4**.

A two factor randomized block design with two genotypes, four soil columns and 8 replicates was used (*n* = 64 plants). Thus, each polyethylene box containing 8 columns was considered as a statistical block (**Fig. 2.3E**). Three sets of analyses of variance (ANOVA) were carried out. The first set of ANOVAs was performed to assess the effect

of genotype, column and block on the expression of each traits at plant level (1 ANOVA/trait). The second set considered the same factors as the first set but was done within each soil level position (6 ANOVAs/trait). A third set considered the same factors as the second one but also level position within the column, and it was done for each genotype separately as a repeated measures ANOVA. For that, each plant was indicated as a "subject" factor, column and block effects as "between subjects factors", and level position as a "within subjects factor" (2 ANOVA/trait). The second and third sets were done only for root traits. Before the analyses of variance, the assumptions of normality and homoscedascity of variances of residuals were evaluated by the Shapiro-Wilks and the Levene tests, respectively. Variables that failed to meet these assumptions were transformed to natural logarithm ($ln(x+1)$). Significant differences among ecotypes were compared by the Tukey test ($P<0.05$) using the R package "agricolae" (Mendiburu 2019). Then, to analyze the relationships among the traits, a correlation analysis was carried out based on Pearson's correlation coefficient between traits.

Soil models and root simulation

In **Chapter 6**, we studied the functional consequences of the plant responses to soil compaction by simulations studies. For that, two soil conditions consisting of two levels of soil density: loose and compacted soil (1.4 and 1.8 g cm^{-3}, respectively) were proposed. To simulate those conditions, a series of mathematical functions based on the formulae proposed by van Genuchten (1980), Saxton and Rawls (2006), Whalley et al. (2007; 2012) and Gao et al. (2012; 2016b) were coded. Soil density, organic matter content, texture and electrical conductivity were used as main inputs. These values were used to calculate the soil water potential and volumetric content, which, together with soil density, were used to estimate penetration resistance as a final output. Additionally, this implementation allows to estimate soil bulk density. To run the simulation, these equations were programmed in R (R Core Team, 2018). The description of those formulae and the R script are found in **Supplementary Material S3** and **S4**, respectively. The simulations of the pot conditions were based on out experimental data from a loam field soil (10% clay, 38.6% silt, and 51.4 sand) compacted at 1.4 and 1.8 g cm^{-3} (**Table 2.5**). The initial nutrient concentration for nitrate, phosphorus and potassium was set at 2, 0.012 and 0.1 µmol mL^{-1}, respectively. In addition, at the beginning of each simulation, this concentration was assumed to be uniformly distributed within the pot.

Table 2.5 - Experimental soil data used in simulations.

Soil condition	Db ($g\ cm^{-3}$)	Silt (%)	Clay (%)	OC (%)	EC ($dS\ m^{-1}$)
Loose	1.4	51.4	10	0.87	0.3125
Compacted	1.8	51.4	10	0.87	0.3125

Db: soil bulk density;
OC: percentage of organic carbon in soil mass;
EC: soil electrical conductivity.

Structural and functional phenotypes of Sorghum plants were simulated in OpenSimRoot (Postma et al. 2017, https://gitlab.com/rootmodels/OpenSimRoot). Plant growth, photosynthesis, nutrient uptake, allocation, and respiration were simulated up to 45 days after germination. The parameterization of phenotype was based on measurements of shoot and root traits (**Chapters 3** to **5**). For those parameters that were not measured, the existing parameterization for maize available in OpenSimRoot was used. A single plant per pot was simulated for each replicate. A cuboid shaped pot of 15×15×23 cm^3 (length×width×height) in volume was design. Later, a seed was germinated in the middle of the top face of the pot (reference level: depth = 0 cm and day = 0).

Three phenotypes were simulated. (i) Reference-phenotype: This is based on the parameterization of phenotypic data of 'SC599' plants (a post-flowering drought tolerant accession from USA) growing in loose soil. (ii) Tolerant-response: this is also based on data of 'SC599' plants but growing in compacted soil. This genotype was labeled as tolerant genotype with low plasticity in **Chapter 4**. This phenotype only shows a reduced length of fine roots (diameter < 0.2 mm, 40% shorter than reference-phenotype) while coarser roots and above-ground traits are not reduced. (iii) Sensitive-response: this is also based on data of 'SC599' plants but considering the degree of plasticity of 'HONEY_DRIP', a sweet-stem line from USA. 'HONEY_DRIP' was labeled as sensitive genotype with high plasticity in **Chapter 4**. Thus, the sensitive-response phenotype shows a shorter root length of fine roots (similar to tolerant-response) than reference-phenotype and above-ground traits are also reduced (15% smaller than reference-phenotype).

As OpenSimRoot can simulate contrasting environments while keeping the plant phenotype constant, we simulated all the combinations of soil treatments and phenotypes.

For example, phenotypes found in compacted soil were placed in loose soils, and those found in loose soil were placed in compacted soils. Simulations were performed in a completely randomized factorial design with two factors (phenotype and soil treatment) and four replicates. The soil simulation in OpenSimRoot was focused only on estimating the consequences of soil compaction in soil water dynamics. The soil water parameters used as input are showed in **Table 6.1**.

3 - PRELIMINARY EXPERIMENTS

As a first step to answer our general research question, a set of preliminary experiments using one genotype of sorghum were done. This genotype was chosen based on kernel phenotyping. Our aims in carrying out this research were (i) to identify in which container type (pots, rhizotrons, pot trays) the effect of soil compaction on plant phenotype is more evident and easier to evaluate in a future screening experiment; (ii) to calculate the minimum number of replicates needed to detect a significant effect of soil compaction on plant phenotype; (iii) to know whether a penetration resistance close to 1.2 MPa is enough to trigger a phenotypic response to soil compaction.

Results

Kernel phenotyping

Kernel size traits measured by image analysis were highly correlated to kernel biomass (**Table 3.1**), specially the projected area and perimeter (r ~ 95 and 94%, respectively). The genotypes 'CSM-63', 'KUYUMA' and 'BN223' were the three most representative phenotypes in average (**Fig. 3.1** and **3.2**, **Table 3.2** and **3.4**). Additionally, 'BN223' expressed the most uniform phenotype among all the genotypes (**Fig. 3.1** and **3.2**, **Table 3.2** and **3.3**). Thus, 'BN223' was selected from the 30 sorghum genotypes for the next preliminary experiments. **Fig. 3.3** shows the high correlation between kernel projected area and biomass. This indicates that kernel area and perimeter may be used as estimators of kernel mass.

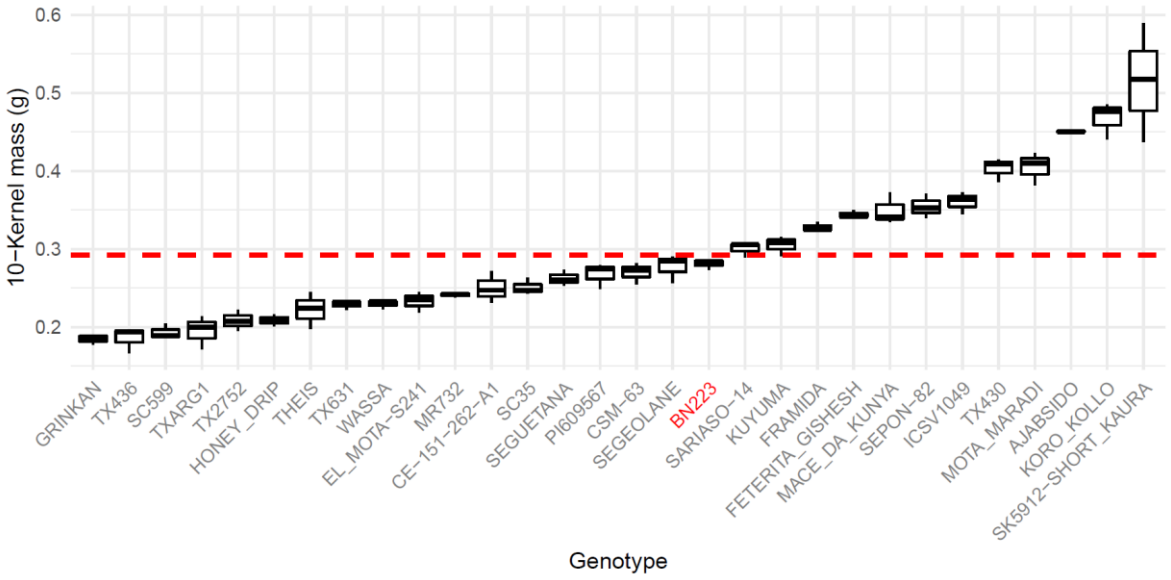


Fig. 3.1 - Distribution of the kernel biomass of 30 sorghum genotypes. Box plots are based on three point of 10 kernels each (30 seeds per genotype). The horizontal, dashed and red line indicates the phenotypic mean among all the kernels. The ‘BN223’ genotype is highlighted in red.

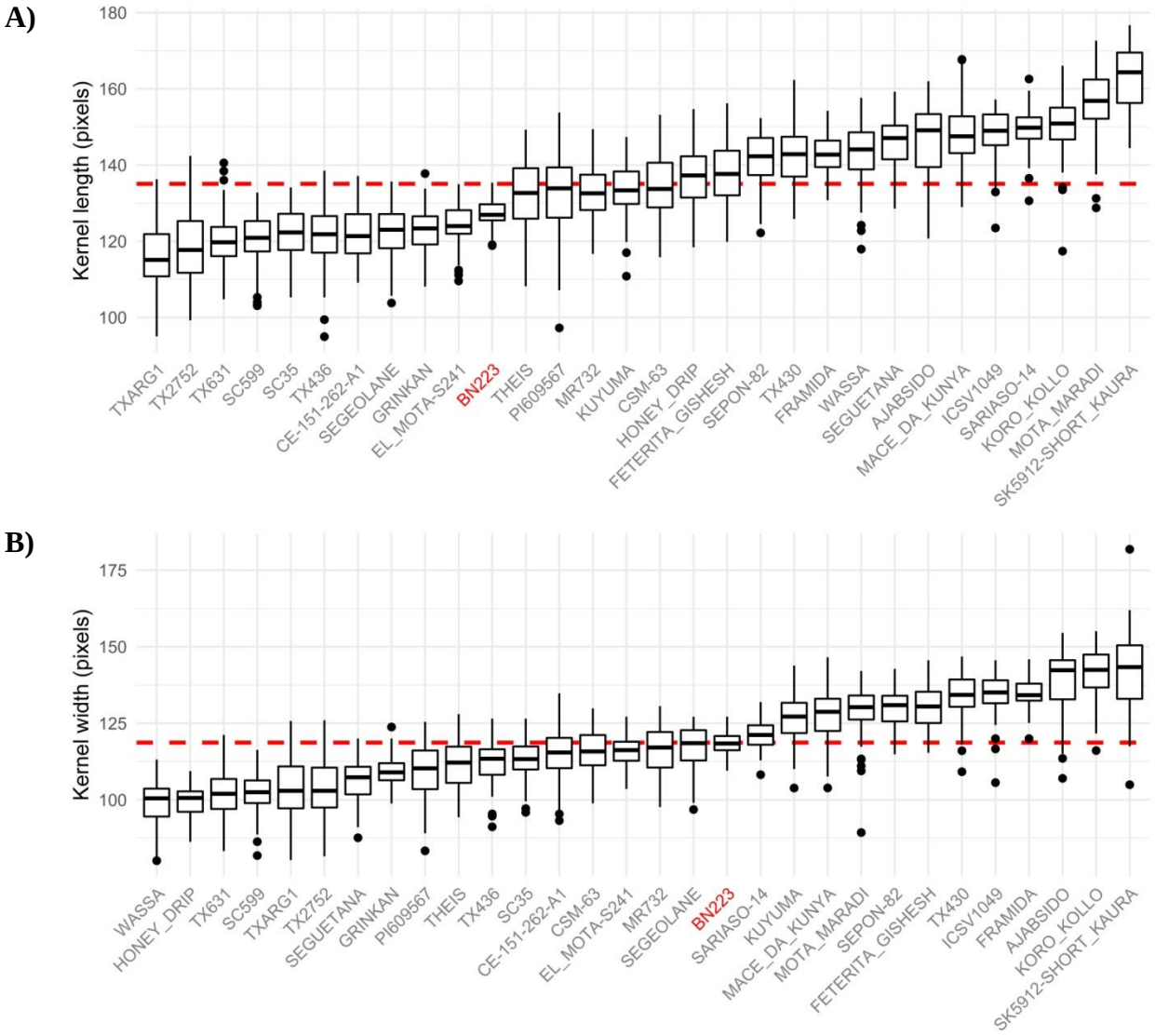
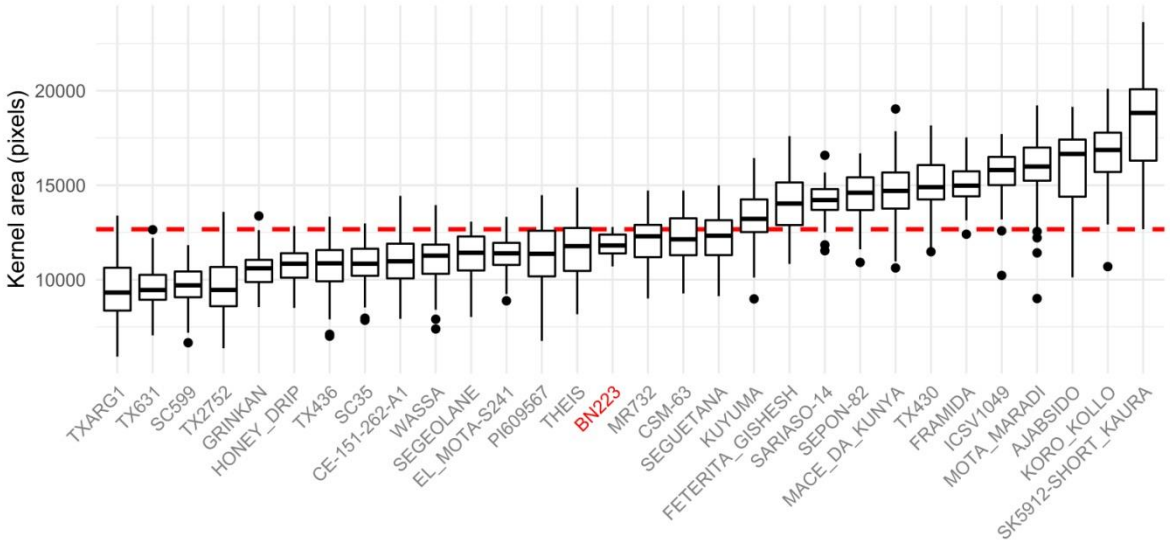


Fig. 3.2 - **Kernel phenotype for each genotype**. Box plots showing the distribution of kernel size traits: **A**) kernel length, **B**) width, **C**) projected area, and **D**) perimeter. The horizontal, dashed and red line indicates the phenotypic mean among all the kernels. The 'BN223' genotype is highlighted in red.

C)



D)

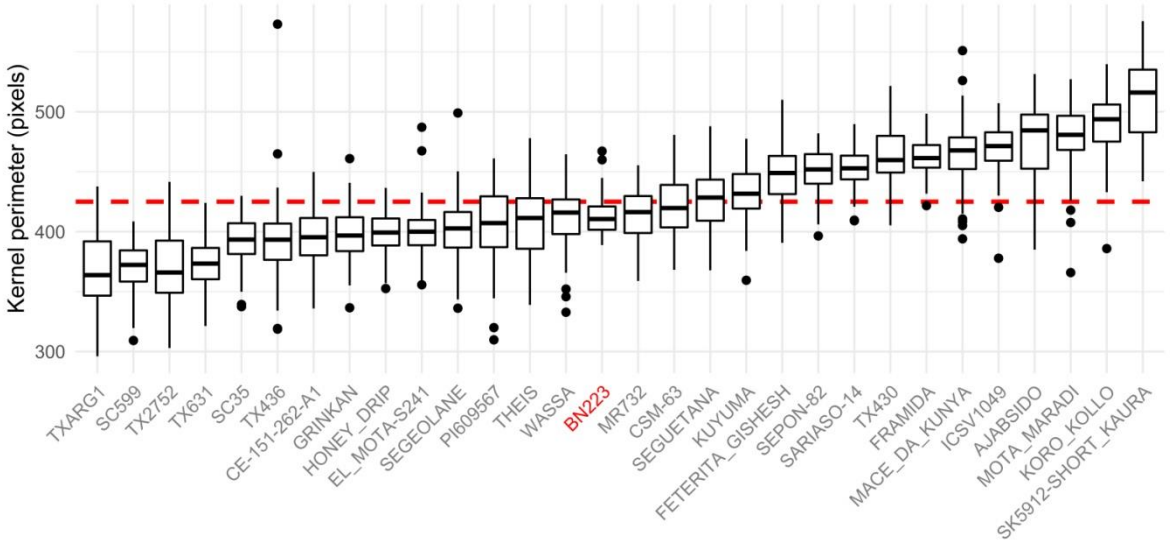


Fig. 3.2. (continued).

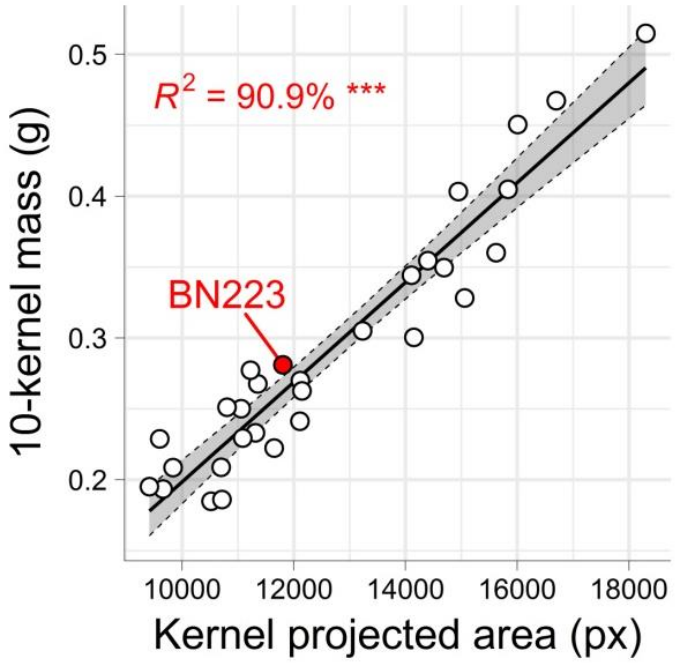


Fig. 3.3 - Correlation between kernel projected area and biomass. White circles: mean projected area in pixels (px) and mean biomass (g) for each genotype. The solid line is the fitted linear regression model. The interval confidence at 95% of the model is in indicated in gray. The 'BN223' genotype and the coefficient of determination (R^2) in percentage are indicated in red. Significance codes according to *F* test of linear regression (*P* value):<0.001 '***'.

Table 3.1 - Phenotypic correlations among kernel traits.

	Width	**Length**	**Area**	**Perimeter**
Length	67.9 ***	-		
Area	93.5 ***	89.3 ***	-	
Perimeter	90.4 ***	92.4 ***	99.4 ***	-
Mass	91.2 ***	81.2 ***	95.3 ***	93.7 ***

Pearson's correlation coefficient (%). *P* values: <0.001 '***'

Table 3.2 - Ranking[1] of genotypes based on the deviation from the mean for each trait

i	Genotype	Width	Length	Area	Perimeter	Mass	Mean[2]
1	CSM-63	6	1	2	2	5	3.2
2	KUYUMA	11	2	3	3	3	4.4
3	BN223	1	10	5	5	2	4.6
4	MR732	3	5	4	4	11	5.4
5	PI609567	13	6	7	8	6	8
6	SEGUETANA	18	13	1	1	7	8
7	SEGEOLANE	2	19	10	10	4	9
8	THEIS	10	7	6	7	19	9.8
9	FETERITA_GISHESH	17	4	9	9	12	10.2
10	EL_MOTA-S241	5	14	8	11	14	10.4
11	SARIASO-14	4	23	11	14	1	10.6
12	SEPON-82	16	8	14	12	15	13
13	CE-151-262-A1	7	20	13	16	10	13.2
14	SC35	8	22	15	18	9	14.4
15	WASSA	27	12	12	6	16	14.6
16	HONEY_DRIP	26	3	17	13	20	15.8
17	MACE_DA_KUNYA	12	17	18	21	13	16.2
18	FRAMIDA	23	11	21	20	8	16.6
19	TX436	9	21	16	17	24	17.4
20	GRINKAN	14	16	19	15	25	17.8
21	TX430	21	9	20	19	26	19
22	ICSV1049	22	18	23	22	18	20.6
23	TX2752	19	27	22	25	21	22.8
24	TX631	25	25	25	23	17	23
25	SC599	24	24	24	27	23	24.4
26	AJABSIDO	28	15	28	24	28	24.6
27	MOTA_MARADI	15	29	26	26	27	24.6
28	TXARG1	20	28	27	28	22	25
29	KORO_KOLLO	29	26	29	29	29	28.4
30	SK5912-SHORT_KAURA	30	30	30	30	30	30

[1]Ranking: genotypes sorted from the lowest to the highest deviation (1 to 30). [2]Mean: average ranking among all the traits of each genotype. The 'BN223' genotype is highlighted in red.

Table 3.3 - Ranking[1] of genotypes based on variance for each trait.

i	Genotype	Width	Length	Area	Perimeter	Mass	Mean[2]
1	BN223	1	1	1	1	7	2.2
2	SARIASO-14	3	2	2	2	10	3.8
3	FRAMIDA	4	4	6	3	5	4.4
4	GRINKAN	2	5	4	11	8	6
5	EL_MOTA-S241	5	3	3	7	15	6.6
6	HONEY_DRIP	6	18	5	4	9	8.4
7	SC35	9	7	8	5	13	8.4
8	MR732	16	8	10	10	2	9.2
9	SC599	8	12	7	8	11	9.2
10	TX631	18	11	9	13	6	11.4
11	ICSV1049	10	6	16	9	18	11.8
12	SEGUETANA	12	14	12	14	12	12.8
13	SEPON-82	7	16	15	6	20	12.8
14	KUYUMA	17	13	19	12	14	15
15	FETERITA_GISHESH	14	20	23	17	3	15.4
16	WASSA	20	19	17	18	4	15.6
17	SEGEOLANE	15	9	11	21	23	15.8
18	CSM-63	11	24	13	15	17	16
19	CE-151-262-A1	21	10	18	16	25	18
20	TX430	19	17	20	19	19	18.8
21	TX436	13	23	14	26	21	19.4
22	KORO_KOLLO	23	15	25	20	29	22.4
23	AJABSIDO	29	28	29	28	1	23
24	MACE_DA_KUNYA	25	21	24	24	24	23.6
25	MOTA_MARADI	24	22	26	22	26	24
26	THEIS	22	27	21	23	28	24.2
27	TXARG1	27	26	22	25	27	25.4
28	TX2752	28	29	27	29	16	25.8
29	PI609567	26	30	28	27	22	26.6
30	SK5912-SHORT_KAURA	30	25	30	30	30	29

[1]Ranking: genotypes sorted from the lowest to the highest variance (1 to 30). [2]Mean: average ranking among all the traits of each genotype. The 'BN223' genotype is highlighted in red.

Number of replicates

Data showed that penetration resistance increases as the soil becomes denser (**Table 2.2**). Pot trays had the highest soil strength at 1.8 g cm^{-3}. The lower resistance to the penetration were found in loose soils for pots and rhizotrons. Additionally, at the same soil density, those substrates with wet black peat (higher organic matter, pots and rhizotrons, **Table 2.2**) have a higher resistance to the penetration than that where dry soil was used alone (pot trays, **Table 2.2**).

Table 2.2 - Experimental setup of the three experiments which was according to a completely randomized design with one genotype ('BN223') of sorghum and two soil density levels.

Container	**Volume** (L)	**Substrate**	**plants per container**	**number of replicates**	W_m (%)	**Soil density [strength]** ($g \cdot cm^{-3}$) [MPa]	
						Loose	**Compacted**
Pots	4	½soil + ½peat	1	6	30	1.0 [0.20]	1.25 [1.20]
Rhizotrons	3.6	½soil + ½peat	2	6	30	1.0 [0.20]	1.25 [1.10]
Pot trays	0.25	soil	2	8	0	1.4 [0.18]	1.80 [1.25]

Volume: container volume in liters

Substrate: mass based-substrate proportion

W_m: gravimetric water content (wet-mass basis).

No significant effect of soil compaction on above-ground traits such as leaf area and biomass was found (**Table 3.4**). However, soil compaction effect on total root length and average root diameter was significant (**Table 3.4**). **Table 3.5**shows the distribution of root length according the root diameter classes. Accordingly, we can conclude that in the three experiments the length of fine roots (that is the length of roots whose average diameters are less than 0.2 mm) decreases significantly under compacted condition. The number of replicates to be used (sample size) increases as the effect of the factor is smaller. Thus, root length of very fine roots (diameter less than 0.2 mm) had the smaller estimated sample size.

Table 3.4 - Phenotype and statistical power for different traits across experiments.

Trait	Experiments	Phenotype (μ ± SEM) [1]		*P*-value	η^2	Effect size	Power	Sample size
		Loose	Compacted					
Leaf area	Pots	272.26±35.06	234.72±37.77	0.492	0.054	0.239	0.117	70
	Rhizotrons	208.85±43.92	237.48±37.51	0.631	0.024	0.157	0.078	161
	Pot trays	29.82±7.60	25.60±3.49	0.902	0.001	0.035	0.052	3249
Leaf dry mass	Pots	0.57±0.08	0.54±0.09	0.795	0.008	0.089	0.059	492
	Rhizotrons	0.54±0.09	0.53±0.09	0.950	0.000	0.021	0.050	9305
	Pot trays	0.09±0.01	0.08±0.01	0.754	0.008	0.089	0.063	501
Number of leaves	Pots	7.37±0.16	7.52±0.13	0.495	0.053	0.237	0.115	71
	Rhizotrons	7.87±0.18	7.59±0.17	0.280	0.115	0.361	0.205	31
	Pot trays	4.58±0.15	4.57±0.13	0.982	0.000	0.006	0.050	93744
Plant height	Pots	74.40±4.23	74.50±4.14	0.987	0.000	0.006	0.050	125507
	Rhizotrons	73.92±3.57	71.42±3.79	0.641	0.023	0.152	0.077	171
	Pot trays	42.54±2.17	43.28±2.29	0.870	0.002	0.046	0.053	1830
Root diameter	Pots	0.35±0.01	0.40±0.01	0.021	0.467	0.935	0.831	6
	Rhizotrons	0.25±0.01	0.26±0.01	0.220	0.146	0.413	0.254	24
	Pot trays	0.23±0.00	0.27±0.00	0.000	0.800	2.003	1.000	2
Root dry mass	Pots	0.12±0.02	0.11±0.02	0.759	0.011	0.106	0.063	353
	Rhizotrons	0.11±0.02	0.09±0.01	0.459	0.056	0.244	0.119	67
	Pot trays	0.04±0.00	0.04±0.00	0.803	0.005	0.071	0.058	784
Stem dry mass	Pots	0.30±0.04	0.26±0.05	0.578	0.036	0.192	0.093	107
	Rhizotrons	0.26±0.05	0.26±0.05	0.971	0.000	0.012	0.050	27323
	Pot trays	0.04±0.00	0.05±0.00	0.852	0.003	0.053	0.054	1411
Total root length	Pots	2720.35±407.47	1852.11±372.78	0.150	0.215	0.524	0.375	15
	Rhizotrons	2810.45±349.21	1970.42±140.02	0.049	0.333	0.706	0.598	9
	Pot trays	1296.28±169.50	902.22±62.23	0.024	0.334	0.708	0.750	9

[1]Phenotypic mean (μ) and standard error of the mean (SEM) under loose and compacted soil treatment of each trait. *P*-value of two sample t-tests (loose versus compacted soil).

η^2: variance explained by soil treatment.

Effect size: $f = \sqrt{\frac{\eta 2}{1-\eta 2}}$.

Statistical power (1 - β) of t-tests at α = 0.05.

Minimum number of replicates recommend for a two sample t-tests at α = 0.05 and 1 - β = 0.8.

Table 3.5 - Phenotype and statistical power for different root length traits across experiments.

Trait	Experiment	Phenotype (μ ± SEM) [1]		*P*-value	η^2	Effect size	Power	Sample size
		Loose	Compacted					
< 0.1	Pots	518.0 ± 82.7	316.9 ± 98.6	0.101	0.27	0.608	0.478	11.7
	Rhizotrons	901.4 ± 149.5	547.2 ± 43.7	0.054	0.323	0.691	0.58	9.3
	Pot trays	374.4 ± 52.1	205.3 ± 15.1	0.001	0.568	1.146	0.946	4.2
[0.1, 0.2)	Pots	844.5 ± 147.3	474.0 ± 101.9	0.052	0.357	0.744	0.643	8.2
	Rhizotrons	1025.7 ± 103.3	677.9 ± 36.9	0.007	0.529	1.06	0.91	4.7
	Pot trays	596.0 ± 75.2	375.5 ± 22.3	0.004	0.487	0.975	0.86	5.3
[0.2, 0.3)	Pots	362.8 ± 57.8	247.9 ± 47.6	0.131	0.234	0.553	0.41	13.9
	Rhizotrons	305.0 ± 43.0	218.8 ± 22.4	0.103	0.244	0.568	0.428	13.2
	Pot trays	150.4 ± 22.3	119.0 ± 9.0	0.189	0.129	0.384	0.226	27.6
[0.3, 0.4)	Pots	312.1 ± 54.1	246.9 ± 46.1	0.352	0.097	0.327	0.177	37.6
	Rhizotrons	208.7 ± 34.9	172.5 ± 20.7	0.537	0.039	0.202	0.097	96.9
	Pot trays	82.9 ± 13.1	94.0 ± 9.5	0.422	0.05	0.23	0.112	75.2
[0.4, 0.5)	Pots	115.7 ± 20.8	89.6 ± 17.1	0.377	0.088	0.31	0.163	41.9
	Rhizotrons	67.3 ± 11.0	55.3 ± 5.8	0.501	0.046	0.221	0.107	81.5
	Pot trays	19.9 ± 2.3	30.2 ± 3.4	0.021	0.348	0.73	0.626	8.4
[0.5, 0.6)	Pots	143.9 ± 25.4	107.3 ± 25.7	0.335	0.104	0.34	0.187	35
	Rhizotrons	82.5 ± 11.8	66.6 ± 7.8	0.295	0.109	0.35	0.195	33.1
	Pot trays	20.2 ± 2.8	28.5 ± 2.8	0.042	0.282	0.626	0.5	11.1
[0.6, 0.7)	Pots	73.0 ± 12.8	49.5 ± 10.2	0.228	0.157	0.431	0.272	22.1
	Rhizotrons	45.8 ± 6.3	37.4 ± 4.7	0.337	0.092	0.319	0.17	39.6
	Pot trays	11.1 ± 0.9	12.5 ± 1.7	0.687	0.013	0.114	0.065	300.7
[0.7, 0.8)	Pots	55.2 ± 6.2	39.8 ± 3.3	0.052	0.357	0.745	0.644	8.1
	Rhizotrons	30.6 ± 5.5	26.4 ± 5.5	0.553	0.036	0.194	0.094	104.9
	Pot trays	11.3 ± 1.2	11.2 ± 1.1	0.925	0.001	0.027	0.051	5497.4
[0.8, 0.9)	Pots	42.9 ± 1.5	35.4 ± 2.3	0.034	0.409	0.832	0.738	6.8
	Rhizotrons	24.6 ± 7.2	21.8 ± 4.7	0.915	0.001	0.034	0.051	3303.3
	Pot trays	11.9 ± 1.8	10.2 ± 1.1	0.532	0.031	0.178	0.087	124.9
[0.9, 1)	Pots	37.1 ± 2.4	28.9 ± 1.9	0.031	0.419	0.849	0.756	6.6
	Rhizotrons	14.1 ± 5.1	13.7 ± 4.0	0.791	0.007	0.086	0.058	528.3
	Pot trays	7.3 ± 1.7	7.2 ± 0.8	0.763	0.007	0.086	0.058	536.9

[1]Phenotypic mean (μ) and standard error of the mean (SEM) under loose and compacted soil treatment of each trait. *P*-value of two sample t-tests (loose versus compacted soil).

η^2: variance explained by soil treatment.

Effect size: $f = \sqrt{\frac{\eta 2}{1-\eta 2}}$.

Statistical power (1 - β) of t-tests at α = 0.05.

Minimum number of replicates recommend for a two sample t-tests at α = 0.05 and 1 - β = 0.8.

Table 3.5 - (continued).

Trait	Experiment	Phenotype (μ ± SEM) [1]		*P*-value	η^2	Effect size	Power	Sample size
		Loose	Compacted					
[1, 1.1)	Pots	36.8 ± 5.9	32.2 ± 5.4	0.637	0.026	0.163	0.08	149.5
	Rhizotrons	6.9 ± 2.3	6.3 ± 2.0	0.971	0	0.012	0.05	27524.8
	Pot trays	4.3 ± 0.9	3.1 ± 0.5	0.397	0.056	0.243	0.119	67.4
[1.1, 1.2)	Pots	19.8 ± 3.5	20.3 ± 4.4	0.871	0.003	0.056	0.054	1269.5
	Rhizotrons	6.0 ± 2.4	6.1 ± 2.1	0.779	0.008	0.091	0.059	475
	Pot trays	3.2 ± 0.9	2.8 ± 0.6	0.955	0	0.016	0.05	15441.4
[1.2, 1.3)	Pots	12.0 ± 2.8	12.8±3.2	0.905	0.002	0.041	0.052	2326.2
	Rhizotrons	2.2±0.8	2.1±0.9	0.925	0.001	0.03	0.051	4227.8
	Pot trays	1.1±0.4	1.0±0.1	0.998	0	0.001	0.05	7394669.9
[1.3, 1.4)	Pots	12.5±2.6	13.9±3.8	0.849	0.004	0.065	0.055	923.4
	Rhizotrons	2.2±0.7	1.8±0.6	0.795	0.007	0.084	0.058	553.1
	Pot trays	0.9±0.3	0.7±0.2	0.711	0.011	0.105	0.063	355.6
[1.4, 1.5)	Pots	3.5±0.6	4.8±1.5	0.825	0.006	0.076	0.057	679.2
	Rhizotrons	0.8±0.3	0.5±0.1	0.341	0.091	0.316	0.168	40.2
	Pot trays	0.3±0.1	0.2±0.0	0.222	0.112	0.356	0.2	32
[1.5, 1.6)	Pots	4.3±0.8	4.9±1.5	0.848	0.004	0.066	0.055	906.6
	Rhizotrons	1.0±0.4	0.6±0.2	0.466	0.054	0.24	0.117	69.4
	Pot trays	0.3±0.1	0.2±0.0	0.315	0.078	0.29	0.149	47.6
[1.6, 1.7)	Pots	2.4±0.5	2.8±0.8	0.948	0	0.022	0.051	7942
	Rhizotrons	0.5±0.2	0.4±0.2	0.736	0.012	0.11	0.064	327.5
	Pot trays	0.1±0.0	0.1±0.0	0.943	0	0.02	0.05	9674.6
[1.7, 1.8)	Pots	2.3±0.3	2.0±0.6	0.48	0.057	0.246	0.121	66
	Rhizotrons	0.6±0.3	0.2±0.1	0.395	0.073	0.281	0.143	50.7
	Pot trays	0.1±0.0	0.1±0.0	0.858	0.003	0.051	0.053	1535.3
[1.8, 1.9)	Pots	0.8±0.1	0.8±0.2	0.866	0.003	0.058	0.054	1171.6
	Rhizotrons	0.2±0.2	0.1±0.0	0.354	0.086	0.307	0.162	42.5
	Pot trays	0.0±0.0	0.0±0.0	0.218	0.114	0.359	0.203	31.5
[1.9, 2)	Pots	1.3±0.2	1.1±0.4	0.576	0.036	0.193	0.093	106
	Rhizotrons	0.5±0.3	0.1±0.0	0.246	0.132	0.389	0.231	26.9
	Pot trays	0.1±0.0	0.0±0.0	0.137	0.162	0.44	0.281	21.3

[1]Phenotypic mean (μ) and standard error of the mean (SEM) under loose and compacted soil treatment of each trait. *P*-value of two sample t-tests (loose versus compacted soil).

η^2: variance explained by soil treatment.

Effect size: $f = \sqrt{\frac{\eta 2}{1-\eta 2}}$.

Statistical power (1 - β) of t-tests at α = 0.05.

Minimum number of replicates recommend for a two sample t-tests at α = 0.05 and 1 - β = 0.8.

Table 3.5 - (continued).

Trait	Experiment	**Phenotype** (Mean ± SEM) [1]		***P*-value**	η^2	**Effect size**	**Power**	**Sample size**
		Loose	**Compacted**					
[2, 2.1)	Pots	0.6±0.1	0.7±0.2	0.841	0.005	0.069	0.055	831.2
	Rhizotrons	0.8±0.8	0.0±0.0	0.303	0.106	0.344	0.19	34.2
	Pot trays	0.0±0.0	0.0±0.0	0.43	0.049	0.226	0.109	77.9
[2.1, 2.2)	Pots	0.3±0.1	0.5±0.2	0.68	0.02	0.142	0.073	195.4
	Rhizotrons	0.2±0.2	0.0±0.0	0.285	0.113	0.357	0.202	31.8
	Pot trays	0.0±0.0	0.0±0.0	0.906	0.001	0.033	0.051	3549.1
[2.2, 2.3)	Pots	0.3±0.1	0.3±0.1	0.762	0.011	0.104	0.062	364.4
	Rhizotrons	0.1±0.1	0.1±0.0	0.501	0.047	0.221	0.107	81.4
	Pot trays	0.0±0.0	0.0±0.0	0.513	0.034	0.186	0.09	114
[2.3, 2.4)	Pots	0.2±0.1	0.2±0.1	0.787	0.009	0.093	0.06	457.8
	Rhizotrons	0.0±0.0	0.0±0.0	0.786	0.008	0.088	0.059	503.9
	Pot trays	0.0±0.0	0.0±0.0	0.869	0.002	0.047	0.052	1814.7
≥ 2.4	Pots	1.1±0.3	1.8±0.7	0.563	0.038	0.2	0.096	99
	Rhizotrons	0.1±0.1	0.1±0.0	0.977	0	0.009	0.05	44815
	Pot trays	0.0±0.0	0.0±0.0	0.168	0.141	0.405	0.246	24.9

[1]Phenotypic mean and standard error of the mean (SEM) under loose and compacted soil treatment of each trait. *P*-value of two sample t-tests (loose versus compacted soil).

η^2: variance explained by soil treatment.

Effect size: $f = \sqrt{\frac{\eta 2}{1-\eta 2}}$.

Statistical power (1 - β) of t-tests at α = 0.05.

Minimum number of replicates recommend for a two sample t-tests at α = 0.05 and 1 - β = 0.8.

Discussion

Penetration (or penetrometer) resistance (pressure or cone index) has been used to provide a relative measure of the resistance offered by soil to the penetration of roots or soil strength (van Huysteen, 1983; Nortjé et al., 2012; Gao et al., 2016a and 2016b) because it has been shown as a good predictor of the ability of roots to penetrate soil, as long as the other soil factors are not restrictive (Gao et al. 2016b). For instance, the decrease of soil organic matter causes a loss of structural stability, causing soils to be more susceptible to compaction (Casanova et al. 2013) and to increase the soil mechanical resistance (To and Kay 2005). It was found that at the same soil density, those substrates with wet black peat (higher organic matter, pots and rhizotrons, **Table 2.2**) have a higher resistance to the penetration than that where dry soil was used alone (pot trays, **Table 2.2**). This may

be explained as follows: if the soil density is held constant, soil mechanical resistance is increased as both organic matter and water potential also are increased. This would be associated with increased cementation within substrate micro-aggregates (To and Kay 2005).

The reduction of shoot dry mass, stem diameter, plant height and total leaf area by effect of soil compaction has been observed in several crops under controlled and field conditions (Masle and Passioura 1987; Grzesiak et al. 2014). These findings contradict our results since no effect of soil compaction on above-ground traits was found whereas fine root were significantly affected in the three experiments, at least, under the root penetration resistance and sorghum genotype used (**Tables 3.6** and **3.7**).

In terms of penetrometer resistance, root elongation is typically affected in soils with values over 0.8-2 MPa and it is severely affected, which could stop completely the root growth, at a resistance of about 5 MPa (Stirzaker et al. 1996; Passioura 2002). According to these antecedents, the penetration resistance applied on these experiments (~ 1.2 MPa) have been showed to be enough to reduce the root length of several crops (**Table 2.2**). Despite that the pot experiment did not have a significant effect of the compacted condition on total root length, it showed a tendency of root length to be reduced (**Table 3.4**). This reduction was confirmed in the rhizotron and pot tray experiments indicating that the detection of this response can be improved as long as the statistical power (under these experimental conditions: number of replicates) is increased.

When a statistical hypothesis is tested, two types of errors that can be made: Type I and Type II errors. Type I error is the probability of making the error of rejecting the null hypothesis, H_0 (no treatment effect), when it is true. It is denoted as α (Cohen, 1992; Sham and Purcell, 2014; Breur, 2016) which, most of the time, is taken to be equal to 0.05 (Cohen, 1992). The probability to make a false negative decision or commit a Type II error is the probability to accept the null hypothesis (H_0) when it is actually false. The false negative rate is denoted as β (Cohen, 1988; Button et al., 2013; Sham and Purcell, 2014; Breur, 2016). Under a decision theory approach, both types of errors should be controlled (Breur, 2016). Thus, the statistical power, $1 - \beta$, can be defined as the probability of correctly rejecting H_0 when it is false and a true treatment effect is present (Cohen, 1992; Sham and Purcell, 2014). As a convention, an enough statistical power is 0.8 (then, $\beta = 0.20$) (Cohen 1992). A study with low statistical power has a reduced chance of detecting a true effect (Cohen, 1992; Button et al., 2013). However, a larger value of β would result in a demand for higher sample sizes that may exceed the investigator's

resources (Cohen, 1992).

Therefore, besides to the *P*-value, it is necessary to look at the statistical power. Both leaf area and root dry mass have low power and excessively high recommended number of replicates (**Table 3.4**). On the other hand, the length of fine roots not only had a significant effect of soil compaction treatment but also a high statistical power. This gave as a result an affordable number of replicates (**Table 3.5**). This indicates that finding similar results in further experiments (reproducibility) may be difficult under the same experimental designs. However, increasing the number of replicates (up 12) is possible to increase the power as well as the reproducibility for fine roots. For rhizotron and pot trays, at least 9 replication are needed (under the same experimental conditions) to have similar results. This may be due to two experimental particularities (**Table 2.2**): (i) the number of replicates and the number of plants per container were higher than the pot experiment. Increasing the number of plants per container may bring some degree of interaction between plants, such as competition. Thus, two plants per container may result in more precise measurements as long as no severe competition occurs. *A priori*, the competition effect can be discarded, at least for the rhizotron experiment, because despite of having less volume per container than pots, the plants grown in rhizotrons had similar size to those found in pots (**Table 3.4**). Based on these results, a good and feasible number of replicates for similar experimental conditions is 12 repetitions and using two plants per container (especially for pot trays). This would help largely to increase both the statistical power and reproducibility of the data.

The main finding of this chapter was found evidence for plasticity of fine roots as a response to soil compaction. Even though the 'BN223' plants did not show any effect of compaction on above-ground traits, root system had a plastic response. This response was manifested by a decreasing of total root length per plant. The plasticity of root length was mainly given by fine roots (average diameter < 0.2 mm) whose length is reduced between 50 and 40%. In addition, the quality of these results is largely dependent on the statistical power. Under these experimental conditions, the statistical power was medium too high to detect the root plasticity (when the effect of compaction was significant). This analysis reveals that much more precise results can be reached increasing the amount of replicates up 12 repetitions, which is a quite doable number. In addition, a root penetration resistance of *ca.* 1.2 MPa is the minimum recommended to apply as soil compaction treatment according to Table 3.5. Otherwise, the effect on roots may be too small to detect with 12 repetitions.

Studying the effect of plant size on root plasticity as a response to soil compaction using 12 replicates on 4L pots is a feasible experiment design when less than 6 genotypes are evaluated. This experiment size would help largely to increase both the statistical power and repeatability of the data. Additionally, pot trays are ideal to screen for plastic responses in the 30 sorghum genotypes. This would be a much easier and cheaper solution than using 4L pots or rhizotrons.

4 - PHENOTYPIC RESPONSE TO SOIL COMPACTION VARIES AMONG GENOTYPES BUT CORRELATES WITH PLANT SIZE

Crops vary in tolerance to soil compaction and roots express various plastic responses. Accordingly, the aim of this chapter is to study whether the genotypic diversity in the degree of responses to soil compaction is more dependent on true plasticity than on plant size. For that, two experiments were conducted (see **Chapter 2**). First, a screening for differential response to soil compaction in a population of 28 sorghum genotypes was carried out. Second, based on the observed genotypic variation in shoot response, six genotypes were selected for in-depth plant phenotypic characterization. Plants were grown under greenhouse conditions in two soil density treatments (1.4 and 1.8 g cm^{-3}). As results, shoot biomass decreased under compaction, but some genotypes showed no plasticity. Phenotypic responses were correlated to plant size, with larger genotypes responding earlier and stronger. In the second experiments, impeded plants produced 35 and 47% less roots in terms of biomass and length, respectively. Plasticity was expressed foremost in nodal root number and fine roots (diameter < 0.2 mm), whereas thick root length was much less or not affected. Finally, as a conclusion sorghum genotypes can vary significantly in terms of their response to soil compaction. Tolerant lines are in general smaller sized genotypes which exhibit plasticity to soil compaction for fine roots only. Finally, we concluded that sorghum genotypes can vary significantly in terms of their response to soil compaction, but that less-sensitive lines are in general smaller sized genotypes which exhibit responses for fine roots only. This may pose challenges in breeding for soil compaction tolerance.

Results

Screening and between-plant phenotyping

Screening for shoot plasticity in young plants, we found most of the genotypes have a decreased shoot biomass under compaction, but some genotypes showed no plasticity at all. To analyze the root response to soil compaction, a longer experiment was

carried out using six of those genotypes with varying shoot plasticity. As a result, impeded plants produced less roots in terms of biomass and length. Roots were more plastic than shoots and plasticity was expressed foremost in fine roots. In overall, compaction reduced plant size and significant genotype-by-treatment interaction existed. Additionally, the response to compaction was correlated to plant size.

Shoot responses

Soil compaction reduced the shoot dry mass of most but not all genotypes (**Fig. 4.1A**, **Table 4.1** and **Supplementary Fig. S2**). The compaction treatment explained 89% of the variation in shoot size, whereas genotype and the genotype-by-treatment interaction explained 6 and 4% respectively.

Half of the genotypes had a significant treatment effect on shoot biomass (t-test, $P<0.05$) and were labeled responsive to compaction (black dots) and the other 14 did not have a significant response and were labeled tolerant (white dots). The tolerant genotypes had on average 28% less shoot dry mass than the responsive (susceptible) ones (0.22 versus 0.3 g). In **Fig. 4.1B**, the response to soil compaction for each genotype was expressed using the proposed plasticity index. The plasticity index is 0 when plants are non-plastic, and negative numbers indicate biomass is smaller under compacted conditions, and -1, indicates biomass in compacted conditions was half that of loose conditions. The compaction index correlates negatively with shoot size under un-compacted conditions, although for example 'AJABSIDO' was a genotype that was relatively large shoot that did not respond to compaction (**Fig. 4.1B**).

Table 4.1 - Effect of soil compaction on shoot mass at population level of 3- to 4-week-old plants of sorghum (screening).

Treatment	Shoot dry mass (g)			CV (%)	*n*	Factor R^2 (%)		
	Mean ± SEM	min	max			Genotype	Treatment	G×T
Loose	0.259 ± 0.008	0.035	0.857	47.7	224	5.6***	88.7***	3.6*
Compacted	0.198 ± 0.005	0.012	0.507	41	224			

SEM, min, max, CV, *n*: standard error of the mean, minimum, maximum, coefficient of variation; number of observations (pot with 2 plants), respectively.

R^2: determination coefficient according to mean square results from two way ANOVA; G×T: Genotype-by-treatment effect. Significant codes (*P*-value):<0.001 '***'; 0.01-0.05 '*'.

Six genotypes were selected with varying response to compaction and varying size. We selected one larger and one smaller genotype in three response classes (marked with a red circle in **Fig. 4.1A-B**). For the highly plastic genotypes, we selected the relatively small 'HONEY_DRIP' (shoots of 0.271 g in loose soil and 43% smaller under compacted soil) and the relatively large 'KORO_KOLLO' (shoots of 0.444 g in loose soil and 59% smaller under compacted soil). For the intermediate responsive genotypes, the relatively small 'SC599' (shoots of 0.205 g in loose soil and 19% smaller under compacted soil) and the relatively large 'BN223' (shoots of 0.356 g in loose soil and 35% smaller under compacted soil) genotypes were chosen. Finally, we selected for the unresponsive (non-plastic) genotypes the relatively small 'TXARG1' (shoots of 0.186 g in loose soil and 7% smaller under compacted soil) and relatively large 'AJABSIDO' (shoots of 0.287 g in loose soil and 0% smaller under compacted soil) genotypes. These genotypes were used for analysis of root traits in '**between-plant phenotyping'**.

Plants grew longer in **between-plant phenotyping experiment** than in **screening experiment** (25 versus 45 days), and consequently the shoot biomass was on average 25 times greater than in **screening experiment**. The shoot size ranking in **between-plant phenotyping** (order of genotypes from the lowest to the highest average shoot dry mass) deviated from the ranking in **screening experiment**, with most notably 'HONEY_DRIP' being relatively larger under both treatments (**Fig. 4.2**).

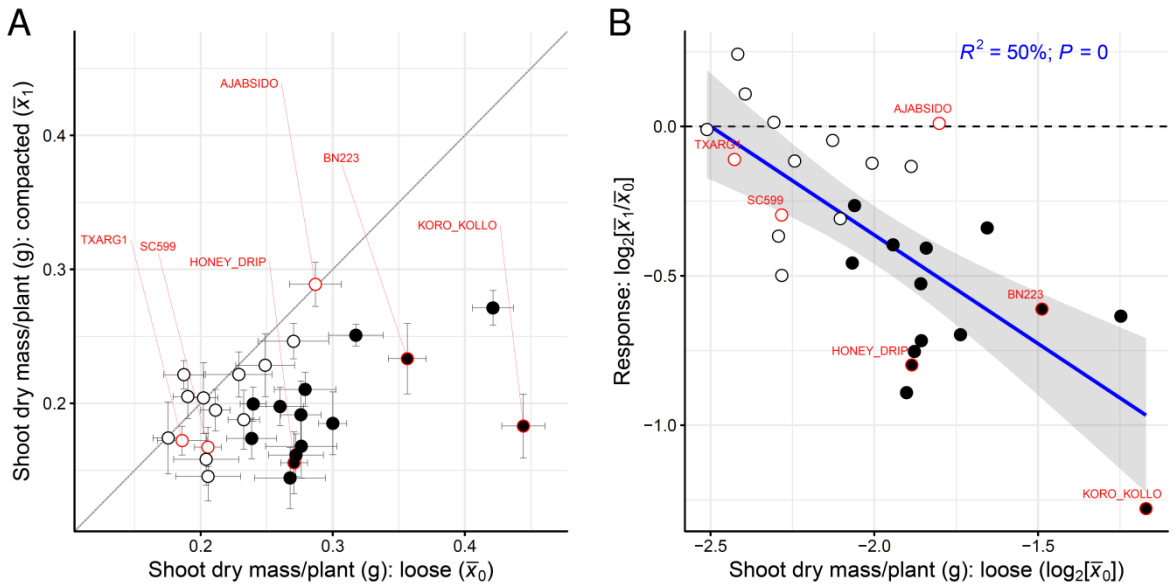


Fig. 4.1- Relative response of shoot dry mass to soil compaction in 3- to 4-week-old plants for 28 genotypes (screening).A) Correlation between shoot biomass under loose and compacted soil. 28 symbols show the mean and standard error of shoot dry mass for 28 sorghum genotypes plant under loose and compacted soil condition: $\bar{x}_0$ and $\bar{x}_1$, respectively. If the t-test between loose and compacted conditions is significant (*P*-value < 0.05), the genotype is labeled as plastic and highlighted with black circles. Otherwise, the genotypes is labeled as non-plastic and plotted with white symbols. Grey line shows1:1 ratio, genotypes close to the grey line are non-plastic (marked white). Whereas plastic lines are far below the grey line (marked black). **B**) Correlation between response to soil compaction and shoot biomass. Response (y-axis) is the fold change of the logarithm base two of the ratio of mean value in compacted and loose soil for each genotype, e.g. negative numbers indicate biomass is smaller under compacted conditions, and -1, indicates biomass in compacted conditions was half that of loose conditions. The blue curve with its confidence interval in gray (at 95%) is the fitted linear regression model between the response and the logarithm base two of the mean value of each genotype under loose condition. For color scheme, see **A**.

Despite that, the ranking of the absolute size of genotypes under loose soil changed (**Fig. 4.2**), the genotypes that were sensitive to soil compaction in the first experiments were also sensitive in the second experiment: 'HONEY_DRIP', 'KORO_KOLLO', and 'BN223'. The only exception was 'AJABSIDO', which was tolerant in **screening**

experiment while in **between-plant phenotyping** it was one of the genotypes most sensitive in terms of shoot biomass response. 'TXARG1' and 'SC599' had the smallest plants and did not respond to soil compaction in either experiment. Importantly, the relationship between plant size and susceptibility to soil compaction was also strong in **between-plant phenotyping** (**Fig. 4.2**): the higher the shoot dry mass in loose soil the higher the effect of soil compaction on shoot biomass, both in absolute and relative terms.

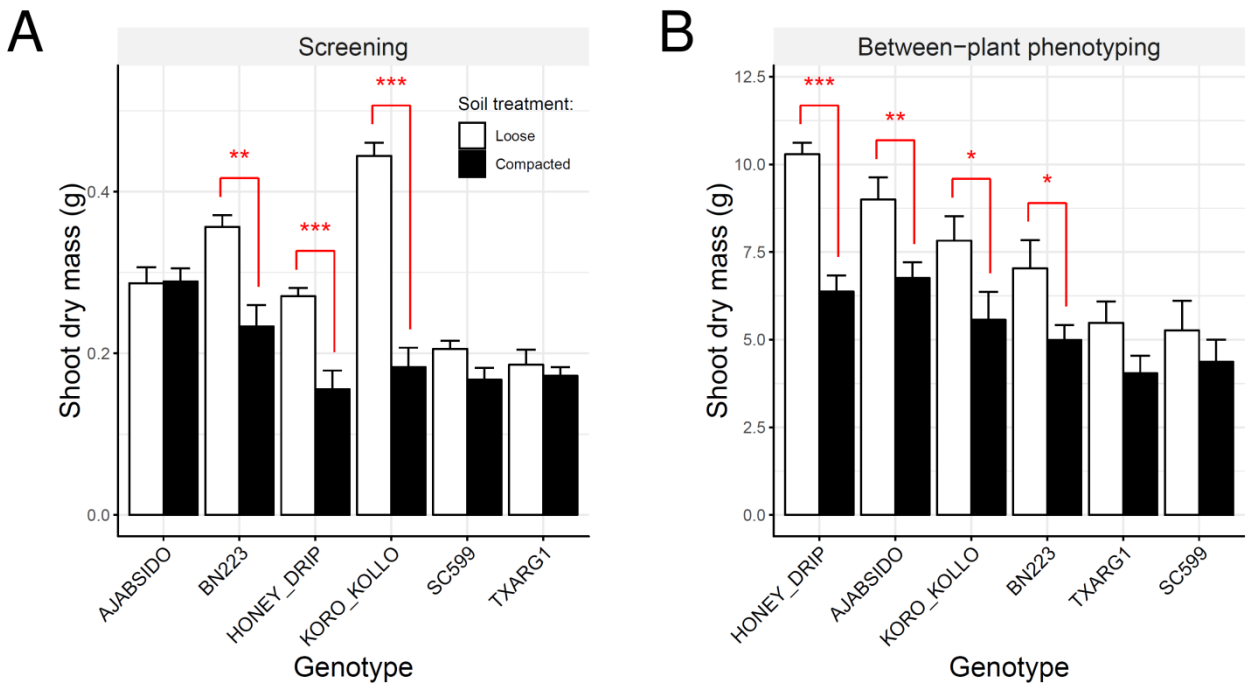


Fig. 4.2. - **Shoot dry mass.** For each experiment (**A**: Screening; **B**: Between-plant phenotyping) a box plot indicates the mean for each genotype and soil compaction condition. The genotypes are sorted on *y*-axis and ranked according their phenotypic mean under loose conditions: from the largest (bottom) to the smallest (top). Error bar: Standard error of the mean. The significant results are highlighted in red according to the t-test between loose and compacted conditions. Significance codes (*P*-value):<0.001 '***'; 0.001-0.01 '**'; 0.01-0.05 '*'.

To track the effect of soil compaction on the development of shoot, leaf area development was estimated non-destructively based on color images of plants. Green pixel count was calibrated against measured leaf area at harvest (R^2 = 99%, RMSE = 156.3,

Supplementary Table S2 and **Fig. S3**). **Fig. 4.3A** shows increase in estimated leaf area over time based on this calibration. As a result, genotypes had different total leaf areas at harvest in loose soil (from the largest to the smallest): 'HONEY_DRIP', 'AJABSIDO', 'KORO_KOLLO', 'BN223', 'TXARG1', and 'SC599'. The four genotypes with significant effect on shoot dry mass in **between-plant phenotyping** (**Fig. 4.2**) also responded to soil compaction in terms of leaf area. In addition to being the biggest genotypes in terms of leaf area and shoot dry mass, 'KORO_KOLLO' and 'HONEY_DRIP' were most affected in terms of leaf area (32 and 29% smaller values under compacted soils; **Fig. 4.3**) and shoot biomass (28 and 38% smaller values under compacted soils; **Fig. 4.2**).

During the first days of growing, the phenotypic variation of leaf area was mainly influenced by genotypic differences, explaining almost 80% of the total variation. The treatment effects on shoots of the larger genotypes became evident during the second week after the transplanting (**Fig. 4.3B**). At this time, the treatment effect became the more important source of variation explaining almost 50% of the variation in leaf area, but the genotype by environment interaction was important. During later stages, genotype became again the most explanatory factor. As in **Fig. 4.1** and **4.2**, larger genotypes had the higher responses in terms of leaf area (**Fig. 4.3C**). The responses of 'KORO_KOLLO', and 'TXARG1' both only had significant treatment effects during relatively late stages. The treatment effect on 'KORO_KOLLO' accelerated during the last week. Accelerated responses were also observed for 'HONEY_DRIP' (**Fig. 4.3C**). As in **screening experiment**, 'SC599' did not show signs of response to the compaction treatment and consequently its plasticity index was very close to zero.

While leaf area and shoot biomass at harvest were highly correlated (r ~ 90%) (**Supplementary Tables S3** and **S4**), specific leaf area was not correlated with shoot biomass. SLA varied significantly among genotypes, but was not significantly affected by treatment (**Supplementary Table S5**).

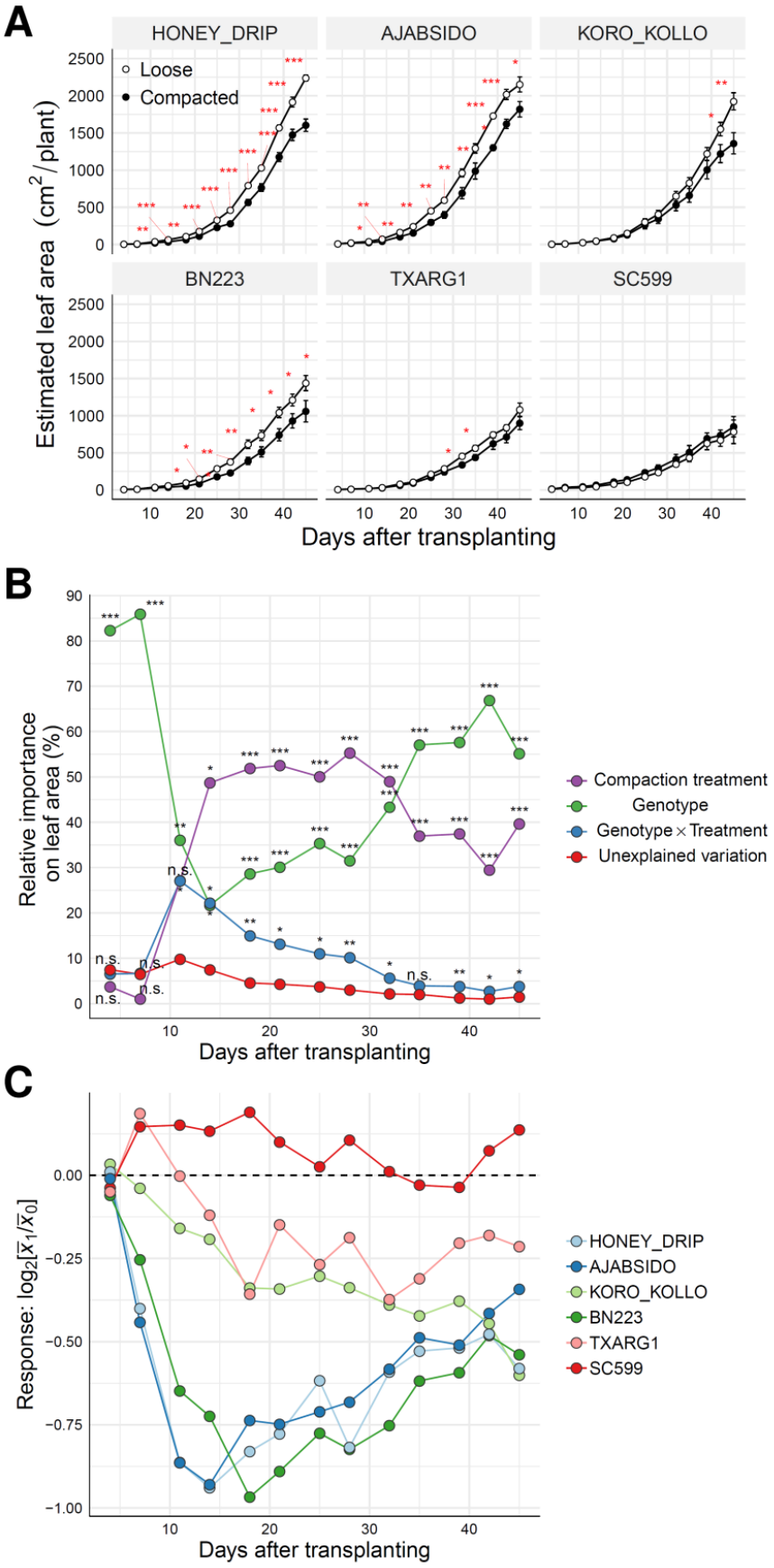


Fig. 4.3 - Response of leaf area to soil compaction over time. (continued on the following page).

Fig. 4.3 -Response of leaf area to soil compaction over time. A) Increase in estimated leaf area over time for the six selected genotypes growing in loose and compacted soils. The measurements were done twice a week yielding in total 13 date points (days after transplanting). Genotypes are sorted according their leaf area at harvest under loose conditions (from the largest to the smallest): 'HONEY_DRIP', 'AJABSIDO', 'KORO_KOLLO', 'BN223', 'TXARG1', and 'SC599'. White and black circles: mean of estimated leaf area (cm^2) for loose and compacted soil conditions, respectively. Error bar: Standard error of the mean. The significant results are highlighted in red according to the t-test between loose and compacted conditions. Significance codes (*P*-value):<0.001 '***'; 0.001-0.01 '**'; 0.01-0.05 '*'. **B**) Relative importance of genotype and soil compaction treatment on leaf area for each date point. The relative importance is based on two-way ANOVA for each diameter class considering genotype, compaction treatment and their interaction (Genotype×Treatment) as factors. The relative importance is calculated by using the mean squares of each of this factors. Significance codes according to *F* test of ANOVA (*P*-value): <0.001 '***'; 0.001-0.01 '**'; 0.01-0.05 '*'; n.s.: not significant (*P*> 0.05). **C**) Variation over time of the leaf area response to soil compaction for each genotype. The response is the fold change of the logarithm base two of the ratio of mean value in compacted and loose soil for each genotype and date point (see **Fig. 4.1**).

Root responses

Root biomass was strongly correlated with shoot biomass, Root/Shoot, leaf area and root length both in loose (*r* ~ 87, 84, 86, 81 and 78%, respectively; **Supplementary Table S3**) and compacted soil (*r* ~ 88, 83, 76 and 86 %, respectively; **Supplementary Table S4**). Under compaction, root biomass was on average reduced by 35% compared to the loose control. Even though genotypes differed in root biomass, they expressed similar levels of absolute responses (significant genotypic effect but non-significant G×T interaction, **Supplementary Table S5**). For example 'SC599', which had a low response in shoot biomass, did have a 23 and 35% reduction in root biomass and total length.

Table 4.2 - Genotypic diversity of total root length (cm) in loose and compacted soil.

Genotype	Loose		Compacted		
	Mean ± SEM[1]		Mean ± SEM		
KORO_KOLLO	38329.7 ± 4976	**A**	16239.1 ± 2253	**ab**	**[2]
HONEY_DRIP	37877.2 ± 4588	**A**	16299.7 ± 2089	**ab**	**
AJABSIDO	29674.6 ± 2891	**AB**	21251.0 ± 1847	**a**	*
BN223	18374.1 ± 1882	**BC**	11808.5 ± 1411	**bc**	*
SC599	14010.4 ± 1512	**C**	9046.5 ± 1047	**c**	*
TXARG1	12416.1 ± 1628	**C**	7012.9 ± 1016	**c**	*

[1] Different letters indicate means ± standard error of the mean (SEM) with statistically significant differences among genotypes according to Fisher's least significant difference test (P<0.05) within each soil treatment level. Upper- and lowercase letter: means under loose and compacted soil, respectively.

[2]The significant codes are according to the t-test between loose and compacted conditions within each genotype (P-value): 0.001-0.01 '**'; 0.01-0.05 '*'.

Compaction reduced Root/Shoot by 11% and most variation was explained by genotypic differences (**Table S4**). The log-log (allometric) relationship of shoot and root biomass across replicates and genotypes (**Table 4.3**) was significantly different between soil conditions. For every decrease (or increase) of one percentage in term of shoot mass is associated with a decrease (or increase) of 1.37 and 1.7% in root mass for compacted and loose soil treatments, respectively (see slopes in **Table 4.3**). Therefore, plants growing in compacted soil, have proportionally less roots than shoots in terms of biomass than non-impeded plants. This decrease in Root/Shoot of impeded plants is accentuated by the fact that the plants are smaller and that smaller plants normally have increased Root/Shoot ratios. This means that genotypes showed different biomass partitioning in favor of shoots under compacted soils pattern.

Table 4.3 - Relative contribution of soil treatment and shoot biomass to the variation of root biomass.

Regression coefficients for each treatment				**Effects on root dry mass (%)** [2]		
Treatment (T)	R^2 (%)[1]	intercept	slope	T	Shoot_DM	T ×Shoot_DM
Loose	85.4	-3.07	1.70	8.0***	90.9***	1.0*
Compacted	76.6	-2.39	1.37			

[1]R^2: determination coefficient according to linear regression model within each treatment: *ln*(Root_DM) = intercept + slope×*ln*(Shoot_DM).

[2]Determination coefficient according to mean square results from ANCOVA

Root length (**Table 4.2**) ranged from 383.3 m of 'KORO_KOLLO' under loose soil to only 70 m of 'TXARG1'in compacted soil. Root length was greatly reduced by soil compaction for all genotypes (46% of reduction on average). For example, roots of 'KORO_KOLLO' and 'HONEY_DRIP' were almost 58% shorter under compacted compared to loose conditions. Even compaction tolerant genotypes such as 'SC599' had an important reduction in root length (35% shorter roots under compacted soil). Additionally, total root length was significantly correlated with plant biomass (*r*= 72 and 83% in loose and compacted soil, respectively) and 38% of the observed phenotypic variation in root length was explained by the variation in plant biomass, and the slopes of the regression dependent significantly on genotype and environment (see for ANCOVA in **Supplementary Table S6**).

Root length was split into five root diameter classes based on a hierarchical clustering (**Fig. 4.4** for root length, **Fig. 4.5** for cluster analysis). The classes with smaller diameters had much greater root length than those with thicker diameters (**Fig. 4.4A**). Unlike leaf area development, all the genotypes responded to the soil compaction treatment with a reduced root length in one or more root diameter class. Genotypes that had large shoot dry mass plasticity, 'HONEY_DRIP' and 'KORO_KOLLO', had also the greatest root length plasticity, and had plastic response for almost all the root diameter classes. On the other hand, the smaller genotypes, 'BN223', 'TXARG1'and 'SC599', had significant effects only on roots with diameter < 0.2 mm. 'AJABSIDO', which was selected as non-responsive in the screening but in this experiment was highly responsive in leaf area and shoot biomass, had treatment effects only on very fine roots. Additionally, the length of roots with diameter < 0.2 mm were one of the traits with a minor effect of

plant size on the phenotypic variation, plant biomass only explained a 33-38%. Root length of thicker roots were more correlated to plant biomass than fine roots. For example, plant biomass explained at least the 65% of the phenotypic variation of the length of roots with diameter > 2 mm (**Supplementary Table S6**).

Fig. 4.4B shows the relative effect of treatment and genotype on the root length for each diameter class. Treatment had the strongest effect with a relative importance of up to 70% for roots whose diameter was less than 0.6 mm. In the case of roots with diameters < 0.2, this effect explained about 70% of the variation of root length. Overall, treatment effect decreased as the root diameter increased. The second most important explanatory variable was genotype, which was stronger in the thicker root diameter classes, even though genotype-by-treatment interaction had a relatively small effect. The interaction was significant in almost all the diameter classes fluctuating between 5 and 15% of the total variation. Similarly, this interaction explained ~8% of the total variation in root length. Therefore, genotypes have a different degree of response to soil compaction and there is genetic diversity in terms of root length response to soil compaction.

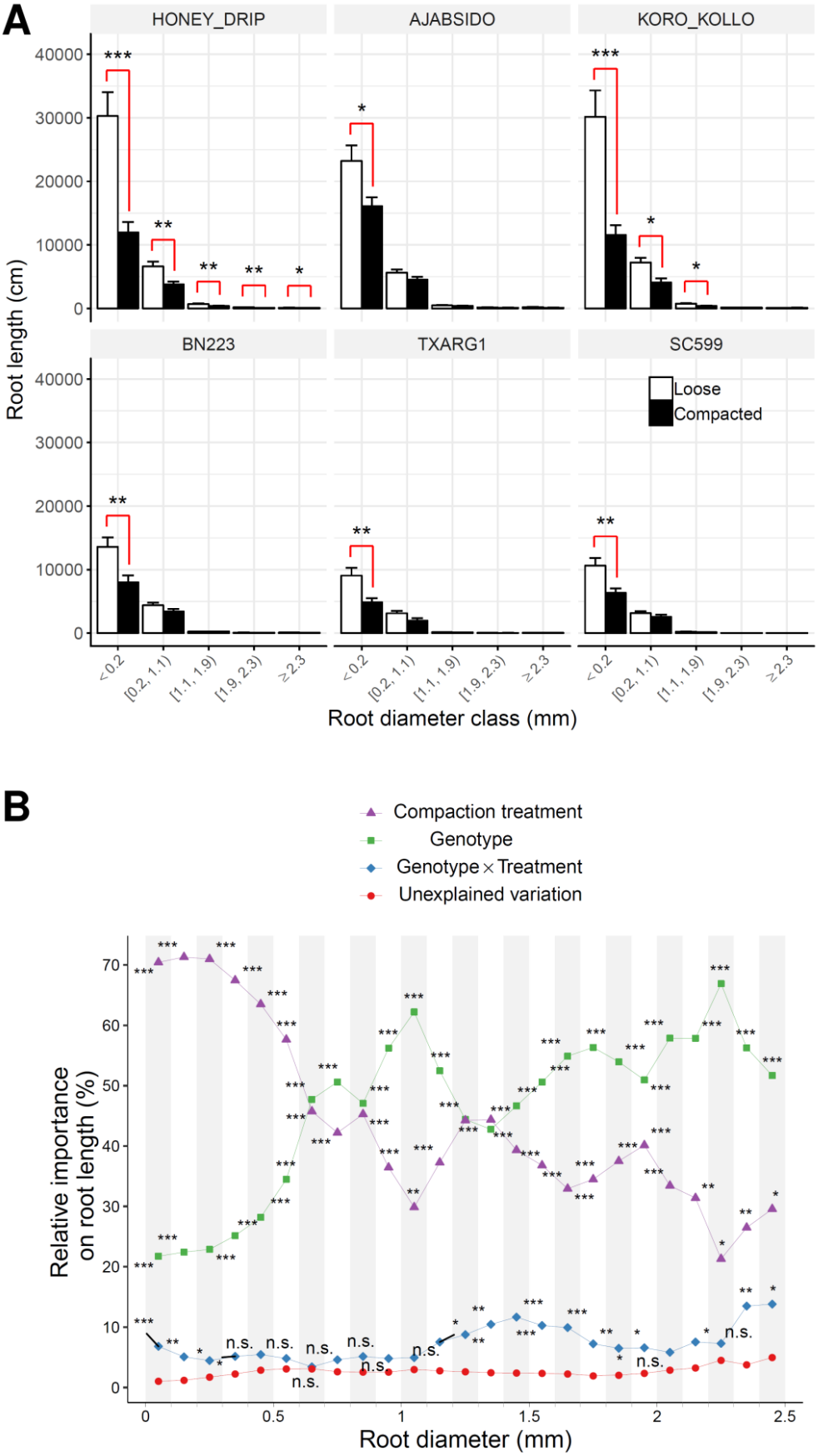


Fig. 4.4 - Response of root length to soil compaction. (continued on the following page).

Fig. 4.4 - Response of root length to soil compaction. A) Distribution of root length according the root diameter classes for the six selected genotypes growing in loose and compacted soils. The root length were recorded in five ranges of root diameter between 0 and 2.5 mm (diameter classes). The five ranges were based on a cluster analysis (see **Fig. 4.5**). Genotype are sorted according **Fig. 4.2**. White and black circles: mean of root length (mm) for each diameter class in loose and compacted soil conditions, respectively. Error bar: Standard error of the mean. The significant results are highlighted in red according to the t-test between loose and compacted conditions. Significance codes (*P*-value):<0.001 '***'; 0.001-0.01 '**'; 0.01-0.05 '*'. **B**) Relative importance of genotype and soil compaction treatment on root length for each diameter class. The root length were recorded in 25 ranges of root diameter between 0 and 2.5 mm. These ranges or diameter classes are indicated as gray or white vertical bands. The relative importance is based on two-way ANOVA for each diameter class considering genotype, compaction treatment and their interaction (Genotype×Treatment) as factors. The relative importance is calculated by using the mean squares of each of these factors. Significance codes according to F test of ANOVA (*P*-value):<0.001 '***'; 0.001-0.01 '**'; 0.01-0.05 '*'; n.s.: not significant (*P*> 0.05).

Overall responses

To summarize the overall effect of soil compaction on plant phenotype, We plotted a heatmap based on the normalized mean response for each genotype and trait (**Fig. 4.5**). On the bottom of the plot, traits were sorted based on a cluster analysis of variables, which in turn yielded four clusters of traits (**Fig. 4.5** topside): Cluster 1 (C1), which is made of length of roots with diameter less than 1.1 mm, leaf area and number of tillers; Cluster 2 (C2), which is only made of length of roots with diameter longer than 1.1 but shorter than 1.9 mm; Cluster 3 (C3), which is made of length of coarse roots (diameter ≥ 2.3), root diameter and collar traits (number of nodal roots, root to shoot ratio, collar dry mass, etc.); and Cluster 4 (C4), which is mainly made of biomass traits. The traits within each cluster are indicated in **Supplementary Table S7**. The average plasticity index for these clusters are: -0.48, -0.47, -0.34 and -0.18 for C1, C2, C3 and C4, respectively.

Depending on the genotype, most of the traits were affected by soil compaction

(as indicated by negative values of plasticity index and dark colors in **Fig. 4.5**) and all of them had a significant genotypic effect (**Supplementary Table S5**). Additionally, genotypes were sorted based on their plasticity index by a hierarchical clustering (right side). The heatmap clearly shows that 'HONEY_DRIP' and 'KORO_KOLLO' were the most plastic genotypes. Sorted by their average plasticity index among all the traits, genotypes are ranked as follows: (1) 'HONEY_DRIP', (2) 'KORO_KOLLO', (3) 'TXARG1', (4) 'SC599', (5) 'AJABSIDO' and (6) 'BN223'(with -0.6, -0.5, -0.36, -0.3, -0.3, and -0.2, respectively).Inside C1, the rank is as follows: (1) 'KORO_KOLLO', (2) 'HONEY_DRIP', (3) 'TXARG1', (4) 'BN223', (5) 'AJABSIDO' and(6) 'SC599' (with an average plasticity index of -0.77, -0.72 -0.5, -0.33, -0.33 and -0.26, respectively). Clustering the genotypes, it was observed that 'HONEY_DRIP' and 'KORO_KOLLO' cluster in a group of sensitive genotypes, whereas this cluster contrasts strongly with the tolerant genotype 'SC599'. The length of roots with diameter <0.2 mm (cluster C1 in purple) were the traits with the highest response (the darkest colors in the heatmap). Their plasticity index was ~ -1.0 on average.

Based on the ANCOVA (**Supplementary Table S6**),I plotted in the bottom panel of **Fig. 4.5** to what extend the trait's plasticity was explained by size related effects (allometric or apparent plasticity), and to what extent it was independent of size and thereby true plasticity. The response of the cluster 1 traits is strongly explained by plastic effects. Within this cluster the length of very fine roots (diameter < 0.1) had a true plasticity effect greater than the apparent plasticity. For example this trait had both a plasticity index of -1.5 in 'HONEY_DRIP' and 'KORO_KOLLO'.

On average, very fine roots under compacted soils were 54% shorter than under loose conditions. On the other hand, biomass-related traits, SLA, plant height, root average diameter per plant, and length of thicker roots (diameter > 1.9 mm) (clusters C3 and C4) were less sensitive to soil compaction than very fine root traits and were mainly given by apparent plasticity. On average, C3 and C4 were the clusters with the higher effect due to apparent plasticity (explaining ~70 and 62% of total variance, respectively) and the lower plastic effect (explaining ~13 and 15% of total variance, respectively). While C1 and C2 had the lower apparent plasticity effect (explaining ~45 and 53% of total variance, respectively) and the higher plastic effect (explaining ~28 and 21% of total variance, respectively; **Fig. 4.5**; **Supplementary Table S6**).

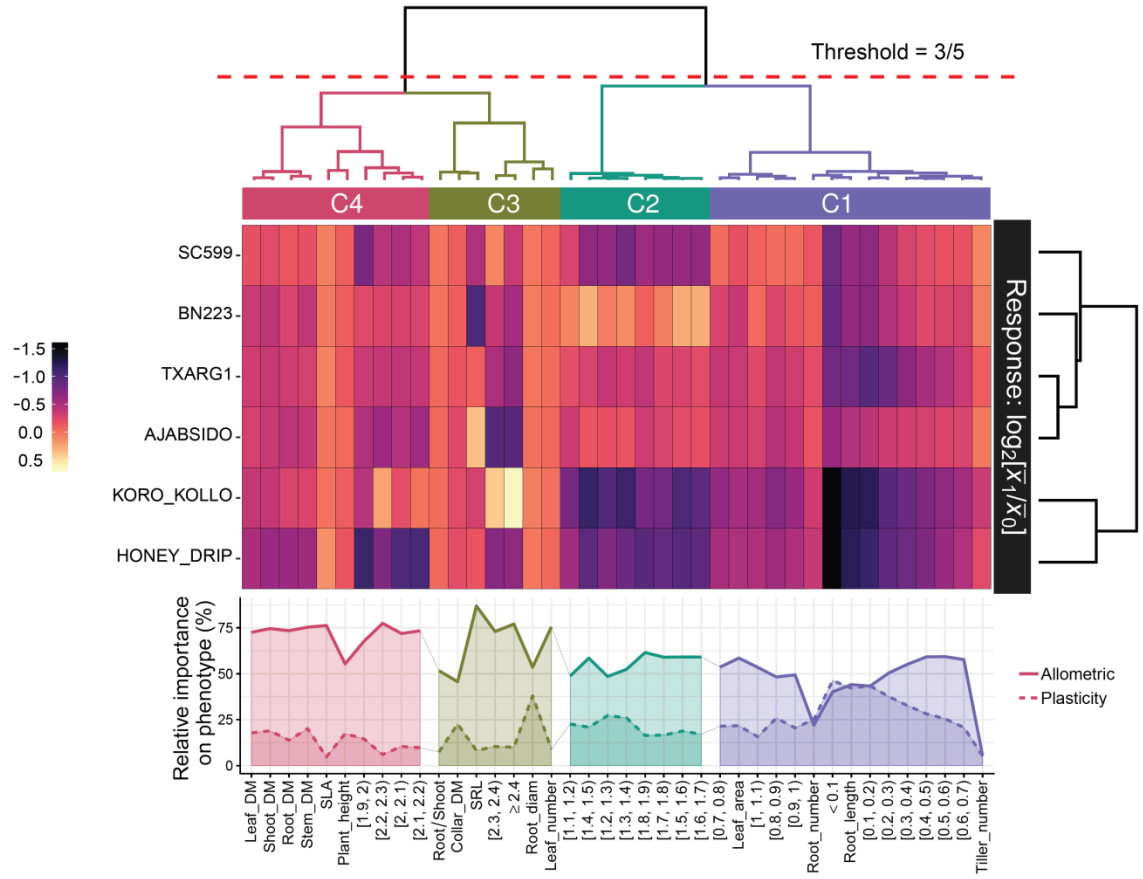


Fig. 4.5–**Phenotypic response to soil compaction in 6-week-old plants of six sorghum genotypes.** Heatmap shows the degree of response expressed as the standardized fold change of the logarithm base two of the ratio of mean value in compacted ($\bar{x}_1$) to that in loose soil ($\bar{x}_0$) soil for each genotype (rows) and trait (columns). Dark and light colors indicate a high and low response, respectively. The relative importance on phenotype of plasticity and allometric effects is based on ANOVA for each trait considering genotype, compaction treatment, plant dry mass (as a covariable) and their interactions as factors. The relative importance is calculated by using the mean squares of each of these factors. Thus, plasticity is the sum of the importance of treatment and treatment-by-genotype interaction effects; allometric effect is the sum of the importance of plant dry mass and all their interactions with treatment, genotype and treatment-by-genotype interaction effects. Traits are sorted according to a variable clustering located on the top of the figure. This clustering yielded four main groups (C1 to C4) using at the threshold of similarity equals to3 (maximum = 5). Genotypes are sorted according to a hierarchical clustering of their response to soil strength located on the right side.

To explore the association of plant size with the phenotypic variation of all the traits a Principal Component Analysis (PCA) was performed. The PCA showed that the first five principal components explained about 90% of the total variation of the data (69, 9.3, 5, 3.8 and 3%, respectively; **Supplementary Material S2**). PC1 was more associated to root length and root biomass (correlation between trait and PC1 > 90%). But plant biomass was also highly correlated with this PC (r ~ 90%). PC2 was mainly correlated with SRL (r ~ 74%). PC3 was more associated with plant height at harvest (Plant_height), average root diameter and Root/Shoot (r ~ 63, 52, 49%, respectively). PC4 and PC5 were correlated to SLA (r ~ 85%) and root diameter (r ~ 53%), respectively.

The PCA-based scatter plots of observations revealed that PC1 and PC3 separates in a better way the soil conditions than PC2, and that plant dry biomass (shoot + root) is highly and positively correlated with the PC1 and to a much lesser extent with PC2 and PC3 (**Fig. 4.6** and **Supplementary Fig. S4-6**).

Additionally, treatment effect was significant on PC1 and PC3 (**Fig. 4.6** and **Supplementary Material S2**); the latter being the largest in terms of relative importance among components (55%). On the contrary, PC2 was not affected by compaction levels. This indicates that treatment effect contributes an important portion of the observed variation. Genotypic effect was significant in the first PCs, especially in PC2 and PC3 where it explained the 22 and 30% of the variation of the component. Even though treatment-by-genotype interaction was significant in PC1 and PC3, it explained a very little portion of total variance of this component.

Even though inconsistencies in terms of shoot dry mass between screening experiment and the phenotypic characterization were found, plastic genotypes found in **screening experiment** were also plastic in **between-plant phenotyping**. The phenotypic variation of root length traits were mostly given by effects of treatment and genotype-by-treatment interaction. Whereas, specific leaf area and leaf number were not affected by soil compaction.

Overall, compaction reduced plant size and significant variation among genotypes existed. Additionally, the response to compaction was correlated to plant size. Finally, we found that the genotype-by-treatment interaction explained a small portion of the observed variation compared to the huge effect of plant size on these traits.

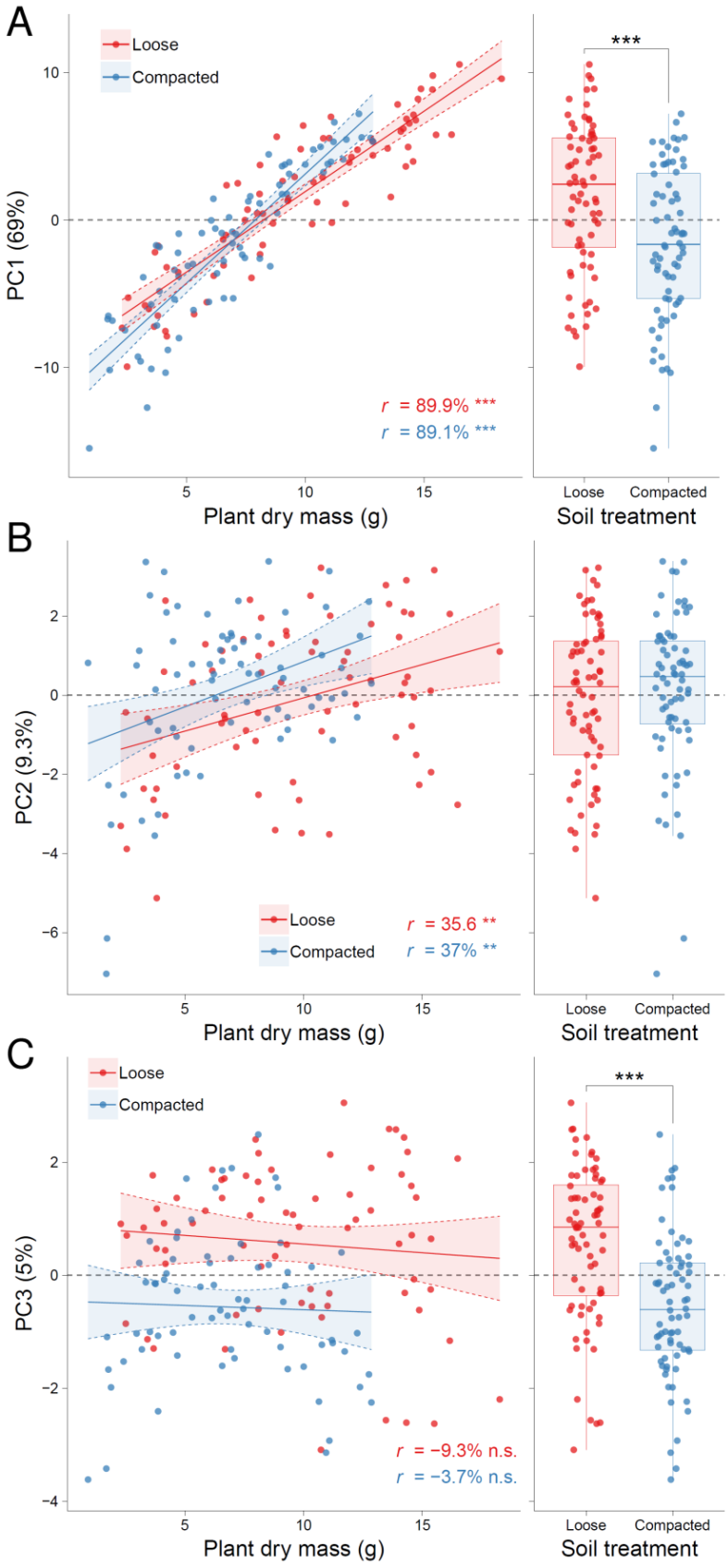


Fig. 4.6 - Results of PCA analysis, with the idea that PC1 mostly represents allometic effects, and the other non-allometric effects. (continued on the following page).

Fig. 4.6 - Results of PCA analysis, with the idea that PC1 mostly represents allometic effects, and the other non-allometric effects. PC3 indeed does not relate to biomass, but contains treatments effects (See discussion). Left plot: scatter plot between the first principal component (PC1) and plant dry mass. Right plot: box plot showing the distribution of PC1 according soil condition. *r*: Pearson's correlation coefficients between PC1 and plant dry mass. Red and blue: loose and compacted conditions, respectively. A t-test between loose and compacted conditions was done ($P < 0.001$ '***').

Discussion

Genetic variation have been observed in both plasticity responses to soil compaction. Some of this variation may be explained by plant size (apparent plasticity). One of the fastest growing sorghum genotypes under compaction, was also among the fastest growing once under controlled conditions, whereas tolerant genotypes that had near equal shoot size under both compacted and control conditions were relatively small. Although this may be perceived as a challenge in breeding for vigorous and soil compaction tolerant lines, we suggest that this requires further research as there is a possibility that the smaller lines at a higher seeding rate may yield as much or more than the larger lines. Having grown plants both in 1 plant and 2 plants per pot, we have no indication that plant density would affect the tolerance to soil compaction, but field research is necessary to confirm these ideas.

Genetic variation was not only observed for plasticity in shoot size related parameters, but also for plasticity in various root traits. Although all the genetic variation in all traits was correlated to over plant size (allometric, and thereby a form of apparent plasticity), especially the number of nodal roots (root number) and (fine) root length had strong true plasticity, which may be promising breeding. Larger trials however are necessary to determine the heritability of true root plasticity to soil compaction.

Shoot responses

Overall, compaction reduced shoot dry mass and leaf area (**Fig. 4.2** and **4.3**). The strength levels applied in both experiments (> 3 MPa) are considered as highly limiting for root growth (Pierce et al., 1983; Passioura, 2002; Bengough et al., 2011) and were high enough to affect the shoot growth of plants younger than four weeks justifying the screening for response to soil compaction (**Fig. 4.1** and **2**; **Table 4.1**).

Significant variation in shoot response to soil compaction existed among genotypes (**Fig. 4.2 and 4.3**). Even though inconsistencies in terms of shoot dry mass between **screening experiment** and **between-plant phenotyping** were found, plastic genotypes in **screening experiment** also were plastic in the **between-plant phenotyping** (Compare **Fig. 4.2A** and **4.2B**). This indicates that the screening was enough to find a consistent response among genotypes in young plants.

There was no clear association between observed shoot phenotype and the genotype's origin or breeding status (data not shown). However, the genotypes with the lowest shoot response, 'MOTA_MARADI' and 'EL_MOTA-S241', have been categorized as "Pre-flowering drought tolerant landraces" (**Table 2.1**). Those genotypes for **between-plant phenotyping** were not selected because their plants were very heterogeneous (variance coefficient ~ 30%). A reduction in shoot and leaf dry mass, and leaf area in response to soil compaction has been observed in several dicot and monocot crops under controlled and field conditions (Masle and Passioura, 1987; Beemster and Masle, 1996; Grzesiak et al., 2014).

The genotypic variation in shoot responses to soil compaction was correlated to shoot size under controlled conditions (**Fig. 4.1B**). Genotypes with large-sized plants under controlled conditions had greater reductions in leaf area than smaller sized genotypes. In general, soil compaction reduces the absorption of water and nutrients by the roots, which in turn results in lower plant biomass and crop yields (Håkansson et al., 1988; Passioura, 2002). In addition, it has been documented that there is genetic diversity in the responses of plants to soil compaction (Materechera et al., 1992; Colombi and Walter, 2017). As far as we are concerned, this is the first study that illustrates how the phenotypic responses to soil compaction correlate with the potential plant size of a genotype (see below for details).

The effects on shoots was evident from the second week after transplanting onward (**Fig. 4.3**).This early response is in agreement with what has been previously

observed in other works on seedlings and young plants growing in compacted soil (Goss and Russell, 1980; Masle and Passioura, 1987; Masle, 1992). The early response has been observed on seedlings and young plants (Goss and Russell, 1980; Masle and Passioura, 1987; Masle, 1992). The early response may be a factor to be considered as early vigor especially under conditions of topsoil compaction. The increased soil strength by soil crusting, when there is a formation of a seal at the soil surface, affects negatively both the seedling emergence and establishment (Awadhwal and Thierstein, 1985; Nortjé et al., 2012). Thus, seedling establishment of highly sensitive genotypes may be severely reduced and possibly may need to be compensated for by higher seeding rates. This is especially relevant for sorghum since it is said to be sensitive to crusting (Awadhwal and Thierstein, 1985).

Root responses

As it has been found in other crops (Pallantet al., 1993; Rengel and Wheal, 1997; Moran et al., 2000; Hund et al., 2009), the root length is dominated mostly by small-diameter roots. In the current study, almost the 75% of the total root length was represented by root whose diameters are less than 0.2 mm (the first two classes). Due to their importance, we refer as 'fine roots' all those roots with diameters ≤ 0.2 mm. These results are in agreement with several previous works that have shown the main influence of higher impedance by soil compaction is the decrease of total root length (Grzesiak et al., 2002; Bingham et al., 2010; Pfeifer et al., 2014) with a coinciding increase in root diameter (Eavis, 1972; Goss, 1977; Popova et al., 2016). Fine roots were the main and more sensitive component of total root length to soil compaction (**Fig. 4.4-6**) and given their functional importance, it possible to assume that their reduction has a great impact on root function. Due to their greater surface area per unit volume, fine roots are the principal pathway for nutrient and water uptake (Eissenstat, 1992; Comas et al., 2013). Additionally, they have significantly higher rates of respiration associated with a higher concentrations of N ([N]) than thicker roots (Eissenstat and Yanai, 1997; Pregitzer et al., 1998) and a relatively short lifespan, rapid turnover and quick decomposition (Jackson et al., 1997). Fine root production may be difficult under compaction due to the low availability of soil resources such as N and oxygen (Håkansson et al., 1988; Passioura, 2002; Tubeileh et al., 2003; Bengough et al., 2011). Based on that, the observed reduction of fine roots, if they are adaptive, may be related to an optimization strategy of carbon and/or soil resources. Plastic genotypes could avoid producing fine roots not only because

of their high cost under impeded conditions but also because they may be less efficient under compaction. Furthermore, keeping stable the SLA may both reduce the negative effects of compaction on carbon assimilation efficiency per biomass unit and may also help to support a thicker root system, which have a greater ability to explore hard soil (Bengough et al., 2011) and whose carbon cost is higher than those of finer roots (Eissenstat and Yanai, 1997).

Genetic diversity in response to soil compaction

Sorghum is recognized to have a wide diversity (Sinha and Kumaravadivel, 2016). In agreement, we found that phenotypic differences among genotypes account for a large portion of the observed phenotypic variation (**Table 4.1**, **Supplementary Tables S5** and **S6**). Accordingly, different sorghum genotypes are expected to differ in response to soil compaction, i.e. genotype-by-treatment interaction. In the **screening experiment**, we found a low correlation between the genotypic means of shoot dry mass in loose and those in compacted soil (n = 28 genotypes, r = 37%, P = 0.053), which is due to the significant genotype-by-environment interaction (**Table 4.1**). However, in the **between-plant phenotyping** experiment, there was no G×T interaction for shoot dry mass (**Supplementary Tables S5** and **S6**). The inconsistency between **screening experiment** and **between-plant phenotyping** may be due to several experimental and statistical factors such as different plant age and fewer genotypes evaluated. Additionally, G×T interaction was found in root length traits in **between-plant phenotyping**. Other traits with G×T interaction were the number of nodal roots (root number) and tillers. In these experiments, very fine roots were more affected than shoots (**Fig. 4.5**). For example, reduction in fine root length, total root length and root biomass were 50, 47 and 35%, respectively; whereas those for shoot biomass and leaf area were 29 and 25%, respectively. Additionally, there was genotypic diversity for response (**Tables 4.1**-**4.3**; **Supplementary Tables S5** and **S6**), which was correlated with biomass. In general, larger genotypes such as 'KORO_KOLLO' and 'HONEY_DRIP' were the more plastic and displayed the higher and earlier response to soil compaction in terms of length of fine roots and leaf area than smaller plant genotypes such as 'TXARG1' and 'SC599'. On the other hand, 'AJABSIDO', a "drought tolerant landrace" from Sudan (**Table 2.1**), was a genotype that was relatively large and had intermediate plasticity responses. Shoot and root biomass of 'AJABSIDO' in compacted soil were reduced by 25 and 35% compared to the loose control, respectively. Since the resistance to the penetration increases as the

soil water potential decreases (Whalley et al., 2005; Bengough et al., 2011), the tolerance mechanisms for compaction and drought may have co-evolved together and / or have the same genetic source (pleiotropy). However, further researches are necessary to establish this. If so, 'AJABSIDO' may be express an interesting "ideotypic phenotype" for both compaction and drought tolerance.

Therefore, the phenotypic responses to soil compaction correlates positively with the size of the plant in sorghum. However, not all the genotypes follow this trend.

Plant size effects on responses

In general, larger genotypes were more sensitive than those genotypes with potentially smaller plant sizes (**Fig. 4.1-4.4** and **4.6**). During the screening, selecting genotypes with different degrees of response had as a consequence that different plant sizes were also co-selected (**Fig. 4.1**).

Since R/S ratio decreases as bulk density increases and is correlated with plant dry mass, it is difficult to distinguish if this response is due to true or apparent plasticity especially when the screening dragged different plant sizes. It is known that smaller or younger plants generally have a greater Root/Shoot ratio (McConnaughay and Coleman, 1999; Weiner, 2004). On the contrary, plants under compaction had lower Root/Shoot ratio despite being smaller than plants in loose soils. Additionally, the number of leaves in the main axis was not significantly affected by compaction. Thus, impeded plants are not ontogenetically more delayed than non-impeded plants but just smaller in this experiment. The log-log relationship of shoot and root biomass (**Table 4.3**) showed soil treatments have different slopes indicating different allocation pattern, e.g. plasticity (Reich, 2002). The slopes mean that every decrease of one percentage in term of shoot mass is associated with a decrease of 1.37 and 1.7% in root mass for compacted and loose soil treatments, respectively.

The analysis of the bivariate log-log relationships can be generalized to multiple traits (Klingenberg, 1996). PCA has been proposed as a multivariate generalization of allometry where the first principle component generally represents the size effect (Jolicoeur, 1963; Somers, 1986). The results of this chapter are in accordance with the latter, since there was a high correlation between plant size andPC1 (**Fig. 4.6**). However, this influence, to a lesser extent, was also observed in PC2. Even though PC1 (69% of total variation) was highly correlated with plant dry biomass (r ~ 90%), it was also affected by soil treatment and genotype-by-treatment interaction (**Fig. 4.6**) revealing

plasticity in terms of PC1 variation. Additionally, the correlation between plant dry biomass and PC1 is affected by treatment effect (significant interaction between treatment and plant biomass, **Fig. 4.6**) indicating different allometric relationships among plants (at the similar developmental stage) at a multivariate level for loose and compacted condition. In other words, the correlation between plant biomass and any given trait varies depending on the degree of soil compaction, i.e., plasticity. PC2 (9% of total variation) did not have an effect of treatment at all. Furthermore, PC2 was given by variation of plant dry mass and genotypic effect. This indicates that 9% of observed variation across traits is explained exclusively by plant size.PC3 was not correlated with plant size, this PC was affected by treatment and interactions (**Fig. 4.6** and **Supplementary Material S2**). This indicates that just 5% of the observed variation across traits was given exclusively by plasticity. Thus, the effect of plant size on phenotype is not negligible and accounts for an important portion of the total variation. Additionally, this indicates there is a strong association between response to soil compaction and plant size. This may mean that they are genetically correlated, which may be attributable either to pleiotropic effects or to close linkage. This may drag consequences for plant breeding process, which should be studied, especially if it is assumed that tolerance is associated with a low shoot response.

Although plant size may explain a large proportion of the data, we conclude that effect of soil compaction and genotype were also important. For all the traits, genotype-by-treatment interaction explained a low proportion of the total phenotypic variation. As results of this, the screening led to different sizes of plants to be correlated with the response. Finally, the observed phenotypic changes in response to soil compaction are complex, both allometry (apparent plasticity) and plasticity are involved.

If these plastic responses were adaptive, they would be in some way linked to specific leaf area (SLA) and carbon metabolism. SLA has been positively correlated with leaf nitrogen (N) and net photosynthesis (Reich et al., 1998; 1999). Specifically, leaf N and SLA levels affect net photosynthesis, at any value of SLA or leaf N, net photosynthesis increases with increasing leaf N or SLA, respectively (Reich et al., 1998). Therefore, by keeping the SLA stable, net assimilation would remain constant, unless the N levels do not change. Root/Shoot and SRL are reduced in the denser soil whereas SLA is stable (**Supplementary Table S6**). In average, the reduction was higher in root biomass (35%) than shoot biomass (29%) and leaf area (25%). This would keep constant the investment of carbon per leaf area while root system tends to accumulate much more

carbon (per unit of length or mass) that they can use to growth, especially in ‘TXARG1’ and ‘SC599’ genotypes. Additionally, due to their high rates of respiration which is related to plant [N] (Eissenstat, 1992; Pregitzer et al., 1998), fine roots may be severely affected under compacted soils. Since the root production is not an option in impeded plants, these available resources can be used in other strategies to survive in compacted layers such as reduction of the friction between the root and soil surface by sloughing of root cells and/or secretion of (Bengough and McKenzie, 1997; Iijima et al., 2008; McKenzie et al., 2013), compensation of the absorbing surface loss by an increased efficiency of nutrient uptake (higher uptake per unit of root length) such as differential expression of high-affinity nutrient transporters. To assess whether these modifications would compensate for the reduction of the length of fine roots, more studies are needed.

5 - WITHIN-ROOT SYSTEM PLASTICITY AS A RESPONSE TO SOIL COMPACTION

In this chapter was tested whether plants are able to compensate the effect of very compacted layers with a higher root proliferation (e.g. a higher root length) where the best condition are found (e.g. looser and more superficial soil layers). To test this hypothesis, it was necessary to produce a set of heterogeneous vertical discrete gradients of soil density (different vertical layers of soil). These gradients were produced by building a system of vertical columns with six layers varying in soil bulk densities ranging from 1.3 to 1.6 g cm^{-3}. Six layers were stacked with either increasing or decreasing compaction with depth. Layers with 1.6 g cm^{-3} had a high soil strength, with a penetrometer reading of > 3.7 MPa. Roots responded locally to a dense soil layer by reducing the length of fine roots by ~ 65%. However, the degree of these responses not only depended on the plant genotype but also on how deep and compacted the soil layer was: deeper and denser soil layers hindered more the root growth. When the very compacted soil layer is located in the middle of the column, tolerant genotype had a high proliferation of fine roots in looser zones above the compacted layer (almost 2 times more roots than loose and homogenous soil columns). While the sensitive genotype did not express any proliferation at all. Additionally, in this chapter, the usefulness of these gradients to test if plants are able to compensate the effect of very compacted layers is discussed.

Results

Each cylinder was filled individually with soil according to the soil bulk density needed (**Fig. 2.3B**). In each compression step, the maximum force applied by the press was obtained. **Fig. 5.1** shows the relationship between the soil bulk density, maximum force applied and the penetration resistance measured *a posteriori* using a hand penetrometer. In average, penetration resistances of 0.22, 1.61, 3.66, 3.71 MPa were registered for densities of 1.3, 1.4, 1.5 and 1.6 g cm^{-3}, respectively.

Between-plant phenotype

At plant level, only differences in terms of genotype were found for shoot traits such as plant height, shoot dry mass, number of leaves, total leaf area and specific leaf area (**Table 5.1**, **Fig. 5.2**). For all the shoot traits, 'HONEY_DRIP' had always higher values than 'SC599'.In average, 'SC599' was 44.6 and 36.6% smaller than 'HONEY_DRIP' in terms of leaf area and shoot dry mass, respectively.

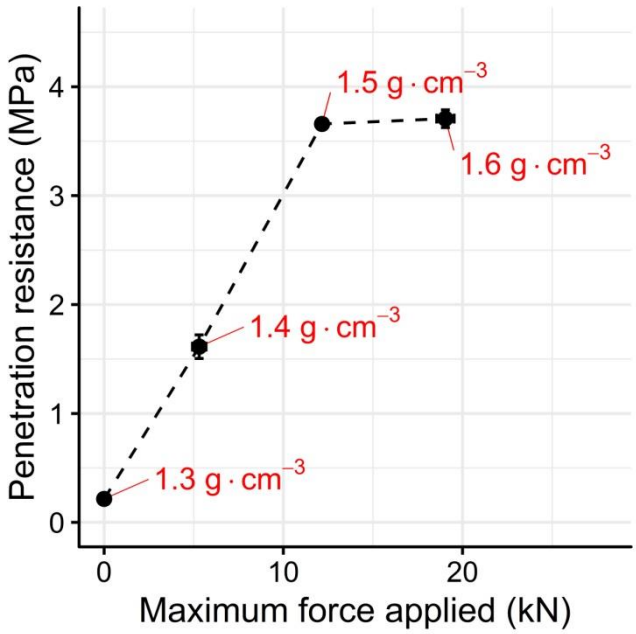


Fig. 5.1 - Resistance to root penetration. Relationship between the maximum force applied by the testing machine and the penetration resistance measured by a hand penetrometer at different soil bulk densities. The soil densities is indicated in red. Each dot is the mean value among 12 data points. Error bars are the standard error of the mean for both axes.

For root traits, column effect was significant for specific root length (SRL) and root diameter (**Table 5.1**, **Fig. 5.2**). The interaction effect between genotype and column was significant only for root length and SRL. Root dry mass was not affected by soil columns and only had differences between genotypes. As in the case of shoot traits, 'HONEY_DRIP' had longer and heavier roots than 'SC599': roots of 'SC599' were 32.2 and 46.2% shorter and lighter than 'HONEY_DRIP' in average (**Table 5.1**, **Fig. 5.2**). For 'HONEY_DRIP', "Control" columns had longer roots than compacted columns whereas 'SC599' the longer roots were found in the "Middle" columns (**Fig. 5.2**). SRL was higher

in “Control” columns for both genotypes. ‘SC599’ has higher values of SRL than ‘HONEY_DRIP’ and its lowest values was found in the bottom column for both genotypes and in the top column for ‘HONEY_DRIP’ (**Fig. 5.2**). Root diameter had a similar but opposite behavior than SRL (**Fig. 5.2**).

Table 5.1 - Relative contribution of Columns, Genotype and their interaction effects to the total variation of each trait

Trait	Variance explained by factors (%) [1]							
	Genotype		Column		G×T		Block	
<0.2	61.5	***	14.4	**	16.1	**	4.9	n.s.
[0.2, 0.8)	74.3	***	6.7	n.s.	6.8	n.s.	8.3	n.s.
≥ 0.8	80.5	***	1.0	n.s.	1.1	n.s.	16.2	***
Collar_DM	76.2	*	1.9	n.s.	5.5	n.s.	5.7	n.s.
Leaf_area	88.9	***	1.6	n.s.	5.1	n.s.	2.1	n.s.
Leaf_DM	88.5	***	1.3	n.s.	3.4	n.s.	4.5	n.s.
Leaf_number	73.1	***	3.5	n.s.	6.7	n.s.	11.6	*
Plant_DM	89.5	***	1.3	n.s.	3.4	n.s.	3.6	n.s.
Root/Shoot	10.7	n.s.	31.0	n.s.	9.4	n.s.	37.6	**
Root_diam	1.9	n.s.	59.5	***	6.9	n.s.	22.3	*
Root_DM	86.0	***	3.5	n.s.	1.5	n.s.	6.7	*
Root_length	66.9	***	8.9	n.s.	14.4	*	6.1	n.s.
Shoot_DM	85.9	***	0.5	n.s.	5.6	n.s.	4.8	n.s.
SLA	60.9	***	4.1	n.s.	13.5	n.s.	10.6	n.s.
SRL	30.1	**	35.4	***	13.3	*	17.4	**
Stem_DM	85.1	***	0.5	n.s.	5.7	n.s.	5.2	n.s.

[1] R^2: determination coefficient according to mean square results from ANOVA; G×T: Genotype-by-treatment effect. Significant codes (*P*-value):<0.001 ‘***’; 0.001-0.01 ‘**’; 0.01-0.05 ‘*’; n.s.: not significant (*P*> 0.05).

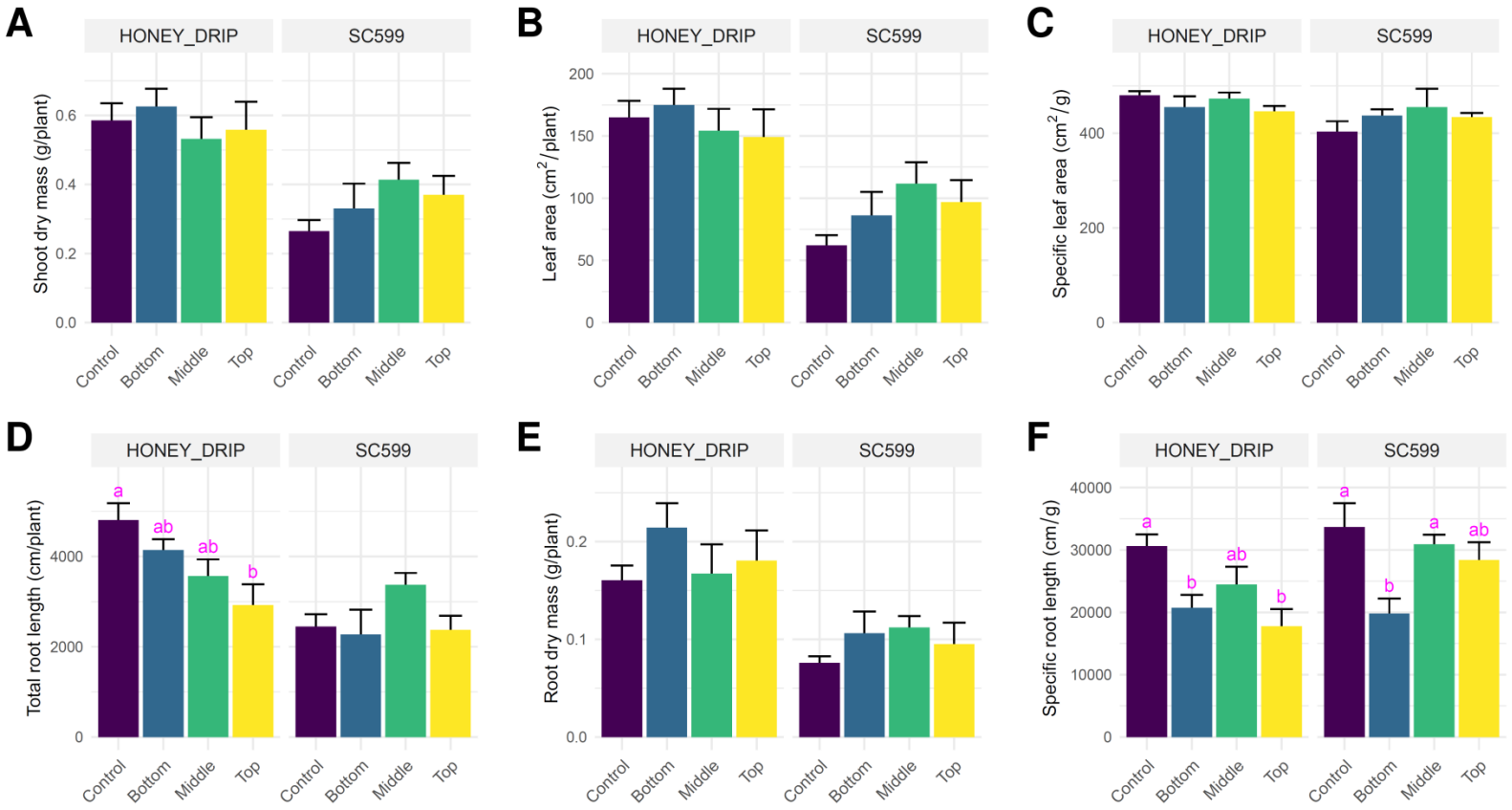


Fig. 5.2 - Between-plant phenotype of 3- to 4-week-old plants old of two sorghum genotypes growing in four soil columns with different soil density distribution each. Genotypes: 'HONEY_DRIP' and 'SC599'. Different letters (in magenta) indicate means ± standard error (error bars) of the mean with statistically significant differences between the soil columns within each genotype according to a two-way ANOVA and Tukey's multiple comparison tests (P<0.05)

By using the hierarchical cluster analysis of variables, three main diameter classes were found (i) length of roots whose diameter is less than 0.2 mm, (ii) length of roots whose diameter is greater than or equal to 0.2 and less than 0.8 mm, and (iii) length of roots whose diameter is greater than or equal to 0.8 mm (**Supplementary Fig. S7**). Root length is mainly made of very fine roots (diameter < 0.2 mm, **Fig. 5.3**). The length of very fine roots was affected by soil columns and their response depends on the genotype (significant interaction between genotype and column, **Table 5.1**). This was not the case for thicker roots (diameter ≥ 0.2 mm, **Fig. 5.3**), which only had genotypic differences (**Table 5.1**). On the other hand, genotypes had statistically equal Root/Shoot and this trait was not affected by soil columns (**Table 5.1**).

Within-root system phenotype

At within-root system level only root traits were evaluated: total root length, root dry mass, root average diameter and specific root length. **Table 5.2** shows the significance and relative importance on the phenotype of level position. For all the root traits, this effect was relatively important in terms of within-root system (or subject) variance. However, the effect of position varied according the column (significant interaction between level position and column). Thus, the observed root phenotype at a given level not only is affect by the position of the cylinder (or level) but also by different distribution of soil density (columns).

'HONEY_DRIP' had longer roots and a higher exploration of lower column levels than 'SC599'. In average, the 75% of the total root length is located in the first three and two levels for HONEY_DRIP' and 'SC599', respectively (**Supplementary Fig. S8**).

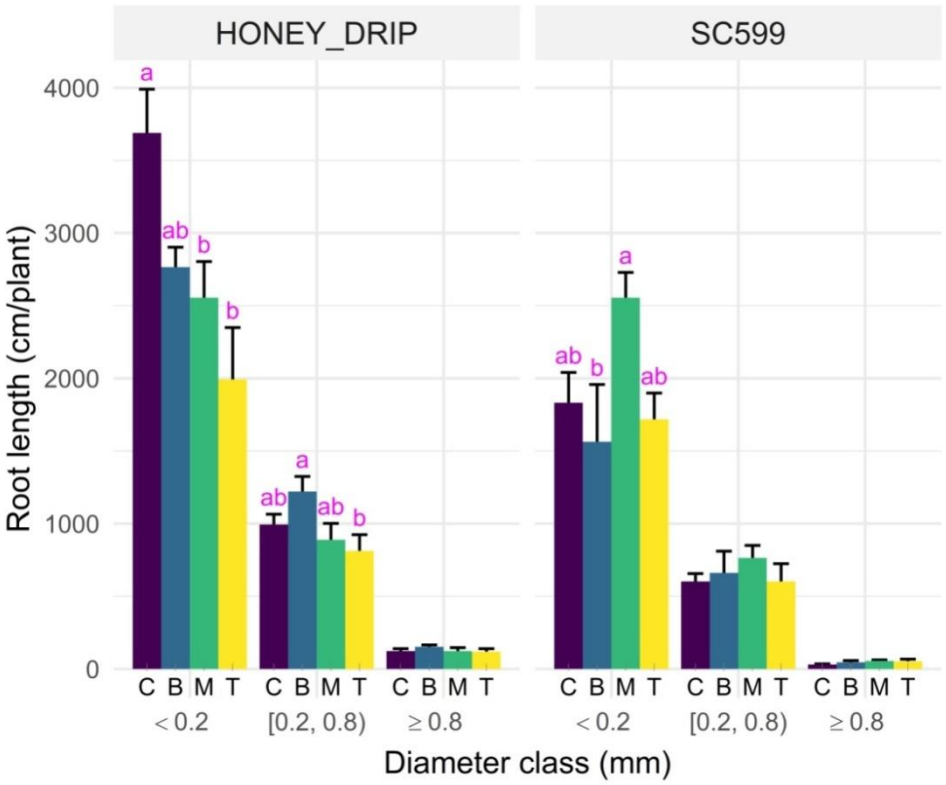


Fig. 5.3 - Distribution of root length according the root diameter classes for the two genotypes growing in four soil columns with different soil density distribution each. Genotypes: 'HONEY_DRIP' and 'SC599'. Columns are indicated as C ("Control"), B ("Bottom"), M ("Middle") and T ("Top"). The root length were recorded in 25 ranges of root diameter between 0 and 2.5 mm (diameter classes) and later it was grouped into three main classes (< 0.2, [0.2, 0.8) and ≥ 0.8) based on a cluster analysis (**Supplementary Fig. S7**). Different letters (in magenta) indicate means ± standard error (error bars) of the mean with statistically significant differences between the soil columns within each genotype according to a two-way ANOVA and Tukey's multiple comparison tests (P<0.05).

For root length, the genotypic effect was significant for all the levels. L2 was where the highest length and column effect were found (**Fig. 5.4**, **Table 5.3**). Significant interactions between genotype and column (Genotype×Column) were only found in L2 (**Table 5.3**). For both genotypes and all the column, the highest concentration of root length is located in L2 followed by L1 (**Fig. 5.4**). When the highly compacted layer is located in the middle (column M), 'SC599' had almost the 60% of total root length in L2 (**Supplementary Fig. S8**), which are even longer than 'HONEY_DRIP' under control conditions at the same column level (**Fig. 5.4**). In addition, control columns had always longer roots than

compacted columns from L4 to L6. In general, root length had the lowest values in those levels where the highest soil density is found. The exception was the "Top" column of 'SC599' (**Fig. 5.4**), which in L2had similar values to those of "Control" and "Bottom" column. However, this genotype had the lowest length (non-significant) in L3 of "Top" column and at lower levels it shows even a sort of recover with longer root than "Bottom" and "Middle" columns.

Table 5.2 - Relative contribution of level position and column treatment on the variation of root traits at within-plant level.

Trait	Genotype	Variance explained by factors (%) [1]	
		Position	Position×Column
Root_length	Honey Drip	96.4 ***	3.0 ***
	SC599	95.8 ***	3.5 ***
Root_diam	Honey Drip	94.0 ***	4.3 **
	SC599	92.4 ***	5.5 **
Root_DM	Honey Drip	91.7 ***	4.7 ***
	SC599	96.1 ***	3.0 ***
SRL	Honey Drip	89.9 ***	7.2 **
	SC599	94.7 ***	3.8 **

[1] R^2: determination coefficient according to mean square results from ANOVA; Significant codes (*P*-value):<0.001 '***'; 0.001-0.01 '**'.

Root diameter increases from L1 to L6 (**Fig. 5.4**). "Bottom" column had the highest values at from L4 to L6. Genotypic effect was in L2 while column effect was found from L2 to L6. Genotype×Column was significant only in L2. In this trait is clear the results of **Table 5.3**, the phenotype not only is affected by level position but also by the degree of soil density.

Root dry mass decreases from L2 to L6 (**Fig. 5.4**). In L2 and L3, the "Control" column had the lightest roots among all the columns. Differences between both genotypes in terms of root dry mass were clear in all the levels (from L1 to L6, **Table 5.3**). Column effect was found only in L2 and L5. No significant Genotype×Column was observed.

In general, SRL decreases from L2 to L6 (**Fig. 5.4**). "Control" columns had the

highest SRL in all the levels but "Middle" column in L2 reached similar values. Differences between columns in terms of SRL were found from L2 to L6 (**Table 5.3**).

In general, for all the levels, genotypic effect was the most important followed by column effect and Genotype×Column (average R^2 among traits ~ 50, 24 and 7.5 %, respectively, **Table 5.3**). Root biomass and length were the traits in which the genotypic effect was the higher (average R^2 among levels per trait ~ 76 and 71%, respectively). In average, L1 had the highest genotypic effect whereas L2 had the lowest one (average R^2among levels ~73 versus 25%, respectively). SRL and root diameter were the traits with the higher column effect (average R^2 per trait ~ 37 and 36%, respectively). L2 and L6 were the levels with the highest and lowest column effect, respectively (average R^2 per level ~42 and 12%, respectively).The highest genotype-by-column interaction effect was found for SRL, root diameter and length in L2 (level R^2 ~ 35, 29 and 19%, respectively).

Fig. 5.5 shows the relative differences between "Control" column and heterogeneous columns in each level (from L1 to L6) in terms of reduction in fine root length (diameter < 0.2 mm). For both genotypes, "Bottom" column had the lower reduction of fines root length in almost all the levels, but in L6 (where the highest soil density is located) had the higher decrease. "Middle" columns had intermediate reductions but also had an increase in L1 and L2, especially for 'SC599', which expressed a very high increase in fine root length: ~ 90% higher than control condition in L2. "Top" column had higher and lower reduction of fine root length in upper and lower levels, respectively.

Table 5.3 - Relative contribution of Soil density level, Genotype and their interaction effects to the total variation of each trait by column level.

Column level	Factor	Variance explained by factors (%) [1]							
		Root_length		Root_diameter		Root_DM		SRL	
L1	Genotype	55.4	*	66.7	**	84.5	***	87.1	***
	Column	13.8	n.s.	11.0	n.s.	4.7	n.s.	2.7	n.s.
	Genotype×Column	10.3	n.s.	4.8	n.s.	0.4	n.s.	3.9	n.s.
	Block	10.9	n.s.	8.9	n.s.	6.6	n.s.	0.0	n.s.
L2	Genotype	35.8	*	0.7	n.s.	62.4	**	2.1	n.s.
	Column	30.3	**	59.5	***	25.6	*	51.8	**
	Genotype×Column	19.3	*	29.1	*	2.2	n.s.	35.5	*
	Block	7.7	n.s.	3.4	n.s.	2.3	n.s.	1.2	n.s.
L3	Genotype	79.1	***	12.7	n.s.	55.8	**	10.1	n.s.
	Column	9.3	*	47.3	**	11.6	n.s.	61.8	***
	Genotype×Column	4.6	n.s.	14.1	n.s.	3.7	n.s.	8.0	n.s.
	Block	3.8	n.s.	14.9	n.s.	24.2	*	11.5	n.s.
L4	Genotype	83.2	***	4.4	n.s.	81.2	***	15.0	*
	Column	10.6	**	47.8	***	6.0	n.s.	60.4	***
	Genotype×Column	2.3	n.s.	8.3	n.s.	3.8	n.s.	3.8	n.s.
	Block	2.3	n.s.	33.8	*	3.2	n.s.	18.5	**
L5	Genotype	89.5	***	17.6	n.s.	86.0	***	57.5	***
	Column	4.4	*	32.5	*	4.7	*	25.3	***
	Genotype×Column	2.2	n.s.	11.2	n.s.	4.4	n.s.	4.1	n.s.
	Block	2.6	n.s.	31.0	*	3.2	n.s.	10.3	n.s.
L6	Genotype	85.4	***	3.0	n.s.	87.0	***	40.5	***
	Column	7.3	**	20.0	**	1.7	n.s.	19.1	**
	Genotype×Column	1.7	n.s.	1.6	n.s.	0.9	n.s.	0.9	n.s.
	Block	4.2	n.s.	71.5	***	8.3	n.s.	36.8	**

[1] Coefficient of determination (R^2) according to mean square results from ANOVA.

Significant codes (*P*-value):<0.001 '***'; 0.001-0.01 '**'; 0.01-0.05 '*'; n.s.: not significant (*P*> 0.05).

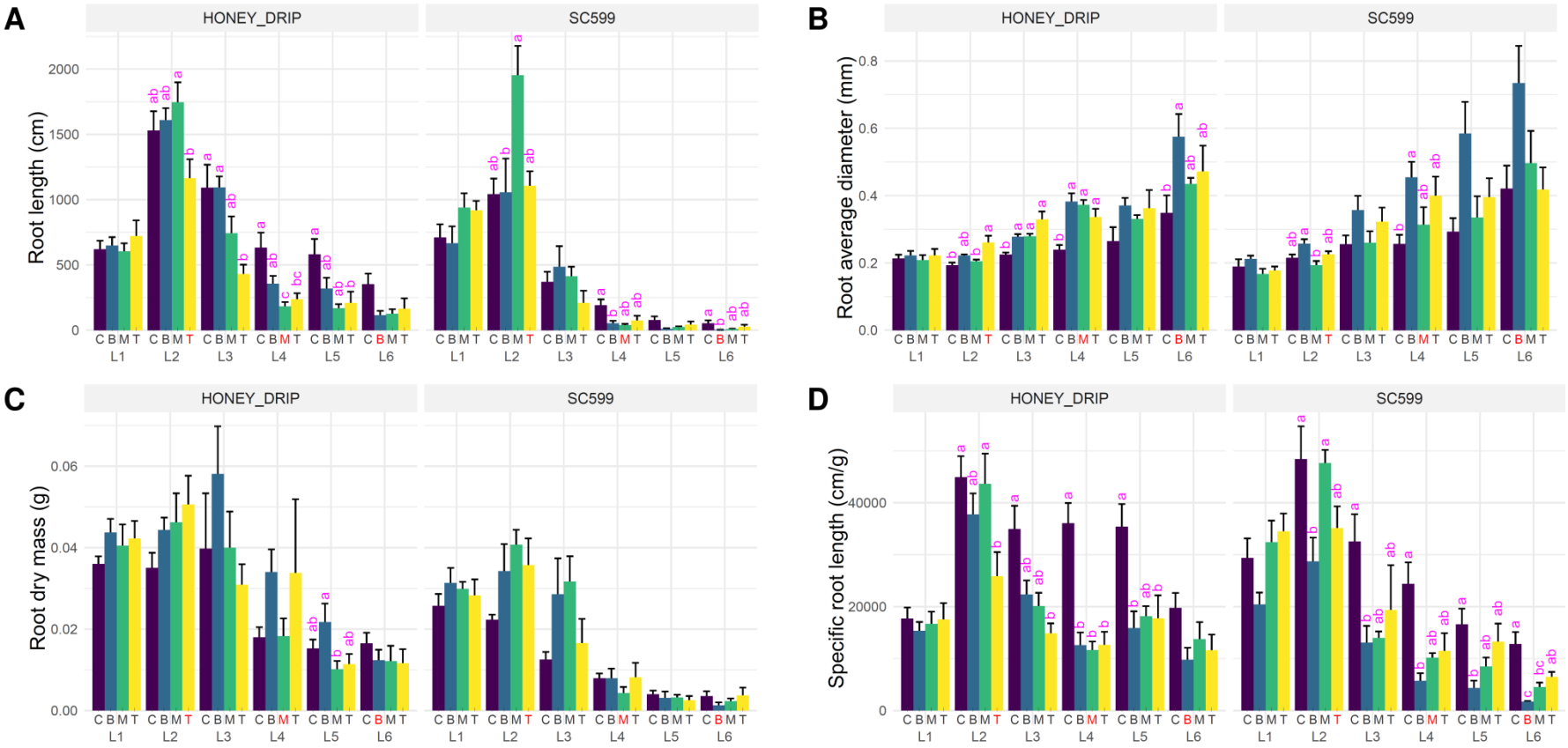


Fig. 5.4 - Within-root system phenotype of 3- to 4-week-old plants old of two sorghum genotypes growing in four soil columns with different soil density distribution each. Genotypes: 'HONEY_DRIP' and 'SC599'. Column levels (cylinders) are indicated from the top to the bottom as L1, L2, L3, L4, L5, and L6 in the *x*-axis. Columns are indicated as C ("Control"), B ("Bottom"), M ("Middle") and T ("Top") within each column level. In red is highlighted the column label in those levels where the highest density layer was located for each column. Different letters (in magenta) indicate means ± standard error (error bars) of the mean with statistically significant differences between the soil columns within each genotype according to a two-way ANOVA and Tukey's multiple comparison tests ($P<0.05$).

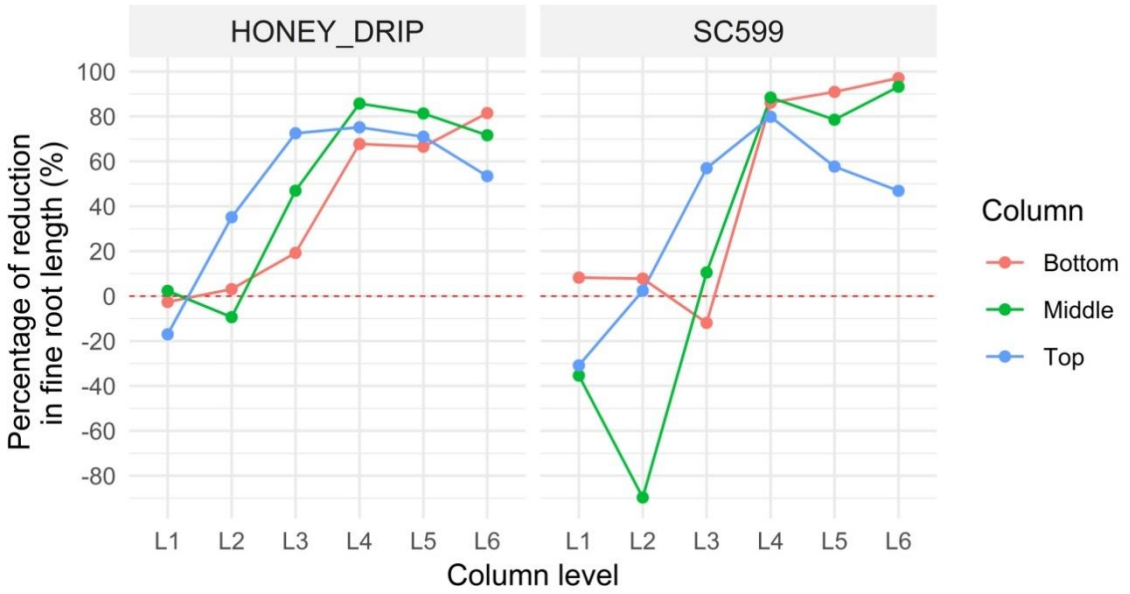


Fig. 5.5 - Relative differences in length of fine root (diameter <0.2 mm) between column "Control" and heterogeneous columns ("Bottom", "Middle" and "Top"). The arithmetic difference in length of the fine root was calculated at each column level and then expressed as a percentage of the length of the fine root in the "Control" column at that level. Thus, if a heterogeneous column has a shorter length of fine roots than the "Control" column at a given level, the difference will have a positive reduction percentage. Genotypes: 'HONEY_DRIP' and 'SC599'. Columns are indicated as B ("Bottom"), M ("Middle") and T ("Top").

Discussion

Using this soil column system, a linear relationship between the maximum force applied by the hydraulic press and the resistance to the penetration registered by a hand soil penetrometer was found. Thus, as the soil density increases, both force and resistance increases linearly (**Fig. 5.1**). However, this trend is broken at 1.6 g cm^{-3}, ~ 20 kN and 3.7 MPa. This resistance level is very close to the maximum values given by the penetrometer (4 MPa), which would explain the broken linear tendency in **Fig. 5.1** and indicate that the actual resistances at 1.6 g $cm^{-3}$are bigger than 3.7 MPa. Additionally, at bigger densities, for example 1.7 g cm^{-3}, the cylinders are broken by the compression at 22 kN. This was

the main reason why we finally used 1.6 g cm^{-3} as a very high soil density level. The resistance levels applied in 1.5 and 1.6 g cm^{-3} (> 3.6 MPa) are considered as highly limiting for root growth (Pierce et al., 1983; Passioura, 2002; Bengough et al., 2011). These values were higher than those found for 1.8 g cm^{-3} (3.2 MPa) using the same soil but in standard pots in previous experiments (Correa, 2019). Unlike those pots, regardless of the density of the soil (≤ 1.6 g cm^{-3}), the cylinders showed no symptoms of having been deformed by the compression. Thus, the resistance of the cylinders to be deformed would be adding more strength to the already soil resistance to the penetration, which would explain these high resistance values. Thereby, the combination of hydraulic compression and modular columns made of PVC cylinders is proposed as a suitable and simple system to study the compaction of soil, with which we could reach very high values of soil resistance and arrange different vertical and discrete gradients of soil density.

Between-plant phenotype

The reduction shoot dry mass, stem diameter, plant height, total leaf area, leaf dry mass and specific leaf area by effect of soil compaction has been observed in several crops such as barley, faba beans, maize, peas, sugar beet, sunflower, tomato, triticale, wheat, among others, under controlled and field conditions (Masle and Passioura, 1987; Beemster and Masle, 1996; Lipiec et al., 1995; Hussain et al., 1999; Grzesiak et al., 2014). However, despite the high levels of soil resistance at the higher soil densities, no effect of soil columns on shoot traits was found (**Table 5.1**, **Fig. 5.2**). Discarding the possibility of having no effects on the roots, there are two possibilities to explain this: (i) the roots that are not affected by the compaction (in looser layers) are able to compensate the negative effect of the highly dense layers; and (ii) the negative effect of very dense layers is minimal compared to the beneficial effect of less dense layers.

The results obtained in this chapter are in agreement with several previous works that have shown a decrease of total root length (Grzesiak et al., 2002; Bingham et al., 2010; Pfeifer et al., 2014) with a coinciding increase in root diameter (Eavis, 1972; Goss, 1977; Popova et al., 2016) as main effects of soil compaction on roots. Unlike shoots, root traits responded to the different gradients of soil densities (column effect, **Table 5.1** and **Fig. 5.2**). In general, 'HONEY_DRIP' had longer and heavier roots than 'SC599' but 'SC599' has higher values of SRL than 'HONEY_DRIP' (**Fig. 5.2D**). Root dry mass was not affected by columns but total root length, root diameter and specific root length (SRL)

were. The degree of response of root length and SRL varied for each genotype (significant genotype-by-column interaction, **Table 5.1**).

'HONEY_DRIP' displayed a clear response pattern: the longest roots were found in "Control" column whereas columns with heterogeneous soil had shorter roots. Additionally, "Bottom" column had longer roots than the "Middle" column, which in turn have longer roots than "Top" column. This indicates that the closer the highly compacted layer to the soil surface is, the shorter the root length per plant is.

'SC599' displayed a different pattern: the shorter roots was found in the "Bottom" column whereas the longest in the "Middle" column. Both "Control" and "Top" columns had intermediate lengths. Even though 'HONEY_DRIP' had longer roots than 'SC599' in almost all the columns, both genotypes have similar root length when the highly compacted layer is located in the middle ("Middle" column). Thus, dissimilar soil density distributions had different consequences in terms of root length.

'HONEY_DRIP' and 'SC599' had similar root diameters (average per plant) and for both genotypes, the largest diameters were found in the "Bottom" and "Top" columns, while the "Control" column had the smaller ones. The opposite was found for specific root length, with "Control" column displaying the higher values and "Bottom" and "Top" columns the lowest ones. This would indicate that there should be a greater colonization of very fine roots in the "Control" column than in the "Bottom" and "Top" columns, thus, the distribution of soil density affects the production of fine root at plant level.

Almost 75% of the total root length per plant is made up of length of very fine roots (diameter < 0.2 mm, **Fig. 5.3** and **Supplementary Fig. S9**). Therefore, it is not surprising that the length of very fine roots showed a similar pattern than total root length per plant for both genotypes (**Fig. 5.2-5.4**). In addition, very fine roots were the only root diameter class with significant differences between columns (**Table 5.1** and **Fig. 5.3**). All this indicates that very fine roots are the most important component both in terms of length and in the response to soil compaction.

Therefore, unlike shoots, roots responses to the presence of a dense layer of soil. In addition, the degree of response depends on distribution of soil density through the column. Very fine roots are an important component of the root system in sorghum, both structurally (in terms of length proportion) and functionally (in terms of responses to different soil density gradients. Additionally, for 'HONEY_DRIP' the closer the more compact layer is to the soil surface, the shorter the root length. The latter does not happen for 'SC599' roots, which surprisingly proliferates more when the highly compacted layer

is located in the middle of the column ("Middle" column).

Within-root system phenotype

Table 5.2 shows that the observed root phenotype at a given level not only is affected by the position of the cylinder (or level) but also by different distribution of soil density (columns). Additionally, the highest root proliferation and effect of columns and genotype-by-column interaction in terms of SRL and length were found in L2 (**Table 5.3**). Thus, the effect of the vertical distribution of soil densities is mainly expressed by phenotypic differences in L2.

Inconsistencies were found between the phenotypic response per plant (**Table 5.1**) and per level (**Table 5.3**). While, at between-plant level, there were no significant Genotype and Genotype×Column effects for root diameter, those effects were found within L1 and L2, respectively. For root dry mass, only genotypic and block effects were found at between-plant level; but L2 and L5 had a significant column effect. Additionally, Columns and Genotype×Column effects were higher in L2 (R^2, **Table 5.3**) than at plant level (**Table 5.1**).This indicates that phenotypic response to soil strength at each column level may be masked when the phenotype is evaluated at plant level. A similar phenomenon was found by Goss (1977) in barley, who found that if the main root apex is exposed to a very compacted layer, the unexposed lateral roots express a much greater length than lateral roots of plants growing in a homogenous loose soil (no gradient). As a result, the total root dry mass was similar between unaffected and the impeded root systems (Goss, 1977).Thus, the negative effect of a very compacted soil layer (for example, poor root growth) may be compensated by a greater root growth in those soil layers with looser soils. Also in barley, Pfeifer et al. (2014) observed that rooting depth of roots under loose soil in split rhizobox (compacted and loose soil) was significantly greater than that under uniform loose condition (loose substrate in both compartments of the split system). Thus, a compensatory root growth may result when a compacted soil layer impedes part of the root system. This is analogous to that observed in other environmental stresses.

Different parts of the root system regulate the total resource uptake of whole root system. Thus, the resource uptake deficit in those root system parts localized in resource-poor patches may be compensated. This compensation is done by an increased uptake rate and/or an enhancement of lateral root proliferation in root system portions localized in resource-rich patches (Crossett et al., 1975; Goss, 1977; Shani et al., 1993; Waisel and

Eshel, 2002; Rubio and Lynch, 2007; Pfeifer et al., 2014; Dara et al., 2015; Wang et al., 2016). This is clear for root length of 'SC599', which expressed a relatively high roots lengths in L2 of "Middle" column (**Fig. 5.4**), even longer than 'HONEY_DRIP' (which has longer roots per plant). Thus, 'SC599' grew 90% more fine roots, in terms of root length, than L2 of "Control" column (**Fig. 5.5**). In some way, those roots were able to detect a very high soil strength at L4 and proliferated in L2 where soil is looser. Additionally, this genotype shows at lower levels of "Top" column a sort of recover with longer root than "Bottom" and "Middle" columns (**Fig. 5.4-5.5**). The same can be seen for 'HONEY_DRIP' but in a much lesser extent. In previous experiments (Correa 2019), using homogeneous soil conditions, shoots of 'SC599' do not response to soil compaction (1.8 g cm^3) while roots have a relatively low response, which is exclusively given by the reduction in length (in a 40.5%) of very fine roots (diameter < 0.2 mm).

The current results suggest that the relatively higher root proliferation of this genotype in L2 in "Middle" columns is not accompanied by a higher percentage of fine roots and that this proportion is similar than those found in "Control" columns (**Supplementary Fig. S9**). Therefore, in sorghum, the length of very fine roots is reduced in conditions of high soil resistance, while more roots are produced in looser layers without favoring any particular root (at least in terms of root diameter classes). Furthermore, the degree of these responses have a clear genetic basis.

In this chapter, we showed that roots are able to respond locally to a dense soil layer by reducing the length of fine roots. However, the degree of these responses not only depended on the plant genotype but also on how the different layers are vertically distributed though the column. Thus, deeper and denser soil layers hindered more the root growth. Additionally, we found that when the very compacted soil layer is located in the middle of the column, a tolerant genotype had a high proliferation of roots in looser zones above the compacted layer (almost 2 times more roots than loose and homogenous soil columns). Based on these findings, we propose that the global plasticity of the root system to soil compaction may be divided into to two kinds. First, (i) local response, where the root system responds locally to the presence of a compact layer of soil reducing the length of fine roots. Second, (ii) response at a distance, situation in which the root system produces more roots in those layers where the conditions for growth are better (looser and more superficial layers) as soon the root detects a compact layer. This would involve a

complex system of sensing and communication between the different components of the root system that should be studied in greater depth in future research.

6 - Consequences of soil compaction for soil properties and plant function

In this chapter, it is tested if those phenotypes with a lower RSA plasticity also express a lower nutrient and/or water uptake per unit of root length than those phenotypes with higher RSA plasticity. For that, a simulation-based research was carried out to study the functional consequence of the structural response to soil compaction. In the previous chapters, an important genotypic variation in term phenotypic plasticity to soil compaction has been reported in the previous chapters. To understand how the different phenotypes influence the ability of the plant to take up nutrients, a simulation study was conducted in which 1) the observed phenotypes were reconstructed *in silico* using the functional and structural model OpenSimRoot, and 2) the water a nutrient uptake by these phenotypes were simulated for different soil compaction scenarios. To achieve this we 1) extended the model to simulate compacted soil, 2) parameterize OpenSimRoot for the various sorghum phenotypes, and 3) ran the simulations for the different compaction treatments.

Results

Soil models

The first consequence of increasing the organic matter content of the soil is the reduction of density (**Fig. 6.1**). This effect is even more marked as the porosity of the soil increases. As the soil is more sandy (higher *S/C*, **Fig. 6.2**), the soil density increases to some extent. After this point, the density begins to decrease as S/C increases. Nevertheless, as the organic matter increases, this relationship is inverted. Additionally, the more sandy the soil, the higher the water conductivity at saturation. A soil with a given texture (expressed as *S/C*), as the organic matter increases, the water conductivity at saturation also increases.

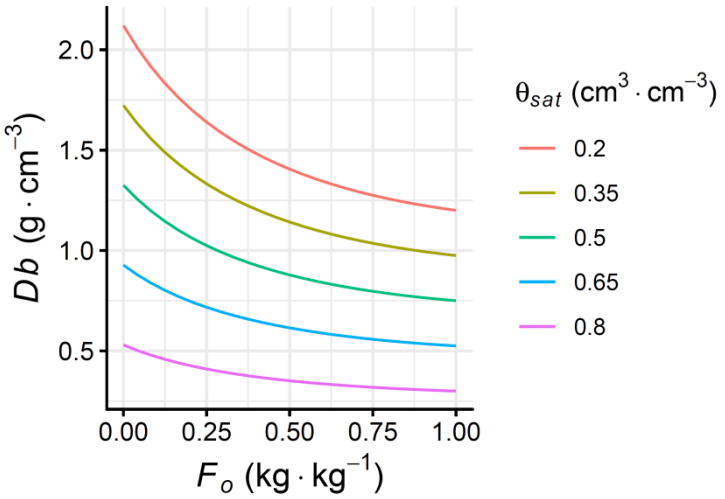


Fig. 6.1 - Effect of soil organic matter and porosity on soil density. *Db*: soil density; F_0: organic fraction of soil mass; θ_{sat}: soil volumetric content at saturation (as an estimation of soil porosity).

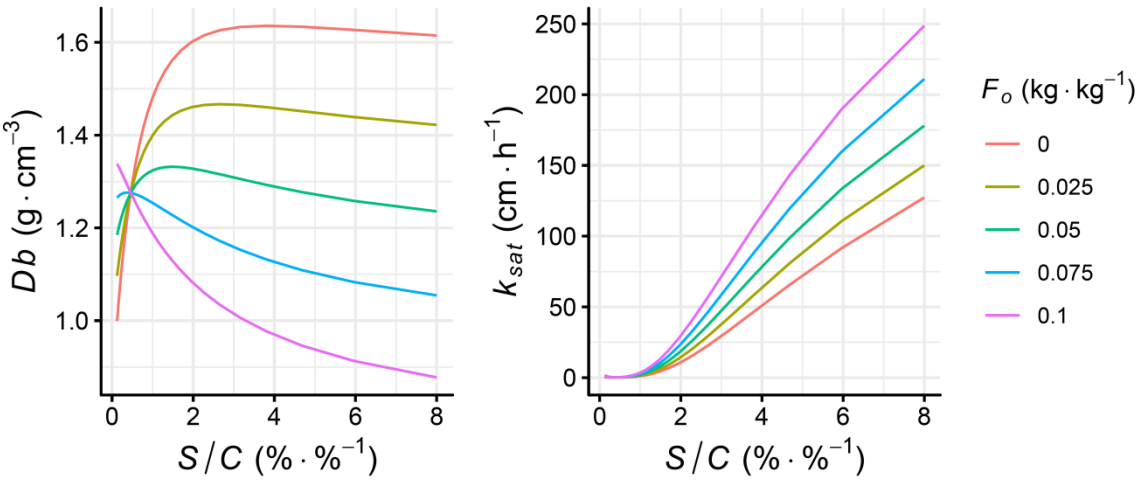


Fig. 6.2-Effect of soil texture and organic matter content on soil density and water conductivity. *Db*: soil density; *S/C*: ratio of sand to clay content (percentage in soil mass); k_{sat}: soil volumetric water content at saturation; F_0: organic fraction of soil mass.

As the soil density increases the volumetric water content at saturation decreases linearly (**Fig. 6.3**). However, at the same density (and texture), as the organic matter increases the water content at saturation decreases. The soil-water characteristic curve (SWCC) for a given soil describes the relationship between water content and potential.

Thus, as the water potential increases (absolute value), the water content should be reduced (**Fig. 6.4** and **Table 6.1**). Those curves were used in the OpenSimRoot simulations.

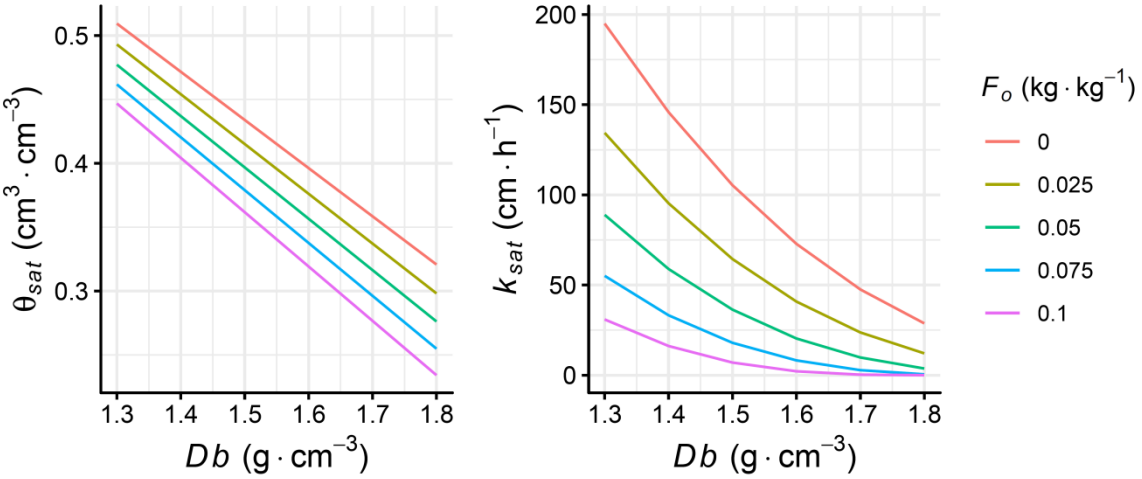


Fig. 6.3 - Effect of soil density and organic matter content on water content and conductivity. *Db*: soil density; θ_{sat}: soil volumetric content at saturation; k_{sat}: water conductivity at saturation; F_0: organic fraction of soil mass.

Table 6.1 - Simulated soil parameters used as input in OpenSimRoot.

Soil condition	k_{sat} (cm h^{-1})	θ_{sat} (cm^3 cm^{-3})	θ_r (cm^3 cm^{-3})	α	n	R^2 (%)[1]
Loose	113.6	0.51	0.09	0.0378	2.3424	99.3
Compacted	17.6	0.47	0.08	0.0663	1.9897	99.0

k_{sat}: water conductivity at saturation; θ_{sat}: soil volumetric content at saturation; θ_r: residual volumetric water content ; α and n: coefficients of van Genuchten's (1980) soil-water characteristic curve; R^2: Coefficient of determination for van Genuchten's (1980) soil-water characteristic curve.

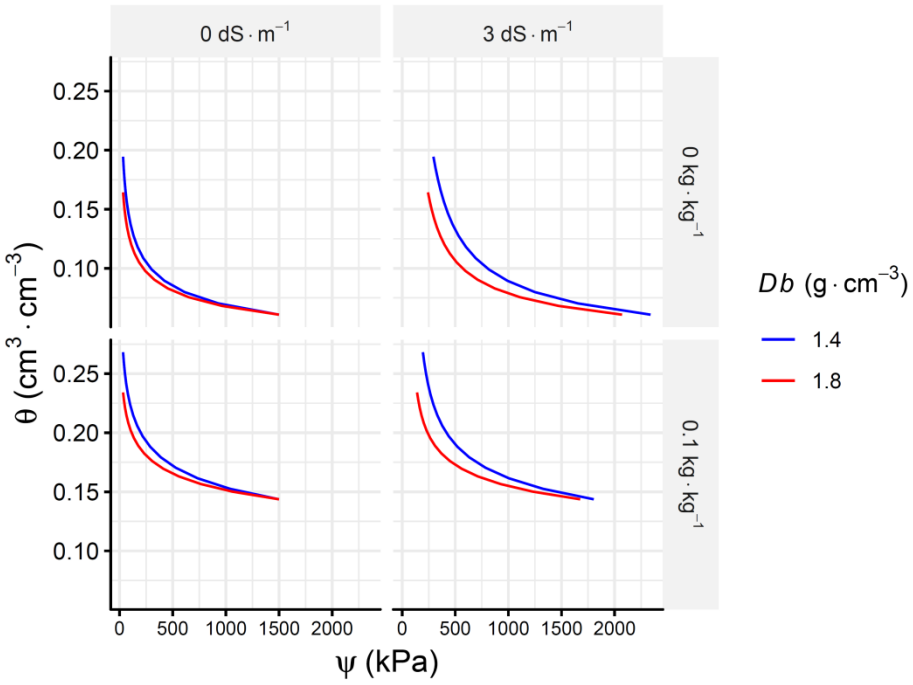


Fig. 6.4 - van Genuchten's (1980) soil-water characteristic curve according to soil density, organic matter content and electric conductivity. θ: soil volumetric water content; ψ: soil water potential; *Db*: soil density. Rows: organic fraction of soil mass. Columns: soil electrical conductivity. Those curves were used in OpenSimRoot simulations (**Table 6.1**).

As water potential increases (absolute value), soil resistance increases (**Fig. 6.5**). The slope of this curve depends largely on the density of the soil. Thus, the increase in resistance for each increase in a unit of water potential is even more pronounced at higher densities. In addition, both organic matter and salinity (electrical conductivity) affect this curve. When soil salinity and / or organic matter is reduced, the differences in soil resistance between loose and compacted soil are minor. The effect of soil salinity is clearly additive by increasing both the minimum and maximum water potential reached by the curve (see **Equation 21** and **22**, **Supplementary Material S3**).

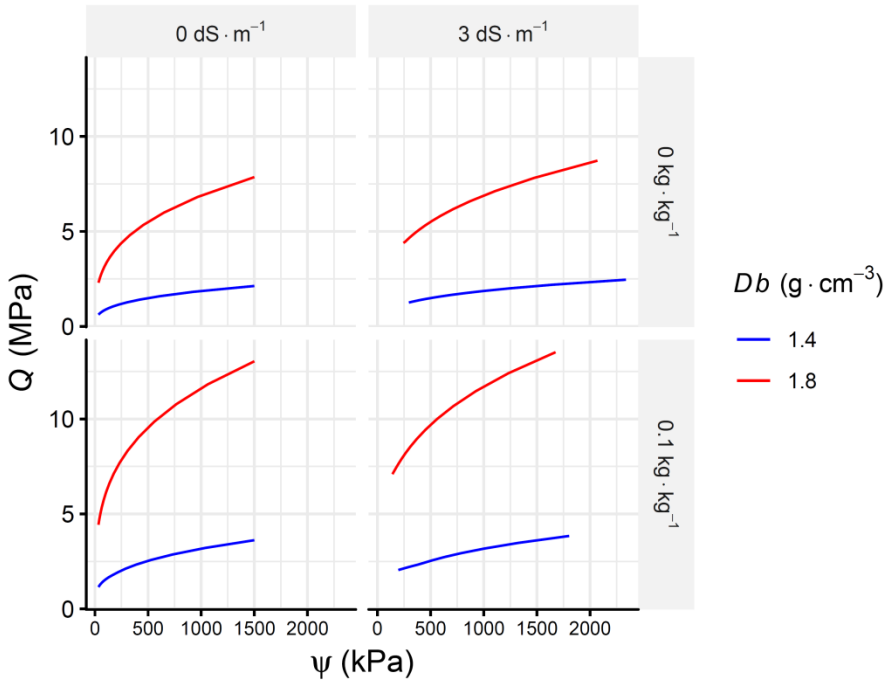


Fig. 6.5 - Effect of soil water potential on soil strength according to density, organic matter content and electric conductivity. *Q*: soil penetration resistance; *Db*: soil density. Rows: organic fraction of soil mass. Columns: soil electrical conductivity. *Q* was estimated based on Gao et al. (2016b).

Root simulations

The effect of soil compaction on phenotype was not significant (**Table 6.2**). Structural traits only showed a significant effect of phenotype. On the other hand, nitrate and phosphorus uptake not only depends on phenotype but also on the degree of compaction (soil-by-phenotype interaction). The effect of this interaction was more important on nitrate uptake. Structural traits such as root length and leaf area did not show effect of compaction, but functional ones did (water and nutrient uptake). This is due to direct effect of soil compaction on the phenotype was not simulated. Instead, the structural phenotype was established *a priori* and placed in loose or compacted soil.

Table 6.2 - Effect[1] of soil compaction on traits of 45-day-old plants of sorghum.

Trait	Soil	Phenotype	Soil × Phenotype
Root length	0.1 n.s.	99.6 ***	0.0 n.s.
Leaf area	8.5 n.s.	79.5 **	0.4 n.s.
Shoot dry mass	2.3 n.s.	89.4 **	0.4 n.s.
Root water uptake	9.7 n.s.	77.7 **	0.4 n.s.
Root nitrate uptake	1.4 n.s.	12.1 *	84.4 ***
Root phosphorus uptake	1.2 n.s.	96.3 ***	2 *

[1]Determination coefficient (R^2) according to mean square results from two way ANOVA. Soil × Phenotype: soil-by-phenotype interaction effect. Significant codes (*P*-value):<0.001 '***'; 0.001-0.01 '**'; *n.s.*: not significant (*P*> 0.05).

At 45 days after germination (DAG), reference-phenotype and tolerant-response plants have similar shoot biomass and leaf area, but they have bigger shoots than sensitive-response ones (**Fig. 6.6**). These differences are clearly visible after 20 DAG. On the other hand, sensitive-response and tolerant-response plants have similar total root length, but they have shorter roots than reference-phenotype ones at harvest day (**Fig. 6.7**). These differences are mainly located in roots growing in the first 10 cm of depth and clearly visible after 35 DAG. Additionally, reference-phenotype have a largest proportion of fine roots among the phenotypes (**Fig. 6.8**). For water uptake, reference-phenotype and tolerant-response plants have similar uptake but higher than sensitive-response ones after 45 days (**Fig. 6.9**). These differences are mainly located in roots growing in the first 5 cm of depth and clearly visible after 20 DAG.

At 45 DAG and under loose soil, sensitive-response plants have higher root nitrate uptake than, reference-phenotype and tolerant-response ones (**Fig. 6.10**). These differences are mainly located in roots growing in the first 5 cm of depth and clearly visible after 40 DAG. However, under compacted soil, reference-phenotype and tolerant-response plants have similar root nitrate uptake but higher than sensitive-response ones. While for phosphorus uptake, reference-phenotype plants have higher root phosphorus uptake than sensitive-response and tolerant-response ones at harvest (**Fig. 6.11**). These differences are mainly located in roots growing in the first 10 cm of depth and clearly visible after 35 DAG.

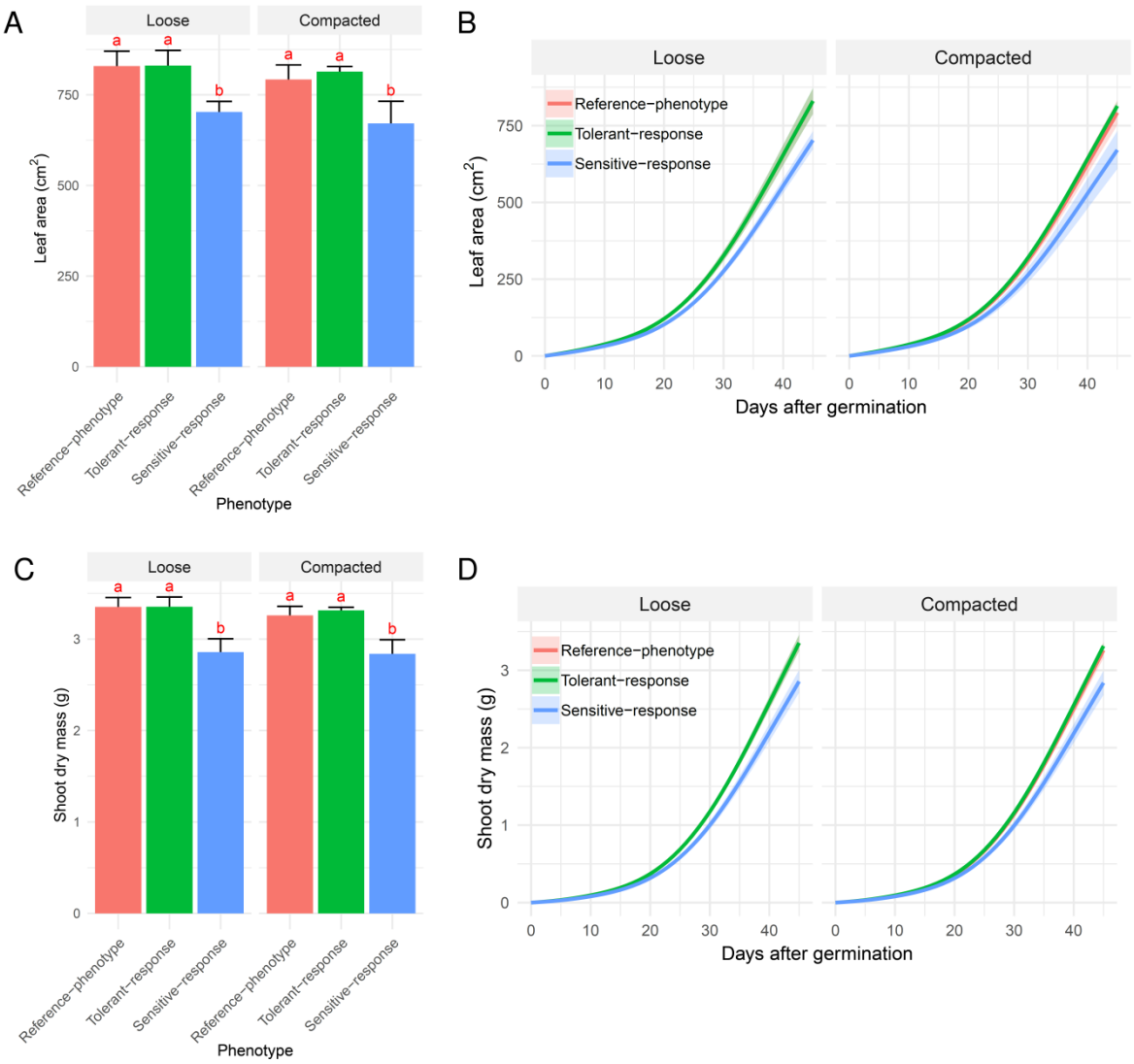


Fig. 6.6 - Simulated above-ground traits for each phenotype under loose and compacted soil. A and **B**: leaf area at harvest time (45 DAG) and over time (from 0 to 45 DAG), respectively. **C** and **D**: shoot dry mass at harvest time (45 DAG) and over time (from 0 to 45 DAG), respectively. Error bars: standard error of the mean (SEM). Different letters indicate means ± SEM with statistically significant differences among genotypes according to Tukey's honest significant differences test (P<0.05) within each soil level. Note that curves of reference-phenotype is being masked by the tolerant-response curves in **B** and **C**.

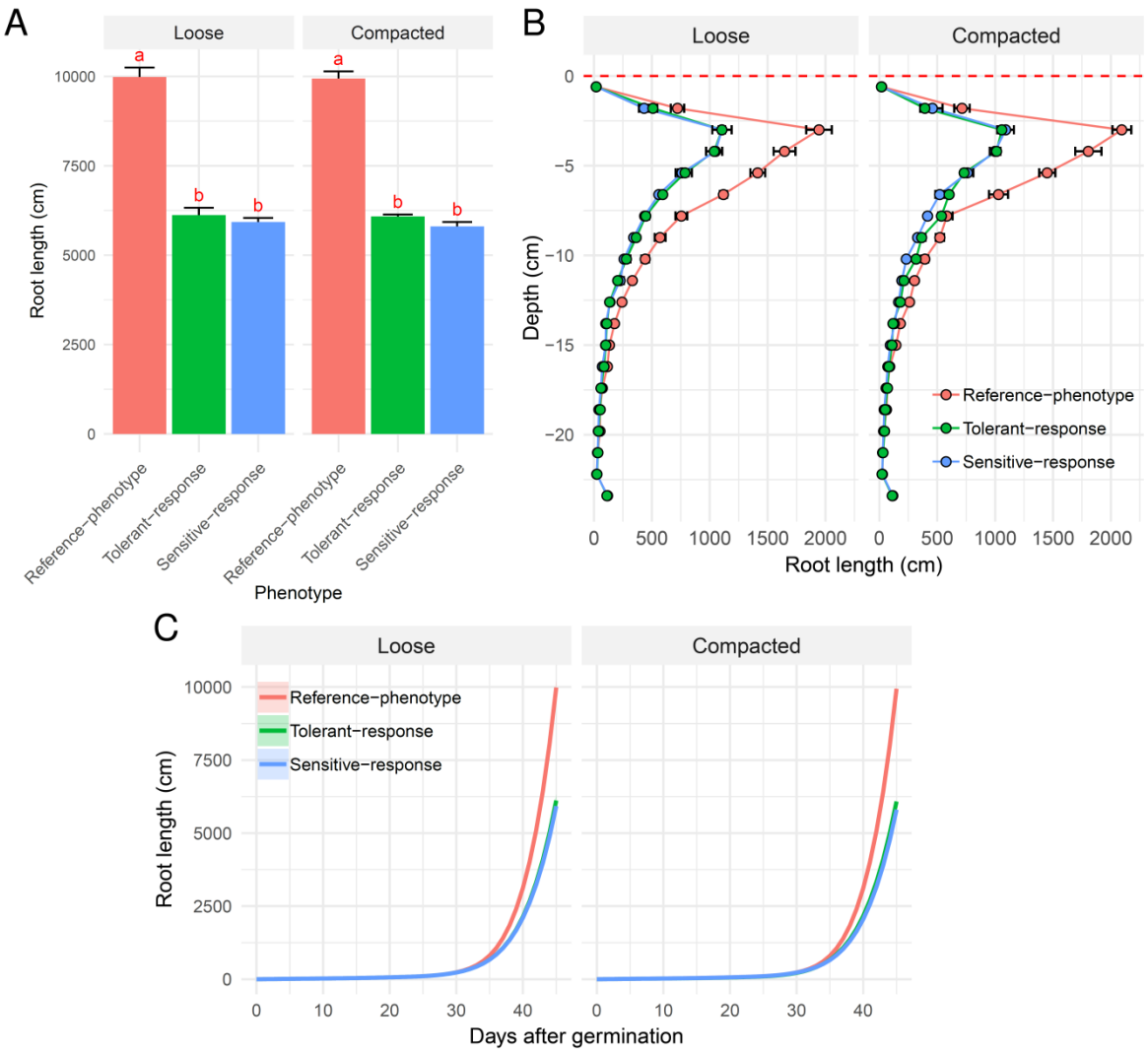


Fig. 6.7 - Simulated root length for each phenotype under loose and compacted soil. **A**: total root length at harvest time (45 DAG). **B**: Root length profile according pot depth (distance from the top to the bottom of the pot) at harvest time. **C**: Total root length over time (from 0 to 45 DAG). Error bars: standard error of the mean (SEM). Different letters indicate means ± SEM with statistically significant differences among genotypes according to Tukey's honest significant differences test (P<0.05) within each soil level. Note that curves of reference-phenotype is being masked by the tolerant-response curves in **B** and **C**.

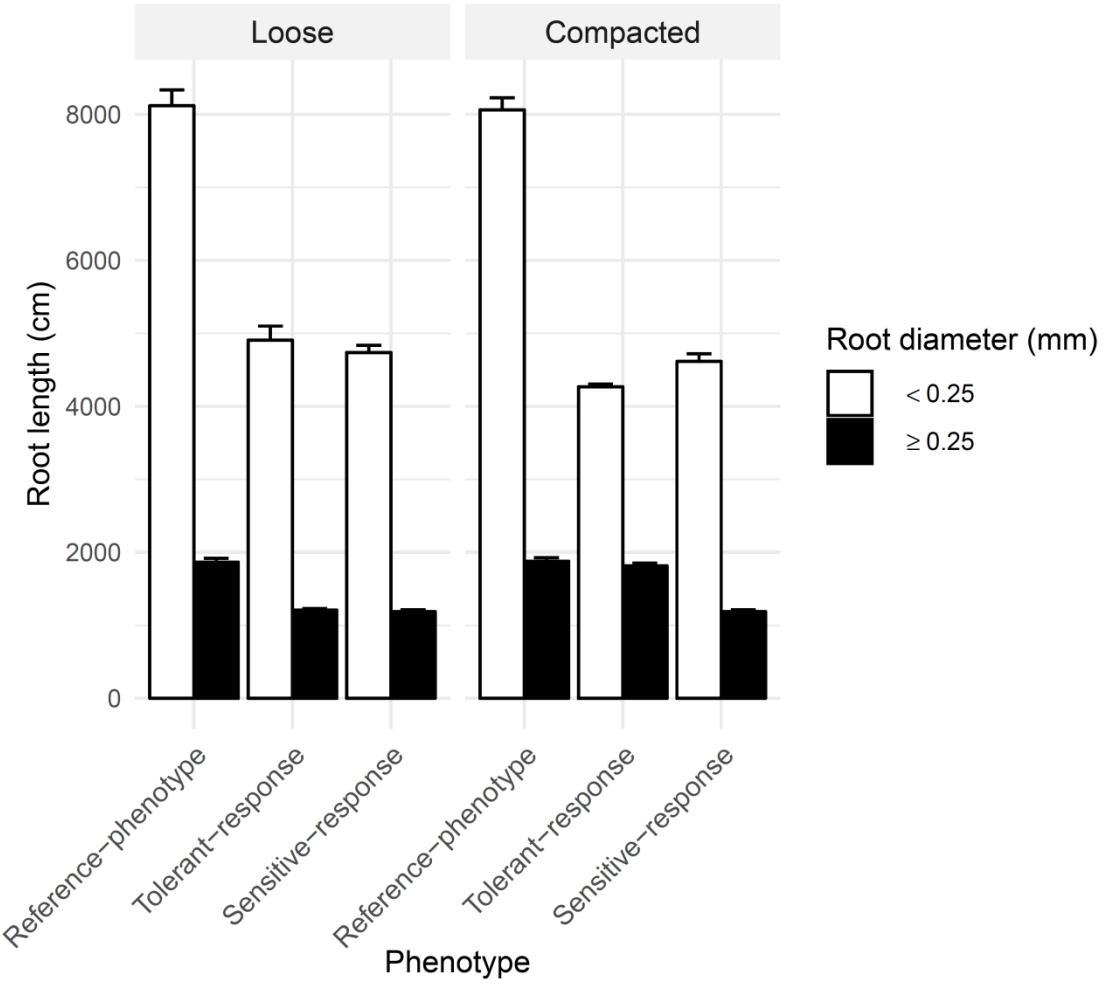


Fig. 6.8 - Distribution of total root length into two root diameter classes for each phenotype under loose and compacted soil. The diameter classes were: (1) fine roots (root diameter is less than 0.25 mm); and (2) coarse roots (root diameter is greater than or equal to 0.25 mm). Error bars: standard error of the mean.

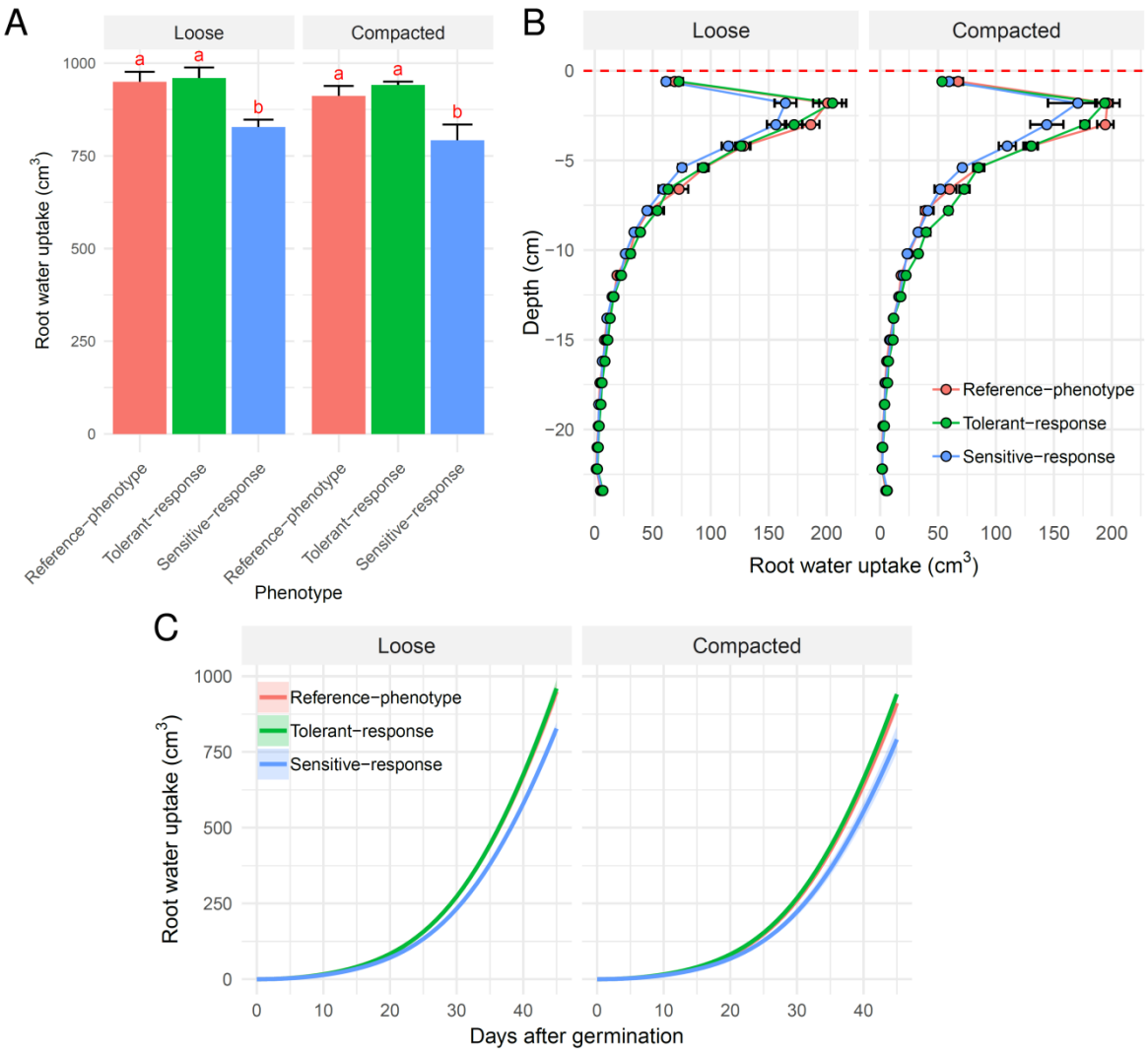


Fig. 6.9 - Simulated root water uptake for each phenotype under loose and compacted soil. A: total root water uptake at harvest time (45 DAG). **B**: Root water uptake profile according pot depth (distance from the top to the bottom of the pot) at harvest time. **C**: Total water uptake over time (from 0 to 45 DAG). Error bars: standard error of the mean (SEM). Different letters indicate means ± SEM with statistically significant differences among genotypes according to Tukey's honest significant differences test (P<0.05) within each soil level. Note that curves of reference-phenotype is being masked by the tolerant-response curves in **B** and **C**.

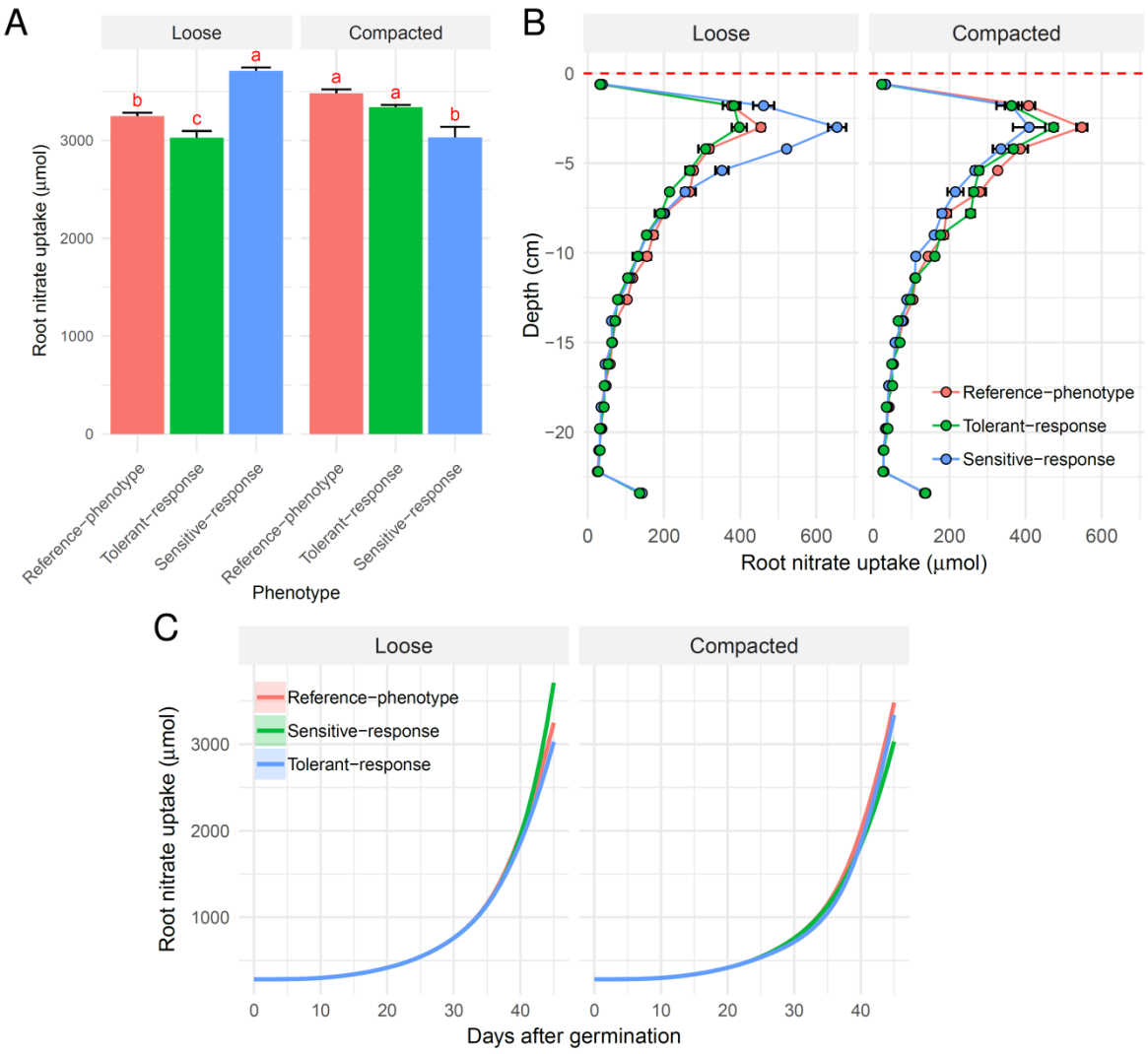


Fig. 6.10 - Simulated root nitrate uptake for each phenotype under loose and compacted soil. A: total root nitrate uptake at harvest time (45 DAG). **B**: Root nitrate uptake profile according pot depth (distance from the top to the bottom of the pot) at harvest time. **C**: Total nitrate uptake over time (from 0 to 45 DAG). Error bars: standard error of the mean (SEM). Different letters indicate means ± SEM with statistically significant differences among genotypes according to Tukey's honest significant differences test (P<0.05) within each soil level.

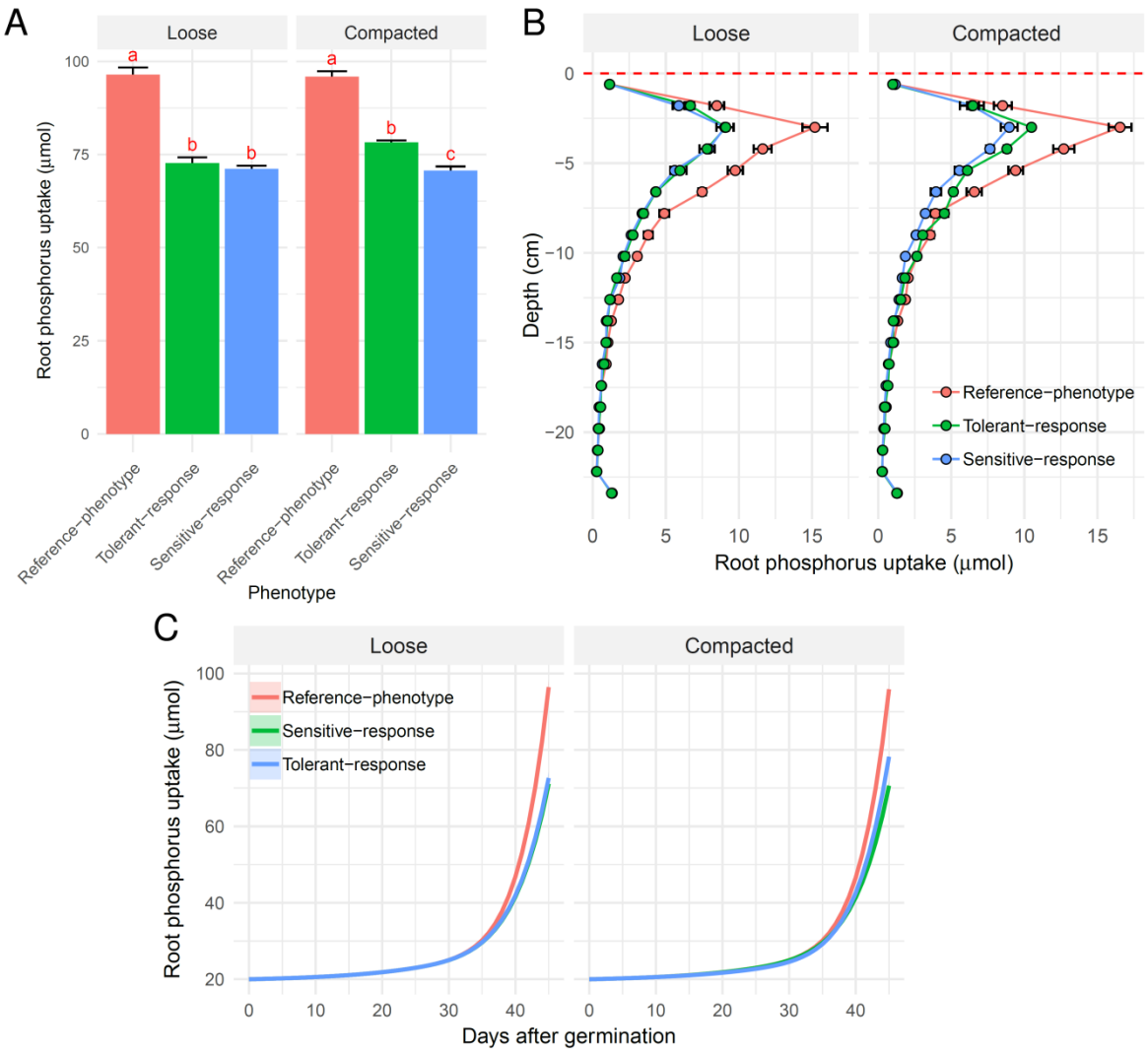


Fig. 6.11 - Simulated root phosphorus uptake for each phenotype under loose and compacted soil. A: total root phosphorus uptake at harvest time (45 DAG). **B**: Root phosphorus uptake profile according pot depth (distance from the top to the bottom of the pot) at harvest time. **C**: Total phosphorus uptake over time (from 0 to 45 DAG). Error bars: standard error of the mean (SEM). Different letters indicate means ± SEM with statistically significant differences among genotypes according to Tukey's honest significant differences test (P<0.05) within each soil level. Note that curves of reference-phenotype is being masked by the tolerant-response curves in 'Loose' condition in **B** and **C**.

Discussion

Soil models

Soil compaction results in deterioration of bulk density, structure, aggregate stability, porosity and mechanical strength (Lal, 1997; Casanova et al., 2013). With the proposed models, our objective was to estimate the effect of various physical properties on soil resistance. We focused mainly on the effect of soil density, water relations, organic matter and others. As **Fig. 6.1** shows, a decrease of soil organic matter is accompanied by an increase of soil density and higher levels of soil mechanical resistance under different ranges of water potential (To and Kay, 2005; Casanova et al., 2013).

In general, soils with high clay content have more resistance to root growth when compacted (Atwell, 1993). However, it has been seen that there is a strong negative correlation between the percentage of clay and the apparent density of the soil (Jones, 1983). However, according to **Fig 5.2**, this would be true only for those soils with a low or no percentage of organic matter. In theory, at the same apparent density, clayey soils have a larger contact area between soil particles per volume of soil than sandy soils, which in turn would increase soil resistance (Mathers et al., 1966). In addition, when the apparent density of the soil remains constant, the mechanical resistance of the soil increases as the content of organic matter increases, especially when the soil is drier. Under these conditions, greater cementation can occur within the substrate micro-aggregates (To and Kay, 2005).

An estimator of soil porosity is the soil volumetric content at saturation (θ_{sat}, **Fig. 6.3**). There are several experimental examples where the increasing bulk density decreases soil porosity (For instance, Douglas and Crawford, 1993; Tubeileh et al., 2003). Furthermore, under compacted soil the proportion of small pores increases and the unsaturated water conductivity and holding capacity are decreased substantially (Douglas and Crawford, 1993; Tubeileh et al., 2003). Those effects are evident from the proposed equations (**Fig. 6.3**).

The soil water content influences soil strength. Thus, soil resistance to the penetration increases with the decrease of the soil water, because the matrix potential becomes more negative as a result of the capillary forces (Gerard, 1965; Mathers et al., 1966; Whalley et al., 2005; Unger and Kaspar, 1993). In the proposed models, the main

effect of increased soil density (compaction) is the reduction of soil porosity (see **Equation 10** and **13**, **Supplementary Material S3**), which in turn affects the soil water content and movement. Those effects are illustrates by van Genuchten's (1980) soil-water characteristic curves in **Fig. 6.4**. The slope of those curves depend on the density of the soil. Thus, the decrease in water content for each increase in a unit of water potential is more pronounced at lower densities. In addition, both organic matter and salinity (electrical conductivity) affect this curve. When soil salinity is increased, the differences in water content between loose and compacted soil at the same water potential are major. However, it should be taken into account that the proposed formulas only describe an additive effect, either by increasing or reducing both the minimum and maximum water potential reached by the curve (see **Equation 21** and **22**, **Supplementary Material S3**).

In general, soil organic matter losses are associated with higher soil densities which in turn increases the soil strength (To and Kay, 2005). However, in **Fig. 6.5**, as the organic matter fraction increases from 0 to 0.1 kg kg^{-1}, soil strength also increases at the same level of water potential. Experimental data shows that when soil density is held constant, soil mechanical resistance increases as the organic matter content and water potential increases (To and Kay, 2005).

The proposed models theoretically explain the experimental findings for several authors. The soil strength is a function of bulk density, soil texture, organic matter content and water content (Unger and Kaspar, 1993; To and Kay, 2005). At the same time, those models illustrate that an increase of bulk density leads an increase of soil strength hindering root growth and development due to low levels of porosity and reduced water and nutrients supply and movement. (Stirzakeret al., 1996; Bengough et al., 2011; Casanova et al., 2013).

Root simulations

My simulations assumed that root uptake is equal to transpiration (no water storage) and relative to root length (Postma et al., 2014). Thus, the longer the root system and higher the leaf area, the greater the water uptake (**Fig. 6.6** to **6.9**). This implies that tolerant-response should have a higher water uptake efficiency than reference-phenotype to keep the same transpiration levels.

On the other hand, the root nitrate uptake may produce areas of nitrogen depletion in the surrounding soil that can lead to competition between the roots, especially at high root densities (Postma et al., 2014). Consequently, N uptake per plant should be greater

in plants with less dense root systems or with less fine roots until a certain point. Under loose conditions (**Fig. 6.10**), the higher proportion of fine roots can lead the competition between roots reducing the N uptake per plant. On the contrary, a thicker root system may be more efficient in those conditions. However, in compacted soils, finer and longer root system are just a little more efficient than a coarser and smaller ones (**Fig. 6.10**). N transport in soil is driven by the water flow and diffusion (Postma et al., 2014), both processes dependent on soil water content and conductivity (Šimunek et al., 1995). Thus, any factor that affects soil water content and movement will ultimately affect N uptake. Therefore, the N uptake is result of the joint effect of the water and N movement, and root length and density. The more nutrient movement is restricted, the more important the root length becomes and the less relevant is the competition among roots.

Since root architecture determines access to nutrients in soil (Rich and Watt 2013) and the extension of the root system is impeded in compacted soil (reduction of both the absorbing root surface and the radial access to soil resources), nutrients uptake also might be seriously affected (Atwell 1990; Hoffmann and Jungk (1995); Rich and Watt 2013). This is especially true for immobile nutrients like phosphorus. However, as in the case of water uptake, tolerant-response phenotype should have a higher nutrient uptake efficiency to keep the same shoot growth levels than the reference-phenotype (**Fig. 6.6** and **6.7**).
Plastic (facultative) resource-feeding strategies of plants can be both structural and functional. For example, root proliferation within soil zones where resources are more abundant, such as nutrients (de Kroon et al. 2009) or water (Bao et al. 2015; Robbins and Dinneny 2015, Lyu et al. 2016), are an example of structural response. An example of a functional response is the increase in the uptake and exudation rates by the root, resulting in higher and faster resource utilization (Dara et al. 2015; Lyu et al. 2016).

Root length density does not necessarily relate to more water extraction because a number of traits can be involved with plant water balance such as leaf conductance, transpiration, and aquaporin activity (Vadez 2014). The aquaporin expression increases root water permeability per unit of root surface (efficiency) to maintain or enhance the root water uptake from the soil (Vadez 2014; Matsunami et al. 2016). Plants are able to modify the activity of nutrient in order to regulate the root nutrient uptake into root and subsequent translocation within the plant body (Aibara and Miwa 2014). In general, the expression high-affinity transporters of nutrients is induced (or depressed) under low substrate availability (Kiba and Krapp 2016). According to these antecedents, root size, at least in terms of mass and length, does not indicate the effectiveness of nutrient and

water uptake per se (Evans 1977; Eavis and Taylor). On the other hand, fine roots have high respiration rates and N concentrations (Eissenstat1992; Pregitzer et al. 1998), and that the oxygen availability is restricted in compacted soil (Fujikawa and Miyazaki 2005). Therefore, fine roots can be severely affected under compacted soils. To maintain this relatively high shoot growth, plants must have mechanisms to compensate a shorter root system by increasing the root uptake efficiency at least in those tolerant genotypes. To assess whether greater root absorption efficiency would compensate for the reduction in fine root length, further studies are needed.

OUTLOOK AND CONCLUDING REMARKS

This thesis was aimed to start and develop a research project endeavored to study the RSA plasticity under a plant breeding context. As a general hypothesis, we test whether a high plasticity of the root system architecture is associated with a greater tolerance to soil compaction in terms of biomass, nutrient acquisition and water absorption.

For this purpose, in **Chapter 1**, we proposed a theoretical framework to support a series of experiment- and simulation-based studies. First, we discussed that soil compaction is a serious global problem and it is a major cause of inadequate rooting and poor yield in different crops around the world. Furthermore, soil physico-chemical and biological properties vary in both time and space, and they interact with each other. Consequently, the plant root system must adjust and compensate its growth and development to such changing and interacting constraints through RSA phenotypic plasticity. As a second point, we proposed that plasticity may have an adaptive value, providing environmental tolerance to soil compaction. However, it is challenging to distinguish developmental retardation (apparent plasticity) or responses to severe stress from those root architectural changes that may provide an actual environmental tolerance (true adaptive plasticity). Finally, we discussed to what extent these responses might be useful for breeding, and which one of them, such as thicker roots and higher tortuosity, enhances the root exploration capabilities in tolerant genotypes. However, we argued that selection in favor of RSA plasticity may be more useful under low-input farming systems or rain-fed agricultural systems. As a conclusion, RSA plasticity in response to soil compaction is complex and can be targeted in breeding to increase the performance of crops under specific agronomical conditions. Accordingly, we propose that a putative tolerant genotype (an ideotype for soil compaction tolerance) should have the following plastic responses for soil compaction under rain-fed agricultural systems: an increased root diameter, a greater number of root axes with steeper root angles, a high degree of tortuosity and a great ability to proliferate roots into soil patches with more favorable soil conditions.

In **Chapter 1**, we explained that phenotypic responses may vary depending of plant growth and/or development by allometric relationships (apparent plasticity). This led me to focus on answering in **Chapter 4** whether the genotypic diversity in the degree of responses to soil compaction is more dependent on true plasticity than on plant size.

As a result, we found that soil compaction reduces the plant size and biomass, but that the degree of plasticity in biomass varies among genotypes. Although some genotypes were tolerant by having low biomass responses, they did have reductions particularly in fine root length. These reductions were not as strong as in the sensitive lines. Additionally, we showed that plant plasticity responses to soil compaction can be explained to a large degree by allometry, that is the sensitive genotypes are relatively large while tolerant ones are smaller. Unfortunately, this apparent plasticity cannot be exploited in breeding, unless high yields can be obtained using less vigorous genotypes at higher planting densities. Nevertheless, size-independent responses (true plasticity) were observed especially for number of nodal roots and root length of fine roots, but also for biomass allocation patterns.

As we stated in **Chapter 1**, soil strength and the other soil physico-chemical properties, which interact and are affected by compaction, are rarely uniformly distributed through the soil profile. Consequently, a plastic root system may direct its growth towards those soil patches with lower mechanical impedance to penetration and where resources are more available than in their surroundings. This proliferation of roots into patches with more favorable soil conditions may be advantageous and a way to compensate for lost root length. However, the results of **Chapter 4** were assessed in roots were not only grown in homogeneously compacted soils but were also phenotyped without distinguishing local responses at within-root level. Thus, we do not know if the relative tolerance present in small plants is associated with greater exploration by the roots in other areas of the soil that have better conditions for growth. This response can be masked by using homogeneous soil conditions and / or when the phenotype is measured at the plant level (e.g., root length per plant). That is why in **Chapter 5**, a study focused on the within-root system plasticity was carried out to test whether plants are able to compensate the effect of very compacted layers with a higher root proliferation where the best condition are found. We found that the effect of a very compacted soil layer not only has a large local impact on the portion of the roots that is being affected but also on the plant as a whole. Based on these findings, the global plasticity of the root system to soil compaction may involve both local and long distance response. This may be a strategy of adaptive plasticity to counterbalance the limited function of an impeded portion of a root system. Thus, plants compensate the lower growth in compacted layers by growing more into the looser zones of the soil. This would involve a complex system of sensing and communication between the different components of the root system that should be

studied in greater depth in future research.

In **Chapter 6**, a simulation-based research was conducted to study the consequence of RSA plasticity as a response soil compaction on the plant performance. Based on that, we tested if those phenotypes with a higher RSA plasticity also express a higher nutrient and/or water uptake per unit of root length than those phenotypes with higher RSA plasticity. In **Chapter 4** and **5**, we showed that tolerant genotypes expressed low to null effect of soil compaction on shoot traits while the length of fine roots was reduced and there was a higher proliferation of roots (e.g. a higher root length) in the looser and more superficial soil layers. Based on the *in silico* experiments, we proposed that a tolerant genotype must have mechanisms to compensate a shorter root system by increasing the root uptake efficiency as long as the shoot is not severely affected. Additionally, we discussed that this higher efficiency must be linked to the facultative expression of high-affinity transporters of nutrients and water. To assess whether greater root absorption efficiency would compensate for the reduction in fine root length, further studies are needed.

…

As a general finding, we showed that less-sensitive genotypes or tolerant are in general smaller sized genotypes. Unfortunately, this apparent plasticity may pose challenges in breeding for soil compaction tolerance. Assuming that a small plant yields less than a larger one, growing small plant genotype for soil compaction tolerance may imply to cultivate a higher plant densities (number of plants per square meter) to compensate for this lower yield. However, some forms of plasticity can be disadvantageous at the population level (Weiner, 2004). For example, increasing population density would increase competition between plants, especially if the response in plasticity is associated with greater proliferation in those areas of the soil with better conditions. Therefore, a strategy of taking advantage of the inherent diversity root plasticity would be the cultivation of heterogeneous crop populations (i.e. genetic mixtures or ‘multilines’) with differential pattern and degree of plasticity. In theory, this may allow the complementary exploitation of distinct soil zones reducing the risk of competition, especially in resource-poor soils (Lynch, 2007b).It is important to emphasize that tolerance is an environment-dependent characteristic, and the list of features that makes a plant tolerant to a specific

constraint may be different for different agricultural conditions.

In conclusion, a high RSA plasticity is associated with a greater tolerance to soil compaction in terms of biomass shoots and leaf area. This RSA plasticity is not only expressed as a reduction of fine root length but also as a greater compensation capacity of root growth. However, this greater tolerance is linked to a smaller plant size and must necessarily be supported by a greater nutrients and water uptake. Additionally, we propose that the understanding of the underlying mechanisms behind RSA plasticity provides a theoretical framework for future cropping techniques or breeding programs focused on minimizing yield penalties where the root plasticity is exploited, which might be of great value for breeding an ‘adaptive’ cultivar in specific low-input farming systems.

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LITERATURE CITED

Anfodillo T, Petit G, Sterck F, Lechthaler S, Olson ME. 2016. Allometric trajectories and 'stress': a quantitative approach. Frontiers in Plant Science 7, 1681.

Armstrong W. 1979. Aeration in higher plants. Advances in Botanical Research 7, 225–332.

Arvidsson J. 1999. Nutrient uptake and growth of barley as affected by soil compaction. Plant and Soil 208, 9–19.

Atkinson JA, Pound MP, Bennett MJ, Wells DM. 2019. Uncovering the hidden half of plants using new advances in root phenotyping. Current Opinion in Biotechnology 55, 1–8.

Atwell BJ. 1993. Response of roots to mechanical impedance. Environmental and Experimental Botany 33, 27–40.

Awadhwal NK, Thierstein GE. 1985. Soil crust and its impact on crop establishment: A review. Soil and Tillage Research 5, 289–302.

Bailey PH, Currey JD, Fitter AH. 2002. The role of root system architecture and root hairs in promoting anchorage against uprooting forces in *Allium cepa* and root mutants of *Arabidopsis thaliana*. Journal of Experimental Botany 53, 333–340.

Barken LR, Bøsrresen T, Njøss A. 1987. Effect of soil compaction by tractor traffic on soil structure, denitrification, and yield of wheat (*Triticum aestivum* L.). Journal of Soil Science 38, 541–552.

Baskerville GL. 1972. Use of logarithmic regression in the estimation of plant biomass. Canadian Journal of Forest Research 2, 49–53.

Bates TR, Lynch JP. 2001. Root hairs confer a competitive advantage under low phosphorus availability. Plant and Soil 236, 243–250.

Batey T. 2009. Soil compaction and soil management—a review. Soil Use and Management 25, 335–345.

Beemster GTS, Masle J. 1996. Effects of soil resistance to root penetration on leaf expansion in wheat (*Triticum aestivum* L.): composition, number and size of epidermal cells in mature blades. Journal of Experimental Botany, 47: 1651–1662.

Bengough AG, Bransby MF, Hans J, McKenna SJ, Roberts TJ, Valentine TA. 2006. Root responses to soil physical conditions; growth dynamics from field to cell. Journal of Experimental Botany 57,437–447.

Bengough AG, Loades K, McKenzie BM. 2016. Root hairs aid soil penetration by anchoring the root surface to pore walls. Journal of Experimental Botany 67, 1071–1078.

Bengough AG, McKenzie BM. 1997. Sloughing of root cap cells decreases the frictional resistance to maize (*Zea mays* L.) root growth. Journal of Experimental Botany 48, 885–893.

Bengough AG, McKenzie BM, Hallett PD, Valentine TA. 2011. Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. Journal of Experimental Botany 62, 59–68.

Bengough AG, McKenzie BM. 1997. Sloughing of root cap cells decreases the frictional resistance to maize (*Zea mays* L.) root growth. Journal of Experimental Botany 48, 885–893.

Bengough AG, Mullins CE. 1990. Mechanical impedance to root growth: a review of experimental techniques and root growth responses. Journal of Soil Science 41, 341–358.

Bingham IJ, Bengough AG, Rees RM. 2010. Soil compaction–N interactions in barley: root growth and tissue composition. Soil and Tillage Research 106, 241–246.

Bingham IJ, Bengough AG. 2003. Morphological plasticity of wheat and barley roots in response to spatial variation in soil strength. Plant and Soil 250, 273–282.

Blum A, Sullivan CY. 1997. The effect of plant size on wheat response to agents of drought stress. I. Root drying. Australian Journal of Plant Physiology 24, 35–41.

Blum A, Sullivan CY, Nguyen HT. 1997. The effect of plant size on wheat response to agents of drought stress. II. water deficit, heat and ABA. Australian Journal of Plant Physiology 24, 43–48.

Blum A. 2016. Stress, strain, signaling, and adaptation—not just a matter of definition. Journal of Experimental Botany 67, 562–565.

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13, 115–155.

Cardoso JA, Rincón J, Jiménez JC, Noguera D, Rao IM. 2013. Morpho-anatomical adaptations to waterlogging by germplasm accessions in a tropical forage grass. AoB Plants plt047. doi: 10.1093/aobpla/plt047.

Casanova M, Salazar O, Seguel O, Luzio W. 2013. The soils of Chile. Cham: Springer.

Chavent M, Kuentz V, Liquet B, Jerome Saracco J. 2017. ClustOfVar: Clustering of Variables. R package version 1.1. https://CRAN.R-project.org/package=ClustOfVar (accessed October 13, 2019)

Chen YL, Palta J, Clements J, Buirchell B, Siddique KHM, Rengel Z. 2014. Root architecture alteration of narrow-leafed lupin and wheat in response to soil compaction. Field Crops Research 165, 61–70.

Chimungu JG, Loades KW, Lynch JP. 2015. Root anatomical phenes predict root penetration ability and biomechanical properties in maize (*Zea mays*). Journal of Experimental Botany 66, 3151–3162.

Clark LJ, Whalley WR, Barraclough PB. 2003. How do roots penetrate strong soil? Plant and Soil 255, 93–104.

Coelho Filho MA, Colebrook EH, Lloyd DPA, Webster CP, Mooney SJ, Phillips AL, Hedden P, Whalley WR. 2013. The involvement of gibberellin signalling in the effect of soil resistance to root penetration on leaf elongation and tiller number in wheat. Plant and Soil 371, 81–94.

Colombi T, Kirchgessner N, Walter A, Keller T. 2017. Root tip shape governs root elongation rate under increased soil strength. Plant Physiology 174, 2289–2301.

Colombi T, Torres LC, Walter A, Keller T. 2018. Feedbacks between soil penetration resistance, root architecture and water uptake limit water accessibility and crop growth—a vicious circle. The Science of the Total Environment 626, 1026–1035.

Colombi T, Walter A. 2016. Root responses of triticale and soybean to soil compaction in the field are reproducible under controlled conditions. Functional Plant Biology 43, 114–128.

Colombi T, Walter A. 2017. Genetic diversity under soil compaction in wheat: root number as a promising trait for early plant vigor. Frontiers in Plant Science 8, 420.

Comas LH, Becker SR, Cruz VMV, Byrne PF, Dierig DA. 2013. Root traits contributing to plant productivity under drought. Frontiers in Plant Science 4, 442.

Correa J, Postma JA, Watt M, Wojciechowski T. 2019. Soil compaction and the architectural plasticity of root systems. Journal of Experimental Botany erz383.

Correa J, Postma JA, Wojciechowski T. 2022. Phenotypic response to soil compaction varies among genotypes and correlates with plant size in sorghum. Plant and Soil 472, 59–76.

Crossett RN, Campbell DJ, Stewart HE. 1975. Compensatory growth in cereal root systems. Plant and Soil 42,673–683.

Dara A, Moradi BA, Vontobel P, Oswald SE. 2015. Mapping compensating root water uptake in heterogeneous soil conditions via neutron radiography. Plant and Soil 397, 273–287.

Des Marais DL, Hernandez KM, Juenger TE. 2013. Genotype-by-environment interaction and plasticity: exploring genomic responses of plants to the abiotic environment. Annual Review of Ecology, Evolution, and Systematics 44, 5–29.

Dewitt TJ, Sih A, Wilson DS. 1998. Costs and limits of phenotypic plasticity. Trends in Ecology & Evolution 13, 77–81.

Dexter AR, Chan KY. 1991. Soil mechanical properties as influenced by exchangeable cations. European Journal of Soil Science 42, 219–226.

Dexter AR, Hewitt JS. 1978. The deflection of plant roots. Journal of Agricultural Engineering Research 23, 17–22.

Douglas JT, Crawford CE. 1993. The response of a ryegrass sward to wheel traffic and applied nitrogen. Grass Forage Science 48, 91–100.

Drew MC, He CJ, Morgan PW. 2000. Programmed cell death and aerenchyma formation in roots. Trends in Plant Science 5, 123–127.

Drew MC, Saker LR, Ashley TW. 1973. Nutrient supply and the growth of the seminal root system in barley. I. The effect of nitrate concentration on the growth of axes and laterals. Journal of Experimental Botany 24, 1189–1202.

Drew MC, Saker LR. 1975. Nutrient supply and the growth of the seminal root system in barley. II. Localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. Journal of Experimental Botany 26, 79–90.

Drew MC. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium, and potassium on the growth of the seminal root system, and the shoot, in barley. New Phytologist 75,479–490.

Eavis BW. 1972. Soil physical conditions affecting seedling growth I. Mechanical impedance, aeration and moisture availability as influenced by bulk density and moisture levels in a sandy loam soil. Plant and Soil 36, 613–622.

Eissenstat DM, Caldwell MM. 1988. Seasonal timing of root growth in favorable microsites. Ecology 69, 870–873.

Eissenstat DM, Yanai RD. 1997. The ecology of root lifespan. Advances in Ecological Research 27, 1–60.

Eissenstat DM. 1992. Costs and benefits of constructing roots of small diameter. Journal of Plant Nutrition 15, 763–782.

El-Soda M, Malosetti M, Zwaan BJ, Koornneef M, Aarts MG. 2014. Genotype×environment interaction QTL mapping in plants: lessons from Arabidopsis. Trends in Plant Science 19, 390–398.

Ericsson T. 1995. Growth and shoot:root ratio of seedlings in relation to nutrient availability. Plant and Soil1 68, 205–214.

Evans GC. 1972. The quantitative analysis of plant growth. Berkeley, CA: University of California Press.

Evert RF. 2006. Esau's plant anatomy, meristems, cells, and tissues of the plant body: their structure, function, and development. Chichester, UK: John Wiley and Sons.

Fishman R. 2016. More uneven distributions overturn benefits of higher precipitation for crop yields. Environmental Research Letters 11, 024004.

Fitter A, Williamson L, Linkohr B, Leyser O. 2002. Root system architecture determines fitness in an Arabidopsis mutant in competition for immobile phosphate ions but not for nitrate ions. Proceedings of the Royal Society B: Biological Sciences 269, 2017–2022.

Fitter AH, Stickland R. 1991. Architectural analysis of plant root systems 2. Influence of nutrient supply on architecture in contrasting plant species. New Phytologist 118, 383–389.

Fitter AH. 1976. Effects of nutrient supply and competition from other species on root growth of *Lolium perenne* in soil. Plant and Soil 45, 177–189.

Fitter AH. 1986. The topology and geometry of plant root systems: influence of watering rate on root system topology in *Trifolium pratense*. Annals of Botany 58, 91–101.

Fitter AH. 1987. An architectural approach to the comparative ecology of plant root systems. New Phytologist 106, 61–77.

Fitz Gerald JN, Lehti-Shiu MD, Ingram PA, Deak KI, Biesiada T, Malamy JE. 2006. Identification of quantitative trait loci that regulate Arabidopsis root system size and plasticity. Genetics 172, 485–498.

Forde BG. 2002. Local and long-range signaling pathways regulating plant responses to nitrate. Annual Review of Plant Biology 53, 203–224.

Fujikawa T, Miyazaki T. 2005. Effects of bulk density on the gas diffusion coefficient in repacked and undisturbed soils. Soil Science 170, 892–901.

Gao W, Hodgkinson L, Jin K, et al. 2016a. Deep roots and soil structure. Plant, Cell & Environment 39, 1662–1668.

Gao W, Whalley WR, Tian Z, Liu J, Ren T. 2016b. A simple model to predict soil penetrometer resistance as a function of density, drying and depth in the field. Soil and Tillage Research 155, 190–198.

Geng YP, Pan XY, Xu CY, Zhang WJ, Li B, ChenJK. 2007. Plasticity and ontogenetic drift of biomass allocation in response to above- and below-ground resource availabilities in perennial herbs: a case study of *Alternanthera philoxeroides*. Ecological Research 2, 255–260.

Gerard CJ. 1965. The influence of soil moisture, soil texture, drying conditions, and exchangeable cations on soil strength. Soil Science Society of America, Proceedings 29, 641–645.

Glimskär A. 2000. Estimates of root system topology of five plant species grown at steady-state nutrition. Plant and Soil 227, 249–256.

Gornall J, Betts R, Burke E, Clark R, Camp J, Willett K, Wiltshire A. 2010. Implications of climate change for agricultural productivity in the early twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences365, 2973–2989.

Goss MJ, Russell RS. 1980. Effects of mechanical impedance on root growth in barley (*Hordeum vulgare* L.): III. Observations on the mechanism of response. Journal of Experimental Botany 31, 577–588.

Goss MJ. 1977. Effects of mechanical impedance on root growth in barley (*Hordeum vulgare* L.): I. Effects on the elongation and branching of seminal root axes. Journal of Experimental Botany 28, 96–111.

Gray SB, Brady SM. 2016. Plant developmental responses to climate change. Developmental Biology 419, 64–77.

Grimm V, Wissel C. 1997.Babel, or the ecological stability discussions: an inventory and analysis of terminology and a guide for avoiding confusion. Oecologia109, 323–334.

Grzesiak MT, Ostrowska A, Hura K, Rut G, Janowiak F, Rzepka A, Hura T, Grzesiak S. 2014. Interspecific differences in root architecture among maize and triticale genotypes grown under drought, waterlogging and soil compaction. Acta Physiologiae Plantarum 36, 3249–3261.

Grzesiak S, Grzesiak MT, Filek W, Hura T, Stabryła J. 2002. The impact of different soil moisture and soil compaction on the growth of triticale root system. Acta Physiologiae Plantarum 24, 331–342.

Håkansson I, Voorhees WB, Riley H. 1988. Vehicle and wheel factors influencing soil compaction and crop response in different traffic regimes. Soil and Tillage Research 35, 239–282.

Haling RE, Brown LK, Bengough AG, Young IM, Hallett PD, White PJ, George TS. 2013. Root hairs improve root penetration, root–soil contact, and phosphorus acquisition in soils of different strength. Journal of Experimental Botany 64, 3711–3721.

Hamamoto S, Moldrup P, Kawamoto K, Komatsu T. 2012. Organic matter fraction dependent model for predicting the gas diffusion coefficient in variably saturated soils. Vadose Zone Journal 11, vzj2011.0065.

Hamblin AP, Tennant D. 1987. Root length density and water uptake in cereals and grain legumes: how well are they correlated? Australian Journal of Agricultural Research 38, 5 13–527.

Hanbury CD, Atwell BJ. 2005. Growth dynamics of mechanically impeded lupin roots: does altered morphology induce hypoxia? Annals of Botany 96, 913–924.

He C, Finlayson SA, Drew MC, Jordan WR, Morgan PW. 1996. Ethylene biosynthesis during aerenchyma formation in roots of maize subjected to mechanical impedance and hypoxia. Plant Physiology 112, 1679–1685.

Ho M, Rosas J, Brown K, Lynch J. 2005. Root architectural tradeoffs for water and phosphorus acquisition. Functional Plant Biology 32, 737–748.

Hoad SP, Russell G, Lucas ME, Bingham IJ. 2001. The management of wheat, barley, and oat root systems. Advances in Agronomy 74, 193–246.

Hodge A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytologist 162, 9–24.

Hoffmann C, Jungk J. 1995. Growth and phosphorus supply of sugar beet as affected by soil compaction and water tension. Plant and Soil 176, 15–25.

Hoque M, Kobata T. 2000. Effect of soil compaction on the grain yield of rice (*Oryza sativa* L.) under water-deficit stress during the reproductive stage. Plant Production Science 3, 316–322.

Huang B, Fry JD. 1998. Root anatomical, morphological, and physiological responses to drought stress for tall fescue cultivars. Crop Science 38, 1017–1022.

Hund A, Ruta N, Liedgens M. 2009. Rooting depth and water use efficiency of tropical maize inbred lines, differing in drought tolerance. Plant and Soil 318, 311–325.

Hussain A, Black CR, Taylor IB, Roberts JA. 1999. Soil compaction. A role for ethylene in regulating leaf expansion and shoot growth in tomato? Plant Physiology 121, 1227–1238.

Hutchings MJ, de Kroon H. 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Advances in Ecological Research 25, 159–238.

Iijima M, Awala SK, Watanabe Y, Kawato Y, Fujioka Y, Yamane K, Wada KC. 2016. Mixed cropping has the potential to enhance flood tolerance of drought-adapted grain crops. Journal of Plant Physiology 192, 21–25.

Iijima M, Higuchi T, Barlow PW, Bengough AG. 2003. Root cap removal increases root penetration resistance in maize (*Zea mays* L). Journal of Experimental Botany 54, 2105–2109.

Iijima M, Higuchi T, Barlow PW. 2004. Contribution of root cap mucilage and presence of an intact root cap in maize (*Zea mays*) to the reduction of soil mechanical impedance. Annals of Botany 94, 473–477.

Iijima M, Morita S, Barlow PW. 2008. Structure and function of the root cap. Plant Production Science 11, 17–27.

In ‘t Zandt D, Le Marié C, Kirchgessner N, Visser EJW, Hund A. 2015. High-resolution quantification of root dynamics in split-nutrient rhizoslides reveals rapid and strong proliferation of maize roots in response to local high nitrogen. Journal of Experimental Botany 66, 5507–5517.

Jackson RB, Mooney HA, Schulze ED. 1997. A global budget for fine root biomass, surface area, and nutrient contents. Proceedings of the National Academy of Sciences, USA 94, 7362–7366.

Jin K, Shen J, Ashton RW, Dodd IC, Parry MA, Whalley WR. 2013. How do roots elongate in a structured soil? Journal of Experimental Botany 64, 4761–4777.

Jin K, White PJ, Whalley WR, Shen J, Shi L. 2017. Shaping an optimal soil by root–soil interaction. Trends in Plant Science 22, 823–829.

Jolicoeur P. 1963. The multivariate generalization of the allometry equation. Biometrics19, 497–499.

Jones AC. 1983. Effect of soil texture on critical bulk densities for root growth. Soil Science Society of America Journal 47, 1208–1211.

Kay BD. 1990. Rates of change of soil structure under different cropping systems. In: Stewart BA, ed. Advances in soil science 12. New York: Springer, 1–52.

Klingenberg CP. 1996. Multivariate Allometry. In: Marcus LF, Corti M, Loy A, Naylor GJP, Slice DE, eds. *Advances in Morphometrics*. Boston, MA: Springer, 23–49.

Kolb E, Legué V, Bogeat-Triboulot MB. 2017. Physical root–soil interactions. Physical Biology 14, 065004.

Kranner I, Minibayeva FV, Beckett RP, Seal CE. 2010. What is stress? Concepts, definitions and applications in seed science. New Phytologist 188, 655–673.

Kuht EJ, Reintam ER. 2004. Soil compaction effect on soil physical properties and the content of nutrients in spring barley (*Hordeum vulgare* L.) and spring wheat (*Triticum aestivum* L.). Agronomy Research 2, 187–194.

Kuijken RC, van Eeuwijk FA, Marcelis LF, Bouwmeester HJ. 2015. Root phenotyping: from component trait in the lab to breeding. Journal of Experimental Botany 66, 5389–5401.

Lal R. 1997. Degradation and resilience of soils. Philosophical Transactions of the Royal Society B: Biological Sciences 352, 997–1010.

Lambers H, Atkin O, MillenaarFF.2002. Respiratory patterns in roots in relation to their functioning. In: Waisel Y, Eshel A, Kafkaki K, eds. Plant roots: the hidden half. New York: Marcel Dekker, 521–552.

Lichtenthaler HK. 1996. Vegetation stress: an introduction to the stress concept in plants. Journal of Plant Physiology 148, 4–14.

Lipiec J, Stępniewski W. 1995. Effects of soil compaction and tillage systems on uptake and losses of nutrients. Soil and Tillage Research 35, 37–52.

Lynch JP, Brown KM. 2008. Root strategies for phosphorus acquisition. In: White P, Hammond J, eds. The ecophysiology of plant–phosphorus interactions. Dordrecht: Springer, 83–116.

Lynch JP, Ho MD. 2005. Rhizoeconomics: carbon costs of phosphorus acquisition. Plant and Soil 269, 45–56.

Lynch JP, Wojciechowski T. 2015. Opportunities and challenges in the subsoil: pathways to deeper rooted crops. Journal of Experimental Botany 66, 2199–2210.

Lynch JP. 1995. Root architecture and plant productivity. Plant Physiology 109, 7–13.

Lynch JP. 2007a. Rhizoeconomics: the roots of shoot growth limitations. Horticultural Science 42, 1107–1109.

Lynch JP. 2007b. Roots of the second green revolution. Australian Journal of Botany 55, 493–512.

Lynch JP. 2013. Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. Annals of Botany 112, 347–357.

Ma Z, Bielenberg DG, Brown KM, Lynch JP. 2001a. Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. Plant, Cell & Environment 24, 459–467.

Ma Z, Walk TC, Marcus A, Lynch JP. 2001b. Morphological synergism in root hair length, density, initiation, and geometry for phosphorus acquisition in Arabidopsis thaliana: a modeling approach. Plant and Soil 236, 221–235.

MacDonald AM, Matthews KB, Paterson E, Aspinall RJ. 1994. The impact of climate change on the soil/moisture regime of Scottish mineral soils. Environmental Pollution 83, 245–250.

MacInnes CB, Albert LS. 1969. Effect of light intensity and plant size on rate of development of early boron deficiency symptoms in tomato root tips. Plant Physiology 44, 965–967.

Masle J, Passioura JB. 1987. The effect of soil strength on the growth of young wheat plants. Australian Journal of Plant Physiology 14, 643–656.

Masle J. 1992. Genetic variation in the effects of root impedance on growth and transpiration rates of wheat and barley. Australian Journal of Plant Physiology 19, 109–125.

Materechera SA, Alston AM, Kirby JM, Dexter AR. 1992. Influence of root diameter on the penetration of seminal roots into a compacted subsoil. Plant and Soil 144, 297–303.

Mathers AC, Lotspeich FB, Laase GR, Wilson GC. 1966. Strength of compacted Amarillo fine sandy loam as influenced by moisture, clay content, and exchangeable cation. Soil Science Society of America Journal 30, 788–791.

McConnaughay KDM, Coleman JS. 1999. Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. Ecology 80, 2581–2593.

McKenzie BM, Mullins CE, Tisdall JM, Bengough AG. 2013. Root–soil friction: quantification provides evidence for measurable benefits for manipulation of root-tip traits. Plant, Cell & Environment 36, 1085–1092.

Mendiburu F. 2012. Agricolae: statistical procedures for agricultural research. R package version 1.1-1. https://cran.r-project.org/web/packages/agricolae/index.html (accessed October 13, 2019)

Mi G, Chen F, Wu Q, Lai N, Yuan L, Zhang F. 2010. Ideotype root architecture for efficient nitrogen acquisition by maize in intensive cropping systems. Science China. Life Sciences 53, 1369–1373.

Moran CJ, Pierret A, Stevenson AW. 2000. X-ray absorption and phase contrast imaging to study the interplay between plant roots and soil structure. Plant and Soil 223, 99–115.

Müller M, Schmidt W. 2004. Environmentally induced plasticity of root hair development in Arabidopsis. Plant Physiology 134, 409–419.

Mullholland BJ, Black CR, Taylor IB, Roberts JA, Lenton JR. 1996. Effect of soil compaction on barley (*Hordeum vulgare* L.) growth. I. Possible role for ABA as a root-sourced chemical signal. Journal of Experimental Botany 47, 539–549.

Munns R. 2002. Comparative physiology of salt and water stress. Plant, Cell & Environment 25, 239–250.

Murren CJ, Auld JR, Callahan H, et al. 2015. Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. Heredity 115, 293–301.

Nakhforoosh A, Bodewein T, Fiorani F, Bodner G. 2016. Identification of water use strategies at early growth stages in durum wheat from shoot phenotyping and physiological measurements. Frontiers in Plant Science 7, 1155.

Negin B, Moshelion M. 2016. The advantages of functional phenotyping in pre-field screening for drought-tolerant crops. Functional Plant Biology 44, 107–118.

Nicotra AB, Davidson A. 2010. Adaptive phenotypic plasticity and plant water use. Functional Plant Biology 37, 117–127.

Nielsen KL, Bouma TJ, Lynch JP, Eissenstat DM. 1998. Effects of phosphorus availability and vesicular-arbuscular mycorrhizas on the carbon budget of common bean (*Phaseolus vulgaris*). New Phytologist 139, 647–656.

Nielsen KL, Eshel A, Lynch JP. 2001. The effect of phosphorus availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris* L.) genotypes. Journal of Experimental Botany 52, 329–339.

Nishiuchi S, Yamauchi T, Takahashi H, Kotula L, NakazonoM. 2012. Mechanisms for coping with submergence and waterlogging in rice. Rice 5, 2.

Nortjé GP, van Hoven W, Laker MC. 2012. Factors affecting the impact of off-road driving on soils in an area in the Kruger National Park, South Africa. Environmental Management 50, 1164–1176.

O'Toole JC, Bland WL. 1987. Genotypic variation in crop plant root systems. Advances in Agronomy 41, 91–145.

Oldeman LR, Hakkeling RTA, Sombroek WG. 1991. World map of the status of human-induced soil degradation: an explanatory note. Wageningen: International Soil Reference and Information Centre.

Olesen JE, Bindi M. 2002. Consequences of climate change for European agricultural productivity, land use and policy. European Journal of Agronomy 16, 239–262.

Orr HA. 2009. Fitness and its role in evolutionary genetics. Nature Reviews. Genetics 10, 531–539.

Osakabe Y, Osakabe K, Shinozaki K, Tran LS. 2014. Response of plants to water stress. Frontiers in Plant Science 5, 86.

Pagès L, Serra V, Draye X, Doussan C, PierretA. 2010. Estimating root elongation rates from morphological measurements of the root tip. Plant and Soil 328, 35–44.

Pallant E, Holmgren RA, Schuler GE, McCracken KL, Drbal B. 1993. Using a fine-root extraction device to quantify small-diameter corn roots (≤0.025 mm) in field soils. Plant and Soil 153, 273–279.

Palmer CM, Bush SM, Maloof JN. 2012. Phenotypic and developmental plasticity in plants. eLS. doi: 10.1002/9780470015902.a0002092.pub2.

Passioura JB. 2002. Soil conditions and plant growth. Plant, Cell & Environment 25, 311–318.

Peterson RL, Farquhar ML. 1996. Root hairs: specialized tubular cells extending root surfaces. Botanical Review 62, 1–40.

Pfeifer J, Faget M, Walter A, Blossfeld S, Fiorani F, Schurr U, Nagel KA. 2014. Spring barley shows dynamic compensatory root and shoot growth responses when exposed to localised soil compaction and fertilisation. Functional Plant Biology 41, 581–597.

Pierce FJ, Larson WE, Dowdy RH, Graham WAP. 1983. Productivity of soils: assessing long-term changes due to erosion. Journal of Soil and Water Conservation 38, 39–44.

Pigliucci M. 2001. Phenotypic plasticity: beyond nature and nurture. Baltimore, MD: John Hopkins University Press.

Poorter H, Nagel O. 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. Australian Journal of Plant Physiology 27, 595–607.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist 193, 30–50.

Poorter H, SackL. 2012. Pitfalls and possibilities in the analysis of biomass allocation patterns in plants. Frontiers in Plant Science3, 259.

Popova L, van Dusschoten D, Nagel KA, Fiorani F, Mazzolai B. 2016. Plant root tortuosity: an indicator of root path formation in soil with different composition and density. Annals of Botany 118, 685–698.

Porporato A, Daly E, Rodriguez-IturbeI. 2004. Soil water balance and ecosystem response to climate change. The American Naturalist 164, 625–632.

Postma JA, Lynch JP. 2011a. Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. Plant Physiology156, 1190–1201.

Postma JA, Lynch JP. 2011b. Theoretical evidence for the functional benefit of root cortical aerenchyma in soils with low phosphorus availability. Annals of Botany 107, 829–841.

Potocka I, Szymanowska-Pulka J. 2018. Morphological responses of plant roots to mechanical stress. Annals of Botany 122, 7 11–723.

Pregitzer KS, Laskowski MJ, Burton AJ, Lessard VC, Zak DR. 1998. Variation in sugar maple root respiration with root diameter and soil depth. Tree Physiology 18, 665–670.

Primack RB, Kang H. 1989. Measuring fitness and natural selection in wild plant populations. Annual Review of Ecology and Systematics 20, 367–396.

R Core Team. 2018. R: A language and environment for statistical computing. https://www.R-project.org/ (accessed August 25, 2019)

Ramalingam P, Kamoshita A, Deshmukh V, Yaginuma, Uga Y. 2017. Association between root growth angle and root length density of a near-isogenic line of IR64 rice with DEEPER ROOTING 1 under different levels of soil compaction. Plant Production Science 20, 162–175.

Rao MS, Datta B, RaoVK. 1989. Sorghum growth and dry matter yield as influenced by soil water, bulk density and temperature. Soil Technology 2, 107–111.

Reich PB, Ellsworth DS, Walters MB. 1998. Leaf structure (specific leaf area) modulates photosynthesis–nitrogen relations: evidence from within and across species and functional groups. Functional Ecology 12, 948–958.

Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD. 1999. Generality of leaf trait relationships: a test across six biomes. Ecology80, 1955–1969.

Reich PB. 2002. Root–shoot relations: optimality in acclimation and adaptation or the 'Emperor's new clothes'? In: Waisel Y, Eshel A, Kafkafi U, eds. Plant roots: the hidden half. New York: Marcel Dekker, 205–220.

Rengel Z, Wheal MS. 1997. Herbicide chlorsulfuron decreases growth of fine roots and micronutrient uptake in wheat genotypes. Journal of Experimental Botany 48, 927–934.

Rich SM, Watt M. 2013. Soil conditions and cereal root system architecture: review and considerations for linking Darwin and Weaver. Journal of Experimental Botany 64, 1193–1208.

Robinson D. 1996. Variation, co-ordination and compensation in root systems in relation to soil variability. Plant and Soil 187, 57–66.

Rogers ED, Benfey PN. 2015. Regulation of plant root system architecture: implications for crop advancement. Current Opinion in Biotechnology 32, 93–98.

Rubio G, Lynch JP. 2007. Compensation among root classes in *Phaseolus vulgaris* L. Plant and Soil 290, 307–321.

Sandhu N, Raman KA, Torres RO, Audebert A, Dardou A, Kumar A, Henry A. 2016. Rice root architectural plasticity traits and genetic regions for adaptability to variable cultivation and stress conditions. Plant Physiology 171, 2562–2576.

Shabala S, White RG, Djordjevic MA, Ruan Y-L, Mathesius U. 2015. Root-to-shoot signalling: integration of diverse molecules, pathways and functions. Functional Plant Biology 43, 87–104.

Shani U, Waisel Y, Eshel A, Xue S, Ziv G. 1993. Responses to salinity of grapevine plants with split root systems. New Phytologist 124, 695–701.

Sharp RE, Silk WK, Hsiao TC. 1988. Growth of the maize primary root at low water potentials: I. Spatial distribution of expansive growth. Plant Physiology 87, 50–57.

Siddique KHM, Belford RK, Tennant D. 1990. Root:shoot ratios of old and modern, tall and semi-dwarf wheats in a Mediterranean environment. Plant and Soil 121, 89–98.

Simms EL. 2000. Defining tolerance as a norm of reaction. Evolutionary Ecology 14, 563–570.

Sinha S, Kumaravadivel N. 2016. Understanding genetic diversity of sorghum using quantitative traits. Scientifica (Cairo) 2016, 3075023.

Sitaula BK, Hansen S, Sitaula JIB, Bakken LR. 2000. Effects of soil compaction on N2O emission in agricultural soil. Chemosphere 2, 367–371.

Smith MS, Tiedje JM. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biology and Biochemistry 11, 261–267.

Smith S, de Smet I. 2012. Root system architecture: insights from Arabidopsis and cereal crops. Philosophical Transactions of the Royal Society B: Biological Sciences 367, 1441–1452.

Soane BD, van Ouwerkerk C. 1994. Soil compaction problems in world agriculture. In: Soane BD, van Ouwerkerk C, eds. Soil compaction in crop production. Amsterdam: Elsevier, 1–21.

Soil Science Society of America. 2008. Glossary of soil science terms 2008. Madison, WI: Soil Science Society of America.

Somers KM. 1986. Multivariate allometry and removal of size with principal components analysis. Functional Ecology 35: 359–368.

Stirzaker RJ, Passioura JB, Wilms Y. 1996. Soil structure and plant growth: impact of bulk density and biopores. Plant and Soil 185, 151–162.

Sultan SE, Spencer HG. 2002. Metapopulation structure favors plasticity over local adaptation. The American Naturalist 160, 271–283.

Sultan SE. 1987. Evolutionary implications of phenotypic plasticity in plants. In: Hecht MK, Wallace B, Prance GT, eds. Evolutionary biology. Boston, MA: Springer, 127–178.

Tabata R, Sumida K, Yoshii T, Ohyama K, Shinohara H, Matsubayashi Y. 2014. Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. Science 346, 343–346.

Taylor HM, Brar GS. 1991. Effect of soil compaction on root development. Soil and Tillage Research 9, 111-119.

To J, Kay BD. 2005. Variation in penetrometer resistance with soil properties: the contribution of effective stress and implications for pedotransfer functions. Geoderma 126, 261–276.

Tracy SR, Black CR, Roberts JA, Dodd IC, Mooney SJ. 2015. Using X-ray computed tomography to explore the role of abscisic acid in moderating the impact of soil compaction on root system architecture. Environmental and Experimental Botany 110, 11–18.

Tracy SR, Black CR, Roberts JA, Sturrock C, Mairhofer S, Craigon J, Mooney SJ. 2012. Quantifying the impact of soil compaction on root system architecture in tomato (*Solanum lycopersicum*) by X-ray micro-computed tomography. Annals of Botany 110, 511–519.

Troughton A. 1956. Studies on the growth of young grass plants with special reference to the relationship between the shoot and root systems. Grass and Forage Science11, 56–65.

Tubeileh A, Groleau-Renaud V, Plantureux S, Guckert A. 2003. Effect of soil compaction on photosynthesis and carbon partitioning within a maize–soil system. Soil and Tillage Research 71, 151–161.

Uga Y, Kitomi Y, Ishikawa S, Yano M. 2015. Genetic improvement for root growth angle to enhance crop production. Breeding science 65, 111–119.

Uga Y, Okuno K, Yano M. 2011. Dro1, a major QTL involved in deep rooting of rice under upland field conditions. Journal of Experimental Botany 62, 2485–2494.

Uga Y, Sugimoto K, Ogawa S, et al. 2013. Control of root system architecture by DEEPER ROOTING1 increases rice yield under drought conditions. Nature Genetics 45, 1097–1102.

Unger PW, Kaspar TC. 1994. Soil compaction and root growth: a review. Agronomy Journal 86, 759–766.

Valladares F, Gianoli E, Gómez JM. 2007. Ecological limits to plant phenotypic plasticity. New Phytologist 176, 749–763.

van Genuchten MT. 1980. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Science Society of America Journal 44, 892–898.

van Huysteen L. 1983. Interpretation and use of penetrometer data to describe soil compaction in vineyards. South African Journal for Enology and Viticulture 4, 59–65.

Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu JK. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. The Plant Journal 45, 523–539.

Via S, Gomulkiewicz R, De Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH. 1995. Adaptive phenotypic plasticity: consensus and controversy. Trends in Ecology & Evolution 10, 212–217.

Waisel Y, Eshel A. 2002. Functional diversity of various constituents of a single root system. In: Waisel Y, Eshel A, Kafkafi U, eds. Plant roots: the hidden half. New York: Marcel Dekker, 157–174.

Walk T, Jaramillo R, Lynch JP. 2006. Architectural tradeoffs between adventitious and basal roots for phosphorus acquisition. Plant and Soil 279, 347–366.

Wallace A. 1986. Definition of stresses in crop production—iron, plant nutrient, and non-nutrient stress interactions. Journal of Plant Nutrition 9, 187–192.

Wang XL, Wang JJ, Sun RH, et al. 2016. Correlation of the corn compensatory growth mechanism after post-drought rewatering with cytokinin induced by root nitrate absorption. Agricultural Water Management 166, 77–85.

Watt M, McCully ME, Kirkegaard JA. 2003. Soil strength and rate of root elongation alter the accumulation of Pseudomonas spp. and other bacteria in the rhizosphere of wheat. Functional Plant Biology 30, 483–491.

Weaver JE. 1926. Root development of field crops. New York: McGraw-Hill.

Weiner J. 2004. Allocation, plasticity and allometry in plants. Perspectives in Plant Ecology, Evolution and Systematics 6, 207–215.

Whalley WR, Dumitru E, Dexter AR. 1995. Biological effects of soil compaction. Soil and Tillage Research 35, 53–68.

Whalley WR, Leeds-Harrison PB, Clark LJ, Gowing DJG. 2005. Use of effective stress to predict the penetrometer resistance of unsaturated agricultural soils. Soil and Tillage Research 84, 18–27.

Wickham H. 2009. ggplot2: Elegant graphics for data analysis. New York: Springer.

Winn AA. 1996. Adaptation to fine-grained environmental variation: an analysis of within-individual leaf variation in an annual plant. Evolution 50, 1111–1118.

Witzel K, Weidner A, Surabhi GK, Börner A, Mock HP. 2009. Salt stress-induced alterations in the root proteome of barley genotypes with contrasting response towards salinity. Journal of Experimental Botany 60, 3545–3557.

Xie J, Tang L, Wang Z, Xu G, Li Y. 2012. Distinguishing the biomass allocation variance resulting from ontogenetic drift or acclimation to soil texture. PLoS One7, e41502.

Yamauchi T, Shimamura S, Nakazono M, Mochizuki T. 2013. Aerenchyma formation in crop species: a review. Field Crops Research152, 8–16.

York LM, Nord EA, Lynch JP. 2013. Integration of root phenes for soil resource acquisition. Frontiers in Plant Science4, 355.

Younginger BS, Sirová D, Cruzan MB, Ballhorn DJ. 2017. Is biomass a reliable estimate of plant fitness? Applications in Plant Sciences 5:apps.1600094.

Zhang H, Forde BG. 1998. An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. Science 279, 407–409.

Zobel RW. 1992. Soil environment constraints to root growth. In: Hatfield JL, Stewart BA, eds. Limitations to plant root growth. New York: Springer, 27–51.

Acknowledgments

My deepest gratitude goes first to Dr. Tobias Wojciechowski and Dr. Johannes Auke Postma, who guided me through my PhD at FZJ. I am very grateful for their advice, support and especially for keeping me motivated thought all these years.

I would also like to thank Prof. Dr. Frank Hochholdinger and Prof. Dr. Uwe Rascher for being willing to join my thesis committee.

I also thank all the member of the Root group and the greenhouse teams of IBG2. Without their help, thus thesis would not have been possible.

I would also like to express my gratitude and appreciation to Dr. Fabio Fiorani. Not only for all the scientific and technical support, but also for integrating me into the life of the institute.

I acknowledge my wife, Carolina, who has blessed me throughout this period with a life full of love, joy and happiness.

Finally, I thank Professor Prof. Dr. Mitch Tuinstra (Purdue University, IN, USA) for selecting and providing a subset of sorghum genotypes from a larger diversity panel.

The Helmholtz Association institutionally funded this thesis.