Leaf area estimation: a first step towards virtual rose breeding

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Introduction

New genotypes (varieties) of hybrid tea roses are introduced at a high rate. At the same time growing conditions like greenhouse climate and cropping systems are also rapidly changing and diversifying, and, varieties may be grown all over the world. For a breeder, the challenge is to efficiently breed the best variety for specific environments. However, at present, breeders and growers lack appropriate tools to find the most suitable combination of greenhouse technology, plant management and genotype other than their own experience and trial and error. A model that integrates all relevant information and that can predict performance of genotypes under a variety of combinations, can serve as an important decision support system for plant breeding.

A crop growth model is an excellent means to predict plant performance under a wide range of conditions, based on a limited number of plant characteristics. However, in order to predict stem production and quality of cut rose, we have to take into account the 3D architecture of the plant.

The aim of this study is to develop a functional-structural model for rose and to parameterize it for a large number of genotypes (150). We also want to find genetic markers that are associated with genotype-specific model parameters. The hypothesis for the latter is that model parameters are more directly linked to genetic information than direct plant measurements of yield as the latter are the final result of complex interactions between different physiological processes, including sink-source. Hence Quantitative Trait Loci (QTL) for these model parameters are expected to be more specific and stable over environments than QTL for those directly measured traits (Van Eeuwijk et al., 2010). The potential of this "gene-to-phenotype" modeling approach was illustrated in a simulation study by Chenu et al. (2009).

In this presentation, different aspects of the virtual rose breeding project will be shown, (1) the currently available functional structural plant model for a single variety; (2) a description of the experiments; (3) the measurements carried out on the genotypes; (4) the genetic markers; (5) the integrative approach, estimating the model parameters from the measurements and finding genetic markers for the model parameters.

To reach the final aim of virtual rose breeding, a huge number of measurements need to be made during growth and development of the genotypes at all locations; such a data collection effort is often referred to as 'large scale phenotyping'. One of the most important measurements to parameterize a crop growth model is leaf area. Hence we need a cheap, fast, unbiased and non-destructive method to measure/estimate leaf area of all the genotypes. In this presentation emphasis will be given on the development of such a method for roses.

A common approach for leaf area estimation is to develop ratios and regression estimators by using easily measurable leaf parameters such as leaf length and leaf width (Schwarz and Kläring, 2001). Various combinations of measurements and various models relating length and width to area have been developed for several crops, such as sunflower (Chanda and Singh, 1997; Bange *et al*., 2000), avocado (Uzun and Celik, 1999), grape (Montero *et al*., 2000; Williams and Martinson, 2003), and hazelnut (Cristofori *et al*., 2007). Since leaf shape (length/width ratio) may vary among different genotypes (Stoppani *et al*., 2003), most statistical models demonstrated a relationship between leaf area and length and width or the product of length and width.

For rose, no such length/width model has been reported. A rose leaf has a pinnate shape with a laminar structure as a central rib with leaflets on each side and one terminal leaflet. A leaf usually consists of one to eleven leaflets. Some rose leaves have ovate leaflets, other rose leaves have lanceolate leaflets, and this may differ between genotype. The number of leaflets per leaf depends on the leaf rank along the stem.

The aim of this experiment, as part of the virtual rose breeding project, is to develop a method for fast non-destructive leaf area measurements, using a statistical model of simple linear measurements such as leaf length and leaf width combined with other parameters such as number of leaflets or leaf position (rank). The model has to be robust for different genotypes and growing conditions.

Virtual rose model
A virtual rose has been developed in the GroIMP modeling environment (Buck-Sorlin et al, 2010). Figure 1 shows a simulated cut-rose production system. The model will be extended and adapted to make it suitable for many different genotypes.
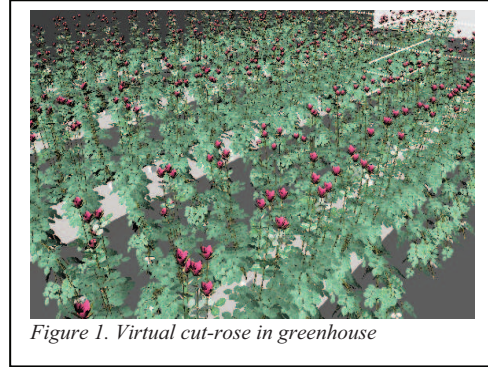

Figure 1. Virtual cut-rose in greenhouse

Large scale experimental setup
One-hundred-fifty genotypes were collected from five different breeding companies in the Netherlands, and were planted mid 2009 at six locations in Ethiopia, Ecuador, Kenya and India. Furthermore, 20 out of these 150 genotypes were also planted in four different locations in the Netherlands for more detailed plant measurements. Currently plant measurements are being taken at every location at regular intervals, including harvest characteristics such as fresh weight, length and diameter of stem.

Molecular markers
DNA was extracted from leaves of all 150 genotypes, and approximately 150 DNA markers will be obtained for all these genotypes. The markers are described in Koopman et al., 2008.

Measurements for estimating leaf area
From the 20 genotypes in the Dutch greenhouse, two to forty shoots from each genotype were harvested at four locations, with eight to 22 leaves on each stem. Only compound rose leaves with at least three leaflets were measured. Of each single leaf, leaf length *(L, in cm)*, leaf width, *(W, in cm)*, number of leaflets (*N*), the position of the leaf on the stem (*absolute rank (AR)*) and leaf area *(A, in cm^2)* were measured. Actual leaf area was measured with the area meter (LI-3100; LICOR). A total of *3446* rose leaves from *310* shoots of *20* genotypes were measured.

Model selection
Different statistical models were fitted, using *A*, log(*A*) and √*A* as response variables, and *L*, *W*, the product *LW*, its root √*LW*, *R*, *N*, *Genotype* and *Location* as explanatory variables. The product of length and width was chosen as this is proportional to the area for many shapes,
The Akaike Information Criterion (AIC) was used as measure for model selection. Bias was calculated as predicted area minus measured area, divided by measured area, averaged over all leaves of a genotype.

Results
The best model according to AIC was: $\sqrt{A}_{ijkl} \propto \sqrt{L_{ijkl}W_{ijkl}} * E_i * G_j * N_k$ where *j* indicates the environment (*E*), *j* indicates the genotype (*G*), *k* indicates the number of leaflets (*N*) of the compound leaf and *l* indicates the stem number.
N, *G* and *E* are treated as factors in the model, i.e. separate coefficients were fitted for every level. The root mean square error associated with this model was 0.3527 and the coefficient of variation was 4.0%. The AIC was 3008.5. The factors *G* and *E* were both significant (P<0.001). When *E* was dropped from the model, the AIC slightly increased to 3542. When *G* was dropped, the AIC increased to 4407 and relative biases over 10% for certain genotypes were observed.
√*A* gave residuals which were more normally distributed than *A* or log*(A)*.

Conclusions

The aim of the project is to develop a genotype-specific functional structural plant model for rose, where molecular markers might be used to estimate genotype-specific parameters One of the most important measurements for calibrating this model is leaf area. In this experiment the aim was to find a simple linear non-destructive measure to estimate the leaf area. The best model was obtained using the product of length and width and number of leaflets per leaf and calibrated per genotype and location. If calibration was only done per genotype (over the locations) still good estimates were obtained. However, a single statistical model to estimate leaf area over all genotypes introduced considerable bias. Therefore we concluded that the statistical model for leaf area needs to be calibrated per genotype.

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