Use of GFP to determine the influence of competition on root dynamics

Jhonathan E. Ephrath[1*] Boris Rewald[1,2], Volkan Oral[1], Ben Asher Jiftah[1], Shimon Rachmilevitch[1]
[1]French Associates Institute for Agriculture and Biotechnology of Drylands
Ben-Gurion University of the Negev, Israel
[2]Forest Ecology, Dept. of Forest and Soil Sciences,
University of Natural Resources and Life Sciences (BOKU), Austria
*yoni@bgu.ac.il

Introduction

Studying root dynamics and belowground plant-plant interactions is essential for understanding the composition of plant communities, the impact of global change, and terrestrial biogeochemistry.
While most soil samples and minirhizotron pictures hold roots of more than one plant individual (Rewald et al. 2012), information about root affiliation to plant individuals is decisive to determine competitive interactions.

Methods

Transgenic *Zea mays* L. plants, expressing green fluorescent protein (GFP), and non-transformed plants of the same variety were grown in a temperature controlled green-house (Fig. 1a).

The GFP-transformed *Zea mays* plants ("Target plants") were replanted three days after germination into the middle of a soil compartment (w 33 × l 80 × h 70 cm). The target plants were either grown in isolation, or were accompanied by one or two non-GFP-transformed plants in the soil ("Competitors", n = 8; Fig. 1b). Each soil compartment (1-3 plants) received the same amount of water and minimal nutrition by automated drip fertigation. In case of no ("Isolation") and one belowground competitor, the plant density above ground was hold constant by placing potted *Zea* plants on top of the soil. A randomized design and buffer plants at the end of each row were used to minimize gradient and edge effects.

Using a special minirhizotron (MR) system able to detect GFP fluorescence and a VIS minirhizotron camera, root systems were photograohed to determine root growth and longevity (Fig. 2). Two MR tubes were installed at equidistance to the GFP plant and potential competitors (Fig. 1b). The pictures will be analyzed, separated by local competitive situation, using WinRhizo Tron MF (Regent, Canada).

Discussion

The identification of root taxa or plant individuals by root traits would allow conducting advanced studies on intra- and interspecific competition in inter-cropping systems and monocultures (Rewald et al. 2012; Rewald and Ephrath 2013). One possibility to do so is the stable expression of green fluorescent protein in root tissues (Faget et al. 2009); the *Zea* seeds used in this study (kindly provided by M. Faget and colleagues) showed a stable expression of GFP. Thus GFP expressing plants can be used in general to determine spatial and temporal rooting pattern in agricultural mixtures. However, thick fibrous roots of GFP expressing *Zea mays* plants showed a strong green fluorescence and could be clearly distinguished from non-GPF-transformed individuals (Fig. 2) while thin maize roots were rather difficult to distinguish. Future research is necessary to determine if GFP expression is less strong in thin *Zea mays* roots or if the detection sensitivity of the digital MR camera system has to be adjusted.

Key Question

Can GPF expression be utilized to identify roots of plant individuals *in situ* and thus to determine belowground competition in monocultures and inter-cropping systems?

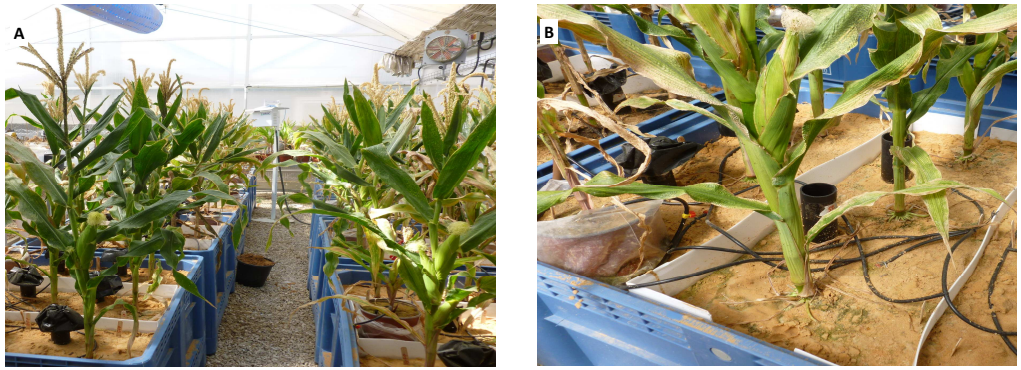

Figure 1. Experimental set-up. GFP-transformed *Zea maize* has been planted in isolation or with one or two non-transformed competitors; aboveground density was hold constant. MR tubes were inserted to determine the root growth of the central plant under intraspecific competition. See method section for details.

← **Figure 2.** Picture of roots of both GFP and non-transformed *Zea mays* plants. The fibrous roots of the GFP-transformed plants show green fluorescence.

Results

The thick fibrous roots of GFP expressing and non-GFP-transformed *Zea mays* plants can be distinguished by the strong, green fluorescence as compared with minor auto-fluorescence in roots non-GPF-transformed individuals (Fig. 2). However, green fluorescence is less pronounced in thin roots.

Further reading

Faget M, Herrera JM, Stamp P, Aulinger-Leipner I, Frossard E, Liedgens M. 2009. The use of green fluorescent protein as a tool to identify roots in mixed plant stands. *Funct. Plant Biol.,* 36:930-937

Rewald B, Meinen C, Trockenbrodt M, Ephrath JE, Rachmilevitch S. 2012. Root taxa identification in plant mixtures – Current techniques and future challenges. *Plant Soil*, doi:10.1007/s11104-012-1164-0

Rewald B, Ephrath JE. 2013. Minirhizotron techniques. In: Eshel A, Beeckman T. (Eds.) *Plant roots: The hidden half*. 4th edition. CRC Press, New York, USA.