

Forward genetic approaches for identifying genes controlling root architecture in maize

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In order to stand firmly and efficiently uptake water and nutrients, plants need complex root stock architectures. The root system in maize is characterized by different root types, each of which contributes to the development and establishment of plants in a distinctive manner. During very early development, the embryonic primary and seminal roots are predominant, together with their post-embryonic lateral roots. These embryonic roots become dispensable later on in the development, when a broad post-embryonic shoot-borne root system forms the majority of the mature root stock. (Hochholdinger et al 2004b). Shoot-borne roots are formed at each consecutive node of the shoot and are called crown roots when they grow from nodes that reside under the soil line, while they are called brace roots when they grow from above ground nodes. Among the different root types, crown and brace roots provide standability and support to plants, while lateral roots provide the major surface water and nutrient uptake.

In our group we are interested in the genetic mechanism underlying root formation and we take different forward genetics approaches to identify genes involved in the molecular pathways that drive root development.

One approach involves the use of Arabidopsis as a model system. We screen T-DNA induced mutant populations for the presence of individuals with increased root mass when grown vertically on plates with several different media compositions including varying nitrogen sources and concentration. The genes responsible for the phenotype are then identified by sequencing the region of the genome flanking the T-DNA insertion. Subsequently, those genes are transformed back in Arabidopsis to validate their functionality in the control of root architecture. Furthermore, their transformation into maize allow us to recapitulate the phenotype in crop plants.

Another approach we are taking involves the use of known maize root mutants. Several monogenic root mutants have been selected for our map-based cloning activities. Two of them control crown and brace root formation. The monogenic recessive *rt1* (*rootless1*) mutant has a variable number of crown roots in the first two nodes, but misses all shoot-borne roots at the higher nodes (Jenkins, 1930). In contrast, the *rtcs* (*rootless concerning crown and seminal roots*) mutant was identified because it lacks the embryonically formed seminal roots and post-embryonically formed shoot-borne roots (Hetz et al, 1996).

Other two mutants we are studying control lateral root formation at the seedling level, in the primary root. Among them, The mutant *lrt1* (*lateral rootless 1*) is impaired in early postembryonic lateral root initiation at the primary and seminal roots (Hochholdinger et al, 1998) and the more recently characterized *rum1* (*rootless with undetectable meristems 1*) mutant is defective in both the initiation of lateral roots from the primary root, and the initiation of seminal roots (Woll et al, 2005). Furthermore, *rum1* shows reduced auxin transport in the primary root,

reduced primary root elongation, delayed gravitropic responses and no lateral root induction by exogenous auxin application.

Here, we present data of the strategy we developed for the positional cloning and characterization of three of the maize genes, two controlling crown root formation: the *RTCS* gene (Taramino et al, 2007), which encodes for a LOB domain protein, and the *RTI* gene, plus the *RUM1* gene, which encodes for a member of the AUX/IAA gene family involved in auxin signalling.

Until recently, positional cloning in maize was considered tedious and not easily achievable. The released of the first draft of the maize genome sequence (announced less than a year ago), the availability of highly polymorphic physically mapped markers, and the exploitation of the high level of micro colinearity among the different cereal genomes, has rendered the task of positional cloning maize genes almost trivial.

Once cloned, validation and characterization of the gene could follow the search for additional alleles through reverse genetic techniques, the use of transgenic techniques or the analysis of the association between different haplotypes at the locus and phenotypes in structured populations.

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