SuperSAGE and SuperTagArray: Technologies for a simultaneous quantitative transcriptome analysis of a host plant and a pathogen

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One of the presently most powerful techniques for **genome-wide quantitative gene expression profiling**, a greatly improved variant of the conventional Serial Analysis of Gene Expression (SAGE; 1,2) technique, stands out for its potential to (i) screen any eukaryotic cell, tissue, organ or organism for all the transcripts genome-wide, (ii) quantify each and every transcript, and (iii) decipher the complete transcriptomes of two (or more) interacting eukaryotic organisms simultaneously and without the physical separation of both, as still is the traditional mandate. This improved SAGE variant, coined **SuperSAGE**, therefore allows to detect and quantify all transcripts present in theinteraction space between a host and a parasite, a host and a pathogen, and a host and a commensal.

SuperSAGE is superior to all conventional SAGE techniques in that it isolates tag sequences (short stretches of DNA characteristic for a cDNA) of 26 bp using the type III restriction enzyme *Eco*P15I as the „tagging enzyme". The longer tags dramatically and reliably identify the corresponding genes and therefore warrants an accurate gene expression analysis. Streamlining the somewhat complicated protocol, simplifying a delicate step, and changing the high-throughput sequencing procedure by GenXPro GmbH transformed SuperSAGE to a relatively fast, accurate and truly quantitative transcript scanning technology. As an „open architecture" platform, SuperSAGE will identify novel genes, splice variants, sense and anti-sense transcripts, that escape conventional expression microarray procedures.

For a proof of principle, we first applied SuperSAGE to *Magnaporthe grisea* (blast)-infected rice leaves to simultaneously monitor gene expression profiles of both the rice host and blast fungus, exploiting the completed genome sequences of both organisms. To that end, laser-capture microdissection of a single apressorium of the fungus and a single attacked host cell (later on undergoing hypersensitive cell death) was employed to prepare appropriate material. Since the RNA isolated from these single cells is not sufficient for an in-depth SuperSAGE analysis, it has to be amplified. SuperSAGE with the amplified cDNA population, roughly comprising 40,000 transcripts (which is a compromise between curiosity and money) revealed, that the rice host cell is reacting with the rapid activation of hundreds of genes, many of them with proven function in defense. The *M. grisea* genome is transcribed into hundreds of messengerRNAs as well, of which one, encoding *hydrophobin* is most abundant, and can be considered an intervention target in future. A series of transcripts encoding secreted proteins of the fungus were characterized as potential pathogenic factors.

Moreover, SuperSAGE allowed to monitor gene expression changes prior to the *h*ypersensitive *r*esponse (HR) in INF1 elicitor-treated *Nicotiana benthamiana*, a „non-model" organism without any DNA database. Again, SuperSAGE allowed rapid identification of leaf genes up- or down-regulated by the elicitor. Surprisingly, many of the down-regulated genes code for proteins involved in photosynthesis (3, 4).

SuperSAGE also reveals dramatic transcript changes in abiotically stressed chickpea leaves and roots. Though drought, salt and cold are major constraints to crop production worldwide, breeding for tolerance to these stresses is hampered by the complexity of the traits. Therefore, deeper insight into stress-responses of crops on the molecular level is required for knowledge-based, targeted cropimprovement. Again, SuperSAGE discovered both quantitative and qualitative, drastic transcript changes of drought- and salt-stressed roots and nodules from chickpea, and cold-stressed stems and leaves from chickpea and lentil. More than 360.000 cDNA signatures representing more than 40.000 single genes from stressed material and non-stressed controls including more than 2000 significantly stress-regulated protein-coding genes were identified. These included SuperTags encoding a series of transcription factors and HMG-proteins exclusively expressed under both stresses, but not at all in non-stressed controls. Also, the members of whole signaling cascades were discovered and displayed a specific transcription pattern in a specific tissue (nodules, roots) in a specific stress situation. However, also regulated and non-regulated (constitutive) signatures from anti-sense transcripts encoding proteins important for osmoregulation (as e.g. aquaporins) or general stress-response (such as peroxidases) were among the transcripts first encountered in the chickpea stress transcriptome.

SuperTag-Array-based dissection of genotype-specific stress-responses corro-

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borated the SuperSAGE transcript analyses. Our data highlight the unmet power of SuperSAGE for transcription profiling in non-model crops (as demonstrated with chickpea), offering a realistic and more comprehensive alternative to frequently unaffordable EST sequencing.

The information content in SuperTags can be exploited for the design of efficient primers for 5'- and 3'-RACE, and the sequencing of the products in various individuals for the generation of expression markers (see the presentation of Horres et al., these proceedings). SuperTags recommend the generation of customized SuperTag-based chips (SuperTag-Arrays) by simply spotting selected 26bp SuperTag oligonucleotides onto solid supports (5). Such tag oligonucleotide microarrays provide the basis for high-throughput transcription profiling in populations and collections. The transcript profiles („expression signatures“) then are expected to reveal the potential of a plant (animal) to react upon a specific environment in a predictable way. SuperSAGE also can guide high throughput gene function analysis by either RNA interference (RNAi) for knockdown of specific genes in animals, or virusinduced gene silencing (VIGS) in plants using the 3’ end of the transcripts to trigger gene-specific VIGS.

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