Marchand, G., Huynh-Thu, V. A., Kane, N., Arribat, S., Varès, D., Rengel, D., Balzergue, S., Rieseberg, L., Vincourt, P., Geurts, P., Vignes, M. and Langlade, N. B. (2014)

Bridging physiological and evolutionary time scales in a gene regulatory network.

New Phytologist 203:685-696

Summary

Gene regulatory networks (GRNs) govern phenotypic adaptations and reflect the trade-offs between physiological responses and evolutionary adaptation that act at different time-scales. To identify patterns of molecular function and genetic diversity in GRNs, we studied the drought response of the common sunflower, Helianthus annuus, and how the underlying GRN is related to its evolution. We examined the responses of 32 423 expressed sequences to drought and to abscisic acid (ABA) and selected 145 co-expressed transcripts. We characterized their regulatory relationships in nine kinetic studies based on different hormones. From this, we inferred a GRN by meta-analyses of a Gaussian graphical model and a random forest algorithm and studied the genetic differentiation among populations (F_{ST}) at nodes. We identified two main hubs in the network that transport nitrate in guard cells. This suggests that nitrate transport is a critical aspect of the sunflower physiological response to drought. We observed that differentiation of the network genes in elite sunflower cultivars is correlated with their position and connectivity. This systems biology approach combined molecular data at different time-scales and identified important physiological processes. At the evolutionary level, we propose that network topology could influence responses to human selection and possibly adaptation to dry environments.

• Marchand, G., Mayjonade, B., Varès, D., Blanchet, N., Boniface, M.C., Maury, P., Andrianasolo Nambinina F., Burger, P., Debaeke, P., Casadebaig, P., Vincourt, P., Langlade N.B. (2013)

A biomarker based on gene expression indicates plant water status in controlled and natural environments.

Plant Cell and Environment DOI:10.1111/pce.12127

Abstract

Plant or soil water status is required in many scientific fields to understand plant responses to drought. Because the transcriptomic response to abiotic conditions, such as water deficit, reflects plant water status, genomic tools could be used to develop a new type of molecular biomarker. Using the sunflower (Helianthus annuus L.) as a model species to study the transcriptomic response to water deficit both in greenhouse and field conditions, we specifically identified three genes that showed an expression pattern highly correlated to plant water status as estimated by the pre-dawn leaf water potential, fraction of transpirable soil water, soil water content or fraction of total soil water in controlled conditions. We developed a generalized linear model to estimate these classical water status indicators from the expression levels of the three selected genes under controlled conditions. This estimation was independent of the four tested genotypes and the stage (pre- or post-flowering) of the plant. We further validated this gene expression biomarker under field conditions for four genotypes

in three different trials, over a large range of water status, and we were able to correct their expression values for a large diurnal sampling period.

Cadic, E.,Coque, M., Vear, F., Grezes-Besset, B., Pauquet, J., Piquemal, J., Lippi, Y., Blanchard, P., Romestant, M., Pouilly, N., Rengel, D., Gouzy, J., Langlade, N., Mangin, B., Vincourt, P. (2013)

Combined linkage and association mapping of flowering time in Sunflower (Helianthus annuus L.)

Theor.Appl.Genet. DOI: 10.1007/s00122-013-2056-2

Abstract

Association mapping and linkage mapping were used to identify quantitative trait loci (QTL) and/or causative mutations involved in the control of flowering time in cultivated sunflower Helianthus annuus. A panel of 384 inbred lines was phenotyped through testcrosses with two tester inbred lines across 15 location × year combinations. A recombinant inbred line (RIL) population comprising 273 lines was phenotyped both per se and through testcrosses with one or two testers in 16 location × year combinations. In the association mapping approach, kinship estimation using 5,923 single nucleotide polymorphisms was found to be the best covariate to correct for effects of panel structure. Linkage disequilibrium decay ranged from 0.08 to 0.26 cM for a threshold of 0.20, after correcting for structure effects, depending on the linkage group (LG) and the ancestry of inbred lines. A possible hitchhiking effect is hypothesized for LG10 and LG08. A total of 11 regions across 10 LGs were found to be associated with flowering time, and QTLs were mapped on 11 LGs in the RIL population. Whereas eight regions were demonstrated to be common between the two approaches, the linkage disequilibrium approach did not detect a documented QTL that was confirmed using the linkage mapping approach.

Rengel, D., Arribat, S., Pierre Maury, P., Martin-Magniette, M.L., Hourlier, T., Laporte, M., Varès, D., Carrère, C., Grieu, P., Balzergue, S., Gouzy, J., Vincourt, P., Langlade, N.B (2012)

A Gene- Phenotype Network Based on Genetic Variability for Drought Responses Reveals Key Physiological Processes in Controlled and Natural Environments.

PLoS ONE 7(10): e45249. doi:10.1371/journal.pone.0045249

Abstract

Identifying the connections between molecular and physiological processes underlying the diversity of drought stress responses in plants is key for basic and applied science. Drought stress response involves a large number of molecular pathways and subsequent physiological processes. Therefore, it constitutes an archetypical systems biology model. We first inferred a gene-phenotype network exploiting differences in drought responses of eight sunflower (Helianthus annuus) genotypes to two drought stress scenarios. Large transcriptomic data were obtained with the sunflower Affymetrix microarray, comprising 32423 probesets, and were associated to nine morpho-physiological traits (integrated transpired water, leaf transpiration rate, osmotic potential, relative water content, leaf mass per area, carbon isotope discrimination, plant height, number of leaves and collar diameter) using sPLS regression. Overall, we could associate the expression patterns of 1263 probesets to six phenotypic traits

and identify if correlations were due to treatment, genotype and/or their interaction. We also identified genes whose expression is affected at moderate and/or intense drought stress together with genes whose expression variation could explain phenotypic and drought tolerance variability among our genetic material. We then used the network model to study phenotypic changes in less tractable agronomical conditions, i.e. sunflower hybrids subjected to different watering regimes in field trials. Mapping this new dataset in the gene-phenotype network allowed us to identify genes whose expression was robustly affected by water deprivation in both controlled and field conditions. The enrichment in genes correlated to relative water content and osmotic potential provides evidence of the importance of these traits in agronomical conditions.

Kane NC, Burke J, Marek ML, Seiler G, Vear F, Baute G, Knapp SJ, Vincourt P, Rieseberg LH. (2012)

Sunflower genetic, genomic, and ecological resources.

Mol.Ecol.Resources 13: 10-20

Abstract

Long a major focus of genetic research and breeding, sunflowers (*Helianthus*) are emerging as an increasingly important experimental system for ecological and evolutionary studies. Here, we review the various attributes of wild and domesticated sunflowers that make them valuable for ecological experimentation and describe the numerous publicly available resources that have enabled rapid advances in ecological and evolutionary genetics. Resources include seed collections available from germplasm centres at the USDA and INRA, genomic and EST sequences, mapping populations, genetic markers, genetic and physical maps and other forward- and reverse-genetic tools. We also discuss some of the key evolutionary, genetic and ecological questions being addressed in sunflowers, as well as gaps in our knowledge and promising areas for future research.

Gascuel Q, Martinez Y, Boniface MC, Vear F, Pichon M, Godiard L (2014). The sunflower downy mildew pathogen *Plasmopara halstedii*

Molecular Plant Pathology DOI: 10.1111/mpp.12164 (in press)

Abstract

Downy mildew of sunflower is caused by *Plasmopara halstedii* (Farlow) Berlese & de Toni. *Plasmopara halstedii* is an obligate biotrophic oomycete pathogen that attacks annual *Helianthus* species and cultivated sunflower, *Helianthus annuus*. Depending on the sunflower developmental stage at which infection occurs, the characteristic symptoms range from young seedling death, plant dwarfing, leaf bleaching and sporulation to the production of infertile flowers. Downy mildew attacks can have a great economic impact on sunflower crops, and several *Pl* resistance genes are present in cultivars to protect them against the disease. Nevertheless, some of these resistances have been overcome by the occurrence of novel isolates of the pathogen showing increased virulence. A better characterization of *P. halstedii* infection and dissemination mechanisms, and the identification of the molecular basis of the interaction with sunflower, is a prerequisite to efficiently fight this pathogen. This review summarizes what is currently known about *P. halstedii*, provides new insights into its infection cycle on resistant and susceptible sunflower lines using scanning electron and light microscopy imaging, and sheds light

on the pathogenicity factors of P. halstedii obtained from recent molecular data.