COST ACTION FA1204

Vegetable Grafting to Improve Yield and Fruit Quality Under Biotic and Abiotic Stress Conditions

STSM Scientific Report

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1. Purpose of the STSM:

Under specific environmental stress conditions or in specific plant genotypes an imbalance between root water uptake and leaf transpiration occurs. A mechanism to avoid stress-induced growth retardation is the modification of root water uptake capacity compared with stomata closure (Matsuo et al., 2009). Chilling sensitivity differences among genotypes are mainly associated with differences in root water uptake rates. Indeed, when

the temperature falls below optimum, root water uptake decreases due to a reduced vapour pressure difference between the leaf surface and the area (Aroca et al., 2003), while water viscosity increases (Bloom et al., 2004). Changes in root morphology may be interpreted as adaptation of nutrient acquisition mechanisms to abiotic stress, aimed at extending the absorbing surface area per unit root weight or length. Thus, the formation of a more extensive root system by tolerant rootstocks in grafted plants and the concomitant increase in root:shoot ratio as stress increases, improves their nutrient and water uptake capacity. As an example an approximately 2.5-fold increase in root:shoot ratio was observed in tomato grafted onto the cold tolerant *S. habrochaites* when these were exposed to sub-optimal T, while only a slight increase was observed when 'Moneymaker', a cold sensitive tomato cultivar (Venema et al., 2008; Ntatsi et al., 2014b) was used as a rootstock.

Linkage mapping of QTL is a common statistical approach in plant genetics where recombinant populations generated from crosses between inbred parent lines are used, in combination with molecular markers, to identify loci associated with variation in continuously distributed traits (Sen et al., 2001). Mapping populations common to QTL analyses are many and include doubled haploids, F2, backcross, nested association mapping and RILs. Mapping QTL for complex traits is now routine, with the typical output being QTL spanning large confidence intervals encompassing many (hundreds or more) possible causal genes (Price et al., 2006).

The steps following QTL identification frequently involve functional validation of the QTL, and refinement of location (fine-mapping) towards the goal of identification of a causal gene – the major challenge in quantitative genetics today. One of the most common approaches for accomplishing these objectives is through the development and phenotypic characterization of NILs. The generation and phenotyping of NILs is considered a laborious and time consuming process, but the robust design leads to a minimal false positive rate.

NILs are lines containing a single or small number of genomic introgressions from a donor parent in a different and otherwise homogeneous genomic background. By homogenizing all genetic factors outside of the focal genomic region, the true effect of the QTL on the phenotype can be estimated relative to the line into which the introgression was introduced (i.e. void of the chromosomal introgression) (Landi et al., 2005). In addition to the simplification of genetic analyses, NILs are considered genetically 'immortal' which allows for replicated experiments across multiple environments resulting in more accurate estimates of effect size for complex traits. NILs have proven to be an effective resource for QTL validation and a logical starting point for the creation of fine-mapping populations (Zhou et al., 2010).

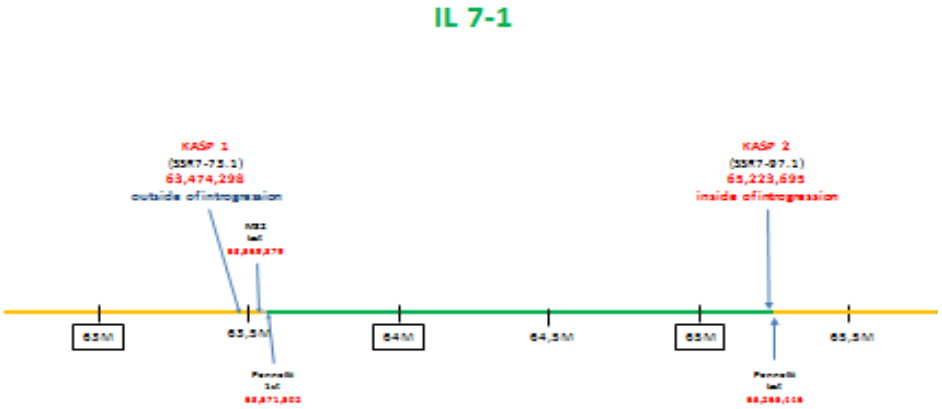
Creation of a single near-isogenic line generally starts by crossing a line carrying the targeted QTL region to one of the parental lines of the population, thus creating a backcross population. Genome-wide genotyping of the backcross progeny is performed to identify recombination events allowing for selection of progeny which carry the target chromosomal introgression derived from the donor and recurrent parent genome elsewhere. Subsequent generations of self-pollination (selfing) are normally required to achieve homozygosity of the introgressed region and the process can take several backcrossing cycles to produce a NIL carrying an introgression of acceptable size and genomic location. An alternative approach has been the use of heterogeneous inbred families (HIFs) where NILs are selected from incompletely inbred lines which still harbor a small amount of heterozygosity at random

intervals across the genome (Loudet et al., 2005). Analysis of a HIF population with molecular markers allows for the selection of lines heterozygous at a candidate genomic location, which in combination with further selfing and genotyping, enables selection of NILs derived from several heterogeneous genetic backgrounds. Producing NILs with smaller introgressions requires greater effort. Large populations are needed to break up small chromosomal segments, and high-density genotyping is required to discover them.

The 6-week project of this STSM was to generate sub-introgressions from IL7-1 which was showed in a previous phenotyping experiment by the group of Dr. Thompson, increased root:shoot ratio of M82 by 40%. These were used to fine-map the position of the QTL.
The seed of the parental lines and the introgression of F1 and F2 generation that were sown on 25th of April were the followings:

- IL7-1
- M82
- M82 x IL7-1 F1
- M82 x IL7-1 F2
- LA0716 (*S. pennellii* parent line) as genotyping controls;

To screen for recombinants we needed to score F2 plants with two KASP markers, one at each end of the IL7-1 introgression and two more in the regions of SSR7-73.1 and SSR7-97.1m as shown in the Figure below:



The strategy that we followed was firstly to look for SNPs in the regions of SSR7-73.1 and SSR7-97.1 (or close to end of chromosome), using the “150 genomes” resource. After that we designed KASP markers for these two positions (LGC genomics help with this process) and order them. Then we checked the markers by using them to score parental lines and known heterozygous material (M82, IL7-1, LA716 and M82 x IL7-1 F1 hybrid). The KASP genotyping assays that were performed were based on competitive allele-specific PCR and enable biallelic scoring of single nucleotide polymorphisms (SNPs) and insertions and deletions (Indels) at specific loci The SNP-specific KASP Assay mix and the universal KASP Master mix were added to DNA samples, a thermal cycling reaction is then performed, followed by an end-point fluorescent read. The KASP Assay mix contained three assay-specific non-labelled oligos: two allele specific forward primers and one common reverse

primer. The allele-specific primers each harbor a unique tail sequence that corresponded with a universal FRET (fluorescence resonant energy transfer) cassette; one labelled with FAM™ dye and the other with HEX™ dye. The KASP Master Mix contained the universal FRET cassettes, ROX™ passive reference dye, taq polymerase, free nucleotides and MgCl2 in an optimized buffer solution. During thermal cycling, the relevant allele-specific primer binded to the template and elongates, thus attaching the tail sequence to the newly synthesized strand. The complement of the allele specific tail sequence was then generated during subsequent rounds of PCR, enabling the FRET cassette to bind to the DNA. The FRET cassette was no longer quenched and emits fluorescence. Bi-allelic discrimination was achieved through the competitive binding of the two allele-specific forward primers. If the genotype at a given SNP is homozygous, only one of the two possible fluorescent signals will be generated. If the genotype is heterozygous, a mixed fluorescent signal will be generated. Therefore, two hundred seedlings were screened for recombination events in an M82 x IL7-1 F2 population using the pair of KASP markers. Finally the recombinant plants were kept for seed production and phenotyping in the next generation.

The results of the present STSM will be important for both working groups WG3 (Rootstock-mediated resistance to biotic and abiotic stresses) and WG4 (Rootstock-mediated improvement of fruit quality) of the COST Action FA1204. Besides, this STSM will provide mutual benefits for the ongoing projects in both groups sharing the same objective of improving heavy metal tolerance in vegetable crops through grafting.

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3. Description of the main results obtained:

Preliminary results have shown that the markers that were tested managed to restrict the position of the QTL that is responsible for the increased root:shoot ratio of M82 by 40%. The design of the KAPS Markers is still in progress since more than 200 seedlings must be screened for recombination events in an M82 x IL7-1 F2 population using several pair of KASP markers. The expected date for finishing the genotyping as well as phenotyping of the 200 hundrend recombinants is the end of June. However, this Study will continue until the

position of the QTL will be restricted enough to allow the identification of specific genes that are responsible for the root:shoot ratio of M82 by 40%.

4. Future collaboration with the host institution (if applicable):

This STSM is the first step of a future collaboration between the School of the Applied Sciences (SAS) of Cranfield University and the Laboratory of Vegetable production of the Agricultural University of Athens. This collaboration will continue in the coming years with a particular emphasis on molecular breeding of vegetable plants and rootstocks and the improvement of abiotic stress tolerance through the use of vegetable grafting. Moreover, in the terms of this collaboration a review entitled “New insights in responses of plants to sub-optimal temperature stress: an example of fruiting vegetables” that is written by Andrew J. Thompson from the University of Cranfield and the Georgia Ntatsi and Dimitrios Savvas from the Agricultural University of Athens will soon be submitted for publication to the *Environmental and Experimental Botany*.

5. Foreseen publications/articles resulting from the STSM (if applicable):

Both sets of work are likely to contribute to high impact publications that will explain the genetic basis of the variation in rootstock traits. In addition, we are also planning to present some results of the current work in the next annual conference of the COST action FA1204 that will be held in Portugal 2014.

6. See PDF File