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# Genetic analysis of wheat root seedlings in a NAM population

Alice Royer

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- d'Ingénieur de l'Institut Supérieur des Sciences agronomiques, agroalimentaires, horticoles et du paysage
- de Master de l'Institut Supérieur des Sciences agronomiques, agroalimentaires, horticoles et du paysage
- d'un autre établissement (étudiant arrivé en M2)

# Genetic analysis of wheat root seedlings in a NAM population

Par : Alice Royer



***Soutenu à Rennes le 12 septembre 2018***

***Devant le jury composé de :***

Président : Maria Manzanares-Dauleux

Maître de stage : Jack Christopher

Enseignant référent : Anne Laperche

Autres membres du jury :

Marie-Laure Pilet, Rapporteur

*Les analyses et les conclusions de ce travail d'étudiant n'engagent que la responsabilité de son auteur et non celle d'AGROCAMPUS OUEST*

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## **Lists of abbreviations/glossary**

AFLP: Amplified Fragment Length Polymorphism  
BARC: Beltsville Agricultural Research Center  
BLUEs: Best Linear Unbiased Estimations  
CAPS: Cleaved Amplified Polymorphic Sequences  
CIMMYT: International Maize and Wheat Improvement Center  
DART: Diversity Arrays Technology  
DHL: Doubled Haploid Lines  
DHPLC: Denaturing High-Performance Liquid Chromatography  
DM root: Dry biomass of the roots (g)  
DM root:shoot ratio: Dry biomass root to shoot ratio  
Emergence1st\_sem: Emergence time of the first seminal root (°Cd)  
EST-SSR: Expressed Sequence Tag – Simple Sequence Repeat  
FM root: Fresh biomass of the roots (g)  
FM root:shoot ratio: Fresh biomass root to shoot ratio  
GEI: Genotype-environment interactions  
GR1st\_sem: Growth rate of the first seminal root (cm °Cd<sup>-1</sup>)  
G-SSR: Genomic - Simple Sequence Repeat  
ICARDA: International Center for Agricultural Research in the Dry Area  
I-SSR: Inter Simple Sequence Repeat  
GMW: Gatersleben Wheat Microsatellite  
GWAS: Genome Wide Association Study  
Meanapdiam\_lat: Mean apical diameter of the lateral roots at 320 °Cd (cm)  
Meansemi\_rank1: Mean diameter of the 1<sup>st</sup> seminal root over time (cm)  
Meansemi\_rank2: Mean diameter of the 2<sup>nd</sup> seminal root over time (cm)  
Meansemi\_rank3: Mean diameter of the 3<sup>rd</sup> seminal root over time (cm)  
MRL: Maximum Root Length (cm)  
MR-NAM: Multi Reference Nested Association Mapping Population  
NAM Population: Nested Association Mapping Population  
Nblat\_layer 1: Number of lateral roots, layer 1 [2 – 6.7 cm]  
Nblat\_layer 2: Number of lateral roots, layer 2 [8.3 – 13 cm]  
Nblat\_layer 3: Number of lateral roots, layer 3 [13 – 17.7 cm]  
Nblat\_layer 4: Number of lateral roots, layer 4 [17.7 cm - ...]  
Nblat\_tot: Total number of lateral roots at 320 °Cd  
Nb\_sem: Number of seminal roots at 320 °Cd  
PV-disc : Paraffin Vaseline Disc  
Px : Percentage of root length  
QTL: Quantitative Trait Loci  
RAPD: Random Amplified Polymorphic DNA  
RIL: Recombinant Inbred Line  
RFLP: Restriction Fragment Length Polymorphism  
RSA: Root System Architecture  
SR: Seminal Root  
SRAP: Sequence Related Amplified Polymorphism  
SSP: Seed Storage Protein  
SSR: Simple Sequence Repeat  
STS: Sequence-Tagged Sites  
TE: Transpiration Efficiency  
UCL: University Catholic of Louvain  
WMC: Wheat Microsatellite Consortium





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Figure 1 : Australian Wheat Belt and states of Australia (Fischer et al., 2014)

## INTRODUCTION

Wheat is a crop of major importance worldwide. It is the most widely cultivated food crop and the one of the most important food grain sources for humans (Curtis et al., 2002). This crop provides 20% of the daily protein and food energy for half of the human population (FAO, 2018). Australia is the 4<sup>th</sup> largest exporter in the world (Curtis et al., 2002), with a wheatbelt extending over 14 million ha (Fischer et al., 2014) and producing an annual 22 million tons of grain (FAO, 2018).

Demand for food is rapidly growing (Lotze-Campen et al., 2008; Tilman et al., 2011) mainly due to the rapid population growth. In order to produce more, more water would normally be needed for crop cultivation. However, population growth as well as urban and industrial demands are both limiting the water supply remaining for agriculture (Borlaug and Dowsell, 2005). In addition, global climate change is predicted to lead to increases in more extreme weather, that could lead to more frequent drought conditions (IPCC, 2013) including in parts of Australia (Watson et al., 2017).

Availability of water and mineral nutrients is a major limitation to crop productivity in many parts of the world and large inputs of fertilizer are required to support high yields (Tilman et al., 2002). In the past, the green revolution boosted crop yields by introducing dwarf genotypes of wheat capable of responding to fertilization without lodging (Lynch, 2007). Presently, we need to improve both the yield and the drought tolerance while breeding new varieties of wheat (Fleury et al., 2010). Drought is a major limitation of the wheat yield in Australia (Chenu et al., 2013). In 2002-2003 drought in Australia resulted in an estimated 30 % reduction in agricultural output (Horridge et al., 2005).

To improve the drought tolerance, breeders have traditionally focused on the above-ground traits, as studying the roots is typically more challenging. However, improving root traits could lead to substantial increase in yield in drought prone environments (Christopher et al., 2013). An improved root system could increase the amount of water available to the plant as well as providing a better distribution of water supply over the plant growth cycle (Passioura, 1983). With the increasing knowledge concerning this underground system and the development of new root phenotyping systems, breeders are now starting to develop an interest in below-ground traits of wheat (Manschadi et al., 2006; Ren et al., 2012).

In recent years, a number of root quantitative trait loci (QTL) have been identified, mainly in recombinant inbred lines (RIL). Among the traits studied are, the total length of all roots, the number of seminal roots and the seminal root angle. In this study, I used existing data on seedling roots to identify QTL in a Nested Association Mapping (NAM) population designed for the improvement of drought tolerance in northern Australia.

In a literature review, I will go through the importance of improving drought adaptation in Australia, then I will focus on the breeding of drought tolerant wheat by improving below ground traits, and to finish, I will discuss the phenotypic and genetic variability of wheat root traits. I will then present the main problems, objectives and strategy of this study. In a second part, I will detail the NAM population that was used, the phenotyping system, and the experiments that were carried out. Thirdly, I will present our results concerning the phenotyping variability and the QTL analysis. Finally, in a fourth part, I will discuss the methodology and findings of this study



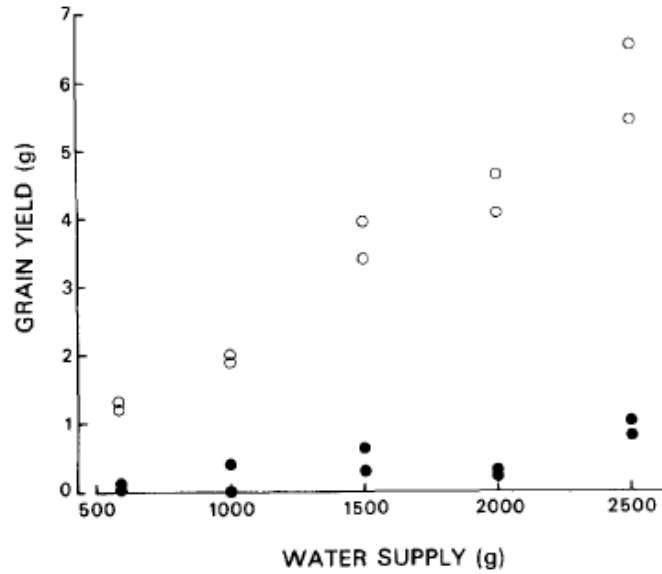


Figure 2: Grain yield as a function of total water supply for individual wheat plants grown in pots. Black dot : all water supplied at sowing; white dot : water metered out throughout the life of the plant (Passioura, 1983)

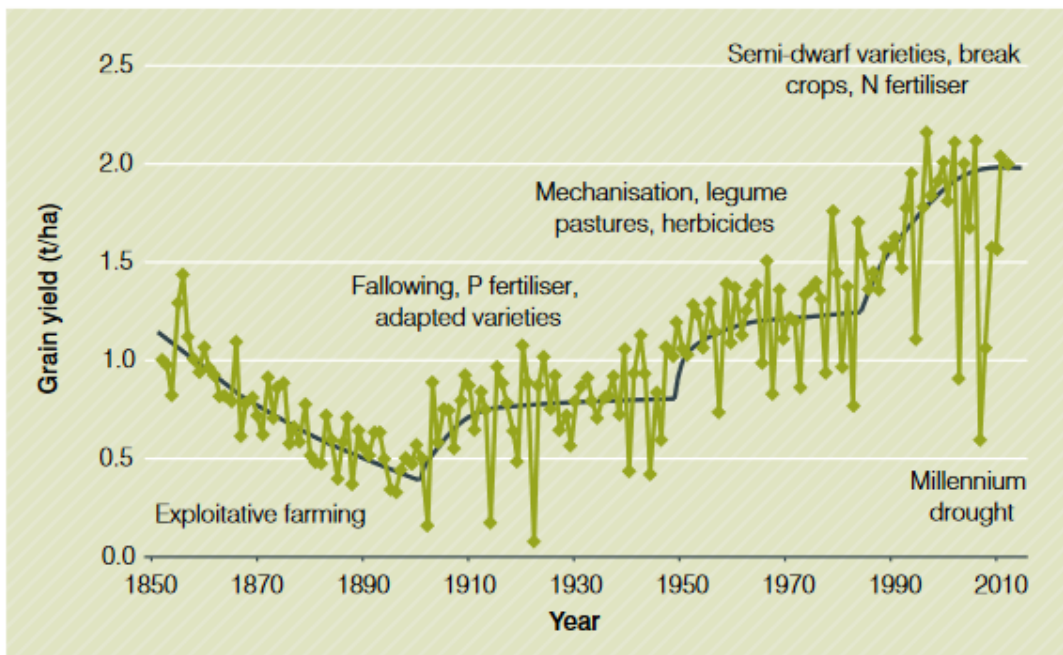


Figure 3: Changes in average farm yield of wheat in Australia from 1852 to 2011 and major drivers of change. P = phosphorus, N = nitrogen (Fischer et al., 2014)

## **I. Literature review**

### **a. Drought adaptation is one of the major targets of Australian wheat breeding**

#### **i. Why drought adaptation as a major trait ?**

Water is a major component of plants, which has essential properties for the crop life. Indeed, water constitutes 80 to 90 % of a monocot plant, it's an excellent solvent used to transport gases or other solutes in the plant, it's a good reactant for the metabolism processes, and finally it maintains the turgidity that is essential for cell growth and enlargement (Kramer and Boyer, 1995). However, water limitation is the greatest production constraint in dryland cropping systems (Christopher et al., 2013), that is known to significantly reduce grain yield (Doorenbos and Kassam, 1979).

The amount of water available to a crop depends both on the amount of water stored in the soil profile at sowing and on the precipitation during the crop cycle (Passioura, 1983). If a water deficit occurs, it can affect both the crop evapotranspiration and yield (Passioura, 1983). It generally reduces the evapotranspiration of the crop, and thus negatively impacts the crop growth and ultimately the crop yield (Doorenbos and Kassam, 1979). In wheat, drought can lead to an increase in the abscisic concentration and lead to pollen sterility (Boyer and Westgate, 2004). However, the way the water stress affects the crop depends a lot on the crop species and the crop cycle period (Doorenbos and Kassam, 1979).

In the northern part of the Australian wheatbelt, the rainfall is summer dominant. Therefore, winter crops like wheat rely heavily on stored soil moisture in the deep clay soil. This can lead to severe terminal moisture stress in seasons with little in-crop rainfall (Chenu et al., 2011, 2013). Hence, severe drought stress is common in production environments of Queensland, representing over 50 % of environments (Chenu et al., 2013; Christopher et al., 2013).

During the 20<sup>th</sup> century, wheat yield in Australia has substantially progressed despite the drought environment due to; mechanization, the use of chemicals, introduction of dwarf varieties as well as other improvements. However, little yield improvement by selection of yield per se has been recorded in the past 20 years (Fischer et al., 2014).

Improving wheat yield under drought conditions in Australia can be done by combining management practices with new varieties that have an improved yield under water limited environments (Passioura, 2006). In the past, irrigation was a solution for water stress, however it is currently set aside due to the present global increasing water demand (Boyer and Westgate, 2004). This restriction has led breeders to focus on the improvement of drought tolerance in wheat, which is defined as the ability of a plant to live, grow and reproduce satisfactorily with limited water supply or under periodic conditions of water deficit (Fleury et al., 2010).

However, drought resistance is complex and involves many morphological (earliness, reduced leaf area, leaf rolling, wax content, efficient rooting system, awn, stability in yield and reduced tillering), physiological (reduced transpiration, high water-use efficiency, stomatal closure and osmotic adjustment) and biochemical components. The different traits involved in the drought resistance of a crop can be either monogenic or polygenic (Mitra, 2001)

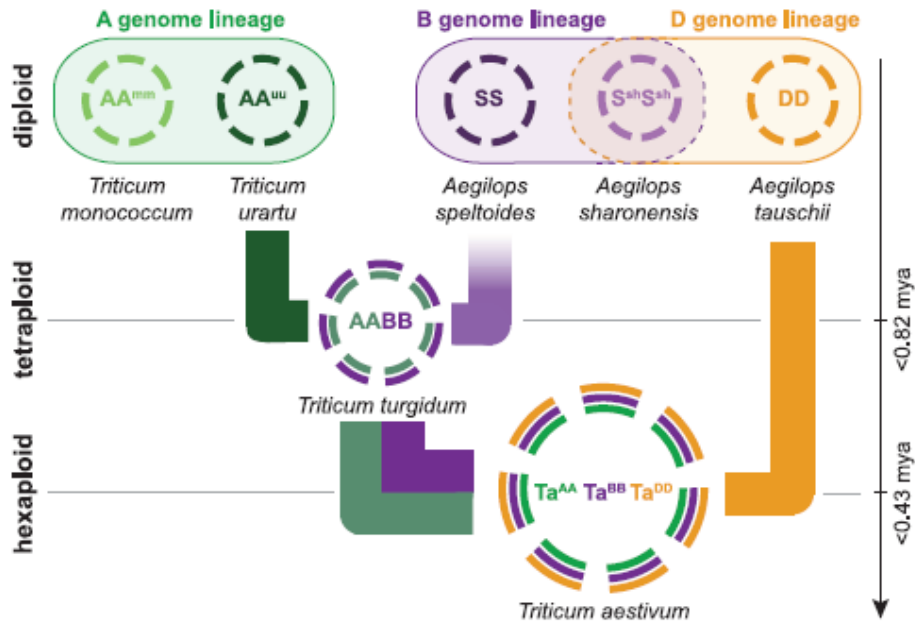


Figure 4: Schematic diagram of the relationships between wheat genomes with polyploidization history and genealogy. Mya : million years ago (Mayer et al., 2014)

Despite the multitude of studies and identification of QTL or genes for improved drought tolerance, only a few varieties have been released that were developed based on such studies. Despite many attempts to use physiological and molecular breeding approaches to improve significant yield under drought conditions, success has been limited. The solution could be to use a multi-disciplinary approach integrating the physiological dissection of drought-tolerance as well as genetic and genomic tools (Fleury et al., 2010).

## ii. Environmental constraints for the breeding of drought tolerance

Selecting a wheat variety for its drought resistance is generally done under drought conditions or under both normal and water limited conditions, to compare the reaction of the cultivar under both conditions (Cattivelli et al., 2008; Fleury et al., 2010). It was demonstrated that some traits allow a good yield under both normal and drought conditions (Richards, 1996). However, breeders have rarely tested in the field the genes they identified; which is essential to characterize correctly the resistance to a specific drought stress (Fleury et al., 2010).

Identifying a gene under drought conditions allows understanding of a small part of the response of a genotype to its environment (Chapman, 2008). Yet in many cases of drought adaptation, the genomic regions controlling the drought tolerance are difficult to identify as their effects are masked by different types of interactions (Chapman, 2008). Among them, the genotype-environment interactions (GEI) constitute an important limiting factor in the estimation of variance components and in the efficiency of selection programs (Hill et al., 1998). GEI are known to limit genetic gain for complex traits such as tolerance to drought. These interactions are intricate and they can considerably reduce the heritability of the yield particularly in drought-prone environments (Chenu et al., 2011).

Thus, it is essential to characterize the different environments in which wheat could be cultivated. Drought stress is influenced by (i) soil characteristics and (ii) rainfall patterns, which both vary spatially and over time; and by (iii) the crop itself (Chenu et al., 2013). Breeding for drought tolerance is also really challenging because several types of abiotic stress can affect the crop at the same time and it becomes harder to identify the cause of a stress resistance (Fleury et al., 2010). In order to be able to understand the effect of drought on crops, it is a challenge to characterize drought over time (Chenu et al., 2013).

Depending on the type of drought involved, the environment may affect different genes and resistance mechanisms (Slafer, 2003; Hammer et al., 2006; Fischer, 2011). Indeed, traits adapted to a specific drought type can confer disadvantage in another drought type. For example, a deep and dense root system is only advantageous if the soil and the water location are deep (Tardieu, 2012), like in northern Queensland where there is a deep soil and low winter rainfall (Manschadi et al., 2006). In Australia, a higher root growth rate leading to a better soil penetration is only beneficial in western Australia due to the deep sandy soils that have poor water holding capacity (Veyradier et al., 2013).

## b. Breeding wheat by improving wheat root systems

### i. Complexity of the wheat genome

Wheat has an allohexaploid genome structure that results from two polyploidization events (Mayer et al., 2014). The first polyploidization is thought to have occurred several hundred thousand years ago and resulted in the cross of two diploid species related to the wild species *Triticum urartu* ( $2n = 2x = 14$ ; AA) with a species that is believed to be related to *Aegilops speltoides* ( $2n = 2x = 14$ ; SS).



This first hybridization formed the allotetraploid *Triticum turgidum* ( $2n = 4x = 28$ ; AABB). A second hybridization occurred between *T. turgidum* and a diploid grass species, *Aegilops tauschii* ( $2n = 2x = 14$ ; DD). It formed the allohexaploid *T. aestivum* ( $2n=6x=42$ ; AABBDD), which has been since cultivated as bread wheat and accounts for over 95 % of the wheat grown worldwide (Mayer et al., 2014). Bread wheat has a big genome size with 17 Gbp (Brenchley et al., 2012), and its whole genome was successfully assembled in 2016 (International Wheat Genome Sequencing Consortium, 2017).

ii. Why improve below ground traits?

The global yield of wheat has increased about two-fold since the first green revolution in the 1960s and 1970s, and the genetic improvement of varieties has largely contributed to this increase (FAO, 2018). During the breeding process, above ground organs have often been used as secondary selection criteria. By contrast, up to recently, below ground organs have been neglected. Our knowledge of root system growth and functioning is limited and the efficiency of root screening methods still being developed (Manschadi et al., 2006). As root architectural traits are important for acquisition of nutrients and water (Gregory, 2007), improving these traits appears as a possible way to evolve towards a more sustainable agriculture.

The root system architecture (RSA) described by the spatial and temporal configuration of the roots in the soil. The ability of a plant to exploit the soil resources is largely determined by RSA. Thus, RSA is an important aspect for plant productivity and yield stability (Ludlow and Muchow, 1990; Lynch, 1995). The shape of the root system defines the distribution of the roots in space and the way the root system occupies the soil. It can be described through the distribution of the root density in the soil (Petrarulo et al., 2015). This system is dynamic, as roots respond to changing moisture, nutrient status, temperature, pH, and they interact with organisms present in the rhizosphere (Bao et al., 2014; Robbins and Dinneney, 2015). Cereals like wheat have a fibrous root system consisting of an extensive mass of thin roots, composed by two types of roots. The seminal roots grow deep in the soil and usually 3 to 6 of them emerge from the seed. The nodal roots emerge later from the coleoptile nodes, and usually remain shallow. Furthermore, lateral roots grow on many main roots. The first order lateral roots arise from the axes and the second order lateral roots that arise from the first order lateral roots (Manske and Vlek, 2002).

Optimizing root architecture can lead to a significant yield advantage in water-limited environments (Manschadi et al., 2006; Veyradier et al., 2013; Christopher et al., 2013). Indeed, a vigorous and efficient root system can help to reduce the negative impacts of drought on plant growth and grain yield (Ehdaie et al., 2016). For example, in subtropical northern Australia with summer dominant rainfall, autumn sown wheat is often highly reliant on stored soil water from previous summer rainfall (Christopher et al., 2013). Thus, root traits are important in northern Australia because they allow plants to adapt to limited water access (Chesnokov et al., 2014). It is important to understand that the amount of water that the plant can access is not the only consideration. The timing of water access is also important. Water is particularly important around and after flowering to set and fill an adequate number of grains (Passioura, 1983). Overall, the study of RSA could help to identify proxy traits for a better adaptation to different types of environments (Veyradier et al., 2013).

**Table 1: References and global information on the population, markers used and QTLs found for the genetic analysis for RSA studies in the literature – Part 1.**

*We could only find the abstract in English for A01, therefore there are few information for this study*

Ref ID	Reference	Parents	Wheat species	Population type and number of lines	Type and number of makers used	Average spacing between markers	Number of root QTL detected
A01	(Zhou et al., 2005)	Hanxuan 10 x Lumai 14	<i>Triticum aestivum</i> L.	150 DHL			11
A02	(Kubo et al., 2007)	Jannah Khetifa x Cham 1	<i>Triticum turgidum</i> L. var. <i>durum</i>	110 RIL	138 RFLPs, 26 SSRs, 134 AFLPs, 5 SSPs and 3 enzyme genes	12 cM	3
A03	(Landjeva et al., 2008)	Opata 85 x W7984	<i>Triticum aestivum</i> L.	114 RIL	1000 RFLPs and 800 SSRs	2 cM	15 (6 in controls, 8 under stressed conditions, 1 tolerance index QTL)
A04	(Landjeva et al., 2010)	Chinese Spring x Synthetic 6x	<i>Triticum aestivum</i> L.	85 RIL			5
A05	(Sharma et al., 2011)	Pavon76 x Pavon 1RS.1BL	<i>Triticum aestivum</i> L. x <i>secale cereale</i>	29 RIL	20 polymorphic physical and molecular markers	2.5 cM	15
A06	(Hamada et al., 2012)	U24 x Ayahikari	<i>Triticum aestivum</i> L.	103 DHL	827 SSRs	9.5 cM	7
A07	(Ibrahim et al., 2012)	Devon x Syn084	<i>Triticum aestivum</i> L.	177 BC <sub>2</sub> F <sub>4:6</sub>	117 SSRs		32
A08	(Kadam et al., 2012)	WL711 x C306	<i>Triticum aestivum</i> L.	206 RIL	169 SSRs + 1 STS + 2 CAPSs + 1 DHPLC	21.2 cM	9
A09	(Ren et al., 2012)	Xiaoyan 54 x Jing 411	<i>Triticum aestivum</i> L.	142 RIL	470 SSRs	7.3 cM	35
A10	(Bai et al., 2013)	Avalon x Cadenza	<i>Triticum aestivum</i> L.	199 DHL			32
A11	(Christopher et al., 2013)	SeriM82 x Hartog	<i>Triticum aestivum</i> L.	184 DHL	841 SSRs	2.4 cM	12
A12	(Liu et al., 2013)	Hanxuan 10 x Lumai 14	<i>Triticum aestivum</i> L.	150 DHL	132 AFLPs + 263 SSRs	9.9 cM	27 (Well-Watered) + 19 (Water-Stressed)
A13	(Sun et al., 2013)	Chuan35050 x Shannong483	<i>Triticum aestivum</i> L.	131 RIL	36 DArTs, 170 SSRs, 100 EST-SSRs, 88 other molecular and biochemical loci	5.57 cM	31
A14	(Zhang et al., 2013)	Weimai8 x Luohan2 Weimai8 x Yannong 19	<i>Triticum aestivum</i> L.	229 RIL 302 RIL	various G-SSR, EST-SSR, ISSR, STS, SRAP, RAPD	8.30 cM (WL), 8.45 cM (WY)	48
A15	(Botwright Acuña et al., 2014)	Halberd x Cranbrook	<i>Triticum aestivum</i> L.	41 DHL	Between 400 and 800 microsatellite, AFLP, DArT, morphological, and biochemical markers		22
A16	(Canè et al., 2014)		<i>Triticum turgidum</i> L. var. <i>durum</i>	183 durum wheat elite accessions and founders genotypes	334 SSRs and 623 DArT markers		18
A17	(Chesnokov et al., 2014)	Opata 85 x W7984	<i>Triticum aestivum</i> L. x ( <i>Aegilops tauschii</i> Coss. X <i>T. turgidum</i> )	110 RIL			5
A18	(Nagel et al., 2014)	Omrabi5 x Belikh2	<i>Triticum turgidum</i> L. var. <i>durum</i>	114 RIL	1072 microsatellites : 159 GWM, 62BARC, 44 WMC		1
A19	(Atkinson et al., 2015)	Rialto x Savannah	<i>Triticum aestivum</i> L. (winter wheat)	94 DHL			29

### iii. Desirable root traits

Depending on the environment, there are variable desirable root traits for wheat. This is not an exhaustive list, but a few traits that come back often in the article. A more exhaustive list of the traits that have been genetically studied is given in the annex 1 and 2.

As nodal roots may stop growing or reduce their growth under water stress, they have not been as commonly studied as seminal roots, which may keep growing under water stress (Manske and Vlek, 2002). You can find below a few root traits that are supposed to play a role in drought adaptation.

- Depth of rooting (Hurd, 1975): Deep roots might provide greater protection against dehydration by extracting water stored in deeper soil horizons (Canè et al., 2014), depending on the type of environment. As mentioned before, this trait is desirable only if the water is located deep in the soil (Tardieu, 2012)
- Root elongation rate (O'Brien, 1979), which is often related to the depth of rooting over time.
- Root distribution at depth (Manschadi et al., 2006) : A thick root system (e.g a root system with a great number of roots at depth) has a positive effect on wheat yield.
- Xylem vessel diameter (Richards and Passioura, 1989): A smaller xylem vessel diameter may allow water to be saved until the post-anthesis period thus allowing more efficiently use of water over the plant cycle especially.
- Angle and number of seminal roots (Nakamoto and Oyanagi, 1994; Manschadi et al., 2008): Root angle is related to the root distribution at depth. A narrow root angle and a high number of seminal roots at the seedling stage can be associated with more roots deep in the soil profile. Seminal root growth angle may be used as a proxy trait for vertical root distribution (Richard et al., 2015)
- Root:shoot dry matter ratio (Siddique et al., 1990): A larger root system generally increases uptake of water and nutrients from the soil but may divert resources from grain production. However in some cases the different distribution of a similar root biomass can result in differences in water uptake (Manschadi et al., 2006).
- Root penetration ability: this trait can be useful either in case of soil compaction that may reduce root access to the deep layers of the soil (Masle and Passioura, 1987) or in where soil has poor water retention properties (Veyradier et al., 2013). Increased root penetration allows the plant to develop more roots in the soil and to access the deeper soil layers.

### c. Phenotypic and genetic variability in wheat root traits

#### i. Phenotyping methods

Studying root traits in fully grown field plants can be challenging for genetic analysis, which requires a large number of plants and genotypes (Bai et al., 2013). Traditional methods for the analysis of root systems such as soil cores, soil columns or large rhizotrons provide high level of detail but are labor intensive, time consuming and are thus often unsuitable for the study of the large mapping populations required for QTL analysis (Blum and Arkin, 1984; Kuecke et al., 1995; Manschadi et al., 2006).



**Table 2: References and global information on the population, markers used and QTLs found for the genetic analysis for RSA studies in the literature – Part 2.**

Ref ID	Reference	Parents	Wheat species	Population type and number of lines	Type and number of makers used	Average spacing between markers	Number of root QTL detected
A20	(Delhaize et al., 2015)	2 MAGIC populations: a 4-way MAGIC pop described by Huang et al., in 2012 ; and an 8-way MAGIC pop resulting from intercrosses between cultivars Westonia, Yitpi, AC Barrie, Xiaoyan 54, Pastor, Alsen, Baxter and Volcani	<i>Triticum aestivum</i> L.	Subpopulation of the 4-way and the 8-way MAGIC RILs			6
A21	(Kabir et al., 2015)	Nongda 3338 x Jingdong 6 Nongda 3331 x Zang 1817	<i>Triticum aestivum</i> L.	216 DHL 217 RIL	274 SSR + 41 EST	9.7 cM	54
A22	(Petrarulo et al., 2015)	Cresox Pedroso	<i>Triticum turgidum</i> L. var. <i>durum</i>	123 RIL	600 (SSR, STS and DArT)	3 cM	57
A23	(Ehdaie et al., 2016)	Iran # 49 x Yecora Rojo	<i>Triticum aestivum</i> L.	120 RIL	173 SSR		28
A24	(Horn et al., 2016)	Spark x Rialto Charger x Badger	<i>Triticum aestivum</i> L.	119 DHL 95 DHL			4
A25	(Maccaferri et al., 2016)	Colosseo x Lloyd Meridiano x Claudio	<i>Triticum turgidum</i> L. var. <i>durum</i>	176 RIL 181 RIL 183 elite cultivars and lines from Mediterranean countries, South Western USA and Mexico	30244 SSR, SNP and DArT	0.09 cM	255 (41 + 67 + 147)
A26	(Iannucci et al., 2017)	Simeto x Molise Colli	<i>Triticum turgidum</i> L. var. <i>durum</i>	136 RIL	9040 markers	0.32 cM	54
A27	(Ma et al., 2017)	Q1028 x Zhengmai 923	<i>Triticum aestivum</i> ssp. <i>Tibetanum</i> (Tibetan semi-wild wheat)	186 RIL	564 DArT + 117 SSR	4cM	15
A28	(Ren et al., 2017)	Xiaoyan 54 x Jing 411	<i>Triticum aestivum</i> L.	142 RIL	470 SSR	7.3 cM	17
A29	(Xie et al., 2017)	Forno x Oberkulmer	<i>Triticum aestivum</i> L. x <i>Triticum spelta</i>	226 RIL	230 RFLP and microsatellites	10.7 cM	38
A30	(Wu et al., 2017)	SHW-L1 x Chuanmai32	<i>T. turgidum</i> ssp. <i>Turgidum</i> x <i>A. tauschii</i> ssp. <i>tauschii</i> x <i>Triticum aestivum</i> L.	138 RIL	120370 SNP, 733 DArT and 119 SSR	0.148 cM	18
A31	(Yang et al., 2018)	H461 x CM107	<i>Triticum aestivum</i> L.	200 RIL	4345 DArT	0.65 cM	7

Faced with the need for robust, high-throughput laboratory screens for genetic analysis, researchers have adopted various artificial media systems to study roots such as hydroponic culture, agarose gel chambers, and systems using germination paper (see examples in Annex 1). However, these growth systems may affect root architecture traits, due to possible differences in seedling development in artificial media, in soil columns or in the field (Wojciechowski et al., 2009).

The observation time and the trial duration of the phenotyping methods varied a lot across the studies, but the trials commonly do not exceed a month. Most of the traits were measured at the seedling stage, for time saving reasons, as seedling root traits are thought to influence the growth and yield of the mature plant (Atkinson et al., 2015).

The observation methods used are mostly manual especially in earlier studies (Table 2). However, in more recent years some software such as WhinRhizo, SmartRoot or RootNav has been developed and used in order to measure the root traits more easily and more rapidly (see annex 1)

## ii. Genetics of root traits

The molecular mechanisms regulating root architecture in the model plant *Arabidopsis* have been well studied over the past 10 years (Péret et al., 2009). However, most of the genes regulating root architecture remain unknown in monocot cereals, which have fibrous root systems distinctly different from the tap root system of *Arabidopsis* (Ren et al., 2012).

Genetic studies of roots traits in wheat are relatively recent, with the first one we could find dating from 2005 (Table 1 and 2). Over the years, the number of publications on root QTL has increased substantially, thus indicating a rising interest for root traits. Most of these articles are studying bread wheat, but a few focused on other wheat species such as durum wheat (Kubo et al., 2007; Canè et al., 2014; Nagel et al., 2014; Petrarulo et al., 2015; Maccaferri et al., 2016; Iannucci et al., 2017; Wu et al., 2017), or on crosses between different species. Various genotypes have been used to create the lines or panels studied., but a few ones are used in different studies. Usually the genotypes used depend on the country where the study was conducted, the genotype characteristics as well as the species.

The objectives of almost all the studies are to improve wheat yield in water-limited environments. A few articles focus also on the improvement of phosphorus or nitrogen assimilation by roots (see Annex 1).

Among the 31 studied listed, a majority is using a linkage analysis to detect the root QTL. Approximately 60% of the linkage analysis are using recombinant inbred lines (RIL), 30 % used doubled haploids lines (DHL), and one study used back-crosses before the self-pollinations. There is only one study using two MAGIC populations (Delhaize et al., 2015), and two studies using panels in order to realize an association analysis (Canè et al., 2014; Maccaferri et al., 2016). The number of lines used for the QTL detection varies across the articles, but generally there are between 100 and 200 lines (Table 1 and 2).

Most studies used different molecular markers (often microsatellites), which makes comparison of marker position between studies difficult. Depending on the articles, from 20 to more than 120 000 markers were used for the genetic map. The average spacing between markers varied from 0.09 cM to 21.2 cM. This distance is highly dependent on the number of markers used, the species of wheat, and on the portion of the wheat genome studied (table 1 and 2).



Between 1 and 255 QTL were detected in the papers listed, which focused on different numbers of traits as well as different numbers of markers and different populations. In total, 913 QTL are listed from the 31 articles (Annex 2). However, it is likely that some of the QTL listed are the same. The chromosomes containing the most QTL are the chromosomes 1B, 2A, 2B, 3B and 4B with 64, 69, 75, 69 and 87 QTL detected, respectively. It seems that most of the QTL are located on the genome B of wheat (see Annex 2).

Some traits were more represented than others (see Annex 2 & 3): total length of all roots (105 QTLs), seminal root number (80 QTLs), seminal root angle (48 QTLs), primary root length (47 QTLs), average root length (43 QTLs). For the seminal root angle, the primary root length and the average root length, it is due to 255 QTLs found by Maccaferri in 2015 that represent many of the genomic regions located. The total length of root and the seminal root number are common traits to study among the root traits and were analyzed in a large number of studies.

#### d. Problems, objectives and strategies of the present study

In this study, I will investigate the genomic regions that are involved in root traits with a view to improving drought tolerance in wheat.

The main objectives are to (i) characterize the phenotypic variability of the different root traits in a NAM population developed for the northern part of the Australian wheatbelt and to (ii) identify genomic markers that are linked to genomic regions coding for root traits and see the reliability of these markers.

To proceed, I studied the literature in order to understand the RSA and the important traits related to drought adaptation. Then, I will examine the aeroponics data of a NAM population issued from an aeroponics phenotyping method in order to evaluate the diversity among the parents and the lines from the NAM population. Finally, I will identify molecular markers that could be of interest for breeders wishing to select for these traits.

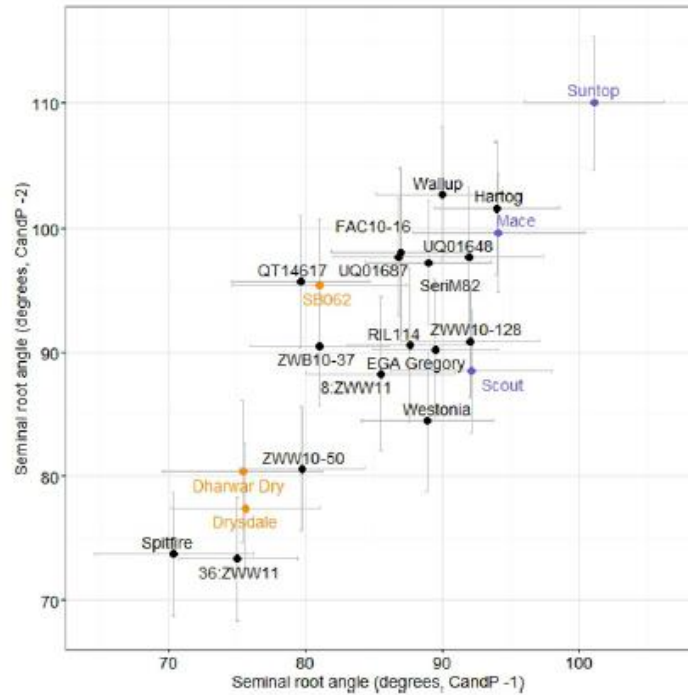


Figure 5 :Mean seminal root angle (BLUPs) for a panel of 22 Australian-adapted wheat lines (Richard et al., 2018). *CandP-1* and *CandP-2* are the 2 experiments in which the mean seminal root was measured

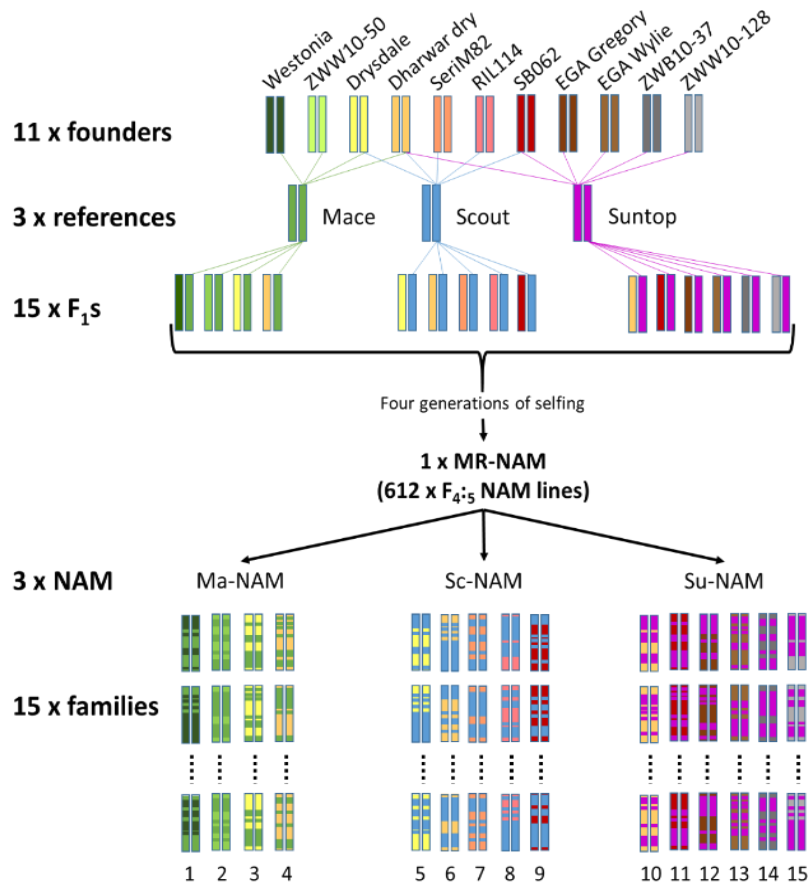


Figure 6: Diagram illustrating the development of the multi-reference parent nested association mapping population (Richard, 2017)  
All the donors, and lines are not represented on this figure.

## II. Materials and methods

### a. Plant material: a NAM population issued from a multi-NAM population

The experiments were carried out using a wheat Nested Association Mapping (NAM) population, subset of a Multi Reference Nested Association Mapping (MR-NAM) population developed at UQ QAAFI. Unlike biparental populations, multiparent populations like MAGIC or NAM have the potential to increase the numbers of alleles evaluated, and due to the higher population size and recombination number the resolution is much better (Rebetzke et al., 2014). NAM populations are an excellent material for genetic study and breeding for adaptation.

The MR-NAM has been created crossing three reference parents (cultivars Mace, Suntop, Scout) with a panel of 10 founder lines. Reference parents were used as females and founders as males. The reference parents Mace, Scout and Suntop, are respectively cultivars well adapted to the Western, Southern and North-Eastern cropping regions of Australia. These three cropping regions of Australia contrast in patterns of abiotic crop stress (Rebetzke et al., 2012). Furthermore, they have been chosen for their high wheat quality as well as a good disease resistance, including to leaf rust and stem rust.

The 10 founders were selected based on their pedigree and origin to incorporate genetic diversity, as well as for specific traits of interest for improving wheat production in diverse environments in Australia: drought and heat tolerance, disease tolerance, acid-soil tolerance and pre-harvest sprouting tolerance (Annex 5). As we can see on figure x, there is a wide range of seminal root angle among founders, which shows a high diversity among the founders. Four of the founders come from the International Maize and Wheat Improvement Center (CIMMYT) in Mexico, four come from different Australian breeding programs, and FAC10-16 was bred by the International Center for Agricultural Research in the Dry Area (ICARDA) (annex 5).

The population used for the experiments is the Su-NAM population, nested subset of the MR-NAM population (Richard et al., 2018, Fig. 6), comprising the progenies of the 10 founder lines and the Suntop reference parent. Suntop is well adapted to the north-eastern region of Australia, which has summer dominant to year-round rainfall and generally heavier soils, with high water holding capacity. Suntop has a high transpiration efficiency (TE) which means that this genotype is efficient in converting transpired water into biomass (Fletcher and Chenu, 2015). Interestingly, Suntop is also known to have a wide seminal root angle (Richard, 2017), while narrow seminal root angle has been proposed as a proxy trait for deep mature roots and increase of water extraction at depth (Manschadi et al., 2006). This trait may thus be improved to further enhanced drought adaptation in the region.

The Su-NAM population was developed by 4 generations of self-fertilization of the 10 F1 progenies to create 507 RILs (Richard et al., 2018, Fig. 6). At the first generation a bulk selection was performed, at the second-generation progeny were selected for plant height and maturity similar to the reference to keep 500 F2 plants per family, and for the last two generations of self-fertilization a single seed descent selection was applied. The number of lines per NAM family is given in the annex 4. In the experiment, the entire Su-NAM population (507 RILs) was studied as well as the 11 parents (10 founders + 1 reference).



Figure 7: Picture of an aeroponic box at the end of an experiment



Figure 8: A strip of an aeroponic box with growing plants

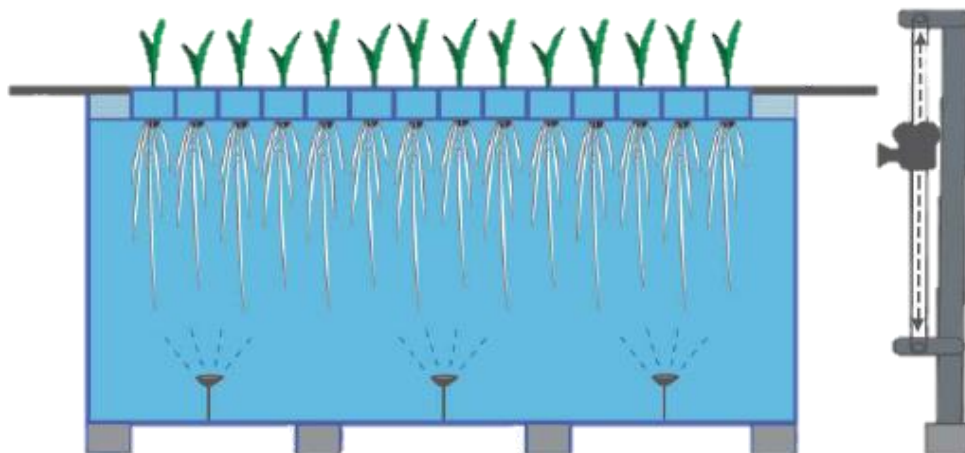


Figure 9: Sideview of an aeroponic platform (Ligeza et al., 2011)

## b. Aeroponic platform

The phenotyping part of the experiment has been carried out by Patrick Thaon in the aeroponic platform of the University Catholic of Louvain (UCL) designed and built by Xavier Draye, in Belgium, in November and December 2016 (1<sup>st</sup> and 2<sup>nd</sup> experiment). The platform comprises two independent aeroponic boxes. Each box has a volume of 11m<sup>3</sup> (1.7m x 1.2m x 5.4m) and conveys 99 strips (30cm x 10cm x 3cm) of 5 slots, so that the platform has a total capacity of 990 plants (2 x 99 x 5) (Fig. 7).

The seeds were sown on the 1<sup>st</sup> of November and the 1<sup>st</sup> of December 2016, and the seedlings were grown in rockwool pieces that fit the size of the slots (figure 8). The protocol consisted in placing each seed in a small cavity drilled in the rockwool plug and adding 1 mL of water. In addition, the seeds were sterilized before sowing by soaking 30 seconds in ethanol (70%), 3 minutes in bleach (5%) and rinsed three times in deionized water.

Problems of germinations were noticed during the first experiment and only a relatively low germination rate was achieved (76.92 % in the first box and 66.87 % in the second box). To resolve this, the protocol was modified in the second experiment and additional seeds were germinated in petri dishes, in order to replace seeds that did not germinate on the rock wool. Eventually the germination rate for the second experiment was 95 % which was much better.

When roots started to emerge from the rock wool (4 days after sowing), a nutritive solution was supplied with the pulverization of droplets through agricultural nozzles placed at the bottom of each box and oriented upwards (Ligeza et al., 2011, Fig. 9). A full-strength Hewitt solution at pH 6 was used (annex 6). To avoid saturating the plugs during germination, spraying occurred for at 5 s at only 30 min interval. After germination, the settings were changed gradually to 20 s spraying at 5 min interval during the day, and at 15 s length and 7 min interval during the night. A 3D structure was located under each plant slot to maintain the roots in place and avoid aggregation.

Plants were grown for 21 days (1<sup>st</sup> nov - 21<sup>st</sup> nov and 1<sup>st</sup> dec – 21<sup>st</sup> dec). At that stage, the longest root was about 40-cm long for the most vigorous lines. A longer time is not suitable since the roots are then too long and numerous for the analysis. The harvest dates (21<sup>st</sup> of nov and 21<sup>st</sup> of dec) correspond to the achievement of 320° Cd. At harvest, the shoot and root part of every plant were weighted, then dried at 80°C for 24 hours. The fresh and dry weights were recorded.

Mean temperatures of 18.28 and 17.79 °C, mean hygrometries of 62.35 and 63.15 % and mean luminosities of 35.9 and 30.38 W m<sup>-2</sup> day were recorded respectively during the November and December experiments.

## c. Experimental design

Seeds of the 521 genotypes were laid in the platform following a partially replicated design for the two experiments. The first experiment occurred from the 1st to the 21st of November 2016 and the second one from the 1st to the 21st of December 2016. In each experiment, each box constituted a design block for replicated entries.

The 11 parental genotypes (references + founder) were replicated six times in each experiment, with three plants in each box. The other genotypes were split into three groups, according to the family size, with different numbers of genotypes randomly selected from each family.



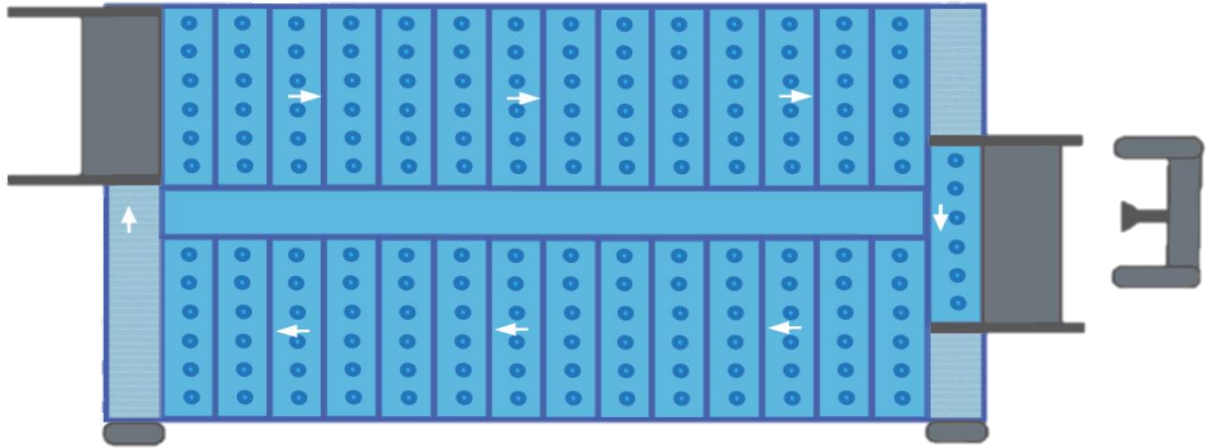


Figure 10: Top view of an aeroponic platform (Ligeza et al., 2011)  
*The camera is visible on the right of the image. The white arrows represent the movement of the strips on the conveyor that brings every plant in front of the camera for the image recording.*

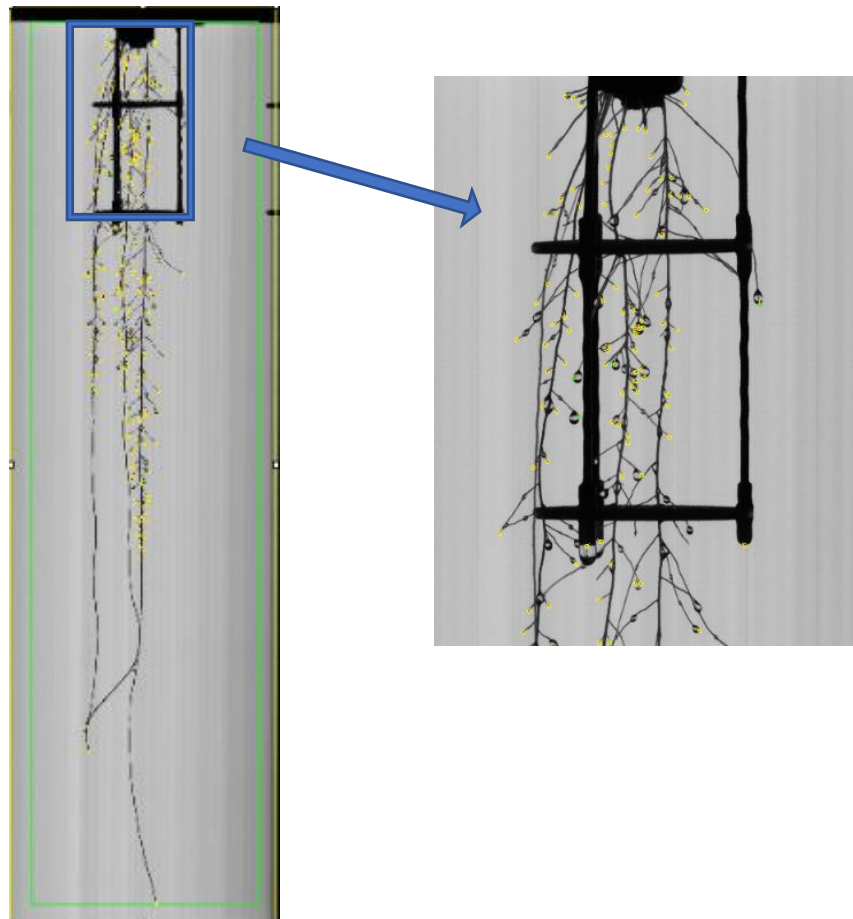


Figure 11: Photo of the root system taken 21 days after sowing. The root tips identified during the image analysis are shown in yellow.

These groups were thus partially balanced across families:

- Group 1: two replications in each experiment
- Group 2: two replications in the first experiment and one in the second
- Group 3: one replication in the first experiment and two in the second

Plants were assigned to a location using an optimal design model (Butler et al., 2008) including terms for the strips, the slot on the strips and the family groups.

#### d. Phenotyping and image processing

##### i. Image capture

The 99 strips were disposed on a conveyor system which brings each strip in front of the imaging station in sequence (Ligeza et al., 2011, Fig. 11). A complete rotation takes approximately 2 hours (i.e. time resolution). Image capture was made with a backlight, which provides a good contrast and a homogeneous background that ease the image processing.

Image capture started when the first roots began to emerge from the rockwool, 4 days after sowing. In optimal conditions, a sequence of almost 200 images was generated for each plant. This means a total of 200 000 high definition images for a 21-day long experiment. Automated procedures are thus needed to analyze these images.

##### ii. Image analysis

The Aeroscan software was developed in the lab to process individual images (fig 11). The application extracts:

- the date
- the time
- the QR code
- the root tips

For the root tips, it computes:

- the 2D coordinates for the tip relative to the rockwool centre
- the growth direction of the root by analyzing the segment upstream of the root tip
- the diameter of the root segment located 1 cm upstream of the tip

Even if Aeroscan was developed for this purpose, a few problems remain:

- A drop of nutritive solution regularly appeared at the root tip and hampers the correct detection or localization of the tip. In this case, Aeroscan uses a specific algorithm to try and guess the correct position. This algorithm fails only for a small fraction of tips.
- The imaging provides a 2D projection of a 3D system. Consequently, root length and angles are biased for most roots.
- The root holder is present on the top 8 cm of the root system (figures 8 and 11). Below 8 cm, roots can aggregate, and tips can be hidden behind other roots. There is no correction for this effect.
- The resolution of the camera might not be sufficient to capture roots with a really small diameter or the nearly transparent ones. Additionally, the diameter difference between the seminal and lateral roots is sometimes subtle.
- Aeroscan does not associate the tips of a given root over time.

**Table 3: Root traits phenotyped with the aeroponic platform**

*Traits from the seminal roots are presented in red, lateral roots in blue and biomass in green*

Root trait	Abbreviation	Unit
Emergence time of the first seminal root	Emergence1st_sem	°Cd
Growth rate of the 1 <sup>st</sup> seminal root	GR1st_sem	cm °Cd <sup>-1</sup>
Maximum root length at 320 °Cd (harvest)	MRL	cm
Mean diameter of the 1 <sup>st</sup> seminal root over time	Meansemi_rank1	cm
Mean diameter of the 2 <sup>nd</sup> seminal root over time	Meansemi_rank2	cm
Mean diameter of the 3 <sup>rd</sup> seminal root over time	Meansemi_rank3	cm
Number of seminal roots at 320 °Cd (harvest)	Nb_sem	-
Mean apical diameter of the lateral roots at 320 °Cd (harvest)	Meanapdiam_lat	cm
Total number of lateral roots at 320 °Cd	Nblat_tot	-
Number of lateral roots, layer 1 [2 cm – 6.7 cm]	Nblat_layer1	-
Number of lateral roots, layer 2 [8.3 cm -13 cm]	Nblat_layer2	-
Number of lateral roots, layer 3 [13 cm – 17.7 cm]	Nblat_layer3	-
Number of lateral roots, layer 4 [17.7 cm - ...]	Nblat_layer4	-
Fresh biomass of the roots	FM root	g
Dry biomass of the roots	DM root	g
Fresh biomass root to shoot ratio	FM root:shoot ratio	-
Dry biomass root to shoot ratio	DM root:shoot ratio	-

### iii. Traits phenotyped

In total, 17 root traits were phenotyped (table 3).

#### iii-1. Phenotyping of seminal root traits

Every tip was assigned to the good root by calculating the Euclidian distance from origin of the tips. This distance allows a better detection of trajectories, because it's less sensitive to horizontal noise. The seminal roots growing almost vertically in the aeroponic platforms, this distance can also approximate their length. It gave us the figure in annex 7.

The seminal root tips of each image belonging to the group A (annex 7) were analyzed and ranked according to their Euclidian distance to the rockwool plug; starting with rank 1 for the 1<sup>st</sup> seminal root, rank 2 for the 2<sup>nd</sup> seminal root... Then the rank values over successive time steps were assembled, and we checked over two successive time steps, that the difference in x and y coordinates of the tips with the same rank was compatible with the seminal root (SR) elongation in 2 hours. In the contrary, the tips were considered as wrongly assigned and removed.

The number of tips in the [A] part was used to calculate the number of seminal roots at 320 °Cd (annex 7). To calculate the emergence time and the growth rate of the first seminal root, a linear regression of Euclidian distance over thermal time for all the SR tips correctly assigned.

$$\text{length of the root} = \text{intercept} + (\text{growth rate} \times \text{thermal time})$$

The tips located too far from the regression line were removed and the growth rate (slope) as well as the time of emergence (intercept on the abscise) were extracted from this regression.

The maximum root length was estimated for every plant by taking the highest Euclidian distance in the population of tips for the plant, at a thermal time of 320 °Cd. In most of the case, it's the value of the first seminal root.

The diameter of the first and second seminal roots were recorded over time at the tip, and it was not significantly changing over time. The mean of the 1<sup>st</sup>, 2<sup>nd</sup> SR and 3<sup>rd</sup> SR diameter were computed as the mean diameter of all its tips over time, after removal of the aberrant values.

#### iii-2. Phenotyping of lateral root traits

The assignment of tips by lateral root was not possible because a majority of lateral root tips appeared near one another. The analysis was therefore restricted to the number of tips in the [B] zone.

The mean apical diameter was calculated by the mean diameter of all tips. The total number of lateral roots over time was calculated by counting the total number of tips in the [B] zone at 320 °Cd. It was then divided in four horizontal layers:

- Layer 1: [2 cm – 6.7 cm]
- Layer 2: [8.3 cm -13 cm]
- Layer 3: [13 cm – 17.7 cm]
- Layer 4: [17.7 cm - ...]

The tips located close to the root holders were removed, this is why there is a gap between some of the layers.



#### e. Statistical design and analysis

Experimental data were influenced both by the structural terms, i.e. experimental layout and by the treatments and by explanatory terms, i.e. the genotype.

Root traits were first analyzed using a first linear mixed model. The model was fitted in ASreml-R in the R-software environment (Butler et al., 2009). Experiment and family were included as fixed effects, while genotype, genotype by experiment interactions and platform by experiment interactions were included as random terms. The design factors within each experiment were also included as random terms by adding them in the residual covariance. The outliers deviating for more than four standard deviations from the mean were removed from further analysis. The mixed model was used to calculate the broad-sense heritability. It was calculated with this formula  $h^2 = 1 - \left(\frac{fm.avsed^2}{2*fm.gvar}\right)$ , where *fm.avsed* is the phenotypic variance and *fm.gvar* is the genotypic variance. The genotypic variance corresponds to the variance of the genotype plus the variance of the genotype to experiment interactions.

In a second step, a second linear mixed model was used to determine if the genotype effects were significant. It was also used to determine whether genotype by experiment interactions were significant, i.e. whether experiments had to be analyzed separately for the considered trait. Experiment, genotype and genotype by experiment interactions were included as fixed effects, while platform by experiment interactions were included as random terms. The design factors were included in the residual covariance as in the precedent model. The Best Linear Unbiased Estimations (BLUEs) were calculated using the effects found by the “fixed model”

#### f. Genetic map construction and QTL analysis

18827 SNPs were available for the analysis. These markers were cleaned for both the parents and the lines before the QTL analysis, in order to use only the most relevant ones. This resulted in 4284 SNPs, i.e. 22.75% of the total SNPs, which were used for the analysis.

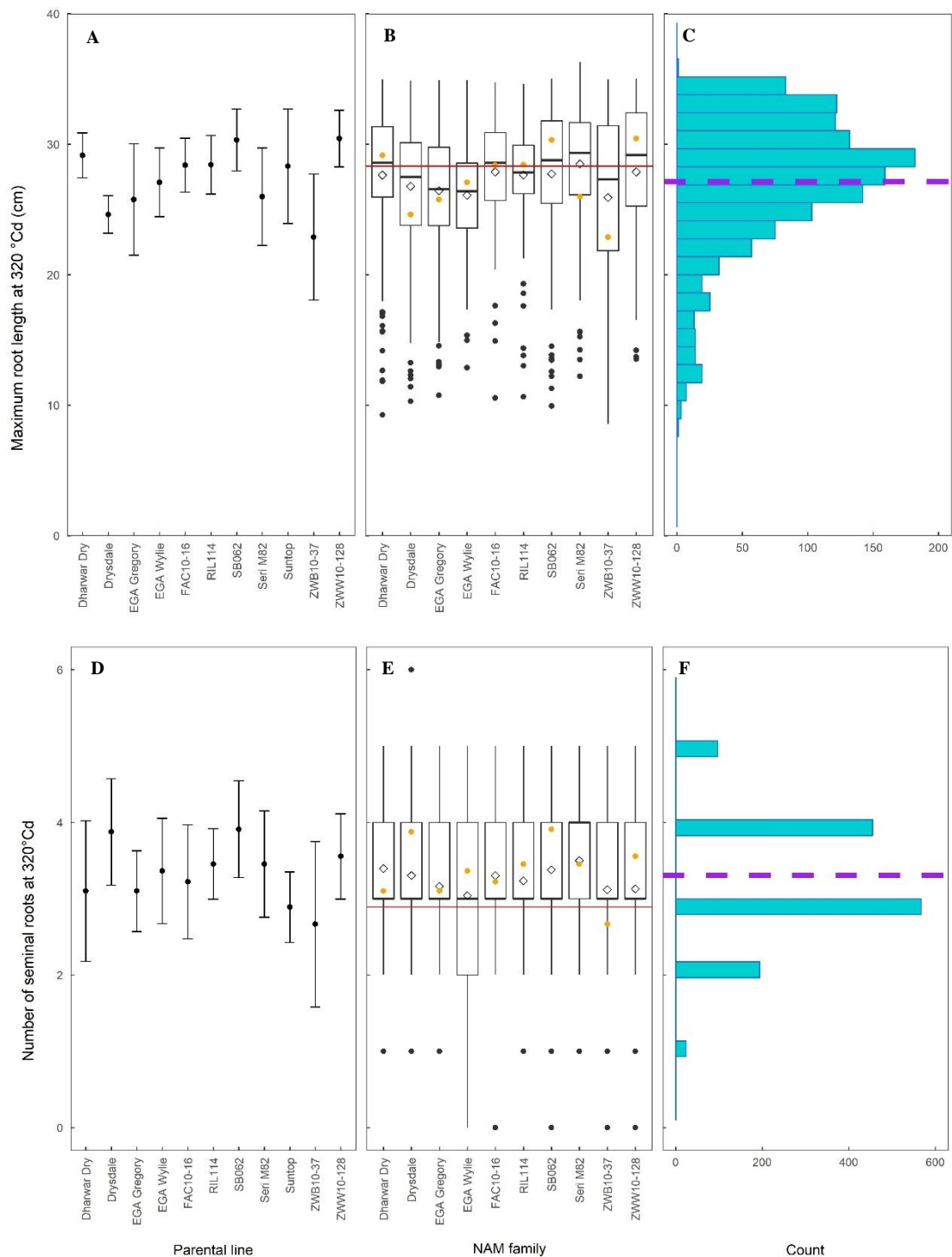
While Genome Wide Association Study (GWAS) is now commonly used for genetic analyses because of the increasing availability of genomic polymorphism data, it is performed using a single locus test to identify association between markers and traits but do not account for any possible population structure. The population studied here was structured as a NAM and using a GWAS analysis that ignore this structure can lead to false positives detection.

Here, to resolve these problems, we used a stepwise mixed-model regression with forward inclusion and backward elimination, called a Multi Locus Mixed Model (MLMM). At each step of the forward inclusion, the genetic and residual variances were re-estimated, and the most significant SNP was added to the model as a cofactor until  $\text{Var}(\alpha_{ij})/\text{Var}(Y_{ij})$  was close to zero. The backward elimination removed at each step the least significant cofactor, allowing to explore more thoroughly the model space. The p\_values Bonferroni-corrected of the SNP are all lower than the threshold (0.05). The model used was the following one (Segura et al., 2012):

$$Y_{ij} = \mu + f_i + \beta X_{ij} + \dots + \delta Z_{ij} + \alpha_{ij} + \varepsilon_{ij}$$

Where  $Y_{ij}$  is the phenotypic value (BLUEs) for the  $j^{\text{th}}$  individual in the  $i^{\text{th}}$  NAM family,  $\mu$  is the general mean of the NAM,  $f_i$  is the fixed effect of the  $i^{\text{th}}$  NAM family,  $\beta$  and  $\delta$  are the fixed coefficients of the markers (as we use a multi-locus mixed-model),  $X_{ijk}$  and  $Z_{ij}$  is the effect of the markers for the  $j^{\text{th}}$  individual in the  $i^{\text{th}}$  family,  $\alpha$  is a vector of random polygenic effects ( $\alpha_{ij} \sim N(0, K\sigma^2_\alpha)$ ), and  $\varepsilon_{ij}$  is the residual term ( $\varepsilon \sim N(0, \sigma^2_\varepsilon)$ ).  $K$  is the kinship matrix, i.e. the parental relationship effects.

**Figure 12 : Parental (first column), family (middle column) and NAM (last column) phenotypic distribution of traits relative to seminal roots, namely the maximum root length (first row) and the number of seminal roots (second row).** Mean values of the parental lines are presented in the first column (A and D) with a confidence interval at  $P = 0.05$ . Distribution for the NAM families are presented as boxplots (second column, B and E). The lower, middle and upper lines of the boxplot indicate 25, 50 (median) and 75% of trait values, respectively. The whiskers correspond to 1.5 times the interquartile range (IQR, the difference between the 75th and 25th percentiles) or to the most extreme observed value, whichever is smallest. Dots above or below the whiskers represent individual values outside this range. The diamonds correspond to the NAM-family means, the solid orange points to the family-donor mean, and the red line to the mean of reference line Suntop. An histogram of the distribution of the value of the trait for all the individual plants within the NAM is presented in the last column (C and F). The purple dashed line represents the mean value of all the individual plants of the NAM.



### III. Results

#### a. Root phenotypic diversity among parents and within the NAM population

##### i. Seminal root traits

While the time of emergence of the first seminal root ranged from  $88 \pm 23$  °Cd (Drysdale) to  $121 \pm 14$  °Cd (SB062) among the parents, no significant differences were observed (Annex 8, graph A). The mean value of all the individuals is smaller than the mean of Suntop ( $106.11$  °Cd against  $119.42$  °Cd). Furthermore, all the NAM families have a smaller mean than their common parent Suntop (Annex 8, graph B). It probably means that the crosses between the donors and Suntop generated recombinations and an accumulation of genes favouring fast emergence of the first root. However, we can see on the distribution (Annex 8, Graph C) that the graph is split in two parts. This might be due to the problems of germinations encountered during the first experiment.

Significant variation in the growth rate of the first seminal root was observed among the parents, with the fastest growth for ZWW10-128 and SB062 ( $0.12 \pm 0.01$  and  $0.13 \pm 0.01$  cm °Cd<sup>-1</sup>, respectively), and the slowest for ZWB10-37 and Drysdale ( $0.09 \pm 0.02$  and  $0.09 \pm 0.01$  cm °Cd<sup>-1</sup>, respectively) (Annex 8, graph D). The mean value of all the individuals was slightly smaller than the mean value of Suntop ( $0.109$  cm °Cd<sup>-1</sup> against  $0.116$  cm °Cd<sup>-1</sup>). Furthermore, the first seminal roots from most of the NAM families grew slower on average than Suntop.

The maximum root length (MRL) measured at 320 °Cd is most likely to correspond to the root length of the first seminal root, which was the first to emerge. Significant differences were observed between the parents, with the deepest roots for ZWW10-128 and SB062 ( $30.4 \pm 2.2$  and  $30.3 \pm 2.4$  cm, respectively), and the shallowest roots for Drysdale and ZWB10-37 ( $24.6 \pm 1.4$  and  $22.9 \pm 4.8$  cm, respectively) (Fig. 12, graph A). The maximum root length values are high in general, and the highest mean (Seri M82) is 9.92 % higher than the smallest family mean (ZWB10-37). As expected, MRL was highly correlated to the growth rate ( $r = 0.8$ , Fig. 14), which means that the plants with a high growth rate had a high MRL. Suntop mean value was greater than the mean of all the individuals ( $28.31$  cm against  $27.14$  cm). Although there are individuals on every families that have longest roots than Suntop, the crossings between Suntop and the donors didn't tend to produce families with longest roots in general (Fig. 12, graph B). In fact, the mean of the families tended to be closer to the mean of Suntop than the one of their donor parents. It means that the crosses didn't allow to accumulate favorable genes in general in the families, except for a few individuals. We can also notice that the distribution is not completely centered (Fig. 12, graph C), mostly due to some plants in most of the families that have shallow first seminal roots, or to the first seminal root which didn't grow properly down, and so the measurement corresponds to another seminal root.

The number of seminal roots measured at 320 °Cd varies from 0 to 6 for the parents and the NAM families, however, it is likely that all the seminal roots haven't been detected. Indeed, after the second seminal it becomes harder to detect correctly the seminal roots. The number of seminal roots is negatively correlated ( $r = -0.24$ , fig. 14) with the emergence of the first seminal root, which means that the longer it takes for the first seminal root to emerge, the less seminal roots the plant will have. Among the parents, SB062 and Drysdale had the most seminal roots ( $3.9 \pm 0.6$  and  $3.9 \pm 0.7$  respectively), and ZWB10-37 and Suntop had the fewest ( $2.7 \pm 1$  and  $2.9 \pm 0.5$  respectively), however there were no significant differences (Fig. 12, graph C). The highest family mean (Seri M82) is 15.13 % higher than the smallest family mean (EGA Wylie).



Figure 13: Mean number of lateral roots in the whole NAM population (right) within the 4 layers (left), and mean depth of the first seminal root (SR) (bottom)

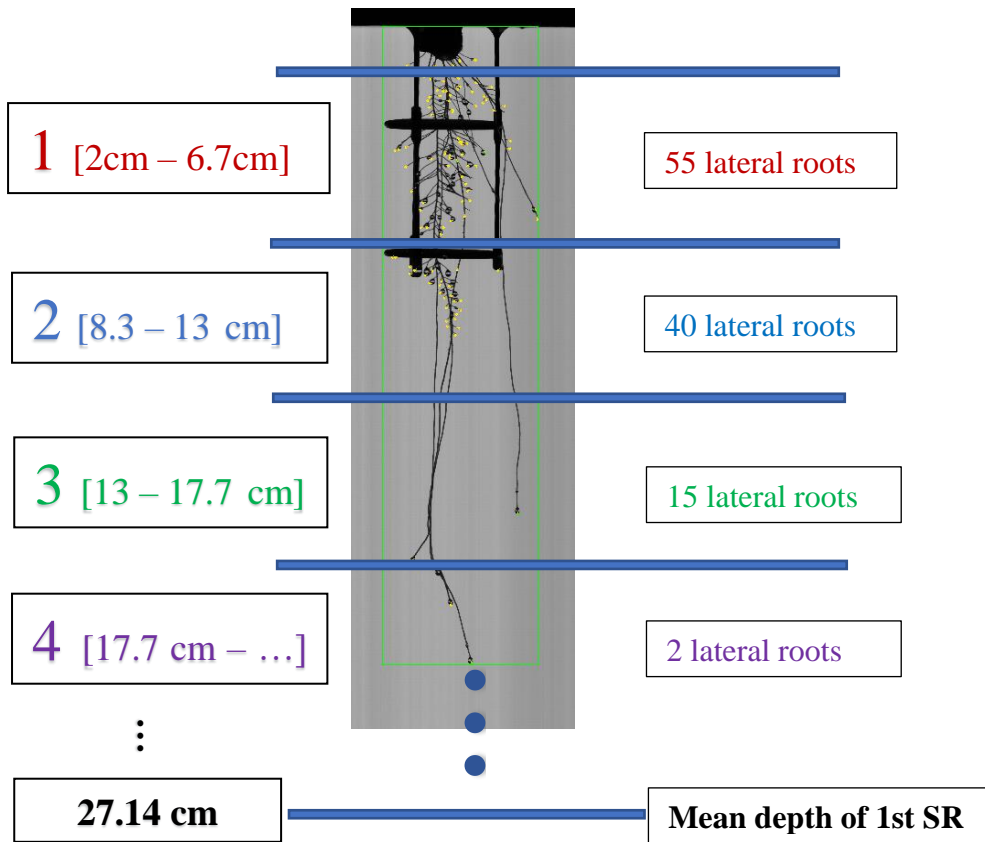
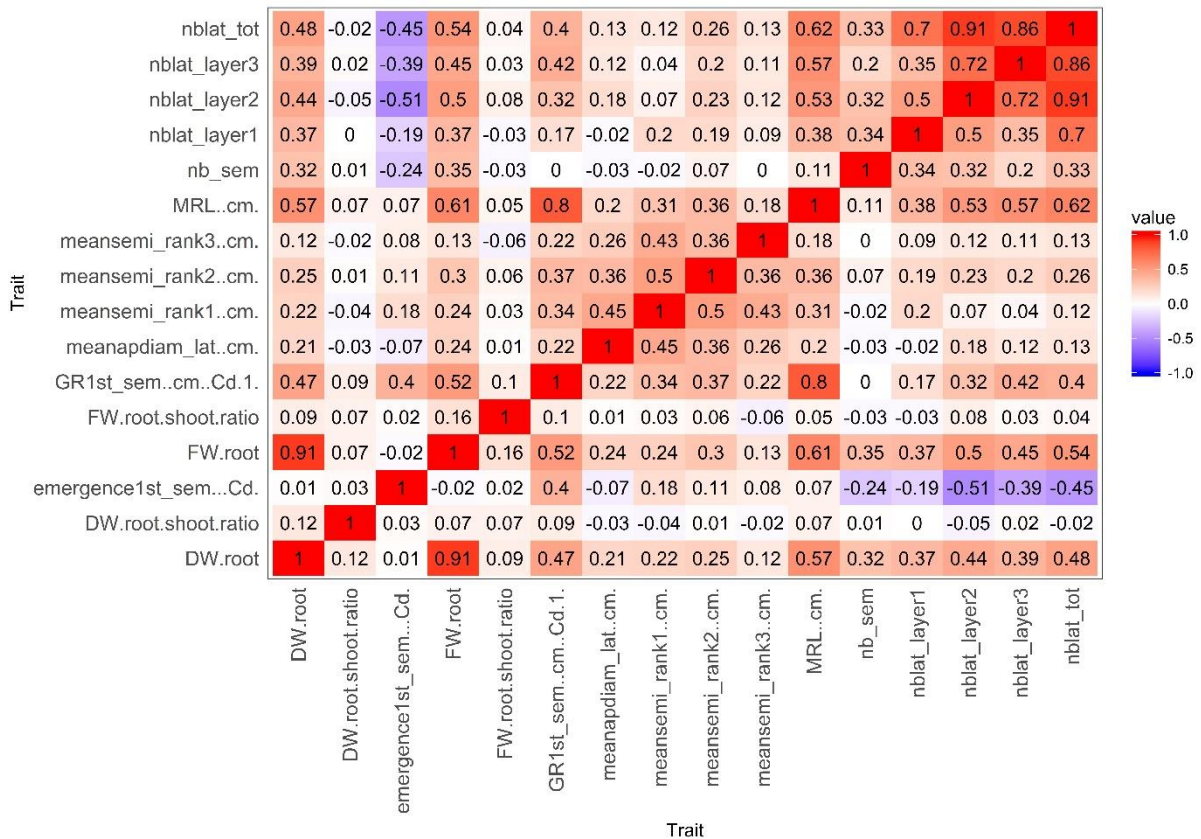


Figure 14 : Correlations between the root variables



Furthermore, all the NAM families have a higher mean than their commun parent Suntop (Fig. 12, graph D), and the mean value of all the individuals was greater than the Suntop mean (3.31 against 2.89).

No significant differences were observed between the parents for the mean diameter of the first, second and third SR. The mean diameter of the first SR varied from 0.0445 to 0.497 with Drysdale having the narrowest 1<sup>st</sup> SR ( $0.0445 \pm 0.0039$  cm) and Dharwar Dry having the thickest 1<sup>st</sup> SR ( $0.0497 \pm 0.0033$  cm) (Annex 8, Graph G). The mean diameter of the second SR varied from 0.0404 and 0.0464 with Drysdale having the narrowest 2<sup>nd</sup> SR ( $0.0404 \pm 0.0038$  cm) and RIL114 having the thickest 2<sup>nd</sup> SR ( $0.0464 \pm 0.0029$  cm) (Annex 8, Graph J). The mean diameter of the third seminal root is less reliable, because there are big confidence intervals and only one value for the parent Suntop (Annex 8, Graph M). The mean of the NAM families for these three traits were very often under the mean of Suntop (Annex 8, Graph H & K). Furthermore, the mean of Suntop is higher than the mean of all individuals for the three traits (1<sup>st</sup> SR: 0.0473 against 0.0465 cm, 2<sup>nd</sup> SR: 0.0449 against 0.0436 cm, 3<sup>rd</sup> SR: 0.0526 against 0.0427 cm). We can also notice that the 2<sup>nd</sup> SR was often narrower than the 1<sup>st</sup> seminal root, but it wasn't true for the third SR. It might be due to some mistakes. Indeed, it can be difficult to localize correctly the third seminal root on the plant.

## ii. Lateral root traits

By contrast to the seminal roots, the lateral roots are shallower because they start growing later. The deepest we go into the soil, the less lateral roots we can find. Indeed, very few lateral roots grow under 17.7 cm whereas the mean depth of the first seminal root is 27.14 cm (Fig. 13).

The total number of lateral roots measured at 320°Cd varied from 91 to 140 for the parents, with EGA Gregory having the less lateral roots ( $91 \pm 41$ ) and SB062 having the more lateral roots ( $140 \pm 32$ ). However, no significant variations between the parents were observed (Annex 9, graph A). The mean of the NAM families were often close to the mean of Suntop. In fact, the mean of the families tended to be closer to the mean of Suntop than the one of their donor parents (Annex 9, graph B). Furthermore, the mean value of all individuals is slightly higher than the mean value of Suntop (112 against 110).

In the first layer, the number of lateral roots measured at 320 °Cd in the first layer varied from 49 to 63 on average for the parents, with EGA Gregory having the less shallow lateral roots ( $49 \pm 16$ ) and Drysdale having the most shallow lateral roots ( $63 \pm 20$ ). However there is no significant variation between the parents (Annex 9, graph D). The means for each of the NAM families were greater than Suntop except for the EGA GREGORY family (Annex 9, graph E). We can notice the mean value of all the individuals is superior than the mean value of Suntop (55 against 52). For the second and the third layers, the parents ZWB10-37 and SB062 are at the extreme values (2<sup>nd</sup> layer  $27 \pm 29$  and  $57 \pm 17$ , 3<sup>rd</sup> layer  $7 \pm 11$  and  $21 \pm 12$ , respectively). No significant variations were observed for the parents for both the 2<sup>nd</sup> and the 3<sup>rd</sup> layers (Annex 9, graph G and J).

In the second layer, a lot of the NAM families have a mean smaller than both Suntop and the respective donors. However, most of the NAM families have a higher mean than there paret-donors. The non-normal distribution for the second and third layers can be explained by the fact than the deepest the roots, the higher is the probability to find no lateral roots. There is a high variation between the NAM families for nblat\_layer3. Indeed, the best family mean (Seri M82) is 100.3% higher than the smallest mean (EGA Gregory).



The graphics of the fourth layer are not represented, as 78 % of the plants had no lateral roots in this layer.

All the traits related to the number of lateral roots were negatively related to the emergence of the first seminal root. These traits were as well positively related to the maximum root length. It means, the number of lateral roots depends on the length of the first seminal root.

Furthermore, very high correlations were observed between the total number of lateral roots and the number of lateral roots in the different layers (Fig.14).

The mean apical diameter of the lateral roots measured at 320 °Cd varied from 0.024 to 0.032 cm for the parents, with ZWB10-37 having the thinnest apical diameter ( $0.024 \pm 0.003$  cm) and EGA GREGORY having the thickest apical diameter ( $0.032 \pm 0.003$  cm). However, no significant differences between the parent were observed (Annex 9, graph M). All the families had thinner lateral roots than Suntop and sometimes their donors (Annex 9, graph N). Furthermore the mean value of all the individuals was smaller than the mean value of the parent Suntop (0.029 cm against 0.030 cm).

### iii. Biomass root traits

The root fresh biomass and the root dry biomass were highly correlated, as expected ( $r=0.91$ , Fig.14). However, the fresh weight root:shoot ratio and the dry weight root:shoot ratio were poorly correlated ( $r=0.07$ , Fig.14). It was mainly due to false value of the shoot weight. Indeed, some genotypes had a high dry weight of the shoot in comparison to their fresh weight. The root fresh weight and the root dry weight were positively correlated to the maximum root length ( $r=0.61$  and  $r=0.57$ , Fig.14) and to the growth rate of the first seminal root ( $r=0.52$  and  $r=0.47$ , Fig.14).

For root dry biomass (DM root) and the dry biomass root:shoot ratio (DM root:shoot ratio), ZWB10-37 was the parent with the smallest biomass and root:shoot ratios ( $0.016 \pm 0.006$  g and  $0.48 \pm 0.1$ , respectively) (Annex 10, Graph D & J). By contrast, EGA Wylie was the parent with the greatest DM root:shoot ratio on average ( $1.21 \pm 0.06$ ), while SB062 had the greatest DM root ( $0.038 \pm 0.005$  g) (Annex 10, Graph D & J). A significant variation between parents for the dry biomass root traits was observed. We can also notice a high number of outliers especially for the DM root:shoot ratio. It is mainly due to errors of measurement of the dry weight of the shoot. Indeed, some dry weight were way to high in comparison to the fresh weight.

For the DM root, the mean value of the NAM lines was similar to the reference parent Suntop (0.0268 against 0.0266 g). Furthermore, the mean value of each family tended to be closer to the mean of Suntop than the mean of the donor, so that the means of all the families were really close to the Suntop mean. The SB062 and Seri M82 families had the greatest root biomass (0.0268 g) (Annex 10, Graph E).

Similarly, for DM root:shoot ratios, the means of the NAM families were close to the Suntop mean. For both traits, the mean value of the individuals was greater than for Suntop (DM: 0.55 against 0.54). The greatest mean ratio was observed for the RIL114 family (0.56), which developed its root system faster than its shoot, at least up to this stage (Annex 10, Graph K).

**Table 4: Heritability of the root traits studied**

The “G P.value” column stands for the p.value of the Genotype effects.

The orange boxes indicate the p.value superior to 0.05.

Trait	Heritability	G P.value
DM root	0.31	6.99.10 <sup>-12</sup>
DM root:shoot ratio, exp 1	0.14	2.2.10 <sup>-16</sup>
DM root:shoot ratio, exp 2	0.07	0.06
Emergence 1st_sem	0.49	1.73.10 <sup>-3</sup>
FM root:shoot ratio	0.19	1.86.10 <sup>-3</sup>
GR1st_sem	0.22	1.57.10 <sup>-9</sup>
Meanapdiam_lat	0.41	3.69.10 <sup>-8</sup>
Meansemi_rank1	0.36	1.78.10 <sup>-2</sup>
Meansemi_rank2	0.03	0.07
Meansemi_rank3, expt 1	0.01	2.01.10 <sup>-3</sup>
Meansemi_rank3, expt 2	4.74.10 <sup>-8</sup>	0.52
MRL	0.14	6.59.10 <sup>-3</sup>
Nb_sem	0.22	1.32.10 <sup>-7</sup>
Nblat_tot	0.25	1.12.10 <sup>-9</sup>
Nblat_layer1	0.21	6.05.10 <sup>-7</sup>
Nblat_layer2	0.20	1.18.10 <sup>-5</sup>
Nblat_layer3	0.22	8.27.10 <sup>-11</sup>

**Table 5: Number of QTLs detected per chromosome**

Chromosome	Number of QTL
1A	7
1B	5
1D	4
2A	4
2B	8
2D	2
3A	11
3B	8
3D	2
4A	2
4B	3
4D	1
5A	8
5B	4
5D	1
6A	3
6B	8
6D	1
7A	10
7B	2
7D	4
TOTAL	98

## b. QTL analysis

For all the traits except DM root:shoot ratio and meansemi\_rank3, the data from both experiments were analyzed at once, as no genotype environment interaction was observed. Results for each experiment were analyzed separately for the DM root:shoot ratio and the meansemi\_rank3. No significant genotype effects were found for these two traits in the experiment 2 (see the orange boxes in the Tab. 4). Furthermore, no significant genotype effect was found for the meansemi\_rank2, which means that there are no significant differences between the NAM families for these traits (see the orange boxes in the Tab. 4). Nblat\_layer4 was not studied as 70% of the results were equal to 0, as well as the FM root, considering we were studying the DM root already.

Most of the heritabilities had a value around 0.2, which means that for the related traits, 20 % of the phenotypic variations are explained by the genotype. However, the heritability for meansemi\_rank3 for the 1<sup>st</sup> experiment is really small (0.01, Tab.4). It means that a smaller part of the phenotypic variability was explained by the genotype for these traits. A small heritability means as well that these traits were going to be difficult to incorporate in future new varieties, because the trait potentially depends more on the environment than on the genes. Furthermore, the highest heritability (0.41) was found for the meanapdiam\_lat.

In total 98 QTLs were found for 14 of the 17 phenotypic traits studied. 1 QTL was found for DM root, 30 QTLs for DM root shoot ratio experiment 1, 7 QTL for emergence1st\_sem, 22 QTLs for FM root shoot ratio, 2 QTLs for GR1st\_sem, 8 QTLs for meanapdiam\_lat, 1 QTLs for meansemi\_rank1, 2 QTLs for meansemi\_rank3 for the first experiment, 3 QTLs for MRL, 5 QTLs for nb\_sem, 4 QTLs for nblat\_layer1, 4 QTLs for nblat\_layer2, 5 QTLs for nblat\_layer3, and 5 QTLs for nblat\_tot. The QTL are mapped on the annex 12.

More QTLs were found for the DM root shoot ratio and the FM root shoot ratio. Significant Genotype x Experiment interactions were found for the DM shoot. It could explain the G x Exp interactions for the DM root shoot ratio. Furthermore, the biomass ratio are complex traits, as they are calculated using two phenotypic traits, and thus errors are more likely to occur. Considering the small number of QTLs found for the other traits, it's likely there is a problem with this result.

The more QTL were located on the chromosomes 3A and 7A (11 and 10 QTLs). We can also notice that fewer QTLs were located on the subgenome D of the wheat genome (Tab.5). Most the QTL detected had a small effect, explaining around 1 to 5 % of the phenotypic variations except a few QTLs. For example, a QTL detected for DM root shoot ratio in the first experiment on the chromosome 4D explained 9.49 % (See the pink box, Annex 11).

Various QTLs seemed to control various root traits at the same time, which was not so surprising considering that some traits are highly correlated (see maroon frames, Annex 11 & map Annex 12). The SNP00334 was collocating on chromosome 1A for nblat\_layer1 and nblat\_tot at 114.82 cM. The SNP08687 was collocating on chromosome 3A for GR1st\_sem and Nblat\_layer2 at 14.37 cM. The SNP04534 was collocating on the chromosome 3B for Nblat\_layer1 and Nblat\_tot at 91.35 cM. The SNP05552 was collocating on the chromosome 4B for Nblat\_layer2 and and Nblat\_tot at 41.96 cM. The SNP08458 was collocating on the chromosome 7A for Emergence1st\_sem and FM root:shoot ratio at 21.48 cM. Finally, the SNP09725 was collocating on chromosome 7D for MRL and Nblat\_tot at 71.72 cM. Nblat\_tot was highly correlated with nblat\_layer1 and nblat\_layer 2. It is likely that these QTL collocating are the same QTL and control in fact different traits.



Furthermore, we can notice on the annex 12 that some QTLs look very close (Annex 12). For example, some QTLs for the DM root:shoot ratio (chromosome 3A, 5B or 6B) or the FM root:shoot ratio (chromosome 5A) are very close and could indeed correspond to the same QTL. It could explain why we found so many QTLs for these two traits.

#### **IV. Discussion**

##### **a. Genetic analysis of root traits in a NAM population phenotyped under aeroponics conditions**

Working on a multi-parent population, such as NAM populations increased the chances of recombination and so the chances to find QTL. It also increases the resolution detection. It didn't only offer possibilities to discover new markers, it also allowed the development and identification of new lines of interest that can be used in a breeding program. In addition, identified makers could easily be introgressed in an elite germplasm (reference parent, Suntop here) relevant for the region. Thus, NAM populations are well adapted to assist crop improvement.

Previous genetic analyses of wheat root used phenotyping methods like hydroponics, agarose gel chambers or soil columns (Table 1 & 2), but none grew the roots in aeroponics. Furthermore, a majority of the studies worked with RILs and only 2 studies used MAGIC population, but none used a NAM population (Annex 1, Table 1 & Table 2). Our study is thus the first genetic analysis of root traits in wheat that considered a NAM population under aeroponics conditions.

The observation time and the trial duration of the root phenotyping methods applied to genetics did not generally exceed a month, just like in our study (21 days). Our traits were measured at seedling stage, for technical reason, to save time, and because seedling root traits are thought to influence the growth and yield of the mature plant (Atkinson et al., 2015). The most recent studies also used softwares and automated algorithm to facilitate and accelerate the phenotyping. For instance, WhinRhizo was used in this study, as in 10 of the 31 articles cited in the annex 1.

##### **b. Genetic analysis of 17 root traits using SNP markers**

4284 SNPs were used in our analysis, which is more than in most of the studies. Using more markers increases the chance to find more QTLs and enhances the accuracy of the positions. 98 QTLs were found in this study, principally on the subgenomes A and B, which is coherent with what was found in the previous studies (Annex 1 & 2).

Among the 17 traits studied, a few have been studied in the literature, such as the maximum root length (Sharma et al., 2011; Kadam et al., 2012; Liu et al., 2013; Zhang et al., 2013; Atkinson et al., 2015; Kabir et al., 2015; Ehdaie et al., 2016; Xie et al., 2017; Ma et al., 2017; Ren et al., 2017), the number of seminal roots (Hamada et al., 2012; Liu et al., 2013; Christopher et al., 2013; Zhang et al., 2013; Canè et al., 2014; Atkinson et al., 2015; Maccaferri et al., 2016; Xie et al., 2017), the number of lateral roots (Ibrahim et al., 2012; Ren et al., 2012; Atkinson et al., 2015; Petrarulo et al., 2015; Kabir et al., 2015; Maccaferri et al., 2016; Iannucci et al., 2017; Ma et al., 2017) and traits related to biomass such as DM root (Kubo et al., 2007; Bai et al., 2013; Sun et al., 2013; Zhang et al., 2013; Maccaferri et al., 2016; Iannucci et al., 2017; Ren et al., 2017), DM root:shoot ratio (Bai et al., 2013; Sun et al., 2013; Zhang et al., 2013; Maccaferri et al., 2016; Ma et al., 2017) or FM root:shoot ratio (Sun et al., 2013; Zhang et al., 2013; Ehdaie et al., 2016).





Concerning the root diameter, Maccaferri *et al.* (2015) looked at the “total root diameter” and Wu *et al.* (2017) studied the “root diameter”. Results on “root diameter” from Wu *et al.* (2017) could probably be compared to the diameter of the first seminal root in our study. However, there are no indications to tell what the “total root diameter” corresponded to. We didn’t find any studies mentioning the mean apical diameter of the lateral roots nor the number of lateral roots at different depths (Annex 2 & 3).

c. Markers of interest for the Northern Australian wheat belt

In the Northern Australian wheat belt, where the rainfall is principally occurring in summer, wheat crops, which grow in winter, typically rely on water stored in the soil. Genotypes able to access water from deep soil have been shown to yield better under the common terminal drought (Manschadi *et al.*, 2006). Breeding of drought tolerant wheat varieties in this region could thus benefit from an increase in the maximum root length, the number of seminal roots in the deeper layer and the number of lateral roots. These three traits had respectively a heritability of 0.14, 0.22 and 0.22 (Tab. 4).

3 QTLs were found in our study for the maximum root length (see blue highlights, annex 11) on chromosomes 2B, 7A and 7D. The QTL located on chromosome 7A explains 7.67 % of the phenotypic variation, while the other two have a much lower effect (4.76 % and 3.18 for the QTLs on chromosome 2B and 7D, respectively). The maximum root length has been studied a lot in the literature, and also referred to as the “main root length” and the “maximum depth of the root system” (annex 2 & 3). QTLs for this trait were previously found on all the chromosomes except the chromosomes 1D and 3D. As we can’t compare the positions of the different QTLs, it’s not possible to say if the QTLs we found are new ones or not.

4 QTLs were found in our study for the number of seminal roots (see green highlights, annex 11) on chromosomes 1D, 2B, 3A and 7B. The QTL located on chromosome 2B explains 5.54 % of the phenotypic variation, which is quite high. Furthermore, the QTL on the chromosome 7B explained 3.18 % of the phenotypic variation. These two QTL could be of interest to improve the number of seminal roots in future varieties.

For the number of seminal roots, previous studies found QTLs on all chromosomes except the chromosomes 1D and 5D (annex 2 & 3). Considering that we found a QTL on chromosome 1D, QTL appears as being a new QTL that hasn’t been found before.

5 QTLs were found in our study for the total number of lateral roots (see orange highlights, annex 11) on chromosomes 1A, 3B, 4B, 7A, 7D. The QTL on chromosome 4B explained 5% of the phenotypic variations. QTLs for the number of lateral roots have previously been identified on all chromosomes except 2B, 2D, 5D and 7B. As the QTLs we found are located on chromosomes were QTLs were found before, we can’t say if our QTLs are new or not.

Studying the total number of lateral roots doesn’t indicate where they are located on the root system. In terms of breeding new varieties for the Northern Australian Wheat Belt, it would be interesting to have plants with more lateral roots in the deep layer of the soil. We found 4, 4 and 5 QTLs in the shallow, mid and deep layers (layers 1, 2, 3, respectively). Some QTLs for the number of lateral roots in the layer 1 or 2 were collocating with QTLs for the total number of lateral roots (maroon frame, Annex 11). Very high correlations were found between all the traits relating to the number of lateral roots (Fig. 14). However, no collocation was observed for the total number of lateral roots and the number of lateral roots in the layer 3, when looking at the same positions and close positions.



This means, that breeding for a higher number of lateral roots in general is going to influence more the number of shallower rather than deeper lateral roots. Indeed, the deeper we go, the less chances there are to find lateral roots. 5 QTLs were found for the number of lateral roots in the layer 3 ([13-17.7 cm]), on chromosomes 2B, 3A, 5A, 6B and 7A (see grey highlights, Annex 11). The QTL on chromosome 7A explained 5.95 % of the phenotypic variations. We didn't find any genetic work that looked at the number of lateral roots for different depths.

The Seri M82 family had the best mean value among all the family for the maximum root length, the number of seminal roots and the number of lateral roots in the layer 3. It means the Seri M82 donor probably had some alleles that are beneficial for these traits. It also means, the crossings between Seri M82 and Suntop produced in general progenies that have a high value for these three phenotypic traits. Thus, these progenies could be interesting to use.

#### d. Limitations and perspectives

The next steps of this study would be to calculate the top and bottom position around each marker, that would allow us to compare the position of the markers between each other. This step of the analysis hasn't been completed because there is at this day no published method for such an analysis.

We didn't talk about the direction of the marker effect or the allele donor for each QTL, as currently a homozygous possessing the alleles from the donor, has an allele that come from the reference used in the sequencing. Work is currently undertaken to consider the allele effect from the NAM reference (Suntop) and the NAM donors.

Other traits have been studied with the Suntop NAM population. For instance, the NAM lines have been grown field trials at different locations, and phenotyped for stay-green and yield. These lines were also screened for TE in a new lysimeter platform (Chenu et al., 2018). It would be interesting to look at collocations between markers across traits, e.g. collocation of root traits with yield under drought conditions.



## CONCLUSION

Our study was the first genetic analysis of wheat root seedling traits using a NAM population phenotyped on an aeroponic platform. 17 root traits were measured on the 507 lines of the wheat NAM population. Among these traits, maximum root length, number of seminal roots and number of deep lateral roots appeared as promising traits for improving drought adaptation in regions where crops rely on deep soil water, such as the Northern Australian wheatbelt. High phenotypic variations were observed between the mean of the NAM families for most traits (e.g. up to 100 % of variation across families for the number of lateral roots in the second layer [8.3 -13 cm]). The Suntop x Seri M82 family had the greatest mean values for the three promising traits (maximum root length, number of seminal roots and number of lateral roots in the layer 3 ([13 – 17.7 cm])). Progenies from this family could be interesting to test in field trials, and use in breeding programs.

In total, 98 QTLs were found for 14 of the 17 root traits, on all the chromosomes of the wheat genome, but with the majority on the subgenomes A and B. A QTL explaining 7.7% of the phenotypic variation was located on the chromosome 7A of the wheat for the maximum root length. Furthermore, a new QTL was detected for the number of seminal roots on the chromosome 1D. Currently, collocation of QTLs was limited to markers with the exact same positions. Research is underway to find new methods to estimate the confidence interval of each QTL in a NAM. Furthermore, work is also on process in regards to potential interesting donors for each QTL. Finally, the NAM population we used has been studied for other traits such as stay-green and yield and it would be interesting to look at potential collocations between QTLs for those traits with QTLs for root traits.



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**Annex 1: Information on the phenotyping methods and traits measured for RSA studies found in the literature.**

*We only found the abstract in English for A01, therefore there are few information for this study*

Ref ID	Growth conditions	Observation method and [timing]	Experimental design	Environmental conditions	Measured root traits
A01	Germination in petri dishes then growth in hydroponics	NA, [5 leaves]			
A02	Pots of vermiculite with a layer of paraffin and Vaseline to simulate a compact layer at 10 cm.	Manually [8 weeks]	Randomized complete block with 3 replications		Root penetration index, root dry weight, number of roots penetratinf the PV disc, total number of seminal and nodal roots
A03	Covered transparent plastic boxes on two layers of filter paper moistened	Manually [8 days]		Osmotic stress	Root length, coleoptile length, shoot length, root:shoot length ratio, tolerance index
A04	Germination in petri dishes	Manually [every 24h from seed imbibation over 7 days]			Root:shoot length ratio
A05	Sand culture in wide PVC tubes	Manually [45 days]	RCBD with 4 replications		Number of roots greater than 30 cm, longest root length, total length of roots greater than 30 cm, shallow root weight, deep root weight, total root weight, root:shoot biomass ratio
A06	Germination in petri dishes and use of the basket method. For hydrotropism, growth on agar agar with a gradient of water	Manually [7 days]	Randomized block design with 3 replications		Number of roots, deep root ratio, root elongation rate, root angle in response to hydrotropism
A07	Quick pots	Manually and WinRHIZO software [4 weeks]		Well-watered conditions or drought stress treatment	Average root diameter, number of root crossings, number of root forks, number of root tips, root volume, surface root area, total root length
A08	15×100 cm PVC pipes	Manually [more than 20 days]	RCBD design with 3 replications over 2 years	Control conditions and drought stress condition	Maximum root length, total root biomass, root biomass up to 30 cm, root biomass bleow 30 cm
A09	Hydroponics	WhinRHIZO software [8 or 12 days]	3 replicates of 3 plants in each pots, with 2 trials		Maximum root length, lateral root length, primary root length, total root length, root rip number, primary root elongation
A10	Germination in petri dishes on filter paper, then seeds placed in a germination paper rolled with the base in a nutrient solution	Manually, and WhinRhizo Pro software [11 days]	Unbalanced block design with 3 replicate rolls per line		Seminal axes length, total root volume, root dry weight, root:shoot ratio...
A11	Gel-filled root observation chambers	Specifically designed computer software [8 days]	Incomplete block design with 3 replicates of 11 batches		Seminal root angle, seminal root number
A12	Agarose gel chambers	ImageJ and WinRHIZO softwares [6 days]		Well-watered or water-stressed regimes	Maximum root length, seminal root number, total root length, project root area, root area surface, seminal root angle
A13	Germination in petri dishes and growth in hydroponics	Manually [30 days]	RCBD with 3 replications	3 NO <sub>3</sub> <sup>-</sup> /NH <sub>4</sub> <sup>+</sup> ratio	Root fresh weight, root dry weight, root:shoot dry weight, root:shoot fresh weight
A14	Growth on filter paper in beakers	Manually [8 days]		2 water conditions	Longest root length, root number, root fresh weight, root dry weight, root:shoot fresh weight ratio, root:shoot dry weight ratio
A15	(1) Pre germination in petri dishes and growth in soil columns (1m H, 0.15 m D) with layers of paraffin and petroleum jelly + (2) field trials on 16 DHL and parents	Manually [56 days]	(1) RCBD with 2 replicates (2) incomplete blocks, 2 sites, parents 2 replicates + check lines, DHL 1 replicate	Contrasting soil type	Roots penetration and roots depth

Ref ID	Growth conditions	Observation method and [timing]	Experimental design	Environmental conditions	Measured root traits
A16	Germination in petri dishes, then growth on a filter paper sheet on a vertical black rectangular polycarbonate plate	Manually and software SmartRoot [9 days]	Randomized block design, 3 replications		Average root length, primary root length, seminal root angle, total root length, total number of roots
A17	In field	Manually [NA]			Root weight, root water content, root dry matter content
A18	Growth in two layers of filter paper in covered transparent plastic box	Manually [8 days]	3 replicates of 15 grains per RIL	Osmotic stress, water or PEG 6000	Maximum root length, root:shoot length ratio
A19	N treatment from 200 to 250 kg N/ha. Moistened germination paper and individual growth pouches	Phenotyping pipeline and RootNav software [9days]	Randomized block design		Angle of emergence between seminal roots, maximum depth of the root system, centre of mass of the root system...
A20	Pre-germinated seeds with 3-6 mm roots planted in pots, or rhizoboxes.	Manually [3 days][11 days for rhizoboxes]	Randomized block design with 6 replications	Different soil types	Rhizosheath
A21	Hydroponics	Manually + WinRHIZO software [10 days]	5 shoots/genotypes and 2 replications/population		Total root length, root surface area, root volume, number of root tips, main root length
A22	Hydroponics	Manually + WinRHIZO software [14 and 28 days]	RCBD with 3 rep/RIL and 5 rep/parent		Root length, root volume, number of tips...
A23	Germination in petri dishes and seedling grown in sand tubes culture. 3 growing seasons.	Manually [45 days for mid-tillering] [maturity for maturity measures]	RCBD with 2 replicates	Well-watered conditions	Longest root, total root length under 30 cm, shallow root weight, deep root weight, total root biomass, ratio of root:shoot, ratio of root to plant
A24	Square plates with cellophane discs	ImageJ software [3 days after germination]			Root hair length
A25	Germination in petri dishes, then growth on a filter paper sheet on a vertical black rectangular polycarbonate plate + field experiment for the panel for root growth angle estimation	Manually and SmartRoot software [9 days]	RCBD with 3 replications for the plates, and RCBD with 2 replications for the field experiments		Total root length, total root volume, average root length, root dry weight, primary root length, percentage of plants with root 6th, lateral root number...
A26	Germination in petri dishes and growth in plastic cylinders	Manually and WhinRHIZO software [5 leaves]	RCBD with 4 replications		Root crossing number, root forks number, root length, root surface area, root volume, number of tips
A27	Germination in petri dishes and growth in hydroponics	Manually and WhinRHIZO software [4 weeks]	RCBD with 3 replications		Maximum root length, total root length, root surface area, average diameter, root volume, root tips number, root:shoot ratio
A28	Hydroponics	Manually [35 days]	RCBD with 3 replications		Root dry weight, maximum root length
A29	"Pouch and wick" high-throughput phenotyping	RootNav software [13 days]	RCBD		Seminal root number, seminal root length, total root length, maximum width, maximum depth, emergence angle of seminal root
A30	Germination in petri dishes and growth in hydroponics	WhinRHIZO [16 days]	RCBD, 4 replications	Phosphorus sufficient and deficient conditions	Root diameter
A31	Hydroponics	WinRHIZO [16 days]	RCBD, 4 replications	Phosphorus sufficient and deficient conditions	Total root surface area, total root volume, number of roottips, number of root forks

**Annex 2: Number and locations of the root QTL on the wheat chromosomes (1, 2, 3 & 4) for the different traits**  
*The green cells the row “total” at the base of the table represent the chromosomes where the most QTL were found*  
*In the different cells, the number of QTL associated is indicated with the reference ID from Annex 1 in parenthesis*

Root traits	Chromosome number and subgenome											
	1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	4D
Above wax seminal dry weight		1(A15)	1(A15)				1(A15)	1(A15)	1(A15)			
Angle of emergence between the outermost seminal roots measured at 95 px								1(A19)				
Angle of emergence between the outermost seminal roots measured at the first quartile of the total length								1(A19)				
Angle of emergence between the second pair of seminal roots measured at 30 px								1(A19)	1(A19)			
Angle of emergence between the second pair of seminal roots measured at 95 px								1(A19)	1(A19)			
Angle of emergence between the second pair of seminal roots measured at the first quartile of the total length									1(A19)			
Angle of emergence between the second pair of seminal roots measured at the second quartile of the total length					1(A19)							
Average root diameter									1(A27)		1(A07)	
Average root length	1(A25)	3(A25)		1(A16), 2(A25)	1(A16), 7(A25)		2(A25)	6(A25)			3(A25)	
Below-wax seminal dry weight		1(A15)	1(A15)		1(A15)			1(A15)		1(A15)		
Centre of mass of the convex hull, vertical co-ordinate												
Centre of mass of the convex hull, horizontal co-ordinate												
Centre of mass of the root system, vertical co-ordinate												
Centre of mass of the root system, horizontal co-ordinate	1(A19)											
Convex hull, area of the smallest convex polygon to enclose the root system												
Deep root ratio		1(A06)										
Deep root weight (roots below 30 cm)		1(A23), 2(A05)				1(A23)	1(A23)			1(A23)	2(A23)	
Emergence angle of seminal root												
Hydrotropism : root angle	1(A06)				1(A06)							
Lateral root length	1(A09)				1(A09)						1(A09)	
Length, diameter class 0.0 < d <= 0.2 mm	1(A22)											
Length, diameter class 0.0 < d <= 0.5 mm											2(A26)	
Length, diameter class 0.2 < d <= 0.4 mm	1(A22)	1(A22)		1(A22)								
Length, diameter class 0.4 < d <= 0.6 mm	1(A22)											
Length, diameter class 0.5 < d <= 1.0 mm											2(A26)	
Length, diameter class 0.8 < d <= 1.0 mm				1(A22)			2(A22)					
Length, diameter class 1.0 < d <= 1.5 mm											1(A26)	
Length, diameter class 1.5 < d <= 2.0 mm												
Length, diameter class 2.0 < d <= 2.5 mm												
Length, diameter class 2.5 < d <= 3.0 mm												
Length, diameter class 4.0 < d <= 4.5 mm												
Main root length				3(A21)				2(A21)				2(A21)
Maximum depth of the root system							1(A29)	1(A29)		1(A29)		

	Chromosome number and subgenome											
Root traits	1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	4D
Maximum root length	1(A14)	1(A23), 2(A05), 1(A12)			1(A28)	1(A23)		1(A14), 1(A27)		2(A14)	1(A14)	
Maximum root length low N					1(A28)							
Maximum root length low P					1(A28)							
Maximum root length at 8 days after transplanting					1(A09)							
Maximum root length at 12 days after transplanting	1(A09)				2(A09)							
Maximum root length under drought stress						1(A12)	1(A12)				1(A08)	
Maximum width of the root system				1(A29)						1(A29)		
Number of lateral roots	1(A22)	1(A21), 1(A22)	1(A07)	2(A21), 1(A22), 2(A25)			1(A09), 3(A21)		1(A09)	1(A07), 1(A21)	1(A07), 1(A09), 1(A26), 1(A27)	3(A21)
Number of root tips under phosphorus deficiency												
Number of roots greater than 30 cm		4(A05)										
Number of tips, diameter class $0.0 < d \leq 0.2$ mm	1(A22)			1(A22)								
Number of tips, diameter class $0.0 < d \leq 0.5$ mm											1(A26)	
Number of tips, diameter class $0.2 < d \leq 0.4$ mm		1(A22)		1(A22)								
Number of tips, diameter class $0.5 < d \leq 1.0$ mm		2(A26)					1(A26)					
Number of tips, diameter class $0.8 < d \leq 1.0$ mm							1(A22)					
Number of tips, diameter class $1.5 < d \leq 2.0$ mm		1(A26)										
Number of tips, diameter class $3.0 < d \leq 3.5$ mm												
Number of root crossings		1(A26)	1(A07)		1(A07)	1(A07)					1(A26)	
Number of root forks		1(A26)	1(A07)	1(A07)		1(A07)					2(A26)	
Number of root forks under phosphorus deficiency									1(A31)			
Number of roots penetrating the PV disc												
Percentage of plants with root 6th				1(A25)	1(A25)		2(A25)				2(A25)	
Primary root diameter					1(A25)		1(A25)				1(A25)	
Primary root elongation					1(A09)		1(A09)					
Primary root length		1(A16), 3(A25)		1(A16), 2(A25)	1(A16), 2(A09), 6(A25)		2(A25)	3(A25)		1(A16), 5(A25)	5(A25)	
Primary root surface								1(A25)			1(A25)	
Primary root volume	1(A25)										1(A25)	
Project root area								1(A12)		1(A12)	1(A12)	
Project root area under water stress					1(A12)			1(A12)				
Rhizosheath size (root hairs)					1(A20)							1(A20)
Root biomass below 30 cm											1(A08)	
Root biomass below 30 cm, drought stress											1(A08)	
Root biomass up to 30 cm											1(A08)	
Root biomass up to 30 cm, drought stress											1(A08)	
Root diameter	1(A30)			1(A30)	4(A30)	2(A30)				1(A30)		
Root diameter under phosphorus deficiency	1(A30)	2(A30)	1(A30)		2(A30)			1(A30)	1(A30)			
Root dry matter content												
Root dry weight	2(A25), 2(A13)	1(A02)	1(A14)	1(A28)	1(A14), 2(A25)	1(A10), 1(A14), 1(A28)	1(A14), 1(A25)	2(A14), 1(A13), 1(A28)		1(A14)	1(A25), 1(A26), 1(A28)	1(A10)

	Chromosome number and subgenome											
Root traits	1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	4D
Root dry weight low N											1(A28)	1(A28)
Root dry weight low P											1(A28)	
Root fresh weight	1(A13)		1(A14), 1(A13)		2(A13)			1(A14)		1(A13)		
Root hair length	1(A24)			1(A24)	1(A24)							
Root length tolerance index under osmotic stress	1(A03)											
Root penetration												
Root surface area	1(A22)		1(A07)	1(A07), 1(A21), 2(A22)		1(A07)	3(A21)	1(A12)		1(A12)	1(A12), 2(A26), 1(A27)	3(A21)
Root surface area under water stress					1(A12)			1(A12)				
Root to plant ratio				2(A23)		2(A23)				1(A23)		
Root:shoot dry weight ratio	2(A13)	1(A13), 1(A27)				1(A14), 2(A13)		2(A14), 2(A13)			1(A25)	1(A14), 1(A10)
Root:shoot length ratio in control				1(A03)		2(A03)			2(A03)			
Root:shoot length ratio under osmotic stress						1(A03)						
Root:shoot fresh weight ratio	1(A13)	1(A13)		2(A23)	1(A13)	1(A14), 1(A13), 1(A23)	1(A13)	2(A14), 1(A13)		2(A13)		
Root volume				1(A07), 1(A22)			1(A07)			1(A07)	1(A21)	3(A21)
Root water content												
Seminal axes length												1(A10)
Seminal axes surface area						1(A10)						1(A10)
Seminal axes volume						1(A10)		1(A10)				
Seminal laterals surface area							1(A10)	1(A10)				
Seminal laterals volume							1(A10)	1(A10)				
Seminal root angle	1(A12)	1(A16), 1(A25)		1(A11), 3(A25)	1(A12), 5(A25)		1(A16), 1(A12), 1(A25)	2(A25)	1(A11)	1(A25)	1(A16), 4(A25)	
Seminal root angle under water stress					2(A12)			1(A12)				
Seminal root length							1(A10), 1(A29)	1(A10), 1(A29)				
Seminal root number	1(A25)	1(A11), 4(A25)		2(A29), 3(A25)	1(A12), 1(A29), 1(A14), 4(A25)	1(A14)	1(A11), 1(A16), 1(A12), 5(A25)	1(A11), 1(A12), 2(A14), 2(A25)	1(A12), 1(A29)	2(A11), 2(A29), 3(A25)	1(A16), 6(A25)	1(A14)
Seminal root number under water stress					1(A12)			1(A12)				
Shallow root weight (roots between 0 and 30 cm)		2(A05)		2(A23)		1(A23)	1(A23)			1(A23)		
Surface area, diameter class 0.0 < d <= 0.2 mm	1(A22)			1(A22)								
Surface area, diameter class 0.0 < d <= 0.5 mm											2(A26)	
Surface area, diameter class 0.2 < d <= 0.4 mm	1(A22)	1(A22)		1(A22)								
Surface area, diameter class 0.4 < d <= 0.6 mm	1(A22)											
Surface area, diameter class 0.5 < d <= 1.0 mm											2(A26)	
Surface area, diameter class 0.8 < d <= 1.0 mm				1(A22)			2(A22)					
Surface area, diameter class 1.0 < d <= 1.5 mm											1(A26)	
Surface area, diameter class 1.5 < d <= 2.0 mm												
Surface area, diameter class 2.0 < d <= 2.5 mm												
Surface area, diameter class 2.5 < d <= 3.0 mm											1(A26)	

	Chromosome number and subgenome											
Root traits	1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	4D
Surface area, diameter class 4.0 < d ≤ 4.5 mm												
Total length of lateral roots												
Total length of seminal roots												
Total length of all roots	1(A22), 2(A25)	2(A12), 1(A22), 5(A25)	1(A07)	1(A16), 1(A07), 2(A21), 2(A22), 3(A25), 2(A29)	1(A16), 1(A09), 2(A21), 5(A25)	1(A07)	4(A21), 4(A25), 1(A29)	1(A10), 2(A12), 1(A21), 3(A25), 1(A29)	1(A29)	1(A21), 3(A25), 2(A29)	1(A09), 1(A12), 2(A25), 2(A26), 1(A27)	1(A10), 2(A21), 1(A29)
Total length of all roots under water stress	4(A03), 1(A12)							2(A12)				
Total root diameter							2(A25)	2(A25)			1(A25)	
Total root length under 30 cm		3(A05)										
Total root volume	1(A25)	2(A25)		1(A25), 1(A26)		1(A10)		1(A10), 1(A25)		1(A25)	1(A25), 1(A26), 1(A27)	1(A10)
Total root volume under phosphorus deficiency			1(A31)			1(A31)			1(A31)			
Total root surface area		1(A25)		1(A10)	2(A25)	1(A10)					1(A25)	1(A10)
Total root surface area under phosphorus deficiency			1(A31)									
Total root weight		1(A15), 2(A05)	1(A15)	1(A15), 1(A23)	1(A15)	1(A23), 1(A08)	1(A23)	1(A15)	1(A15)	1(A15), 1(A23)		
Total root weight under drought stress						1(A08)					1(A08)	
Volume, diameter class 0.0 < d ≤ 0.2 mm	1(A22)			1(A22)								
Volume, diameter class 0.0 < d ≤ 0.5 mm											2(A26)	
Volume, diameter class 0.2 < d ≤ 0.4 mm	1(A22)	1(A22)		2(A22)								
Volume, diameter class 0.4 < d ≤ 0.6 mm	1(A22)											
Volume, diameter class 0.5 < d ≤ 1.0 mm											2(A26)	
Volume, diameter class 0.8 < d ≤ 1.0 mm				1(A22)			2(A22)					
Volume, diameter class 1.0 < d ≤ 1.5 mm											1(A26)	
Volume, diameter class 1.5 < d ≤ 2.0 mm												
Volume, diameter class 2.0 < d ≤ 2.5 mm												
Volume, diameter class 2.5 < d ≤ 3.0 mm											1(A26)	
Volume, diameter class 4.0 < d ≤ 4.5 mm												
Width-depth ratio												1(A19)
TOTAL of QTL found per chromosome	42	64	14	69	75	33	60	69	16	41	87	26





Root traits	Chromosome number and subgenome									TOTAL
	5A	5B	5D	6A	6B	6D	7A	7B	7D	
Maximum width of the root system	1(A29)					1(A19)		1(A29)	1(A29)	6
Number of lateral roots	1(A25)	1(A07)		1(A09), 1(A22), 1(A26)	1(A09), 1(A22), 1(A26), 1(A25)	1(A07), 1(A09), 1(A19)	1(A21), 1(A27)		1(A07), 1(A19)	42
Number of root tips under phosphorus deficiency									1(A31)	1
Number of roots greater than 30 cm										4
Number of tips, diameter class 0.0 < d ≤ 0.2 mm				1(A22)	1(A22)					4
Number of tips, diameter class 0.0 < d ≤ 0.5 mm				1(A26)	1(A26)					3
Number of tips, diameter class 0.2 < d ≤ 0.4 mm										2
Number of tips, diameter class 0.5 < d ≤ 1.0 mm										3
Number of tips, diameter class 0.8 < d ≤ 1.0 mm										1
Number of tips, diameter class 1.5 < d ≤ 2.0 mm										1
Number of tips, diameter class 3.0 < d ≤ 3.5 mm		1(A26)								1
Number of root crossings							1(A07)			6
Number of root forks									1(A07)	7
Number of root forks under phosphorus deficiency									1(A31)	2
Number of roots penetrating the PV disc				1(A02)						1
Percentage of plants with root 6th	1(A25)			1(A25)	1(A25)			1(A25)		10
Primary root diameter							1(A25)			4
Primary root elongation			1(A06)					1(A09)	1(A06)	5
Primary root length	3(A25)			3(A25)	3(A25)		2(A25)	1(A09), 3(A25)		47
Primary root surface				1(A25)	1(A25)			1(A25)		5
Primary root volume				1(A25)			1(A25)			4
Project root area									1(A12)	4
Project root area under water stress		1(A12)								3
Rhizosheath size (root hairs)	1(A20)	1(A20)		1(A20)			1(A20)			6
Root biomass below 30 cm										1
Root biomass below 30 cm, drought stress										1
Root biomass up to 30 cm										1
Root biomass up to 30 cm, drought stress										1
Root diameter										9
Root diameter under phosphorus deficiency									1(A30)	9
Root dry matter content		1(A17)				1(A17)				2
Root dry weight		1(A13)		1(A25)		1(A14)	1(A14)			28
Root dry weight low N				1(A28)						3
Root dry weight low P										1
Root fresh weight	1(A14)			2(A13)						10
Root hair length				1(A24)						4
Root length tolerance index under osmotic stress										1
Root penetration				1(A02)						1
Root surface area	1(A21)	1(A07)	1(A07)	2(A22)		1(A21)			1(A07), 1(A12)	27
Root surface area under water stress		1(A12)								3
Root to plant ratio										5
Root:shoot dry weight ratio	1(A14),1 (A13)		1(A14)	1(A14)		2(A14)	1(A14), 2(A13), 1(A27)	1(A13)	1(A14)	26
Root:shoot length ratio in control			5(A04)							10
Root:shoot length ratio under osmotic stress						1(A03)				2
Root:shoot fresh weight ratio	2(A14)						1(A13)			14
Root volume		2(A21)	1(A07)							11
Root water content		1(A17)				1(A17)				2
Seminal axes length				1(A10)						2
Seminal axes surface area	1(A10)									3
Seminal axes volume	1(A10)									3
Seminal laterals surface area		1(A10)								3
Seminal laterals volume		1(A10)		1(A10)						4

	Chromosome number and subgenome									
Root traits	5A	5B	5D	6A	6B	6D	7A	7B	7D	TOTAL
Seminal root angle		2(A25)	1(A11)	1(A16), 1(A11), 4(A25)	2(A16), 2(A11), 2(A25)		6(A25)	1(A25)	1(A12)	48
Seminal root angle under water stress										3
Seminal root length		1(A10)								5
Seminal root number	1(A12),2 (A29), 1(A06), 1(A14), 3(A25)	1(A29), 1(A14), 4(A25)		1(A11), 1(A25)	5(A25)	1(A14)	1(A12), 1(A29), 1(A19), 1(A25)	1(A29), 1(A25), 2(A14)	1(A29)	80
Seminal root number under water stress							1(A12)			3
Shallow root weight (roots between 0 and 30 cm)										7
Surface area, diameter class 0.0 < d ≤ 0.2 mm				1(A22)						3
Surface area, diameter class 0.0 < d ≤ 0.5 mm				1(A26)						3
Surface area, diameter class 0.2 < d ≤ 0.4 mm				1(A22)						4
Surface area, diameter class 0.4 < d ≤ 0.6 mm										1
Surface area, diameter class 0.5 < d ≤ 1.0 mm										2
Surface area, diameter class 0.8 < d ≤ 1.0 mm										3
Surface area, diameter class 1.0 < d ≤ 1.5 mm										1
Surface area, diameter class 1.5 < d ≤ 2.0 mm					1(A26)					1
Surface area, diameter class 2.0 < d ≤ 2.5 mm					1(A26)					1
Surface area, diameter class 2.5 < d ≤ 3.0 mm					1(A26)					2
Surface area, diameter class 4.0 < d ≤ 4.5 mm								1(A26)		1
Total length of lateral roots						1(A19)			1(A19)	2
Total length of seminal roots						1(A19)			1(A19)	2
Total length of all roots	2(A21),2 (A25),2(A29)	1(A10), 1(A03), 1(A07), 1(A25), 1(A29)	1(A12)	1(A10), 1(A09), 2(A22), 4(A25)	1(A16), 1(A09), 4(A25)	1(A19), 1(A09), 1(A21)	1(A07), 1(A25), 1(A27), 1(A29)	1(A29)	1(A19), 1(A07), 1(A12)	105
Total length of all roots under water stress		1(A12)				1(A03)			1(A03)	10
Total root diameter					1(A25)			1(A25)		7
Total root length under 30 cm					1(A23)					4
Total root volume	1(A10)									14
Total root volume under phosphorus deficiency										3
Total root surface area				1(A25)			1(A25)	1(A25)		10
Total root surface area under phosphorus deficiency										1
Total root weight		1(A17)				1(A15)	1(A15)		1(A15)	18
Total root weight under drought stress										2
Volume, diameter class 0.0 < d ≤ 0.2 mm				1(A22)						3
Volume, diameter class 0.0 < d ≤ 0.5 mm										2
Volume, diameter class 0.2 < d ≤ 0.4 mm				1(A22)						5
Volume, diameter class 0.4 < d ≤ 0.6 mm										1
Volume, diameter class 0.5 < d ≤ 1.0 mm										2
Volume, diameter class 0.8 < d ≤ 1.0 mm										3
Volume, diameter class 1.0 < d ≤ 1.5 mm										1
Volume, diameter class 1.5 < d ≤ 2.0 mm					1(A26)					1
Volume, diameter class 2.0 < d ≤ 2.5 mm					1(A26)					1
Volume, diameter class 2.5 < d ≤ 3.0 mm					1(A26)					2
Volume, diameter class 4.0 < d ≤ 4.5 mm								1(A26)		1
Width-depth ratio										1
<b>TOTAL</b>	<b>39</b>	<b>35</b>	<b>15</b>	<b>55</b>	<b>51</b>	<b>26</b>	<b>35</b>	<b>38</b>	<b>27</b>	<b>913</b>

Annex 4: Number of lines per founders in the Su-NAM population

Founders	Number of lines
Wylie	40
ZWW10-37	41
ZWW10-128	42
Dharwar dry	52
Drysdale	52
Gregory	42
FAC10-16	42
RIL114	42
SB062	52
SeriM82	52

Annex 5: Physiologic and agronomic characteristics of the 10 wheat founders of the Su-NAM population

Name	Reason for selection	Target traits of interest
<b>Dharwar Dry</b>	Drought adaptation features	Adapted to rainfed wheat production in India, deep root system, stay-green phenotype (Manske and Vlek, 2002; Manschadi et al., 2008)
<b>Drysdale</b>	Heat tolerance	High transpiration efficiency (Condon et al., 2004; Tausz-Posch et al., 2012)
<b>EGA Gregory</b>	Adaptation to nematodes	Moderate tolerance to root lesion nematodes ( <i>Pratylenchus thornei</i> ), (Grains Research and Development,)
<b>EGA Wylie</b>	Disease resistance	Moderate levels of <i>P. thornei</i> and black point tolerance as well as <i>Fusarium</i> crown rot resistance (Grains Research and Development,; Zheng et al., 2014)
<b>FAC10-16</b>	Drought adaptation	High levels of resistance to leaf rust (Riaz et al., 2016) and moderate resistance to yellow spot (Dinglasan et al., 2016).
<b>RIL114</b>	Pre-harvest sprouting tolerance	High levels of grain dormancy donated by DH70 parent (Hickey et al., 2009)
<b>SB062</b>	Heat tolerance	High levels of water soluble carbohydrates (Olivares-Villegas et al., 2007; Dreccer et al., 2009)
<b>SeriM82</b>	Drought adaptation features	Deep root system (Manschadi et al., 2006, 2008), stay-green phenotype (Olivares-Villegas et al., 2007; Christopher et al., 2008; Manschadi et al., 2010)
<b>ZWB10-37</b>	General adaptation	High yielding in CAIGE <sup>2</sup> trials conducted in South Australia
<b>ZWW10-128</b>	General adaptation	High yielding in CAIGE <sup>2</sup> trials conducted in South Australia

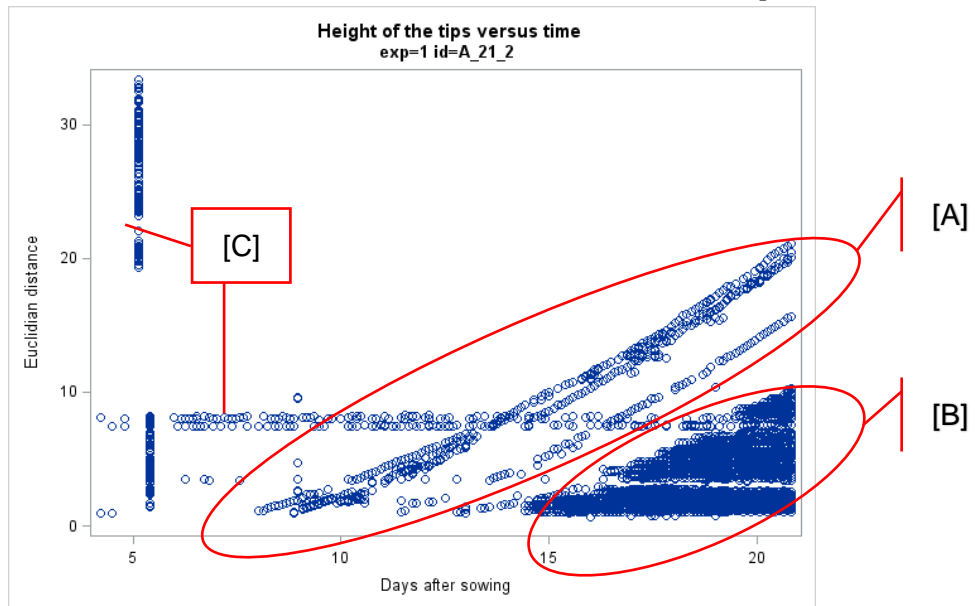
<sup>2</sup>CIMMYT-Australia-ICARDA Germplasm Evaluation (CAIGE)

Annex 6: Composition of the full-strength Hewitt solution

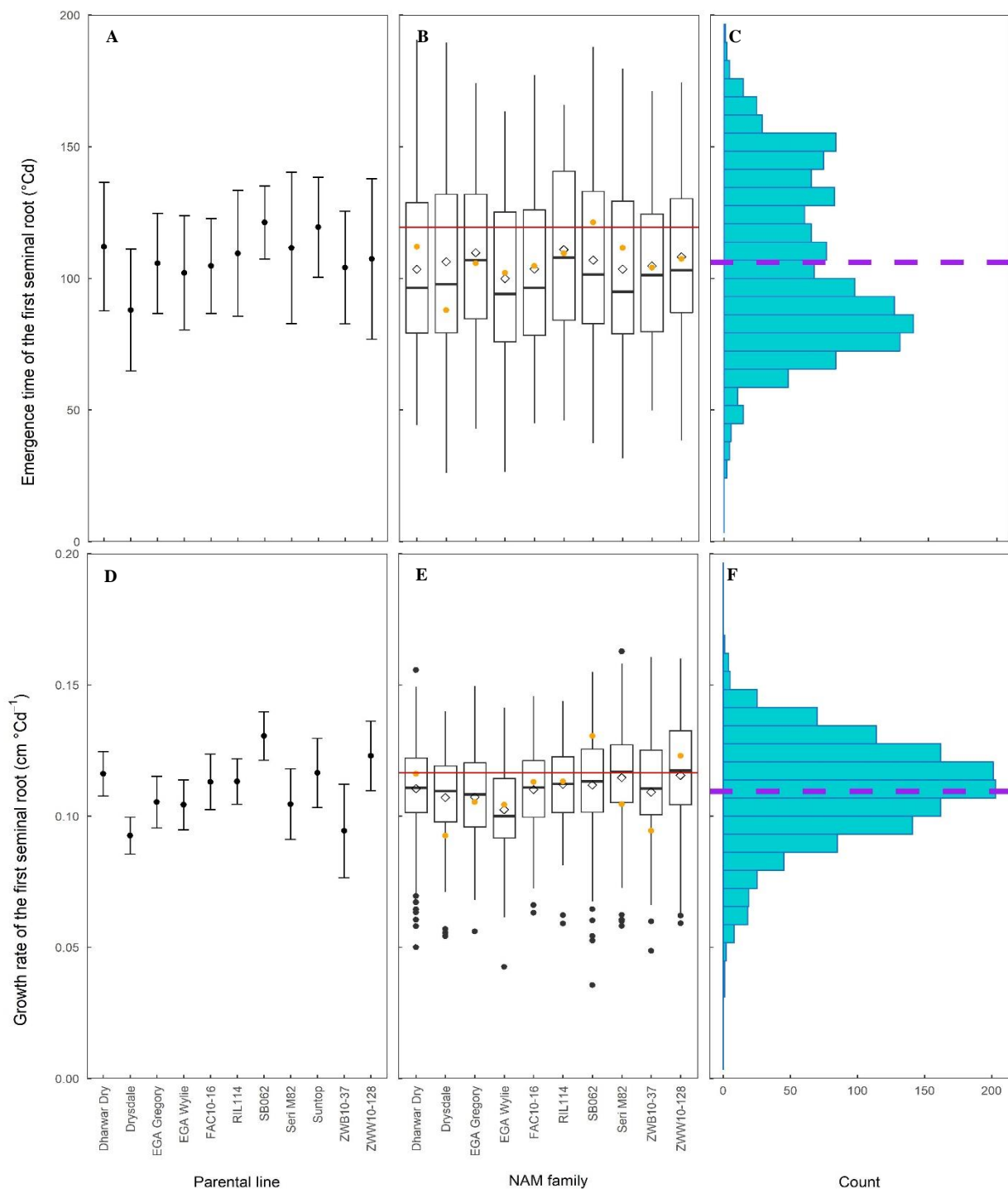
Nutrient	Concentration (g.L <sup>-1</sup> )
KNO <sub>3</sub>	202.20
Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	472.28
MgSO <sub>4</sub> .7H <sub>2</sub> O	184.77
NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> O	103.75
FeEDTANa	18.35
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.990
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.125
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.144
H <sub>3</sub> BO <sub>3</sub>	1.546
NaCl	2.922
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub>	0.044

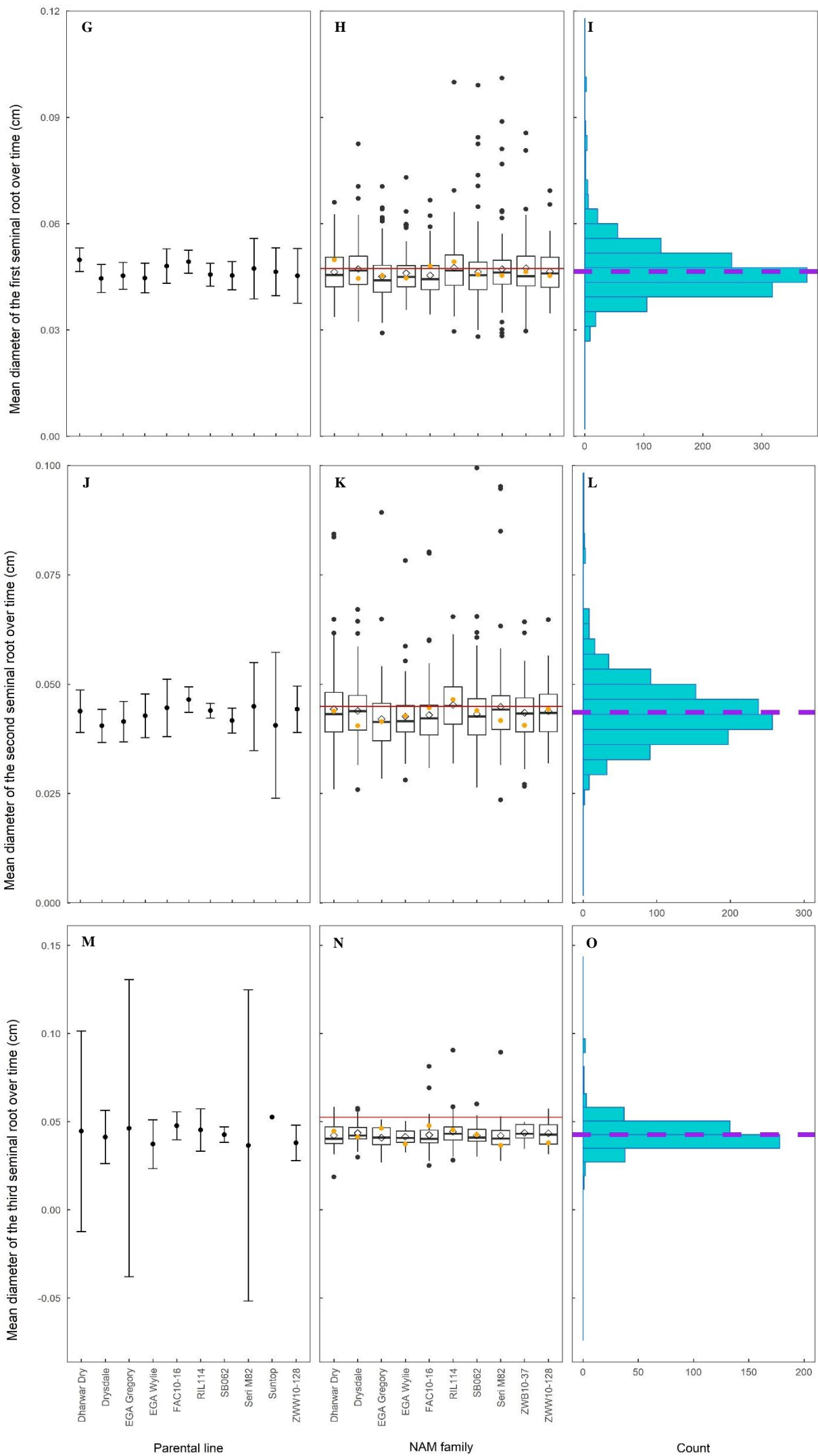
Annex 7: Euclidian distance of the plant tips in the aeroponics through time.

*A: section with the seminal roots, B: section with the lateral roots, C: tips to be removed*

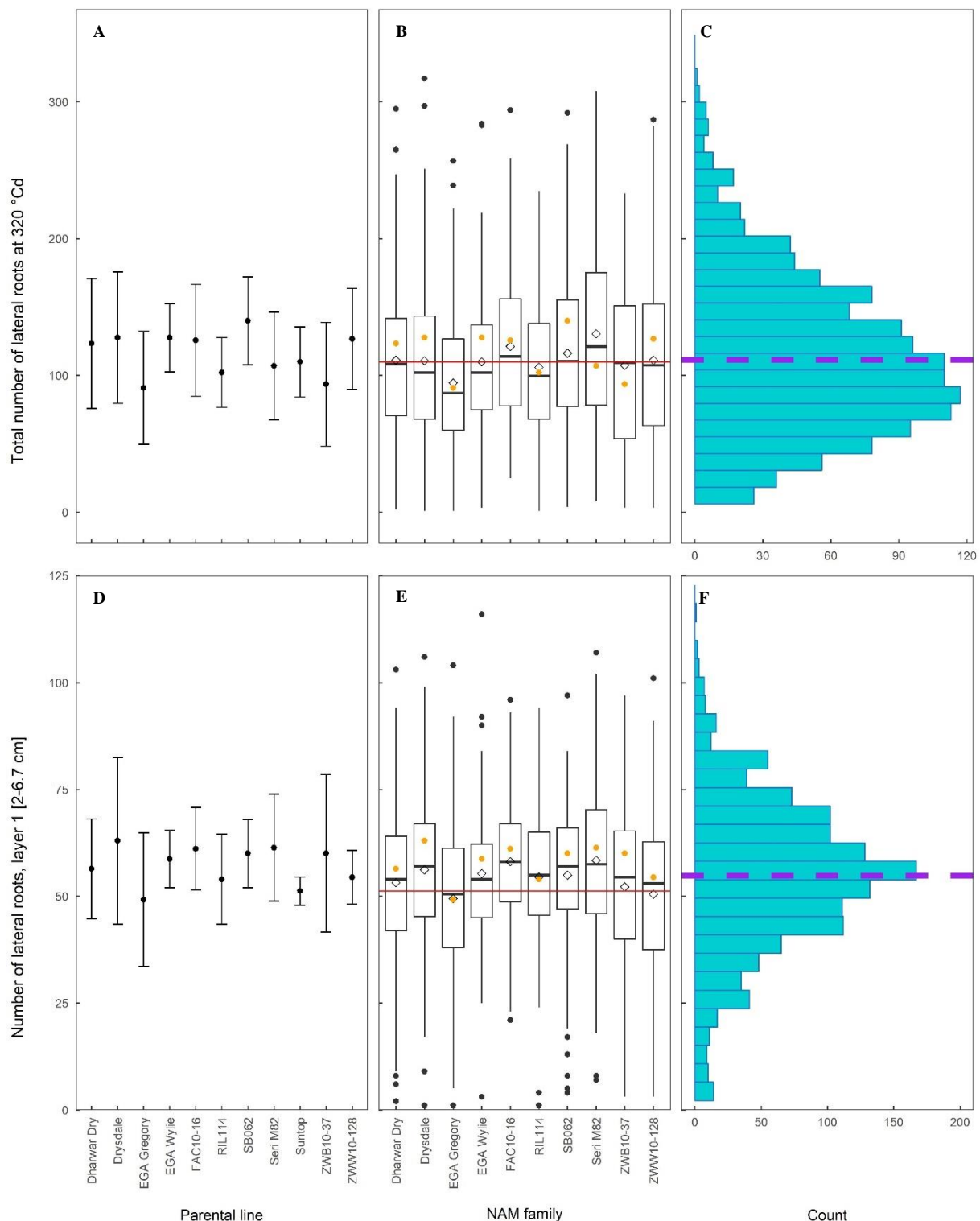


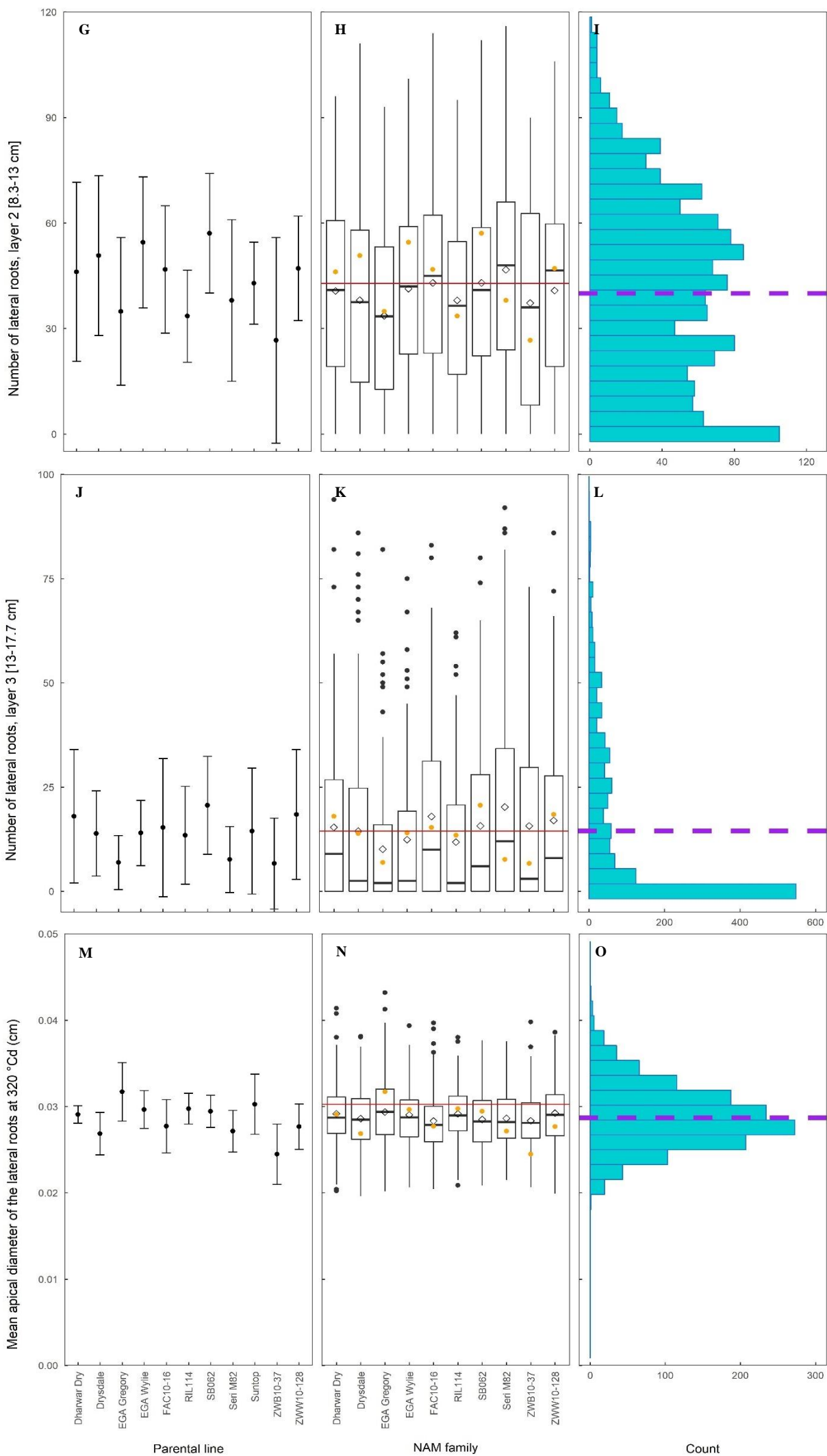
**Annex 8: Parental (first column), family (middle column) and NAM (last column) phenotypic distribution of traits relative to seminal roots, namely the emergence of the first seminal root (first row) the growth rate of the first seminal root (second row), the mean diameter of the first seminal root over time (third row), the mean diameter of the second seminal root over time (fourth row), and the mean diameter of the third seminal root over time (fifth row). Mean values of the parental lines are presented in the first column (A, D...) with a confidence interval at  $P = 0.05$ . Distribution for the NAM families are presented as boxplots (second column, B, E...). The lower, middle and upper lines of the boxplot indicate 25, 50 (median) and 75% of trait values, respectively. The whiskers correspond to 1.5 times the interquartile range (IQR, the difference between the 75th and 25th percentiles) or to the most extreme observed value, whichever is smallest. Dots above or below the whiskers represent individual values outside this range. The diamonds correspond to the NAM-family means, the solid orange points to the family-donor mean, and the red line to the mean of reference line Suntop. An histogram of the distribution of the value of the trait for all the individual plants within the NAM is presented in the last column (C, F...). The purple dashed line represents the mean value of all the individual plants of the NAM.**





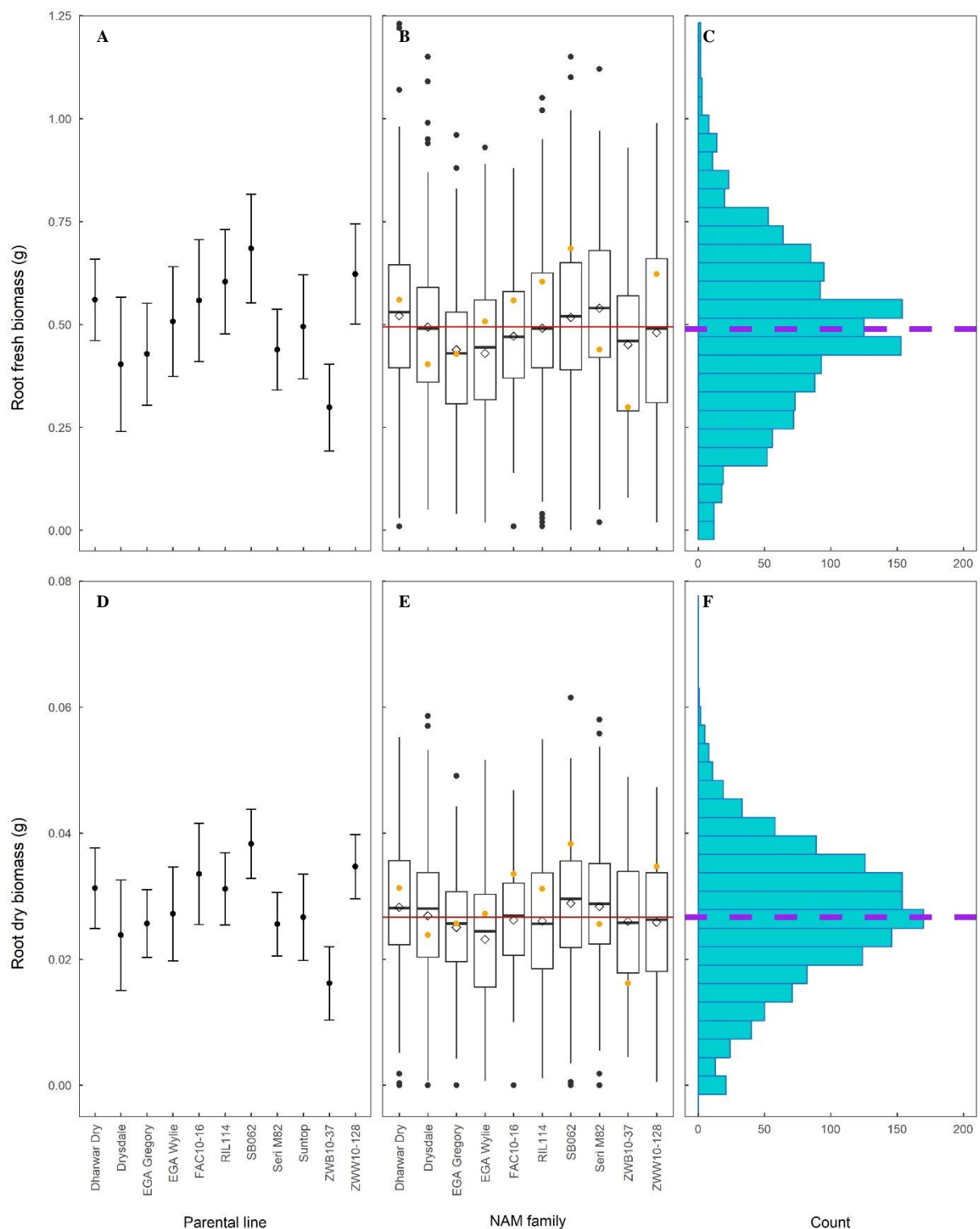
**Annex 9: Parental (first column), family (middle column) and NAM (last column) phenotypic distribution of traits relative to lateral roots, namely the total number of lateral roots (first row) the number of lateral roots in the layer 1 (second row), the number of lateral roots in the layer 2 (third row), the number of lateral roots in the layer 3 (fourth row), and the mean apical diameter of the lateral roots (fifth row). Mean values of the parental lines are presented in the first column (A, D...) with a confidence interval at  $P = 0.05$ . Distribution for the NAM families are presented as boxplots (second column, B, E...). The lower, middle and upper lines of the boxplot indicate 25, 50 (median) and 75% of trait values, respectively. The whiskers correspond to 1.5 times the interquartile range (IQR, the difference between the 75th and 25th percentiles) or to the most extreme observed value, whichever is smallest. Dots above or below the whiskers represent individual values outside this range. The diamonds correspond to the NAM-family means, the solid orange points to the family-donor means, and the red line to the mean of reference line Suntop. An histogram of the distribution of the value of the trait for all the individual plants within the NAM is presented in the last column (C, F...). The purple dashed line represents the mean value of all the individual plants of the NAM.**

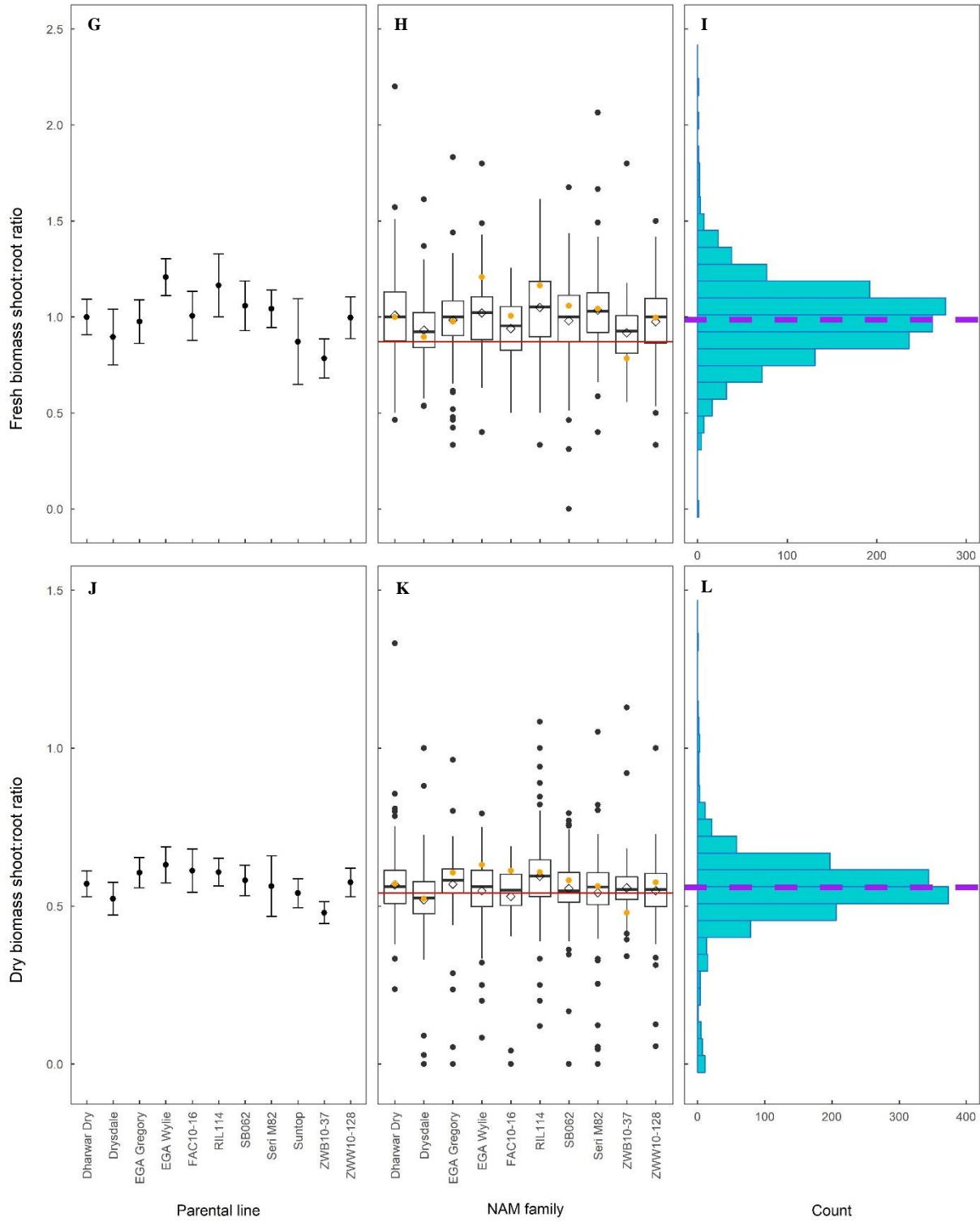






**Annex 10: Parental (first column), family (middle column) and NAM (last column) phenotypic distribution of traits relative to the root biomass, namely the root fresh biomass (first row) the root dry biomass (second row), the root:shoot fresh biomass ratio (third row), and the root:shoot dry biomass ratio (fourth row).** Mean values of the parental lines are presented in the first column (A, D...) with a confidence interval at  $P = 0.05$ . Distribution for the NAM families are presented as boxplots (second column, B, E...). The lower, middle and upper lines of the boxplot indicate 25, 50 (median) and 75% of trait values, respectively. The whiskers correspond to 1.5 times the interquartile range (IQR, the difference between the 75th and 25th percentiles) or to the most extreme observed value, whichever is smallest. Dots above or below the whiskers represent individual values outside this range. The diamonds correspond to the NAM-family means, the solid orange points to the family-donor means, and the red line to the mean of reference line Suntop. An histogram of the distribution of the value of the trait for all the individual plants within the NAM is presented in the last column (C, F...). The purple dashed line represents the mean value of all the individual plants of the NAM.





Annex 11: Table of the QTLs found for the root traits

The “SNP” column indicates the SNP in disequilibrium with the QTL, the “Effect” column indicates the effect of the QTL, the “Chr” column indicates the chromosomes on which the QTL is located, the “Pos (cM)” column indicates the position of the SNP, the “% expl” column indicates the percentage of the phenotypic variation explained by the QTL, the “Trait” column indicates the trait for which the QTL is related to, the “Unit” column indicates the unit of the trait related to the QTL. The QTLs for the MRL are highlighted in blue, the QTLs for the nb\_sem are highlighted in green, the QTLs for the nblat\_tot are highlighted in orange and the QTLs for the nblat\_layer 3 are highlighted in grey. The value in pink is the highest % expl. The marron frames indicate the QTLs collocating.

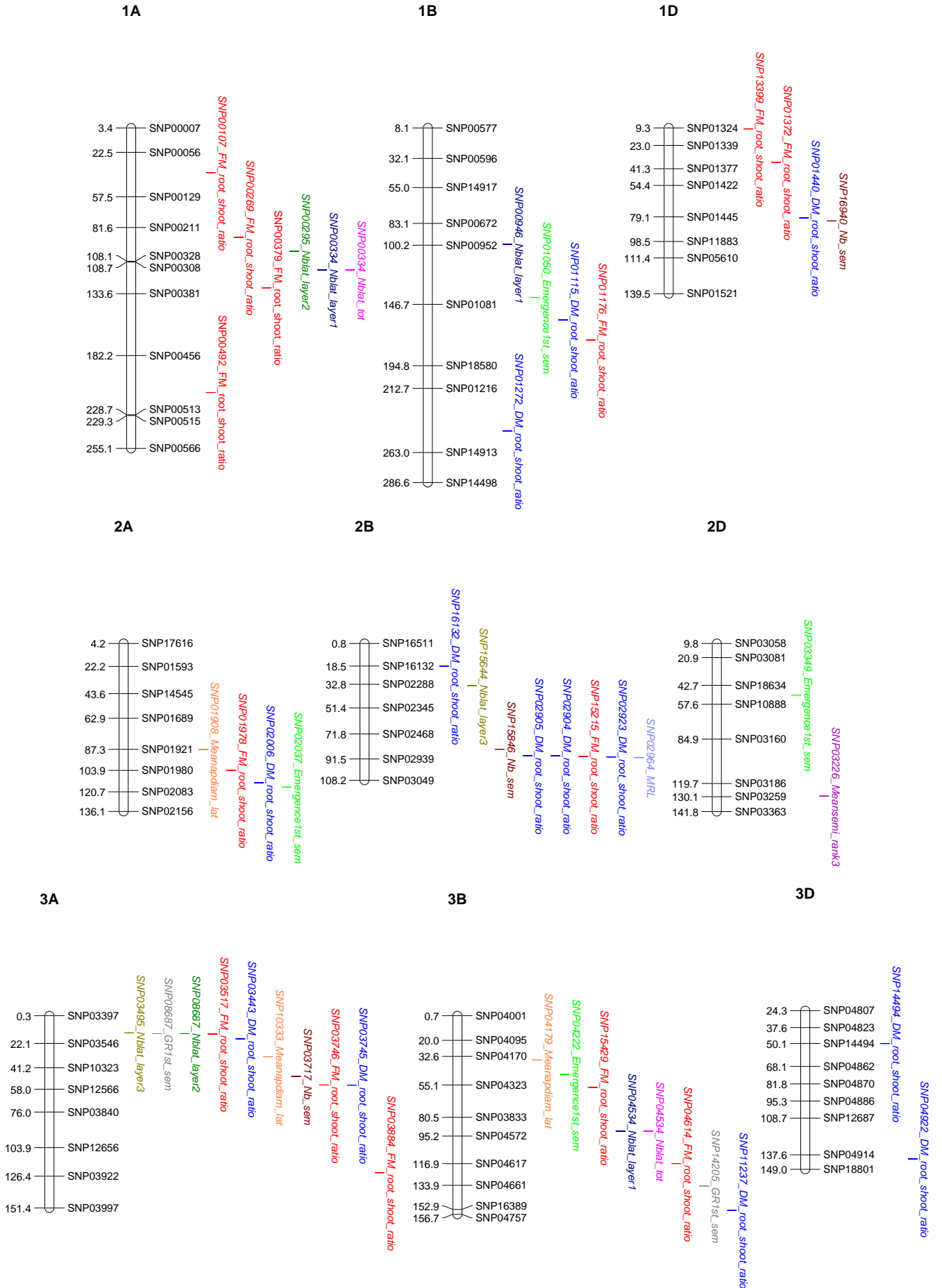
SNP	Effect	Chr	Pos (cM)	% expl	P.value	Trait	Unit
SNP00107	-0.0285	1A	38.47	1.88	4.72E-06	FM root shoot ratio	-
SNP00269	-0.0376	1A	89.33	1.31	1.95E-03	FM root shoot ratio	-
SNP00295	-2.8709	1A	100.16	5.83	6.56E-05	Nblat_layer2	-
SNP00334	2.1607	1A	114.82	2.43	1.46E-03	Nblat_layer1	-
SNP00334	5.8791	1A	114.82	4.85	1.44E-04	Nblat_tot	-
SNP00379	0.0649	1A	128.99	3.84	4.30E-06	FM root shoot ratio	-
SNP00492	0.0232	1A	211.19	1.41	6.03E-04	FM root shoot ratio	-
SNP00946	3.9924	1B	99.52	3.09	7.86E-04	Nblat_layer1	-
SNP01050	9.5053	1B	141.21	2.37	1.55E-03	Emergence1st_sem	°Cd
SNP01115	0.0223	1B	158.93	1.77	6.19E-04	DM root shoot ratio, exp 1	-
SNP01176	0.0280	1B	174.61	3.76	2.99E-04	FM root shoot ratio	-
SNP01272	-0.1251	1B	246.05	-0.35	1.76E-14	DM root shoot ratio, exp 1	-
SNP13399	-0.0613	1D	10.02	1.70	2.37E-06	FM root shoot ratio	-
SNP01372	0.0287	1D	36.20	1.82	2.58E-03	FM root shoot ratio	-
SNP01440	-0.0176	1D	79.96	0.63	1.56E-03	DM root shoot ratio, exp 1	-
SNP16940	-0.2024	1D	82.42	2.94	1.47E-04	Nb_sem	-
SNP01908	-0.0008	2A	87.32	1.87	4.53E-04	Meanapdiam_lat	cm
SNP01978	0.0359	2A	103.65	1.47	3.46E-04	FM root shoot ratio	-
SNP02006	0.1197	2A	113.54	8.14	2.16E-08	DM root shoot ratio, exp 1	-
SNP02037	3.0218	2A	117.05	2.77	1.82E-04	Emergence1st_sem	°Cd
SNP16132	0.0156	2B	18.50	0.54	2.92E-03	DM root shoot ratio, exp 1	-
SNP15644	-1.1208	2B	34.01	5.51	1.92E-04	Nblat_layer3	-
SNP15846	-0.6037	2B	83.84	5.54	3.21E-06	Nb_sem	-
SNP02905	-0.2910	2B	88.98	1.14	4.13E-05	DM root shoot ratio, exp 1	-
SNP02904	0.3533	2B	88.98	0.64	4.76E-06	DM root shoot ratio, exp 1	-
SNP15215	-0.0204	2B	89.38	1.72	5.75E-04	FM root shoot ratio	-
SNP02923	0.1336	2B	89.88	0.37	8.98E-04	DM root shoot ratio, exp 1	-
SNP02964	-1.3452	2B	90.36	4.76	9.20E-05	MRL	cm
SNP03349	1.9257	2D	50.25	1.78	4.36E-03	Emergence1st_sem	°Cd
SNP03226	0.0029	2D	129.66	6.45	6.54E-05	Meansemi_rank3, exp 1	cm
SNP03397	-0.0326	3A	0.27	1.57	2.56E-05	FM root shoot ratio	-

SNP	Effect	Chr	Pos (cM)	% expl	P.value	Trait	Unit
SNP03495	-1.5397	3A	13.77	3.86	8.52E-04	Nblat_layer3	-
SNP08687	0.0024	3A	14.37	5.41	2.71E-06	GR1st_sem	cm °Cd <sup>-1</sup>
SNP08687	1.9544	3A	14.37	2.36	1.30E-03	Nblat_layer2	-
SNP03517	-0.0173	3A	14.59	1.47	8.82E-04	FM root shoot ratio	-
SNP03443	0.0709	3A	18.37	2.58	1.27E-02	DM root shoot ratio, exp 1	-
SNP10333	0.0018	3A	32.41	2.45	1.71E-05	Meanapdiam_lat	cm
SNP03717	-0.2043	3A	48.14	2.69	5.55E-04	Nb_sem	-
SNP03746	-0.0910	3A	54.73	1.82	9.81E-09	FM root shoot ratio	-
SNP03745	0.1458	3A	54.83	6.11	7.77E-10	DM root shoot ratio, exp 1	-
SNP03884	0.0612	3A	123.47	1.98	2.43E-06	FM root shoot ratio	-
SNP04179	0.0004	3B	35.54	2.47	3.01E-04	Meanapdiam_lat	cm
SNP04222	-2.3722	3B	46.82	2.43	2.01E-03	Emergence1st_sem	°Cd
SNP15429	0.0728	3B	57.03	2.68	2.92E-05	FM root shoot ratio	-
SNP04534	-4.9255	3B	91.35	4.04	4.38E-06	Nblat_layer1	-
SNP04534	-10.2918	3B	91.35	2.03	1.80E-03	Nblat_tot	-
SNP04614	-0.0284	3B	116.79	1.40	4.27E-04	FM root shoot ratio	-
SNP14205	0.0026	3B	134.56	2.87	3.84E-04	GR1st_sem	cm °Cd <sup>-1</sup>
SNP11237	-0.0653	3B	153.71	2.55	1.23E-09	DM root shoot ratio, exp 1	-
SNP14494	0.1560	3D	50.07	0.31	1.13E-14	DM root shoot ratio, exp 1	-
SNP04922	-0.0108	3D	140.83	0.7	5.82E-03	DM root shoot ratio, exp 1	-
SNP16525	0.0383	4A	19.80	1.42	2.05E-04	FM root shoot ratio	-
SNP05172	-8.6044	4A	88.61	2.53	1.37E-03	Nblat_layer2	-
SNP05552	3.9419	4B	41.96	7.13	6.24E-06	Nblat_layer2	-
SNP05552	7.1248	4B	41.96	6.00	1.25E-05	Nblat_tot	-
SNP05619	-0.0257	4B	62.86	0.58	2.82E-03	DM root shoot ratio, exp 1	-
SNP05716	0.1941	4D	73.73	9.49	9.61E-23	DM root shoot ratio, exp 1	-
SNP14047	0.1950	5A	40.51	4.13	2.54E-12	FM root shoot ratio	-
SNP05871	-0.1402	5A	44.49	1.54	2.58E-07	FM root shoot ratio	-
SNP05899	-0.0008	5A	48.67	3.81	1.43E-07	Meanapdiam_lat	cm
SNP06033	-2.5397	5A	68.47	4.05	3.89E-05	Nblat_layer1	-
SNP16827	2.7516	5A	79.47	2.83	1.15E-04	Nblat_layer3	-
SNP06113	0.0019	5A	91.64	2.79	7.25E-04	DM root	g
SNP06118	-0.0004	5A	100.94	2.72	1.49E-03	Meanapdiam_lat	cm
SNP06172	-0.0175	5A	129.44	1.40	2.25E-04	FM root shoot ratio	-
SNP09227	-0.0182	5B	32.63	1.02	1.08E-04	DM root shoot ratio, exp 1	-
SNP01379	-0.0999	5B	32.95	3.82	1.01E-06	DM root shoot ratio, exp 1	-
SNP06868	0.1157	5B	151.80	2.16	1.69E-12	DM root shoot ratio, exp 1	-
SNP06872	-0.0942	5B	152.32	1.27	6.59E-05	DM root shoot ratio, exp 1	-
SNP11125	-0.1266	5D	141.35	3.79	3.51E-09	DM root shoot ratio, exp 1	-
SNP07265	6.3020	6A	52.34	4.73	9.82E-04	Emergence1st_sem	°Cd
SNP07371	-0.0012	6A	87.43	2.99	8.33E-04	Meansemi_rank1	cm

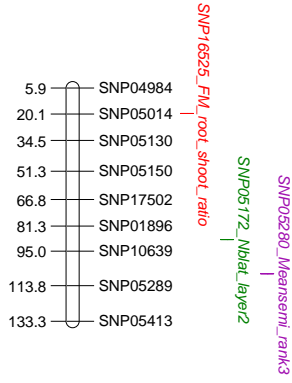
SNP	Effect	Chr	Pos (cM)	% expl	P.value	Trait	Unit
SNP07389	-0.0003	6A	89.90	4.48	1.89E-04	Meanapdiam_lat	cm
SNP07519	0.1339	6B	3.35	7.48	8.62E-05	DM root shoot ratio, exp 1	-
SNP07568	-0.4105	6B	10.84	2.49	2.22E-10	DM root shoot ratio, exp 1	-
SNP07577	0.2683	6B	11.19	0.65	1.96E-14	DM root shoot ratio, exp 1	-
SNP07585	0.2482	6B	12.06	0.69	2.99E-05	DM root shoot ratio, exp 1	-
SNP07629	-0.1088	6B	14.76	1.39	2.43E-10	DM root shoot ratio, exp 1	-
SNP07865	0.0300	6B	32.72	0.75	6.14E-04	DM root shoot ratio, exp 1	-
SNP16080	-2.4995	6B	66.36	2.16	1.15E-03	Nblat_layer3	-
SNP14502	-0.0011	6B	80.29	5.41	1.25E-03	Meansemi_rank3, exp 1	cm
SNP08304	0.0004	6D	97.64	1.85	3.42E-03	Meanapdiam_lat	cm
SNP08458	-6.5631	7A	21.48	2.43	2.94E-07	Emergence1st_sem	°Cd
SNP08451	-0.0218	7A	21.48	1.87	6.30E-05	FM root shoot ratio	-
SNP08485	2.9357	7A	28.06	2.37	8.14E-04	Emergence1st_sem	°Cd
SNP08543	0.0495	7A	44.16	2.10	6.29E-05	FM root shoot ratio	-
SNP08594	0.0002	7A	61.23	2.08	2.40E-03	Meanapdiam_lat	cm
SNP13581	0.0332	7A	67.10	0.93	5.65E-05	DM root shoot ratio, exp 1	-
SNP12649	-0.5348	7A	78.47	7.67	1.34E-06	MRL	cm
SNP08724	4.2514	7A	88.87	2.74	2.72E-04	Nblat_tot	-
SNP13689	3.4241	7A	90.33	5.95	2.42E-05	Nblat_layer3	-
SNP08800	-0.1072	7A	96.07	3.7	2.85E-10	DM root shoot ratio, exp 1	-
SNP09016	-0.1524	7B	0.29	4.35	2.29E-17	DM root shoot ratio, exp 1	-
SNP09131	0.3418	7B	31.04	3.18	1.88E-04	Nb_sem	-
SNP09725	-0.9810	7D	71.72	3.18	1.39E-04	MRL	cm
SNP09725	-7.3506	7D	71.72	3.41	1.09E-03	Nblat_tot	-
SNP14035	-0.1436	7D	112.99	2.12	3.23E-14	DM root shoot ratio, exp 1	-
SNP09855	0.0560	7D	115.30	2.95	4.92E-05	FM root shoot ratio	-

## Annex 12: QTL map of the 14 root traits analyzed

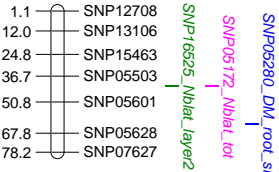
*The positions of the markers/QTLs are indicated in cM. Each color represents a root trait.*



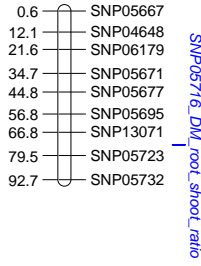
4A



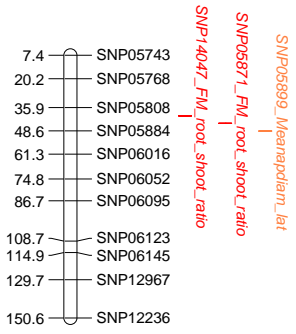
4B



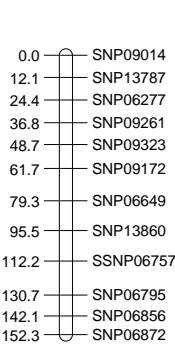
4D



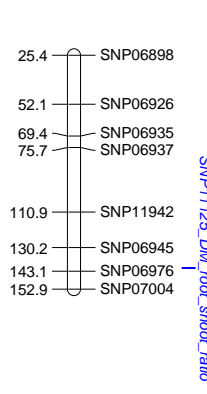
5A



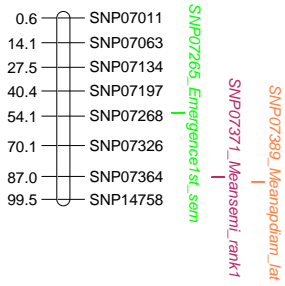
5B



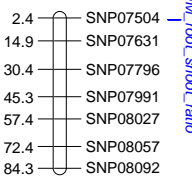
5D



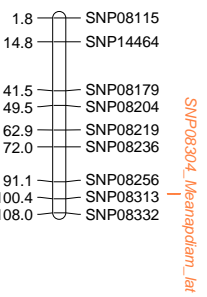
6A



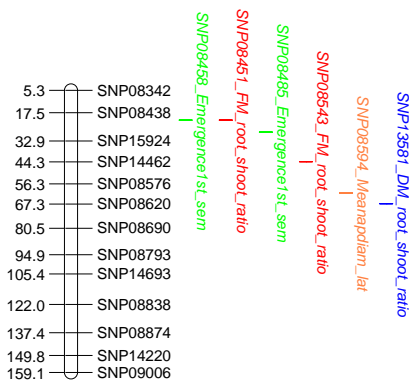
6B



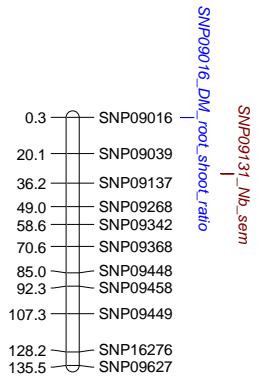
6D



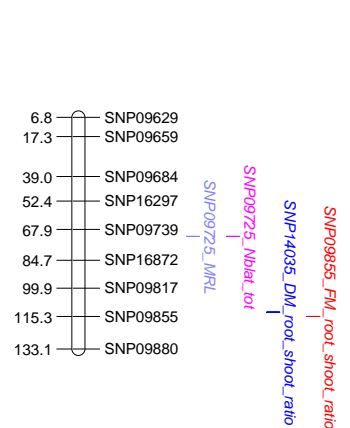
7A



7B



7D









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Titre français : Analyse génétique de traits racinaire chez le blé dans une population NAM

Titre anglais : Genetic analysis of wheat root seedlings in a NAM population

Résumé : Le blé est une culture et une ressource alimentaire majeure dans le monde. Cependant, la sécheresse est un facteur limitant important réduisant le rendement, dans des régions de culture telles que le nord de l'Australie. La création de nouvelles variétés de blé en améliorant des traits racinaires pourrait conduire à une amélioration du rendement en condition de sécheresse. Dans ce but, une analyse génétique a été réalisée sur une population NAM créée pour assister à la création de variétés plus tolérantes à la sécheresse dans le Sud-Est du Queensland. Plusieurs traits racinaires ont été phénotypés sur une plateforme aéroponique, en Belgique, en 2016. Au total, 98 QTLs ont été trouvés pour 14 traits racinaires. Parmi les traits étudiés, trois étaient particulièrement intéressants pour la création de nouvelles variétés dans le sud-est de l'Australie où les plantes reposent sur des ressources en eau profondes dans les périodes critiques de sécheresse : la longueur maximale du système racinaire, le nombre de racines séminales et le nombre de racines latérales profondes. Compte tenu des études précédentes traitant d'analyses génétiques chez le blé, il est très probable que l'un des QTLs identifié sur le chromosome 1D pour le nombre de racines séminales soit un nouveau QTL. Des QTLs avec des effets intéressants ont été identifiés et pourraient être utilisés dans des travaux de sélection pour améliorer la tolérance à la sécheresse dans la région du Queensland.

Abstract: Wheat is a major culture and food resource around the world. However, drought considerably reduces yield in major regions, such as in Northern Australia. Wheat breeding by improving root traits could lead to significant yield improvements for drought-prone environments. Thereby, a genetic analysis was realized using a NAM population designed to assist breeding for better drought tolerance in South-Eastern Queensland. Various seedling root traits were phenotyped using an aeroponic platform in two experiments in Belgium in 2016. In total, we found 98 QTLs for 14 root traits. Among the traits studied, three were of major interest for the breeding of new varieties in South-Eastern Queensland, where crops heavily rely on deep soil water storage in terminal drought: the maximum root length, the number of seminal roots and the number of deep lateral roots. Considering the previous QTL studies, it is likely that one of the QTLs identified on chromosome 1D for the number of seminal roots is a new QTL. QTLs with interesting effects were identified and could potentially assist breeders improve drought tolerance in the region.

Mots-clés : génétique, création variétale, amélioration des plantes, NAM, QTL, tolérance à la sécheresse

Key Words: genetics, breeding, plant improvement, NAM, QTL, root traits, drought tolerance

\* Élément qui permet d'enregistrer les notices auteurs dans le catalogue des bibliothèques universitaires