

# Semi-automated image analysis and genome-wide association studies of root architecture and early root development in faba bean and white clover

Report on methods and experimental design

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## Introduction

Agriculture and food industry of Northern European countries, including Denmark are greatly depending on import of crop products providing protein resources – mainly soybean and processed soybean products. To diminish dependence on soybean import, there is an increasing interest in the Nordic countries for expanding local production of protein crops, like faba bean and clover. However, significant expansion of the production area of protein crops is challenged by the sub-optimal climatic conditions in these regions, especially by the increasing probability of year-to-year fluctuation of extreme weather conditions (cold winters, dry summers) due to global climate changes. To overcome these challenges, development of new climate-resilient varieties suitable for growing under Northern-European conditions are needed. Root architecture and early root development, as well as adequate phenotyping technologies for these traits are crucial factors of breeding such varieties. To facilitate effective screening for early root development, we conducted greenhouse rhizobox experiments using 180 faba bean and 180 white clover genotypes, including standard varieties and breeding lines.

## Experimental design

Plants of faba bean and white clover were grown in custom-made plastic rhizoboxes (Internal dimension: 36x18x2.5 cm). These boxes were filled with 1.8 L substrate (a mix of turf, local topsoil and sand) and supplemented with 300 ml water. In each box two seeds were sown (faba bean) or two young lateral shoot segments were planted (white clover). The boxes were placed in the greenhouse on racks that ensured the boxes to be inclined at 60° to facilitate root growth along the transparent side. During growing, the transparent plexiglas front side of the boxes was covered with a black foil to avoid light penetration. For both plant species, experiments were conducted in three subsequent batches in randomized blocks throughout a 12-month period in the same greenhouse section equipped with an automatic shading system during summer and additional lighting and heating (16/8h day/night at 21/18°C) during winter. Depending on the season, plants were grown for 18 to 25 days.



*Foto 1. Rhizoboxes with Vicia faba seedlings in the greenhouse.*

Root images were taken by a horizontal scanner (Epson Perfection V700) with a modified lid. Image analysis was conducted by the Rootpainter software<sup>[1]</sup>. The analysis procedure involved manual annotation of clear foreground and background regions and applying corrective annotations to refine the segmentation model. The final model was used to automatically segment the entire root dataset. In addition, segmented root images were exported for further quantification by the RhizoVision<sup>[2]</sup> software.

In case of faba bean, Single primer enrichment technology (SPET)<sup>[3]</sup> was applied to identify genetic variants on *Vicia faba* pseudo-chromosomes of the Hedin1 assembly of the Faba Bean Genome Consortium<sup>[4]</sup>. Quality filtering (Mapping Quality >20, Minor Allele Frequency >0.05, Read Depth >3) and subsequent imputing resulted in 323,007 biallelic SNPs. Bayesian Variable Selection (BVS) [5] was applied to detect root trait QTLs using a mixture prior on marker effects<sup>[6]</sup> using the software BayZ<sup>[6]</sup>.

Images were used for segmentation model training with RootPainter<sup>[1]</sup> to distinguish root biomass from soil (Figure 1). We extracted data for four root-related traits: total root length, root surface area, average root diameter, and number of branching points. The phenotypic distribution of the traits in three batches of *Vicia faba* rhizobox experiments can be seen in Figure 2. Their heritability of the traits range from 0.138 to 0.179 indicating that it is possible to select for and improve root-related traits.

Figure 1. *Vicia faba* rhizobox images. (a): Original scan; (b): Segmentation image after RootPainter training.

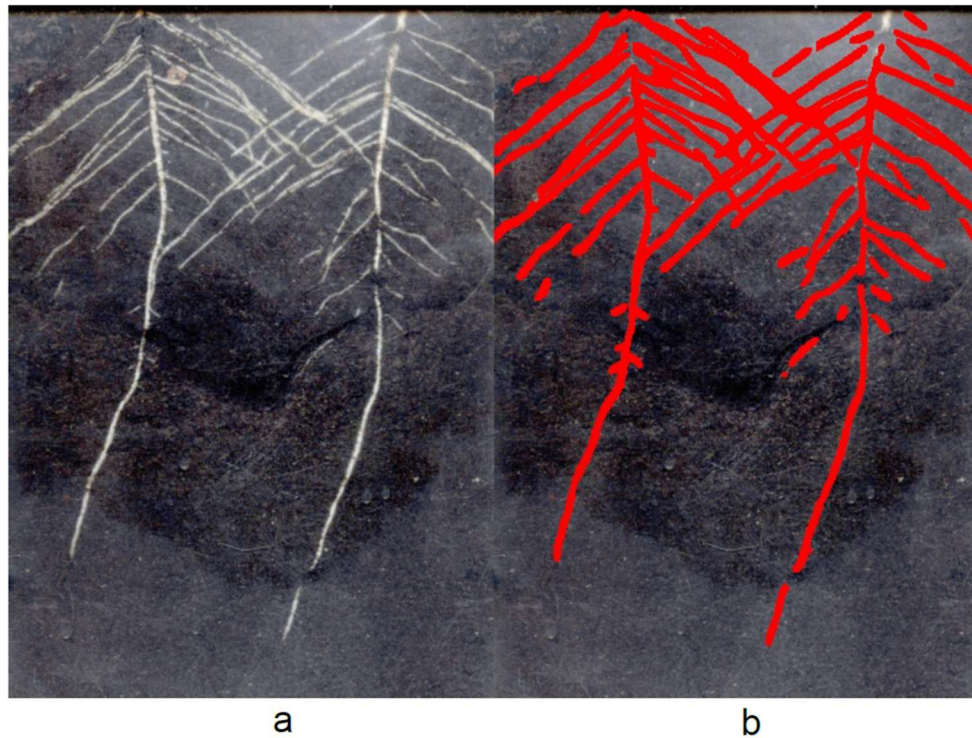
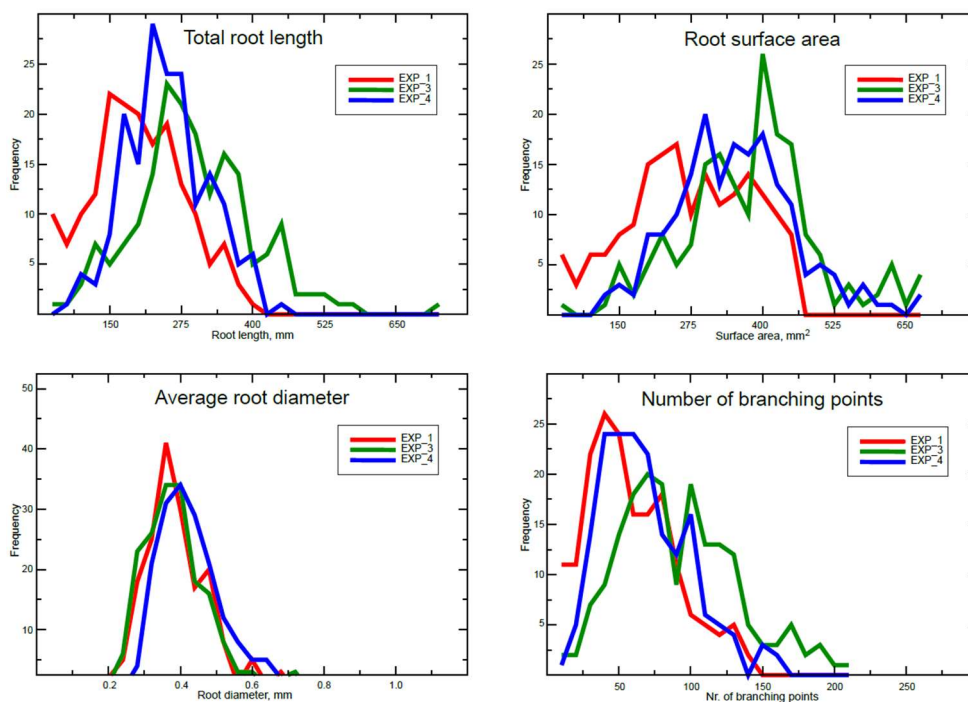


Figure 2. Distribution of quantitative root traits in three batches of *Vicia faba* rhizobox experiments.



*Tabel 1. Narrow-sense heritability values of quantitative root traits for Vicia faba, extracted from the rhizobox experiments.*

Trait	$h^2$
Total root length, mm	0.179
Total root volume, mm <sup>3</sup>	0.148
Branching frequency per mm	0.138
Nr. of branching points	0.166

## References

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- [4] <https://projects.au.dk/fabagenome/genomics-data>
- [5] George EI, McCulloch RE (1993): Variable Selection via Gibbs Sampling. *J. American Statistical Association* 88:881–889
- [6] Heuven HCM et al. (2010): Bayesian multi-QTL mapping for growth curve parameters. In: *BMC Proceedings* doi: 10.1186/1753-6561-4-s1-s12.449
- [6] <http://bayz.biz/>

## Aknowledgements

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