



Work Programme 2012 "COOPERATION"

Theme 2: FOOD, AGRICULTURE AND FISHERIES, AND BIOTECHNOLOGY

Activity 2.3: Life sciences, biotechnology and biochemistry for sustainable non-food products and processes

Area 2.3.5: Environmental biotechnology

KBBE.2012.3.5-03

Biotechnological waste water treatments and reuse in agronomical system

Call: FP7-KBBE-2012-6

## Integrating biotreated wastewater reuse and valorization with enhanced water use efficiency to support the Green Economy in EU and India

Grant agreement no.: 311933

Funding scheme: Collaborative Project

Coordinator: Dr Antonio Lopez, IRSA-CNR (Italy)

### Water4Crops - EU

#### Work Package n°: 4

Improving WUE and drought tolerance of maize, sorghum, millet and tomato via genomics approaches and modelling

#### Deliverable 4.2

Information on different root architectures (brace and seminal roots) on maize WUE and yield stability

Due date: month 48

Actual submission date: month 48

Start of project: 1/08/2012

Deliverable Lead contractor: UNIBO

Participants: UNIBO – HORTA

Contact for queries: [silvio.salvi@unibo.it](mailto:silvio.salvi@unibo.it)

Dissemination level: PU



## CONTENTS

### Paragraphs

1. Introduction.....	pag.	3
2. Materials and Methods.....	pag.	4
3. Results.....	pag.	7
4. Discussion.....	pag.	13
REFERENCES.....	pag.	14

## 1. INTRODUCTION

The study of root system architecture (RSA) has become increasingly important in the last years (Hochholdinger and Tuberosa 2009; Tuberosa 2012; Cobb J.N. *et al.*, 2013; Lynch 2013; Uga *et al.* 2013; Meister *et al.* 2014; Rogers *et al.*, 2015; Lynch *et al.*, 2015; Zhan *et al.*, 2015; Pestova *et al.*, 2016).

The maize root system is composed of embryonic primary and seminal roots (Abbe and Stein, 1954) and postembryonic shoot-borne and lateral roots. Shoot-borne roots formed at underground nodes are called crown roots, while those formed at above-ground nodes of the shoot are called brace roots (Hochholdinger *et al.*, 2004). Embryonic roots are relevant for early establishment of the plant while postembryonic roots are important later in development and can have a significant influence on grain yield in water-limited conditions (Hochholdinger and Tuberosa 2009). It is well established that drought and nutrient deficiency modulate root growth (Sharp *et al.*, 2004; Chun *et al.*, 2005; Hermans *et al.*, 2006). Additionally, ideal root ideotypes have been formulated (Lynch 2013, Meistaer *et al.*, 2014). Moreover, the historical yield increase of the last decades seems more correlated with changes in RSA rather than with canopy architecture (Hammer *et al.* 2009).

Despite the importance of roots, high throughput phenotyping remains a bottleneck for genetic analysis of RSA. The evaluation of RSA in field-grown plants is very difficult, expensive and time-consuming, given the large number of genotypes necessary for QTL analysis (Richards 2008). For this reason, many laboratory and greenhouse techniques for the screening of RSA at early stage have been described (James *et al.*, 1985; Hetz *et al.*, 1996; Bengough *et al.*, 2004; Sanguineti *et al.* 2007; Hargreaves 2009; Iyer-Pascuzzi 2010). However, the evaluation of RSA of adult plants grown in the field provides a true representation of root growth in an agriculturally relevant context. A relatively fast method to visually analyse root architectural traits of adult maize plants in the field was proposed (Shovelomics. Trachsel *et al.* 2011). In our experiment within W4C WP4, in order to further increase the accuracy and speed of the scoring method, we coupled 'Shovelomics' with digital image analysis. In the last decade many software dedicated to root image analysis have been developed (WinRHIZO™, Arsenault *et al.* 1995, DART, Le Bot, *et al.*, 2010; RooTrak, Mairhofer *et al.*, 2012; SmartRoot, Lobet *et al.*, 2011; RootNav, Pound *et al.*, 2013; RootScape, Ristova *et al.*, 2013; Gia-Roots, Galkovskyi *et al.*, 2014). While most of these softwares are dedicated to root image analysis at the seedling level, two softwares have been developed specifically to aid shovelomics in maize: DIRT (Bucksch *et al.* 2014) and REST (Root Estimator for Shovelomics Traits, Colombi *et al.*, 2015).

While the first one phenotypes just the outer brace and crown roots, REST generates a more detailed information about the internal structure of the root system. In this study, the approaches described by Trachsel *et al.* (2011) and the software REST were combined to study the variability for maize RSA and water use efficiency in a collection of introgression lines developed from the cross between B73 and Gaspé Flint. B73 is an important elite line while Gaspé Flint is an old landrace of no current commercial or agronomic interest. The phenotypic differences between the two materials make their cross ideal for the genetic dissection of agronomic traits. The population has already been screened for flowering time, number of leaves, plant height and root architecture at early stage (Salvi *et al.*, 2011 and 2016).

## 2. Materials and Methods

The plant material consisted of the B73 x Gaspé Flint introgression library (Salvi *et al.*, 2011). B73 is an elite inbred line of the Iowa Stiff Stalk Synthetic heterotic group and is reference genome for maize (Schnable *et al.*, 2009). Gaspé Flint is a Canadian landrace belonging to the Northern Flint maize race group (Vigouroux *et al.*, 2008) with a phenotype very different to B73. The IL population includes 73 lines originated by the cross B73 x Gaspé Flint followed by five cycles of marker-assisted backcross using B73 as recurrent parent, and two cycles of selfing. During backcross and selfing cycles, SSR markers were used to introgress in each line, and to fix as homozygous, a different portion of the Gaspé Flint donor genome (Salvi *et al.* 2011).

The 73 ILs and the two parents (B73 and Gaspé Flint) were genotyped using a SNP array based on the ILLUMINA Infinium technology. Samples were analyzed in duplicate. Raw SNP data produced by the Infinium analysis were treated at UNIBO with the ILLUMINA software GenomeStudio™. By comparing B73 and Gaspé Flint, approx. 39% of the SNPs resulted polymorphic and used for the analysis of results. Bin map was constructed for the 73 IL lines based on individual SNPs. Consecutive SNP sites with the same genotype were lumped into blocks and a breakpoint was assumed at the transition between two different genotype blocks.

The 73 IL lines were evaluated in replicated (3 reps) field trials at two water regimes (well watered - WW, and rain-fed or water-stressed - WS) at Horta, Ravenna (Italy) during summer 2013.

Thirty-two traits covering phenology, plant architecture, yield, and root architecture were analyzed (Table 1).

	Acronym	Trait descriptions	unit	
roots	REST	RAR	Root angle - REST	°
		FFA	Fill factor on arc - REST	
		MFD	Mean fractal dimension - REST	
		AREA	Area - REST	cm <sup>2</sup>
		MW	Maximal width - REST	cm
	visual score	TPSL	Total projected structure length - REST	cm
		RMS	Root mass - score	visual score
		DS	Depth - score	visual score
		NBR	total number of brace root	count
		NBRN	number of brace root nodes	count
morphological	RDW	Root dry weight	g	
	NDRE	NDRE	index	
	SPAD	SPAD	index	
	ST	Stress	visual score	
	MF	Male flowering	gdd	
	FF	Female flowering	gdd	
	ASI	ASI	gdd	
	SC	Stomatal conductance	mmol m <sup>-2</sup> s <sup>-1</sup>	
	TL	Transition leaf	count	
	SG	Stay green	visual score	
yield related	PH	Plant height	cm	
	PN	Plant nodes	number	
	EW	Ear weight	g	
	EPP	Ear per plant	count	
	KR	kernel row per ear	count	
	EL	Ear length	cm	
	ED	Ear diameter	cm	
	CD	Cob diameter	cm	
	KNPE	Kernel number per ear	number	
	GY	Grain yield	t/ha	
TKW	Thousand kernel weight	g		

Table 1- list of phenotypic traits evaluated by Horta on the collection of IL lines

For underground root analysis (Shovelomics. Trachsel *et al.* 2011), the root apparatus of three representative plants per plot were considered by manually uprooting each plant, and subsequent washing and phenotypic analysis. A total of 1,350 samples (= plants) were evaluated. For each root, digital photos and dry matter weight were assessed. The 18 available photos for each genotype were cleaned and analyzed with the software REST (Colombi *et al.* 2015) and brace root number at each whorl counted and multiplied by two. Moreover, traits related to root system architecture (mass and depth) were measured (visual score) in order to validate software results. QTL analysis was carried out using the program QTL IciMapping, version 4.0.6.0 (Meng *et al.*, 2015) and using the method

“RSTEP-LRT-ADD” (Stepwise regression based likelihood ratio tests of additive QTL, (Wang *et al.*, 2006) enabling to deal with segment substitution (CSS) lines (a synonymous for introgression library).

Based on the results of 2013 experiment, five IL lines (and the parental line B73) were selected to be tested as F1 hybrids with commercial lines in order to validate the QTLs in other genetic background. In addition to the selected ILs for agronomic and brace roots characteristics, the IL 94-6-1-6 (ILqSR) was tested as F1 hybrids with the same commercial line. IL 94-6-1-6 is a line carrying the introgression (substitution) of Gaspé Flint at a QTL region on chromosome 1 that, at seedling stage, was shown to produce 0-1 seminal roots while the reference background line B73 was shown to develop approx. three seminal roots (Salvi *et al.*, 2016). The field experiments have been carried out for the two seasons (2014 and 2015) in two locations of the Po Valley: at Horta (Ravenna) and UNIBO (Bologna). At Horta, F1 hybrids and IL lines were replicated four times in the irrigated area and four times in the rainfed area in 2014 and three times in 2015. In Bologna, the experiment was carried out in an artificially sloped field with a homogeneous gradient of soil moisture as a function of the water table depth (WTD). The 80-m long field has an artificial slope (2%) along its length, which leads to a linearly increasing distance from the water-table. The field was subdivided into four 20-m-long sections, which allowed us to evaluate the maize hybrids at four average WTD. Traits covering phenology (flowering time, plant height), stress index (stomatal conductance, stress score), yield, and yield related traits were measured. Shovelomics was carried out for the field in Horta and Unibo: for each genotype, 42 pictures of clean roots are available (3 plants x 3-4 rep x 2WR x 2 year) for Horta and 24 (3 plants x 4 rep x 2 year) for Unibo. Root dry weight was measured and pictures have been analyzed with software REST.

Two IL lines (ILqSR and one selected based on the results of field trial 2013) were tested at seedling stage in rhizotron boxes at three water regimes in order to test a drought response at the root level. The same two lines have been tested also at adult stage in bigger rhizotrons (1 m<sup>3</sup>) at three water regimes for a deeper screening of rooting characteristics (shovelomics method), root water-uptake rate and efficiency.

### 3. Results

We identified high plasticity among the IL lines in terms of RSA and yield response to drought. Even if it was not possible to identify a direct correlation between yield response to drought and a single root architecture parameter, it was possible to identify some trends. Most of the IL lines characterized by low yield decrease in WD conditions showed wider root angle, bigger roots in terms of area and root dry weight and lower depth. Drought stress effect on yield was quite low also for IL lines characterized by high plasticity of root angle (i.e. 37-9-1 that in WD conditions reduce root angle of 14% and increase depth of 20%). IL lines most affected by drought in terms of yield are instead characterized by big roots but quite superficial (i.e. IL 22-1-3, IL 29-1-4 and IL 100-2-1-19).

Based on the 2013 field analysis, we identified several regions involved in the control of root morphology that collocate with yield and yield related traits or drought related (see also deliverable 4.1). Some of these regions were further investigated in 2014 and 2015 by testing IL lines per se and as F1 hybrids (hybrids were produced using commercial tester lines).

1. One of the most interesting region is located on chromosome 2 bin 2.04: this region is involved in the control of root angle, number of brace root and ear per plant in WW conditions. The Gaspè Flint allele is associated with an increase of all traits. This region was already indicated as involved in the control of both root angle and yield for the *Root\_ABA1* locus (Giuliani *et al.*, 2005) and collocated with the MQTL Rt6 for root traits (Hund *et al.*, 2011) for Root pulling Force (Lebreton *et al.*, 1995). The IL line that significantly differed from B73 based on Dunnett's test and carries the introgression detected by ICI-mapping is the line 159-7-1-2. In this IL line all roots (crown and brace) are characterized by a higher angle compared to B73, as shown in Figure 1.

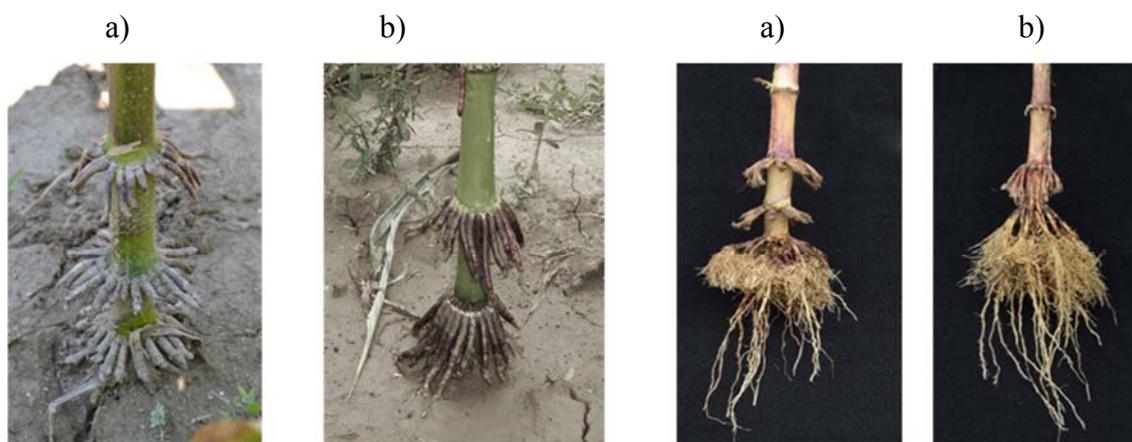


Figure 1: brace root and complete root system of IL 159-7-2-1 (a) compared to the reference B73 (b).

Brace roots in maize are important for lodging resistance and for water and nutrient uptake during the later stages of plant growth (Varney and Canny, 1993; Wang *et al.*, 1994). Very little is known about the molecular mechanisms specific to brace root initiation however, there are some evidence that they are under hormonal and genetic control (Hetz *et al.*, 1996). Moreover, the angle of emergence of brace and other adventitious root growth has been shown to become steeper under water- and nitrogen-limiting conditions in certain maize lines (Trachsel *et al.*, 2013).

The line 159-7-2-1 was also tested in F1 hybrid combination in 2014 and 2015. In most of the hybrid combination tested the general direction was an increase on root angle compared to same tester crossed with the reference B73 and in three hybrid combination in 2015 a significant difference of + 20° for root angle (Dunnet test,  $P > 0.05$ ) was detected in WS condition. The significant difference in root angle identified in in 2015 was also positively correlated with yield variation (0.63).

The higher root angle, root area and root dry weight of IL 159-7-2-1 compared to B73 was also confirmed in the big rhizotron experiment in WW conditions while in drought stress conditions the difference was not significant.

Considering the behavior of IL 159-7-2-1 in the water table experiment the higher root angle compared to B73 is confirmed both in WW and also at the increasing of the water table depth (Figure 2). The highest yield is confirmed in WW and moderate stress conditions. Yield drastically decreased at severe water stress condition.

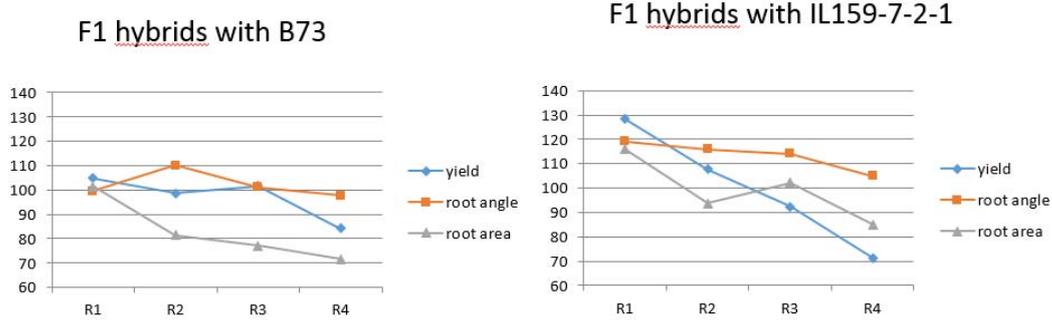


Figure 2. F1 hybrids of B73 and IL 159-7-2-1 tested in 2015 by UNIBO in water table field

On the contrary, at seedling stage IL 159-7-2-1 showed a significantly lower root dry weight compared to B73 and was not affected by drought. Shoot dry weight instead increased significantly in drought conditions in both the IL line and the reference B73.

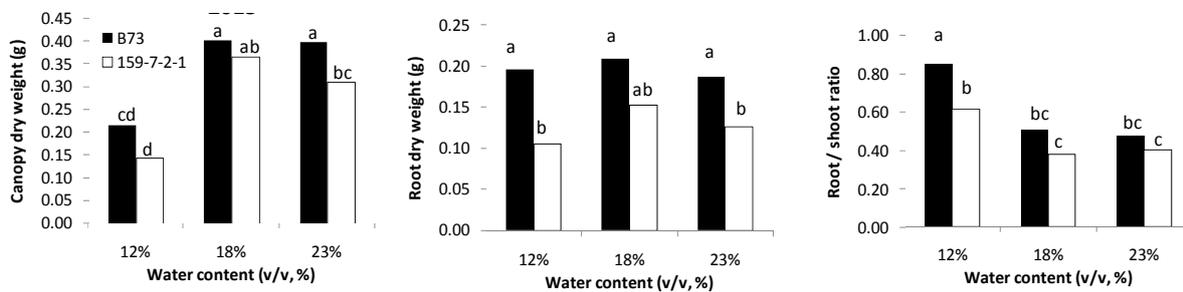


Figure 3: analysis of IL line 159-7-2-1 at seedling stage in rhizotron at three water regimes.

2. The main QTL for total number of brace root (NBR) was identified on chromosome 1 bin 1.02 ( $R^2$  24.4, LOD 6). In the same region we identified a QTL for TKW which comapped with the MQTL1 for yield and yield related traits (Martinez *et al.*, 2016). The Gaspé Flint allele was associated with a reduction of both root number and TKW. This region is introgressed in the IL 94-6-1-6 (ILqSR). This line was already known to be characterized by the absence of seminal roots while the parental line B73 is characterized by 3 seminal roots. Fine mapping of this QTL was also addressed in W4C project (see deliverable 4.3). The effect of root reduction was confirmed also when tested as

F1 hybrids. The QTL effect was detected even when in combination with line (Mo17, Lo106) characterized by relatively large root system in WS condition. Moreover, we identified a positive strong correlation between the reduction in root dry weight and yield in both 2014 and 2015 (0.94 and 0.89). The yield reduction was confirmed also in the water table experiment were in WW condition the F1 hybrids of the line 94-6-1-6 showed a higher yield compared to the F1 hybrids of the control B73. A drastic yield decrease was associated decreasing water table depth.

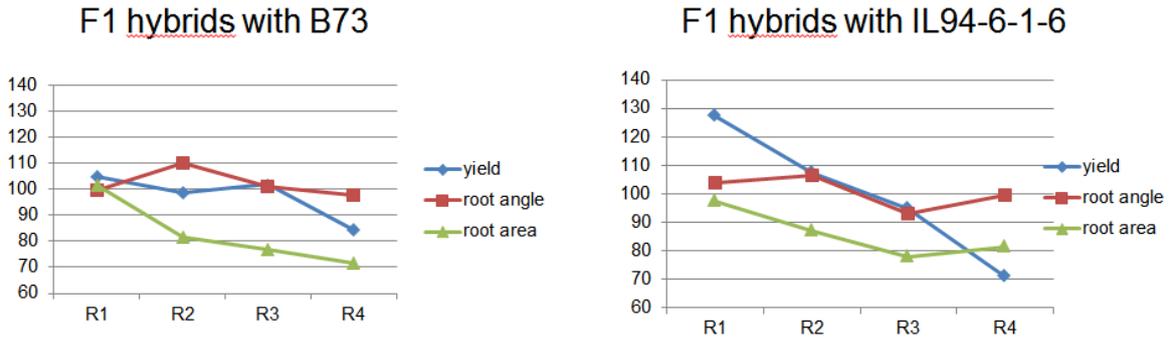


Figure 4. F1 hybrids of B73 and IL 94-6-1-6 tested in 2015 by UNIBO in water table field

The screening of IL 94-6-1-6 at seedling stage, showed that the IL in comparison with the control B73 activated several drought adaptation strategies, focused primarily on a thinner but longer root system.

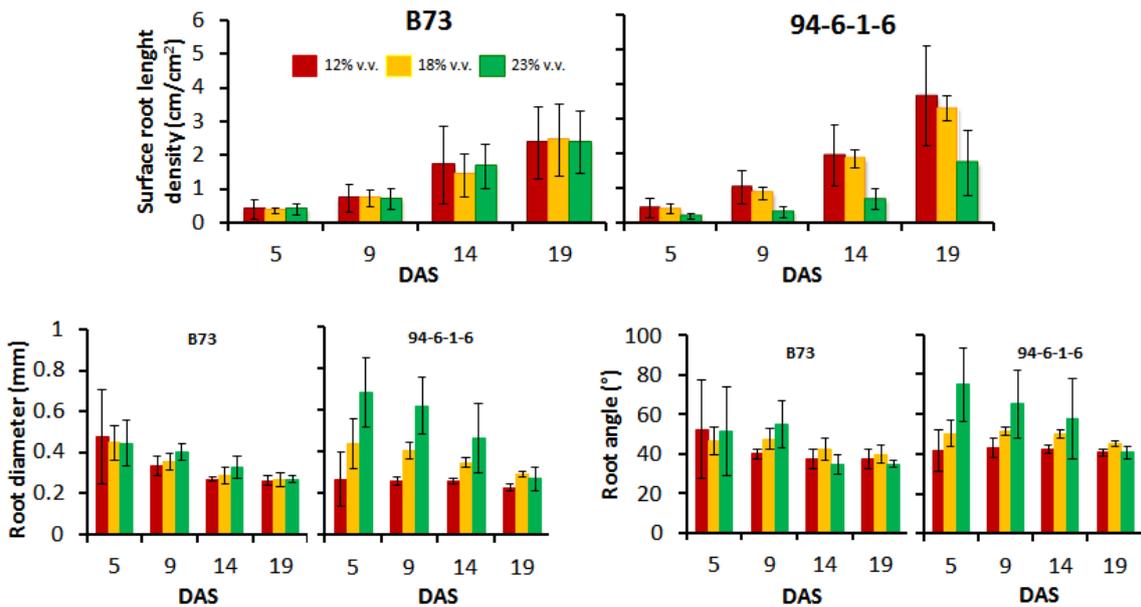


Figure 5. Results of characterization of IL 94-6-1-6 at seedling stage in zhitron box

3. Another interesting region is on chromosome 3 bin 3.03. In this region Gaspé Flint allele is responsible for an increase in grain yield, ear weight, ear length, ear diameter, cob diameter and kernel number per ear in WS condition. This region correspond to the MQTL 21 for grain yield and yield related traits (Martinez *et al.*, 2016). The two IL lines with only this region introgressed were also characterized by a higher root angle and width compared to B73 even if not significative different based on Dunnet test in 2013 experiment (Figure 6). Anyhow, they were selected for a further screening in 2014 and 2015 as IL line per se and as F1 hybrids. Based on this other 2 years experiment it was possible to identify a significative difference of this two lines compared to B73 for both grain yield related traits and root traits as described in table 2.



Figure 6. Example of root of IL 18-4-1 and 130-2-8-2 compared to control B73

<b>IL 18-4-1</b>											
IL QTL analisys (task 4.2a)	Gaspé effect			Dunnett 2014 (P 0.05)	Gaspé effect			Dunnett 2015 (P 0.05)	Gaspé effect		
	WW	WD	Mean		WW	WD	Mean		WW	WD	Mean
thousand kernel weight			+	yield			+	yield	+	+	+
ear weight		+		kernel n. per ear			+	kernel numeber per ear	+	+	+
ear lenght		+	+					ear per plant		+	
ear diameter		+	+					thousand kernel weight	+	+	+
cob diameter		+	+								
				root dry weight			+	root dry weight	+		
				root filling factor			+	root width		+	
								root angle	+	+	+

130-2-8-2													
IL QTL analysis (task 4.2a)	Gaspé effect			Dunnett 2014 (P 0.05)				Gaspé effect			Dunnett 2015 (P 0.05)		
	WW	WD	Mean		WW	WD	Mean		WW	WD	Mean		
yield	+		+	yield	+			yield			+		
kernel n. per ear		+	+	ear/plant			+	th. Kernel weight			+		
ear weight		+		kernel n./ear			+						
ear length		+	+										
th. Kernel weight			+										
cob diameter		+											
ear diameter		+	+										
				root angle	+								
				root width	+								

Table 2: screening of IL line per se: IL 18-4-1 and 130-2-8-2 at adult stage. “+” sign indicates a statistically significant difference (Dunnett test, P>0.05) of the IL line compared to B73 for the corresponding trait.

By the screening of the two IL lines as F1 hybrids at Horta it was possible to confirm the effect on root angle for IL 18-4-1 in WS conditions with a significant (Dunnett test, P>0.05) increase of the corresponding hybrid compared to B73 in WS condition while the effect on yield was usually negative. The behavior was confirmed also in the water table experiment (figure 7) where the IL showed an increasing of root angle and root area at the decreasing of water table depth associated with a yield reduction. Also for IL line 130-2-8-2, when tested as F1 hybrids at Horta, the effect on root was small and, above all in 2014, the effect on yield in the hybrid combination tested showed a significant lower yield both in WW and WD compared to the same tester crossed with the reference B73. Such a reduction of yield was not confirmed in water table experiment where the F1 hybrids of line 130-2-8-2 showed a behavior similar to the B73 F1 hybrids and with a higher yield also at lower water table depth (figure 7). This was probably due to the different soil characteristics of the two environment tested and/or the different type of stress of the two environment.

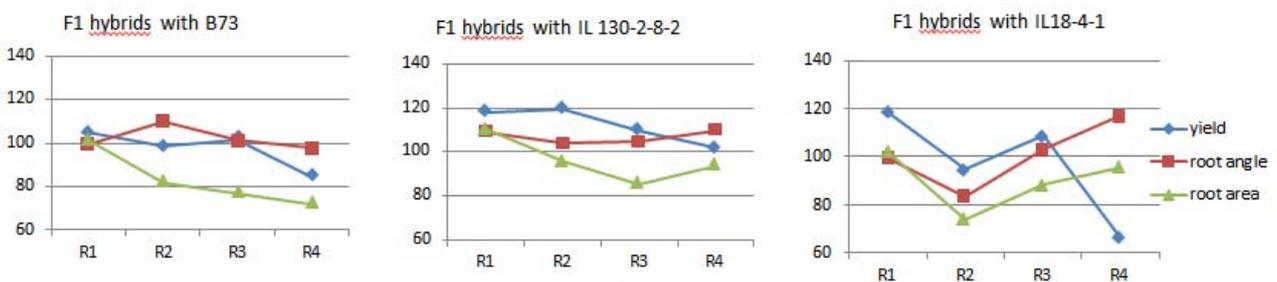


Figure 7. F1 hybrids of B73 and IL 130-2-8-2 and 18-4-1 tested in 2015 by UNIBO in water table field

4. Other two lines were selected for further investigation because of their plasticity in drought stress response. IL 45-15-1 showed a severe yield reduction (70%) in the 2013 experiment confirmed also in 2014 (35%) and 2015 (60%). This line has also a QTL for root density with the Gaspé allele associated with a decrease in root density in WS condition. Interestingly, by the screening of the F1 hybrids we found a good correlation between thousand kernel weight in 2014 and root area, root depth and density (0.54-0.69-0.74) and yield in 2015 with the same root traits (0.65-0.63-0.58).

5. The last interesting region is located on chromosome 8. This chromosome carries the highest number of QTLs for root characteristics. The Gaspé allele is associated with a constitutive decrease of number of brace root node and with a decrease of total number of brace root in water deficit condition. It also decrease root mass and root dry weight in WW conditions and depth at which maximal width is located in a constitutive way. This region is also involved in phenology as QTLs for plant height, node number and female flowering have been mapped in the same interval. As this region is involved in the constitutive control of root and not associated with yield or yield related QTLs and also all the IL lines carrying the root QTLs has also this second region introgressed involved in the control of plant phenology it has not been selected for further study.

#### 4. Discussion

The IL lines collection showed high plasticity in terms of root system architecture and yield response to drought. The high throughput screening method that coupled shovelomics and image analysis with REST software let us to screen more than 5,000 roots within the water4crops project.

Despite the highly complex nature of drought resistance and root traits, we identified the presence of root QTL clusters that coincide for both traits, implicating the potential genetic improvement for yield increase in drought condition in maize. Some of the QTL identified have a positive effect both *per se* and at hybrid level representing valuable targets for marker assisted selection in commercial breeding program. Currently, it is not possible to understand whether the relationships identified (eg. root size vs. yield) were due to pleiotropy or linkage but the use of an introgression library would enable to make available a large collection of nearly isogenic pair of lines for eventually fine mapping and positional cloning the genes.

## References

- Abbe E.C., Stein O.L. (1954) The growth of the shoot apex in maize: embryogeny. *American Journal of Botany* 41: 285–93.
- Abendroth L.J., Elmore R.W., Boyer M.J., and Marlay S.K. (2011) Corn growth and development. 459 PMR 1009. Iowa State University Extension, Ames, Iowa.
- Arsenault, J.-L., S. Pouleur, C. Messier, and R. Guay (1995) WinRHIZO™, a root-measuring system with a unique overlap correction method. *HortScience* 30: 906
- Bengough A.G. Gordon D.C., Al-Menaie, H. *et al.* (2004) Gel observation chamber for rapid screening of root traits in cereal seedlings *PLANT AND SOIL* 262 (1-2): 63-70
- Bucksch A., BurrIDGE J., York L.M., Das A., Nord., Weitz J.S., Lynch J.P. (2014) Image-based high-throughput field phenotyping of crop roots. *Plant Physiol.* 166 (2) 470-486
- Chun L., Mi G.H., Li, J.S. *et al.* (2005) Genetic analysis of maize root characteristics in response to low nitrogen stress *PLANT AND SOIL* 276 (1-2): 369-382
- Cobb J. N., DeClerck G., Greenberg A., *et al.* (2013) Next-generation phenotyping: requirements and strategies for enhancing our understanding of genotype-phenotype relationships and its relevance to crop improvement *THEORETICAL AND APPLIED GENETICS* 126 (4): 867-887
- Colombi T., Kirchgessner N., Le Marié C.A., York L.M., Lynch J.P., Hund A., (2015) Next generation shovelomics: set up a tent and REST *Plant and Soil*, 388 (1): 1-20
- de Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X. (2007) Root system architecture: opportunities and constraints for genetic improvement of crops *Trends Plant Sci.* 12(10):474-81.
- Galkovskyi T., Mileyko Y., Bucksch A., Moore B., Symonova O., Price C.A., Topp C.N., Iyer-Pascuzzi A.S., Zurek P.R., Fang S., *et al.* (2012) GiA Roots: software for the high throughput analysis of plant root system architecture *BMC Plant Biol.* , 12: 116
- Giuliani S., Sanguineti M.C., Tuberosa R. *et al.* (2005) Root-ABA1, a major constitutive QTL, affects maize root architecture and leaf ABA concentration at different water regimes *JOURNAL OF EXPERIMENTAL BOTANY* 56 (422): 3061-3070
- Hammer G.L., Dong Z., McLean G., Doherty A., Messina C., Schussler J., Zinselmeier C., Paszkiewicz C., and Cooper M. (2009) Can Changes in Canopy and/or Root System Architecture Explain Historical Maize Yield Trends in the U.S. Corn Belt? *CROP SCIENCE* 49: 299-312

- Hargreaves C.E., Gregory P.J., Bengough A.G. (2009) Measuring root traits in barley (*Hordeum vulgare* ssp *vulgare* and ssp *spontaneum*) seedlings using gel chambers, soil sacs and X-ray microtomography *PLANT AND SOIL* 316 (1-2): 285-297
- Hermans C., Hammond J.P., White P.J. *et al.* (2006) How do plants respond to nutrient shortage by biomass allocation? *TRENDS IN PLANT SCIENCE* 11 (12): 610-617
- Hetz W., Hochholdinger F., Schwall M., Feix G. (1996) Isolation and characterization of *rtcs*, a maize mutant deficient in the formation of nodal roots. *Plant J.*10:845–857.
- Hochholdinger F., Woll K., Sauer M., Dembinsky D. (2004) Genetic dissection of root formation in maize (*Zea mays* L.) reveals root-type specific developmental programmes. *Ann Bot (Lond)* 93: 359–368
- Hochholdinger F., Tuberosa R., (2009) Genetic and genomic dissection of maize root development and architecture *CURRENT OPINION IN PLANT BIO*
- Hund A., Reimer R., Messmer R. (2011) A consensus map of QTLs controlling the root length of maize *PLANT AND SOIL* 344 (1-2): 143-158
- James, B.R., Bartlett, R.J., Amadon, J.F. (1985) A root observation and sampling chamber (rhizotron) for pot studies. *Plant Soil* 85:291–293.
- Iyer-Pascuzzi, A.S., Symonova, O., Mileyko Y. *et al.* (2010) Imaging and Analysis Platform for Automatic Phenotyping and Trait Ranking of Plant Root Systems *PLANT PHYSIOLOGY* 152 (3): 1148-1157
- LEBRETON C., LAZICJANCIC V., STEED A. *et al.* (1995) Identification of QTL for drought response in maize and their use in testing causal relationship between traits *JOURNAL OF EXPERIMENTAL BOTANY* 46 (288): 853-865
- Le Bot, J. *et al.* (2010) DART: a software to analyse root system architecture and development from captured images *Plant and Soil*, 326(1-2), pp.261–273.
- Lobet G., L. Pagès, X. Draye, (2011) A novel image-analysis toolbox enabling quantitative analysis of root system architecture. *Plant Physiol.* 157(1), 29
- Lynch J P. (2013) Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems *ANNALS OF BOTANY* 112 (2): 347-357
- Lynch J.P. (2015) Root phenes that reduce the metabolic costs of soil exploration: opportunities for 21st century agriculture *PLANT CELL AND ENVIRONMENT* 38 (9):1775- 1784

- Mairhofer S., S. Zappala, SR. Tracy, C. Sturrock, M. Bennett, SJ. Mooney, T. Pridmore, (2012) RooTrak: automated recovery of three-dimensional plant root architecture in soil from x-ray microcomputed tomography images using visual tracking. *Plant Physiol.* , 158(2), 561
- Martinez A.K., Soriano J.M. Tuberosa, Roberto; *et al.* (2016) Yield QTLome distribution correlates with gene density in maize *PLANT SCIENCE* 242:300-309
- Meister, R; Rajani M. S.; Ruzicka, D; *et al.* (2014) Challenges of modifying root traits in crops for agriculture *TRENDS IN PLANT SCIENCE* 19 (12): 779-788
- Meng L., Li H., Zhang L., Wang J. (2015) QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental population *The Crop Journal* 3(3) 269–283
- Pestsova E., Lichtblau D., Wever C. *et al.* (2016) QTL mapping of seedling root traits associated with nitrogen and water use efficiency in maize *EUPHYTICA* 209 (3) 585-602
- Pound M.P., AP. French, J. Atkinson, DM. Wells, MJ. Bennett, TP. Pridmore, (2013) RootNav: Navigating images of complex root architectures. *Plant Physiol* 162 (4) 1802-1814
- Richards R.A. (2008) Genetic opportunities to improve cereal root systems for dryland agriculture *PLANT PRODUCTION SCIENCE* 11(1):12-16
- Ristova D., U. Rosas, G. Krouk, S. Ruffel, KD. Birnbaum, GM. Coruzzi, (2013) RootScape: a landmark-based system for rapid screening of root architecture in *Arabidopsis*. *Plant Physiol.*, 161(3), 1086
- Rogers E.D.; Benfey, P.N. (2015) Regulation of plant root system architecture: implications for crop advancement *CURRENT OPINION IN BIOTECHNOLOGY* 32: 93-98
- Salvi S., Tuberosa R. (2005) To clone or not to clone plant QTLs: present and future challenges *Trends Plant Sci.* 10(6):297-304.
- Salvi S., Corneti S., Bellotti M., Carraro N., Sanguineti M.C., Castelletti S., Tuberosa R. (2011) Genetic dissection of maize phenology using an intraspecific introgression library *BMC Plant Biology* 11:4
- Salvi S., Giuliani S., Ricciolini C., *et al.* (2016) Two major quantitative trait loci controlling the number of seminal roots in maize co-map with the root developmental genes *rtcs* and *rum1* *JOURNAL OF EXPERIMENTAL BOTANY* 67 (4) 1149-1159
- Sanguineti M. C., Li S., Maccaferri M., *et al.* (2007) Genetic dissection of seminal root architecture in elite durum wheat germplasm *ANNALS OF APPLIED BIOLOGY* 151 (3): 291-305

- Schnable P., *et al.* (2009) The B73 Maize Genome: Complexity, Diversity, and Dynamics Science 1112:326
- Sharp R.E., Poroyko V., Hejlek L.G. *et al.* (2004) Root growth maintenance during water deficits: physiology to functional genomics JOURNAL OF EXPERIMENTAL BOTANY 55 (407): 2343-2351
- Trachsel S., Kaeppler S.M., Brown K.M., Lynch J.P. (2011) Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. Plant Soil 341: 75–87
- Trachsel S., Kaeppler S. M., Brown K.M.; *et al.* (2013) Maize root growth angles become steeper under low N conditions FIELD CROPS RESEARCH 140: 18-31
- Tuberosa R.; Giuliani S.; Parry M. A. J.; *et al.* (2007) Improving water use efficiency in Mediterranean agriculture: what limits the adoption of new technologies? ANNALS OF APPLIED BIOLOGY 150 (2): 157-162
- Tuberosa R. (2012) Phenotyping for drought tolerance of crops in the genomics era FRONTIERS IN PHYSIOLOGY 3 Article Number: UNSP 347
- Uga Y., Sugimoto K., Ogawa S. *et al.* (2013) Control of root system architecture by DEEPER ROOTING
- Varney G.T., Canny M.J. (1993) Rates of water-uptake into the mature root-system of maize plants NEW PHYTOLOGIST 123 (4): 775-786
- Vigouroux Y., Glaubitz J.C., Matsuoka Y., Goodman M.M., Sánchez J.G., Doebley G. (2008) Population structure and genetic diversity of new world maize races assessed by DNA microsatellites American Journal of Botany 95(10): 1240–1253
- Wang X.L., McCully M.E., Canny M.J (1994) The branch roots of *zea*.4 the maturation and openness of xylem conduits in first-order branches of soil-grown roots NEW PHYTOLOGIST 126 (1): 21-29
- Wang J., Wan X., Crossa J., Crouch J., Weng J., Zhai H., Wan J. (2006) QTL mapping of grain length in rice (*Oryza sativa* L.) using chromosome segment substitution lines. Genet Res 88:93–104
- Zhan A., Schneider H., Lynch J.P. (2015) Reduced Lateral Root Branching Density Improves Drought Tolerance in Maize 168 (4): 1603-U885