INTEGRATING BIOTREATED WASTEWATER REUSE AND VALORIZATION WITH ENHANCED WATER USE EFFICIENCY TO SUPPORT THE GREEN ECONOMY IN EU AND INDIA

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Collection of segmental isolines useful for cloning a QTL for seminal roots in maize

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Paragraphs

1. INTRODUCTION

Roots are the principal water-absorbing organs of a plant and they have a key role in the anchorage of the plant and in nutrients uptake.

Advances in root genetics and physiology provide the opportunity for breeding programs to design and introduce new root architectural and physiological ideotypes optimized for improved crop adaptation to a range of environmental stresses and increased sustainability of cropping systems (Hammer *et al.* 2009; Tardieu and Tuberosa, 2010, White *et al.* 2013; Lynch 2013).

Previous modeling studies indicated that changes in root architecture and water acquisition capacity directly affect biomass accumulation and yield increases (Hammer *et al.* 2009).

The root system of maize can be divided into an embryonic root system (Abbe and Stein, 1954) consisting of a single primary root and a variable number of seminal roots, and a post-embryonic root system which is made up by shoot-borne roots. Shoot-borne roots formed at consecutive underground nodes are called crown roots, while the respective roots formed at consecutive above-ground nodes of the shoot are called brace roots (Hochholdinger *et al.*, 2004). Primary root is the first to emerge at germination at the basal end of the embryo. Seminal roots are also laid down endogenously in the embryo between 22 and 40 days after pollination and emerge from the scutellar node at germination contemporarily or soon after the primary root (Sass, 1977; Hochholdinger, 2009). It is not completely clear whether primordia for all seminal roots are differentiated before germination (Feldman, 1994). Intriguingly, seminal roots appears to be a unique feature of maize among Poaceae, and do not form in the closely related sorghum (Singh *et al.* 2010). Depending on genotypes and/or growing conditions, primary and seminal roots may persist throughout the life cycle of the plants or dye out after the formation of the shoot-born system (Hochholdinger, 2009).

Two maize mutant for seminal root have been described: *rtcs1* and *rum1*. The first one codes for a LOB-domain while the second for an Aux/IAA protein (Hetz *et al.* 1996; Woll *et al.* 2005; Taramino *et al.* 2007; von Behrens *et al.* 2011). They are both characterized by absence of seminal root; *rum1* also affects the initiation of lateral roots in the primary root, while *rtcs1* abolishes the initiation of both crown and brace roots.

Whether variation in number and architecture of seminal roots does affect crop performance in terms of final yield is still unclear. It has been shown that nitrogen uptake efficiency had significant phenotypic correlations with root system architecture (RSA), particularly with seminal roots (Li *et*

al., 2015) and a role for the variation of seminal root architecture in determining adaptation to reduced soil P availability has been proposed (Baker *et al.* 1970; Lynch 2011).

QTLs for seminal root number have been identified in a collection of introgression lines developed from the cross between the maize reference line B73 used as background line and the landrace Gaspé Flint as donor (Salvi *et al.* 2016). In order to proceed with the fine mapping of the QTL *qSRN-1.02*, identified on chromosome 1, bin 1.03 near marker *umc1685* (25.8 cM), a collection of recombinant lines was developed and phenotyped for seminal root number.

2. MATERIALS AND METHOD

A cross between the two inbred lines NILqSRN and B73 was produced in summer/fall 2012. The B73 parental line usually develops three seminal roots while the other parental line, NILqSR, is characterized by absence or one seminal root (Figure 1).

Figure 1. Root morphology of the parental lines at the seedling stage (one week after seed imbibition) as observed using the 'paper-roll' protocol.

The level of isogenicity of two nearly isogenic lines (NILqSR, and B73) was studied using a 50,000 SNP array. DNA of the two lines (ILqSR and B73) was prepared using standard methods from leaf tissue, quantified and quality-checked. Samples were analyzed in duplicate. Row SNP data produced by the Infinium analysis were treated at UNIBO with the ILLUMINA software GenomeStudio™.

F1 plants NILqSR x B73 were grown and selfed during spring-summer 2013 at UNIBO facility. From these selfing crosses, approximately 6,000 F_2 seeds were produced. In autumn/winter 2013 a total of 720 F_2 plants were grown using the paper roll technique and genotyped with two molecular markers. The paper roll method is a simple and useful method to study the root system at early stage (Hetz *et al.*, 1996): seeds were sown between two sheets of light wet filter paper that was rolled up and put vertically into a 3L beaker filled with 300 ml of water. After two weeks the paper-rolls were unrolled on a table and the root system was visualized in order to count the number of seminal root for each of the F_2 plant. Paper roll with the two inbred lines NILqSR and B73 were grown contemporary as control. During the two weeks, plants were also genotyped with one SSR markers. Recombinant plants have been transplanted from paper roll to pots filled with peat in order to self them and genotyped with 4 additional SSR markers.

In summer 2014, in order to identify additional recombinants, a total of 4,830 F_2 plants were grown in the field. Recombinants were identified and seeds were sent to a winter nursery service in Chile. During winter 2014-2015 homozygous recombinants have been produced: a leaf sample have been received from the winter nursery for molecular marker screening in order to identify homozygous recombinant plants to be selfed. We identified 189 homozygous recombinants plants (64 independent recombination events). Seeds of the homozygous recombinants were received in June 2015 and sown at theUNIBO experimental station in Cadriano (BO) for seed increase. Paper roll screening of homozygous recombinants was carried out in winter nursery during the 2015-16 winter.

3. RESULTS

Investigation of the level of isogenicity of two nearly isogenic lines (NILqSR, and B73) indicated that the two lines differed for a introgressed (substituted) region of 12 cM, corresponding to approx. 1.44 Mb in physical size (based on the IBM2 2008 Neighbors 1,www.maizegdb.org). The rate of physical vs genetic distance appeared favorable as it was 1 cM = 0.12 Mb (mean value in maize is approx. 1 cM = 1 Mb). Other introgressions were identified on chromosome 4 (6 Mb), on chromosome 6 (10 Mb) and on chromosome 8 (two introgressions of 65 Mb) that, based on previous studies, are regions not involved in seminal root morphology.

The preliminary screening of the 720 F_2 showed that the Gaspé allele was present only in plants characterized by less than two seminal roots while the B73 allele was mainly associated with two or three seminal roots confirmed the importance of the region in the control of seminal root number.

The collection of homozygous recombinants lines was assembled in 2015 and screened for seminal root number using the paper roll technique as reported in figure 2. QTL analysis was performed (Fig. 3) and, based on the results, the region involved in the control of seminal root number was located between umc1685 and umc1222. In that region is located the well known root developmental gene *rtcs* (Taramino *et al.*, 2007). The *rtcs* (*rootless concerning crown and seminal roots*) mutant is impaired in the initiation of the embryonic seminal roots and the post-embryonic shoot-borne root system. It encodes for a 25.5-kDa protein with a single LOB domain. So far, gene products containing LOB domains have been implicated in various aspects of plant development including leaf venation in Arabidopsis (Iwakawa*et al.*, 2002), patterning of axillary meristems in maize (Bortiri *et al.*, 2006a), and adventitious root formation in rice (Inukai *et al.*, 2005; Liu *et al.*, 2005). Another LOB domain gene (*rtcn*) is located in the same region. However, based on expression patterns results reported in Taramino *et al.* (2007), this gene is not likely to be involved in root development..

We identified one recombinant event between *rtcs* and *rtcn* gene. Phenotyping of this recombinant line seems to indicate that also in this material the *rtcs* gene is responsible for the lower number of seminal root.

The phenotype observed for Gaspé Flint and NILqSR is quite different from maize root mutant *rtcs*, since NILqSR has a reduced number of seminal roots but a normal adult root apparatus, while *rtcs* mutant shows a strong reduction of both seminal and adult roots thus indicating a different allele of Gaspé Flint at the *rtcs* gene region.

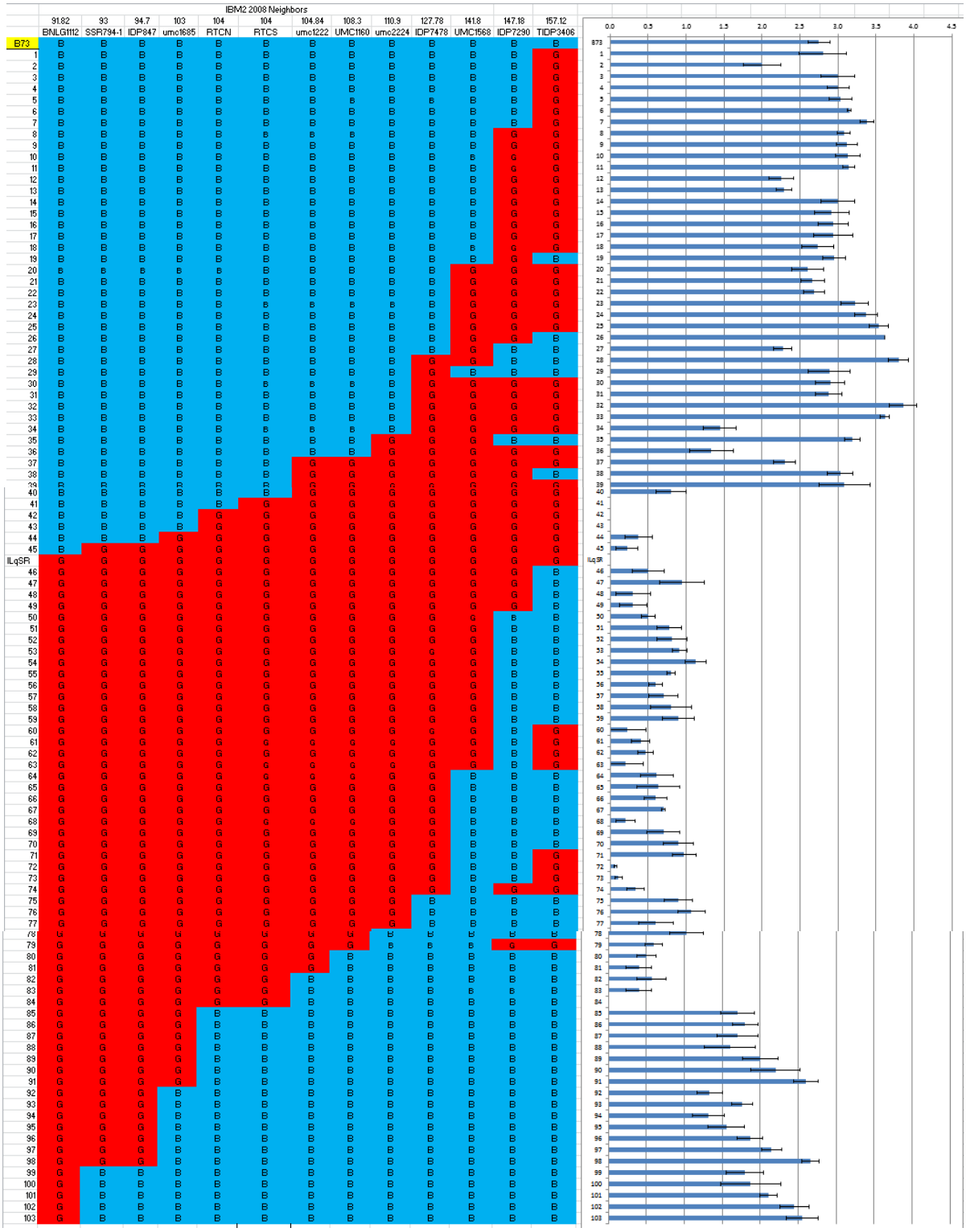

Figure 2: genotype of the homozygous 103 families of recombinant plant identified and seminal root number scored for each family with paper roll technique.

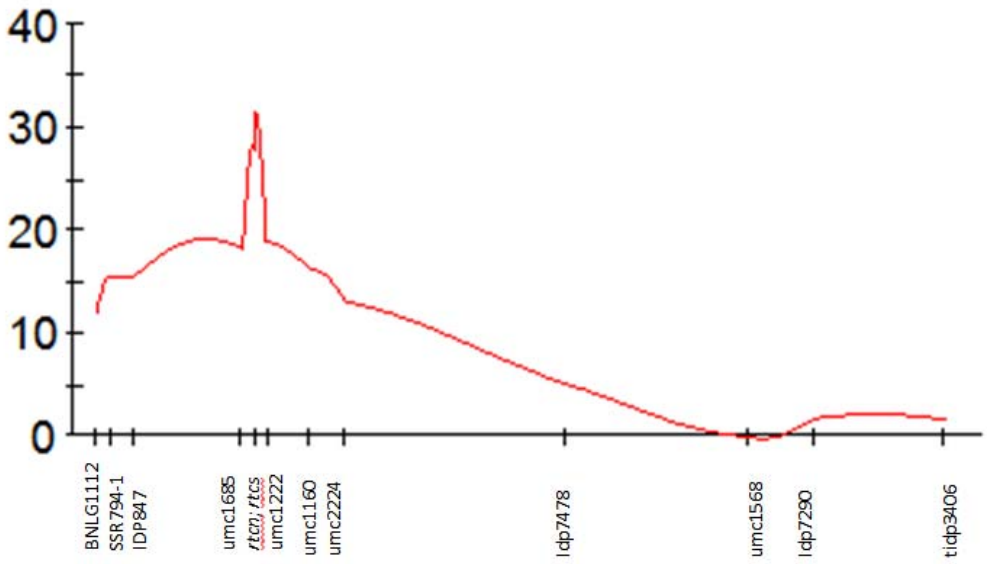

Fig 3. QTL analysis result for seminal root number for the 103 homozygous recombinants families.

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