Transnational Access Report

1. General Information

Project Acronym (ID):	RootREG
Project Title	Regulation of root growth and development by cell cycle – comparative study on mono- and dicot plants
Installation used	UNOTT MicroCT
Name of Group Leader	Michal Slota
Name of organization	University of Silesia

2. Project summary (max. 250 words)

The aim of the project was to elucidate the role of the activity of selected cell cycle regulatory elements during the development of the root system in mono- and dicotyledonous plants. The proposed research strategy involved the investigation on root growth dynamics of mutant lines induced mutations in genes acting at conserved regulatory mechanisms that control both the cell cycle progression and the development of the root system. The candidate genes which were chosen for the analysis comprised of genes which activity is specifically associated with inhibition of independent components of the cell cycle. Cyclin dependent kinase inhibitor *KRP2* (*KIP-related protein 2*) is associated with D-type cyclin regulated inhibition of the G1 to S phase transition of cell cycle. The loss-of-function *krp2* Arabidopsis mutant is characterized by increased lateral root density (Sanz et al. 2011). *MYB59* (*Myb-related protein 59*) transcription factor functions as an inhibitor of the B-type cyclins activity which jointly with the *FZR2* (*FIZZY-related 2*) gene are associated with G2/M phase transition checkpoint. Transgenic Arabidopsis overexpressing *AtMYB59* exhibited shorter roots, whereas *myb59* knockout plants possessed longer roots in comparison to control, indicating that *AtMYB59* may in physiological conditions inhibit root growth (Mu et al. 2009). *CKS1* (*CDK-associating subunit 1*) is a cyclin dependent protein kinase catalytic subunit involved both in G1-to-S and G2-to-M transition. The precise characterization of a root phenotype will possibly contribute to the completion of knowledge on the regulation and cell cycle genes function in the cereal root morphogenesis.

3. Main achievements (max. 250 words)

The conducted project allowed for the precise characterization of the chemically-induced mutant line of a *FZR2* (*FIZZY-related 2*) gene. That gene is associated with G2/M phase checkpoint, which is crucial during the transition process from extensive mitotic divisions in root meristem to the further elongation and the differentiation of the cells. The root phenotype of the mutant was depicted in a non-destructive manner during the growth of soil-grown plants. The volume of the root system of a 7-day-old seedling of a *fzr2.c* mutant was approximately 120 mm^3 with a corresponding convex hull volume of 4500 mm^3. The elongation rate of the longest root was approximately 14,2 mm/ day, which is slightly lower in comparison to the value of 15,2 mm/day which was measured earlier for this mutant grown in hydroponics culture. The obtained data complies with previously measured 2D characteristics of a root system of *fzr2.c* mutant using WinRHIZO software. The total root system volume of a 7-day-old seedling was 94,8 mm^3 for hydroponic- grown plants analysed using WinRHIZO system, in comparison to 122 mm^3 for soil-grown plants analysed using MicroCT.
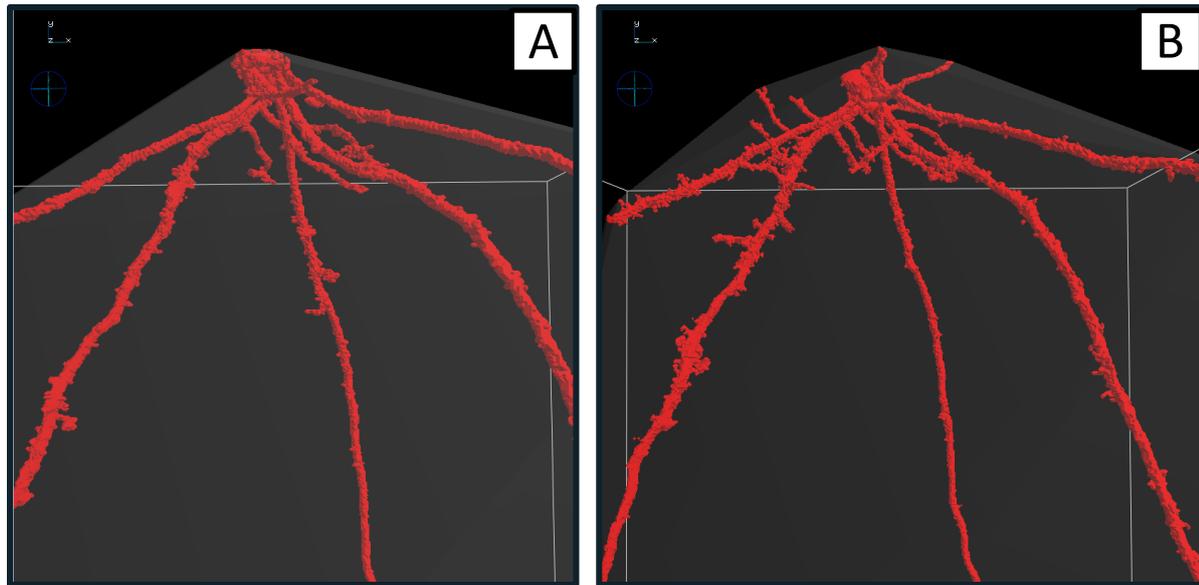
Fig. 1 Segmented root images of 7-day-old seedling of a *fzr2.c*. Image segmentation was carried using a RooTrak (A) and VGStudioMAX (B) software.

The comparison of two methods of root image segmentation was accomplished. It was shown that for the early stages (day 3 and 5) of the growth of barley seedling the root system parameters computed using a RooTrak and VGStudioMAX software packages are highly comparable. The differences are considerably higher if the 7-day-old seedlings are considered. Root system parameters computed after the segmentation of CT images using a VGStudioMAX were increased in comparison to RooTrak-segmented images. The changes can be attributed to the omission a certain portion of lateral roots whih grow shoot-ward or parallel to the soil surface. On the other hand RooTrak software enables a definitely quicker segmentation of root systems of plants grown in soil columns requiring a minor user interaction.

Tab. 1 The comparison of the parameters of root system of 3, 5 and 7-day old seedling of fzr2.c mutant computed using VGStudioMAX and RooTrak software.

FZR2_R2	Day 3		Day 5		Day 7	
Software package	**VGStudioMAX**	**RooTrak**	**VGStudioMAX**	**RooTrak**	**VGStudioMAX**	**RooTrak**
Volume [mm³]	13,359	12,832	47,871	50,332	122,01	107,31
Area [mm²]	137,402	141,707	454,74	496,65	1348,67	1258,94
Convex Hull Volume [mm³]	809,915	826,6	14448,5	15420,6	55759,4	48825,28
Depth [mm]	22,62	21,9	58,74	60	79,38	80,33
Width [mm]	26,4882	26,4392	50,093	48,694	50,18	50,58