# Designing the platform for phenotyping stem cuttings and clones of ornamental woody plants

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Plant phenomics is relatively new field of bioinformatics that refers to a quantitative description of the plant's morphological and physiological properties. Recent development of phenomics has revolutionised the field of plant biology and agriculture because it allowed to make a high-throughput automated quantitative analyses of plant growth, development, productivity and stress resistance under variable conditions. In about 30 countries, a number of phenotyping research platforms has been established since 1990's. Plant phenotyping involves both laboratory- and field-based methodologies, using a number of non-invasive techniques, such as RGB visible imaging, imaging spectroscopy (multispectral and hyperspectral remote sensing), thermal infrared, fluorescence, 3D and tomographic imaging (MRT, PET and CT). Various plant organs have been successfully phenotyped including shoot and root systems in hundreds of species. Here, we propose to develop a platform for phenotyping stem cuttings and juvenile clones used for vegetative propagation of ornamental woody plants. At the moment plant biotechnologists and horticulturists do not have tools based on the image analysis to distinguish rooted and dead green cuttings during rooting in the soil substrate. Moreover plants propogated *in vitro* by microcloning pass through vulnerable stage of adaptation and rooting in *ex vitro* conditions, when many of them fade and die.

Thus, from the point of view of both industry and fundamental science, the development of phenomic analyses for juvenile arboreal plants will be really interesting. In this study, the focus will be on *Forsythia* × *intermedia*, *Cotoneaster lucidus*, *Physocarpus opulifolius* and *Hydrangea arborescens*. Millions of these ornamental shrubs are produced in Belarus and EU every year. The plant material of these species and cultivars is abundant in the nursery of Belarusian State University. Green stem cuttings will be prepared in the late Spring and transferred to phenomic cultivation trays for rooting. Systems with fully controlled growth environment, such as programmed illumination, humidity, water supply, mineral status and organic substrate composition, will be assembled and automated. The environmental parameters will be constant during rooting phenotyping. Success of rooting will be monitored manually at days 50 and 100. One phenomic tray (PT) will contain 20 green cuttings. Up to 10 trays will be monitored by visible (RGB) imaging every 24 h. To this purpose five RGB cameras will used simultaneously, which will be placed under different angles and distances to cover all green cuttings in each PT. Original images (IM) will be taken and stored in databases ($DB_{1\text{-}10}$) and then analysed. Five IM will correspond to 5 different views: front ($IM_F$), right ($IM_R$), left ($IM_L$), back ($IM_B$) and top ($IM_T$). This means that, at day 100, each DB will contain 100 $IM_F$, $IM_R$, $IM_L$, $IM_B$ and $IM_T$. All IM will be analysed using pattern recognition and image analysis algorithms previously developed in Belarusian State University.

Overall, the analysis will include seven major stages that are as follows:

1) The images of plant will be separated from the background and stored as individual background-free images (I), $I_F$, $I_R$, $I_L$, $I_B$ and $I_T$, in background-free databases (D), $D_{1\text{-}10}$. As plant tissues do not pass and do not reflect the blue light, the blue spandex fabric will be used to cover substrate in PT. This will facilitate background detection and obtaining $I_F$, $I_R$, $I_L$, $I_B$ and $I_T$.

2) The segmentation will be carried out using $I_F$, $I_R$, $I_L$, $I_B$ and $I_T$ from $D_{1\text{-}10}$. Segmentation algorithms will detect and separate images of stems (non-photosynthesising organs) from leaves (photosynthesising organs) based on difference in their spectral properties. Photosynthesising organs absorb hundred times less in green/yellow part (500-600 nm) of the visible electromagnetic spectrum than in blue and red parts (400-500 nm and 600-700 nm, respectively). Background-free images of leaves (IL), $IL_F$, $IL_R$, $IL_L$, $IL_B$ and $IL_T$ and corresponding images of stems (IS), $IS_F$, $IS_R$, $IS_L$, $IS_B$ and $IS_T$ will be obtained at this stage of the image analysis. They will be stored in leaf background-free databases ($DL5_{1\text{-}10}$) and stem background-free databases ($DS5_{1\text{-}10}$).

3) $IL_F$, $IL_R$, $IL_L$, $IL_B$ and $IL_T$ of individual plants in each PT will be recognised, then extracted and included in one bigger image (IL). Therefore IL will summarize all-side views of one plant in one day of measurements. ILs will be numbered by the number of the plant in PT ($IL_{1\text{-}20}$). The same procedure will be conducted on stem images (IS). Obtained images will be stored in leaf background-free databases ($DL_{1\text{-}10}$) and stem background-free databases ($DS_{1\text{-}10}$).

4) $IL_{1\text{-}20}$ and $IS_{1\text{-}20}$ will be obtained every day during 100 days and will be named as $1IL_{1\text{-}20}$ - $100IL_{1\text{-}20}$ and $1IS_{1\text{-}20}$ - $100IS_{1\text{-}20}$, respectively. They will be stored in databases ($1\text{-}100DL_{1\text{-}10}$) and stem background-free databases ($1\text{-}100DS_{1\text{-}10}$), respectively.

5) $1IL_{1\text{-}10}$ and $1IS_{1\text{-}10}$ will be used as initial patterns; they will be compared with $2\text{-}100IL_{1\text{-}10}$ and $2\text{-}100IS_{1\text{-}10}$ (images obtained at days 2-100). The comparison will show numerical change in intensities of different parts of RGB spectrum (with 10 nm step) and identify correlations between these changes and rooting at day 50 and day 100

(rooting will be examined manually). These data will be presented as spectral bar histograms of distribution of intensities (34 bars: 370-710 nm) monitored every day and stored in histogram databases (HD).
6) Patterns of averaged specific histogram changes related to death, survival (callusogenesis) or rooting will be identified and stored. They will be used as patterns for early recognition of physiological fate of green cuttings and making recommendations to horticulturists.
7) Obtained patterns will be used for monitoring and predicting rooting effectiveness during three sequential seasons. This will help to verify and adjust developed algorithms and refine the protocol of phenomic measurements.
The developed platform will be used for the research onto various aspects of ornamental plant biology and will have practical outputs in horticulture and biotechnology. Moreover, with modular architecture, the platform can be adapted for measuring important crop plants, such as tomatoes and potatoes.

**Keywords:** phenomics, phenotyping, plant physiology, image analysis.