



Pre-breeding strategies for obtaining new resilient and added value berries

NEW PHENOTYPING METHODS –

Strawberry root phenotyping

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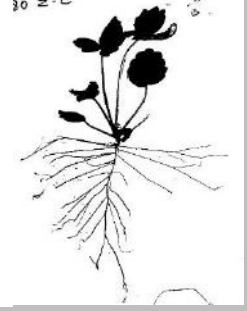
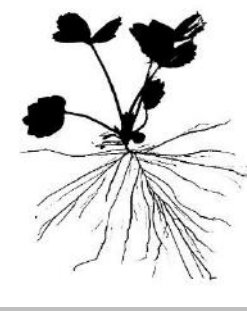
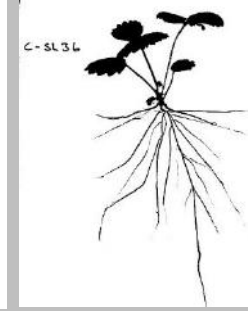
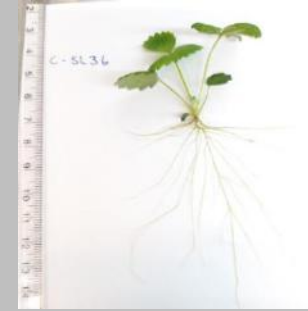


The BreedingValue project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000747.

The initial developmental stages in soilless and soil cultures

In vitro (seedlings only)

Phytigel



Hydroponic system

Nutrient film (NFT)



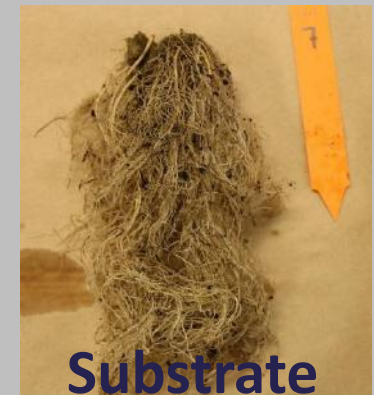
Substrates:

What kind of substrate is washable from strawberry roots?

- ➔ Peat : sand mixture (1:1)
- ➔ Sieving the components



Rockwool



Substrate

What are the needs from breeding research?

- For genetic studies **High** to **medium** throughput to make sufficient replication possible
 - Quantitative traits: Imaging-based
- For clonal plants = for adventitious root systems
 - For young plants ~ transplantation stage
- For different architectures
 - Versatility of measured traits
- Scalable to actual growing systems: hydroponic or substrate-based
- Not laborious

Root parameters from hydroponic system vs field traits?

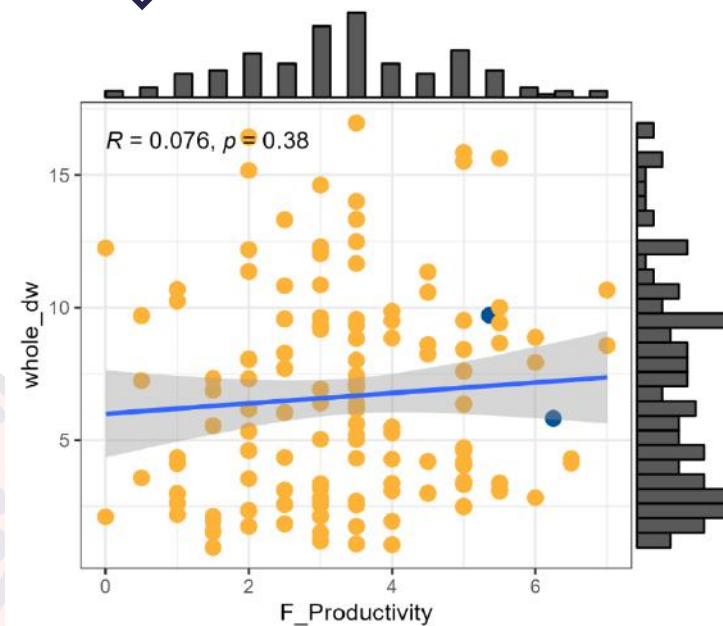
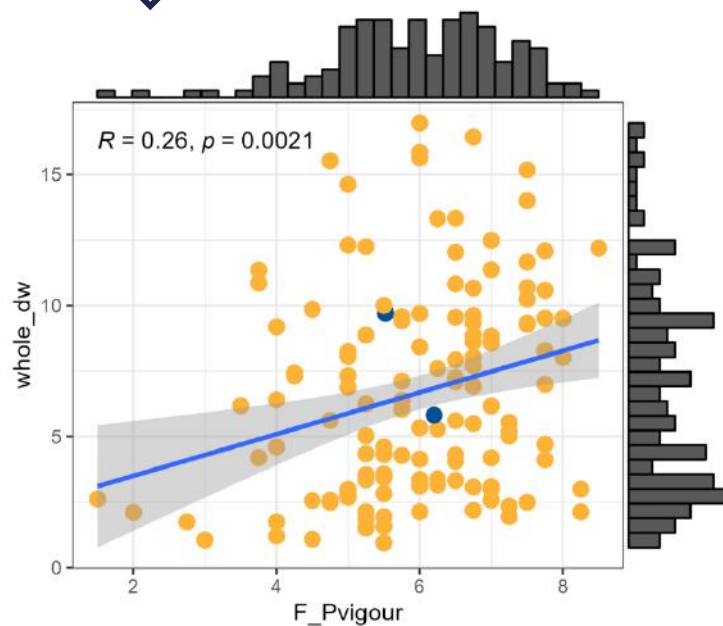
- ✓ Reconstructed *F. virginiana* × *F. chiloensis* F1 population + control cultivars



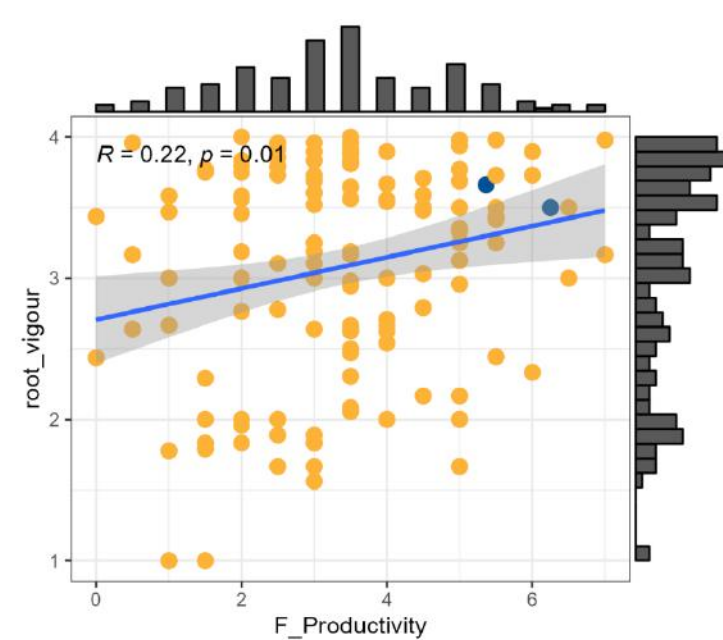
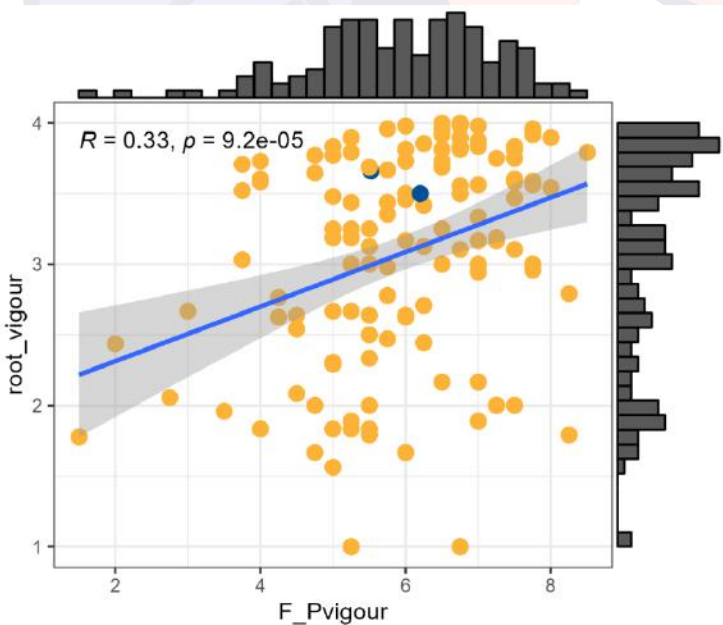
Field:
Plant vigour

Field:
Productivity

Hydroponic:
Plant dry
weight



Hydroponic
Root vigor



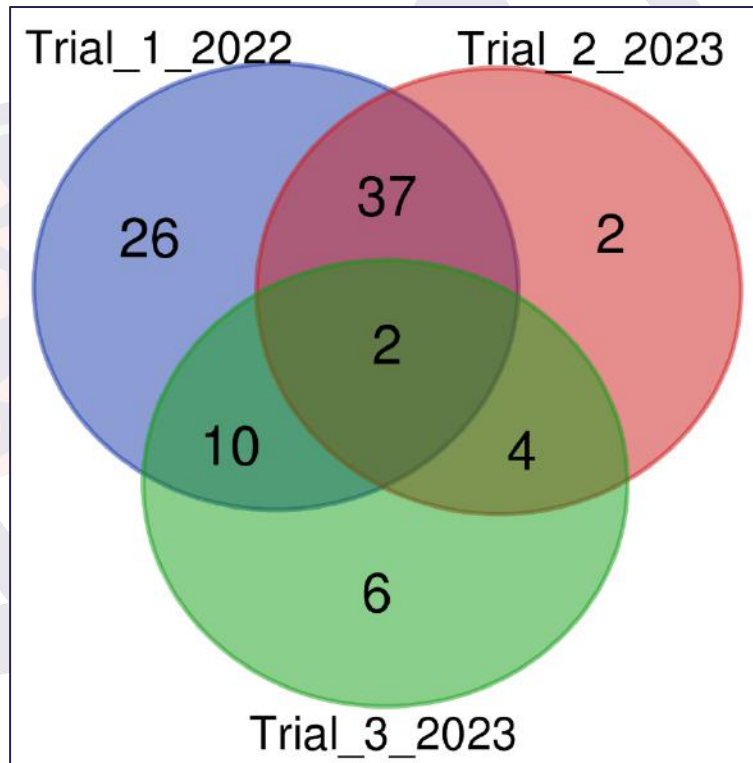
From hydroponic to substrate-based growing system





Experimental setup 2022, 2023

- RCBD design with 5-7 blocks in total
- Each block contained 2 trays (2x 5x5 plants)
- In total, 87 cultivars and population individuals



- No. of leaves (start & end point)
- Leaves fresh weight
- Leaves dry weight (LDW)
- Roots dry weight (RDW)
- Root Images

Data analysed using linear regression



Trial termination: Washing the roots



In-house root drying and imaging



Rhizovision Explorer (*Seethepalli & York, 2020, Seethepalli et al., 2021*)



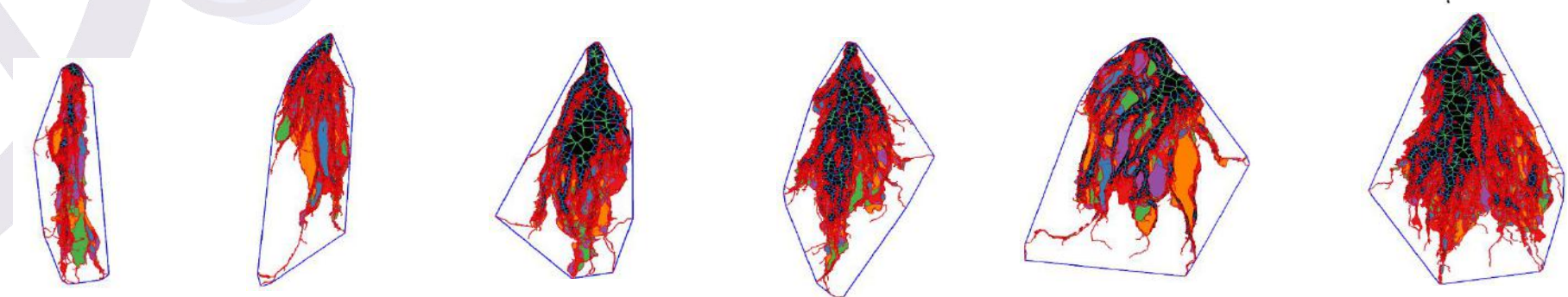
Raw Image



Segmented Image



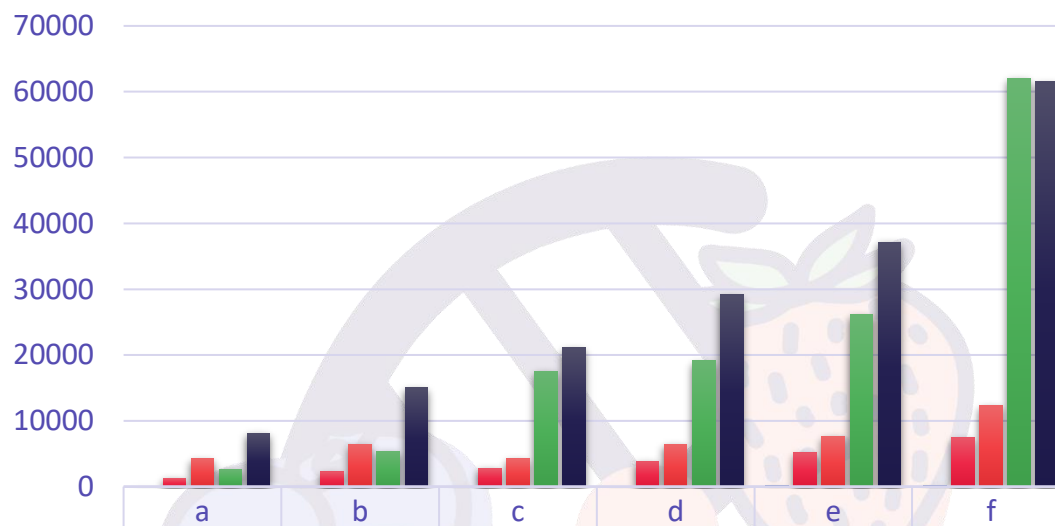
Traced Image



Extracting
different root
Parameters



Rhizovision root parameters from example images:



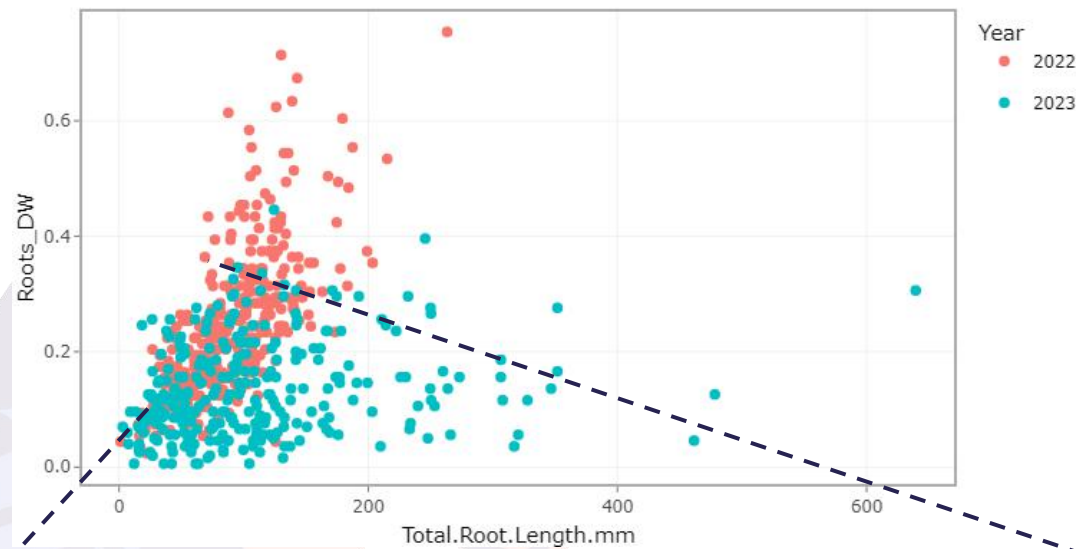
RhizoVision Explorer provides more than 35 different root parameters from each image

Selection of parameters?
Their correlation with root dry weight
Significant differences between cultivars and genotypes



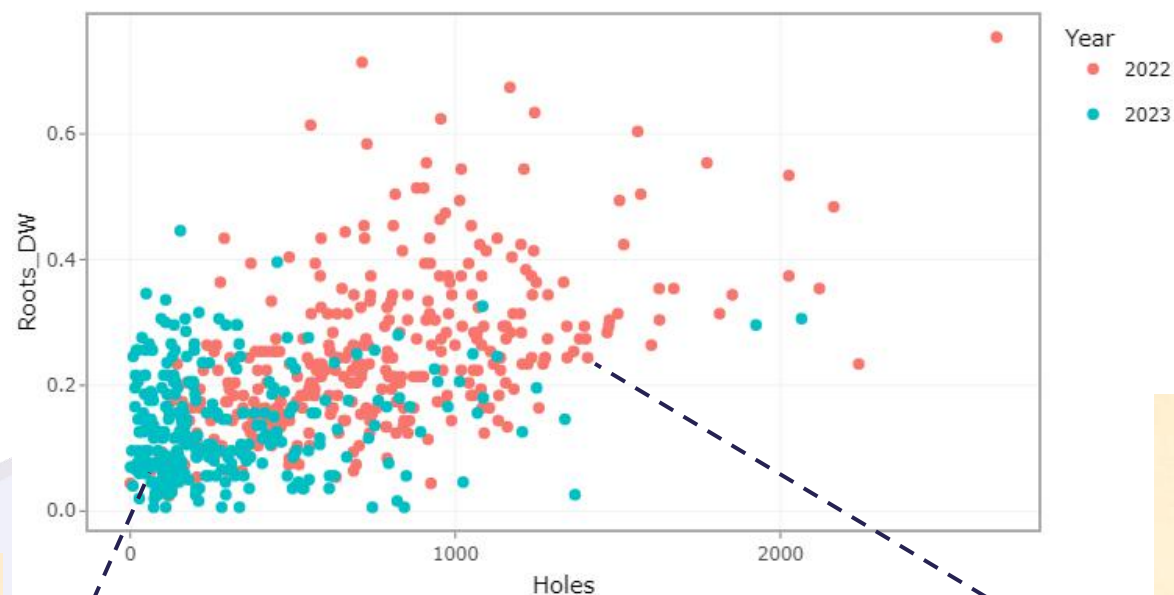
RHIZOVISION PARAMETERS: TOTAL ROOT LENGTH vs *root dry weight*

Computed by counting the total number of pixels in the skeletonized image.



RHIZOVISION PARAMETERS: HOLES *vs* root dry weight

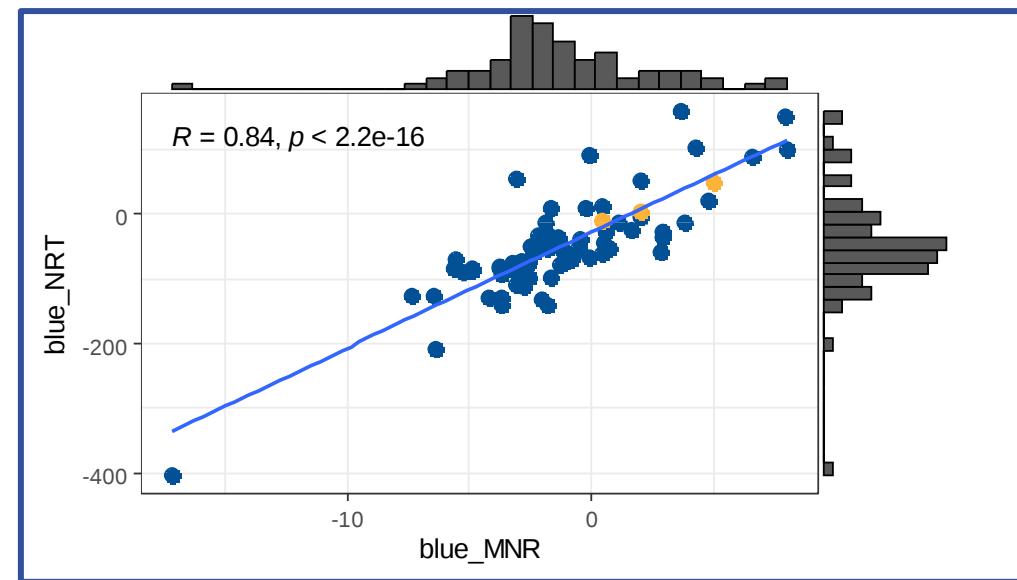
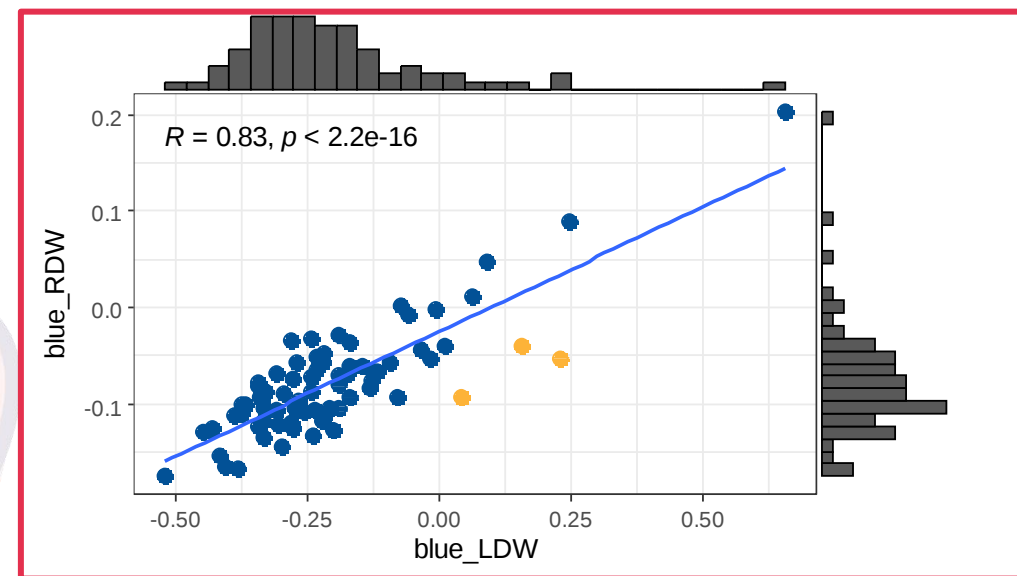
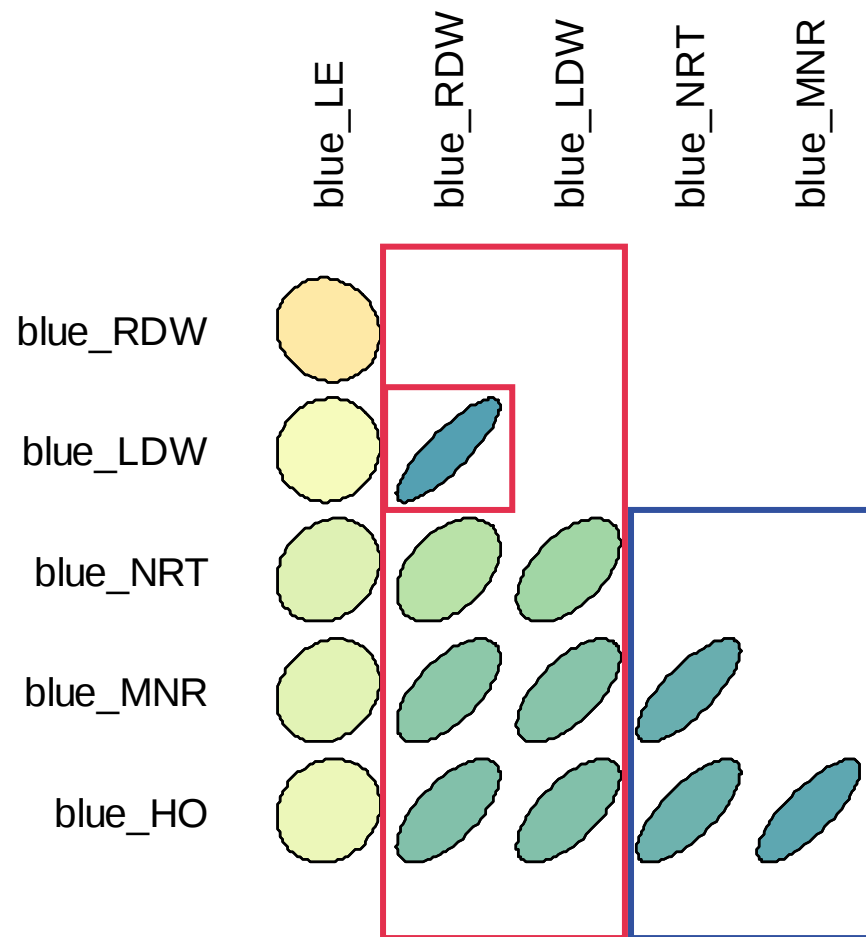
Holes are the disconnected background components and indicative of root branching and complexity.



Root system parameters that we selected for comparison to field performance:

- **Maximum.Number.of.Roots MNR** The number of roots is counted by performing horizontal line scans from left to right in each row through the segmented image. The maximum number is taken from the values of all horizontal scans per image
- **Number.of.Root.Tips NRT** Computed by counting the total number of tip pixels in the skeletonized image with topology
- **Holes HO** Holes are the disconnected background components and indicative of root branching and complexity.

Root system parameters were positively correlated to leaf and root dry weight and to each other



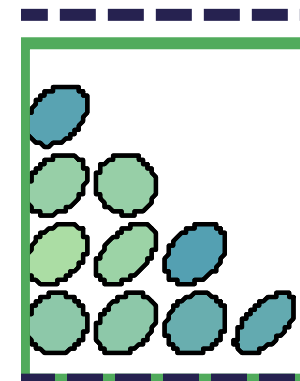
Comparison of root system data to field data



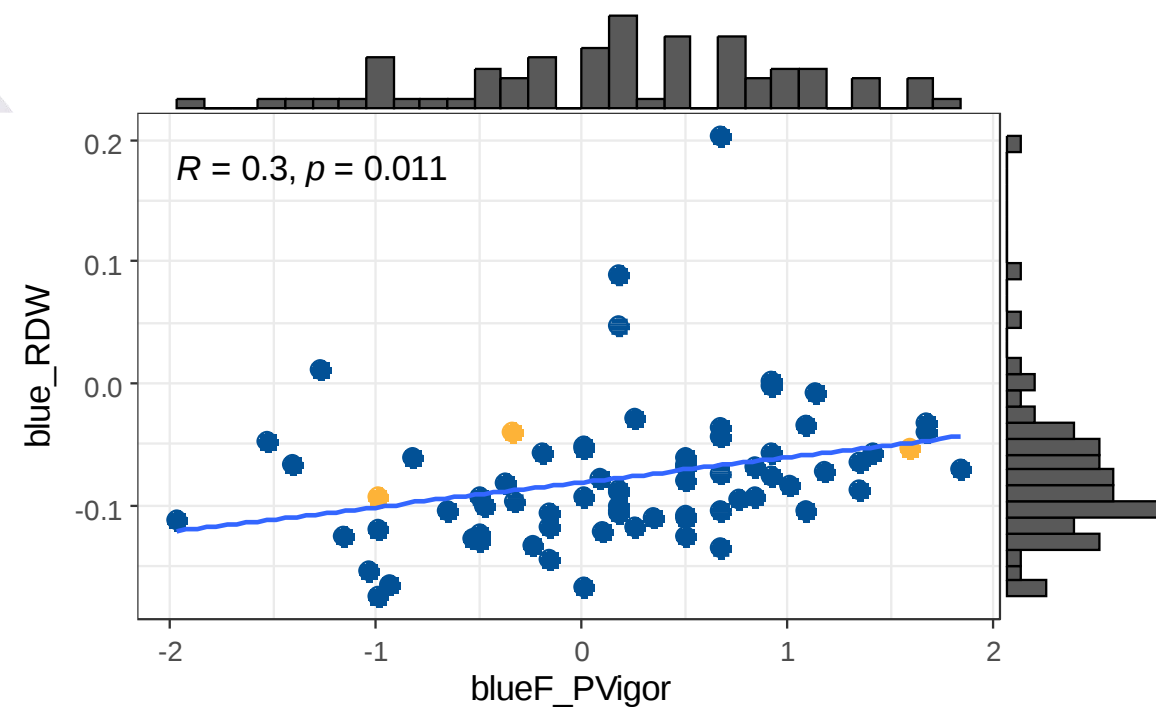
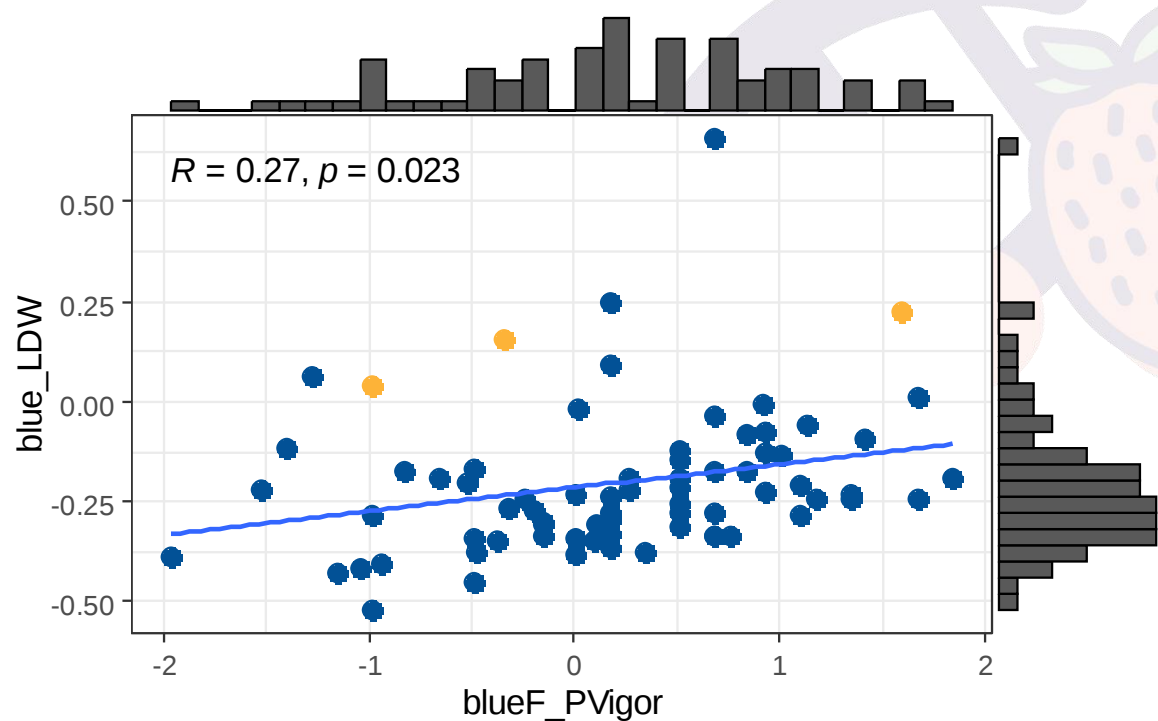
Field dataset: resiliency indicators and plant characteristics

blue_HO
blue_LDW
blue_LE
blue_MNR
blue_NRT
blue_RDW
blueF_Overw
blueF_PDensity
blueF_Productivi
blueF_PSize

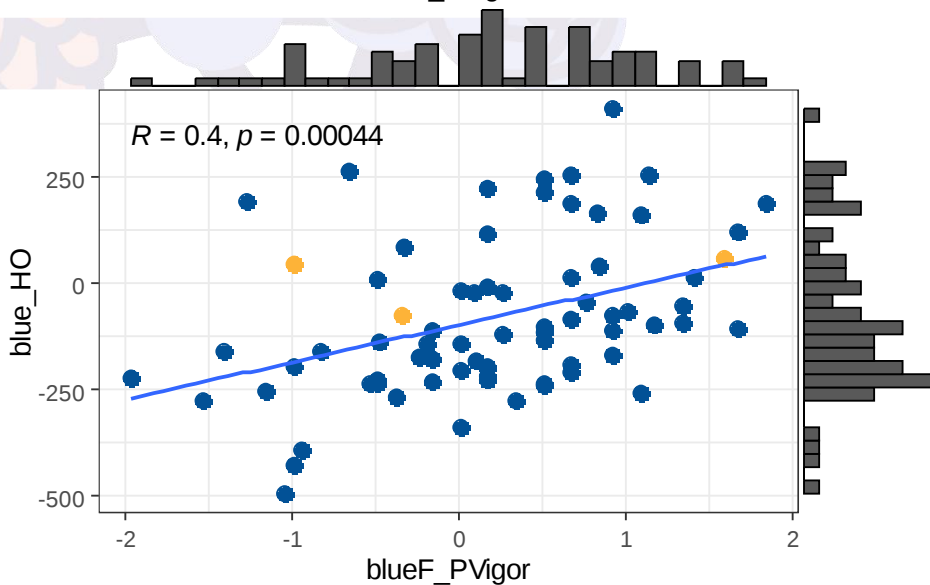
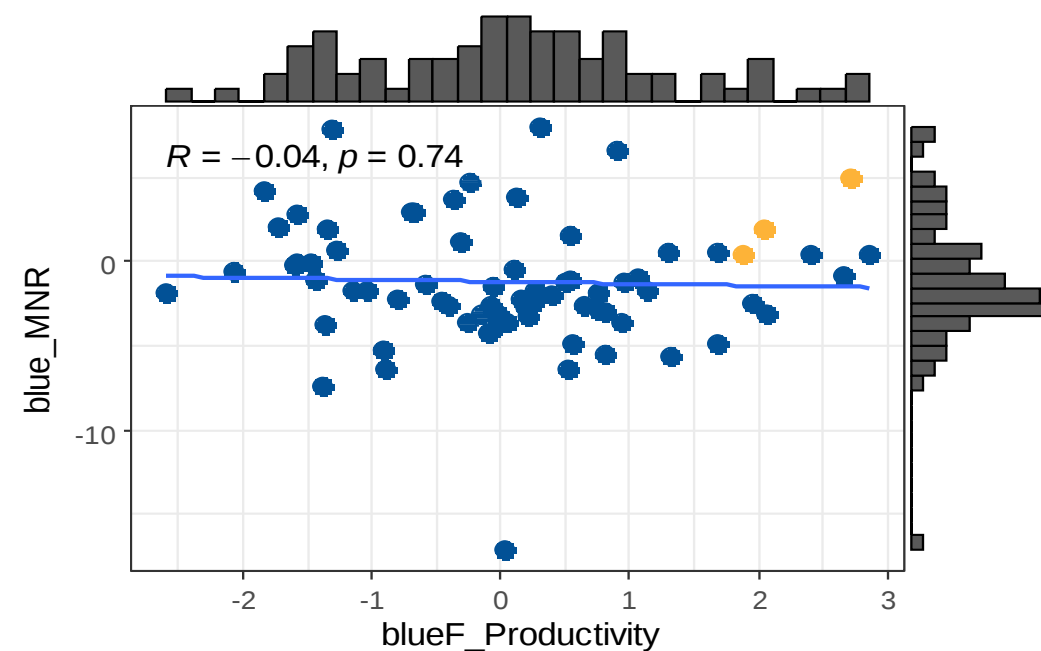
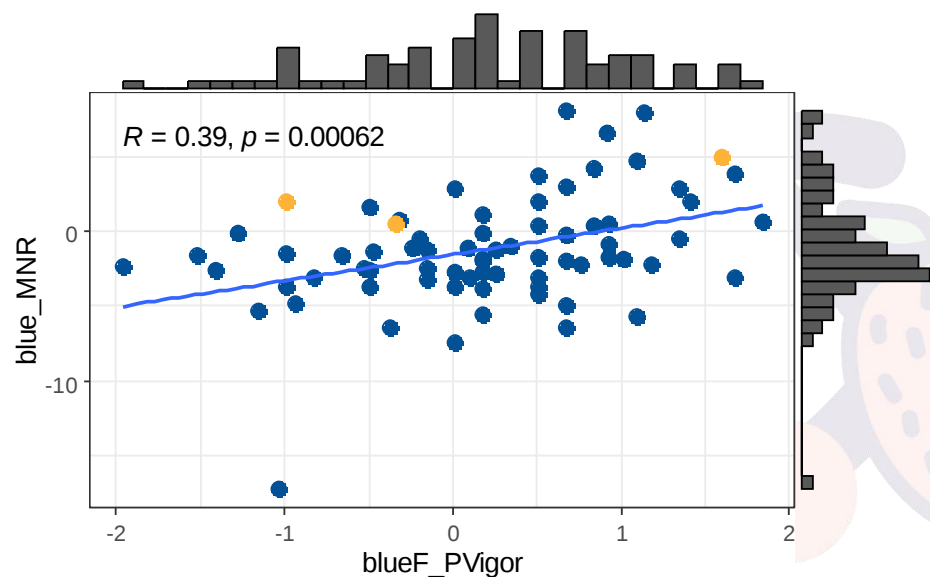
blue_LDW
blue_LE
blue_MNR
blue_NRT
blue_RDW
blueF_Overw
blueF_PDensity
blueF_Productivity
blueF_PSize
blueF_PVigor



Plant size in the field correlated with leaf and root dry weights in the greenhouse



Complexity-related root system parameters correlated with Plant vigour in the field



but no clear associations
to Productivity (Yield) 😊

Applications – demonstration and results

- Multiple root system traits can be quantified using image analyses
- Identification of breeding materials and cultivars with strong root systems
- Exploration of the phenotypic associations of root system architecture to other resiliency and adaptation traits



Future options:

- Applications of the root phenotyping method for research questions related to cultivar responses to different growing and stress conditions

