

Efficient cell reprogramming as a target for functional-marker strategies? Towards new perspectives in applied plant-nutrition research

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Summary—Zusammenfassung

The review aims at visualizing and strengthening approximation of current strategies in plant breeding, plant nutrition, and molecular biology. Innovations in new breeding strategies on quantitative traits are based on the development of functional DNA markers. This requires knowledge on robust physiological key reactions or parameters in view of the desired agronomic trait. To understand the significance of adaptive molecular-physiological factors for the expression of agronomic traits in quantitative terms, systems analyses have to demonstrate the phenotypic effect of differential gene activities. The logistic to advance in applied systems biology is currently being strongly discussed. In the present contribution, identification of target cells, which are important for agronomic traits, is stressed as a key for future modeling and virtual experimentation. Integration of target cells in systems analysis should allow to link top-down approaches, that start at the whole-plant level, with bottom-up approaches, that come from the molecular level. To illustrate the importance of adaptive cell reprogramming for agronomic traits, reprogramming of rhizodermic cells to trichoblasts is pointed out in its role for nutrient efficiency (NE). The nature of molecular factors, which may serve as functional markers in breeding, is discussed in view of future marker developments.

Key words: stress adaptation / cell reprogramming / functional marker / global genome regulation / nutrient efficiency / systems biology

1 New strategies in marker-based plant-breeding research require functional biology

Progress in breeding for quantitative traits, such as mineral-nutrient efficiency (NE), is important to support sustainable and cost-effective plant production. However, molecular strategies to assist conventional breeding methods need to be improved. Whereas breeding had been successful during the past without any deeper insights into molecular-physiological mechanisms underlying abiotic-stress tolerance, plant nutritioners as well as molecular biologists are convinced that this knowledge is crucial for future progress in modern breeding. Nevertheless, DNA markers that may assist the selection of quantitative trait loci (QTLs) are normally phenotypic neutral. Also, QTLs are genetically mapped and quantitatively defined

Effiziente Umprogrammierung von Zellen als Ziel funktionaler Markerstrategien?

Dieser Übersichtsartikel zielt darauf ab, die Annäherung zwischen Strategien in Pflanzenzüchtung, Pflanzenernährung und Molekularbiologie sichtbar zu machen und zu verstärken. Innovative Züchtungsstrategien für quantitative Merkmale basieren auf der effizienten Entwicklung funktionaler DNA-Marker. Dies erfordert die Kenntnis robuster physiologischer Schlüsselreaktionen oder Parameter im Hinblick auf das erwünschte agronomische Merkmal. Um die Bedeutung adaptiver molekular-physiologischer Faktoren für die Ausprägung agronomischer Merkmale quantifizieren zu können, müssen Systemanalysen durchgeführt werden, die den Effekt von differenziellen Genaktivitäten auf den Phänotyp ermitteln können. Die logistische Vorgehensweise hierfür wird zur Zeit heftig diskutiert. In dem vorliegenden Beitrag wird der Identifizierung von Zellen, die agronomisch wichtige Merkmale maßgeblich beeinflussen, eine Schlüsselrolle für zukünftiges Modellieren und virtuelles Experimentieren beigegeben. Die Einbeziehung solcher Zielzellen in die Systemanalyse sollte die Verknüpfung von „top-down“-Ansätzen, die von der Gesamtpflanze ausgehen, mit „bottom-up“-Untersuchungen, die von der molekularen Ebene herkommen, ermöglichen. Um die Wichtigkeit adaptiver Zellprogrammierung für agronomische Merkmale zu illustrieren, wird die Umprogrammierung von Rhizodermiszellen zu Trichoblasten in ihrer Bedeutung für die Nährstoffeffizienz (NE) herausgestellt. Die Natur molekularer Faktoren, die als funktionale Marker für die Züchtungsarbeit in Frage kämen, wird im Hinblick auf zukünftige Markerentwicklungen diskutiert.

by their genetic contribution to the final goal, yield or quality. Definition of such loci occurs without direct link to the molecular information underlying localized DNA sequences. So breeders do not need the expertise of plant physiologists/nutritioners? In contrast to earlier expectations, mapped linkages between DNA markers and trait loci are restricted to defined genotypes. They may be lost through recombination and normally do not serve as general diagnostic tools across diverse populations. That is why recent attention towards DNA-marker-assisted breeding is increasingly focusing on appropriate candidate genes for functional-marker (FM) development (Andersen and Lübberstedt, 2003; Neale and Savolainen, 2004). Genetic and statistical mapping methods can provide narrow candidate loci on chromosomes, that may be cloned (“positional cloning”) and sequenced. Analyses of differential expression of gene sequences at the position of QTLs, transgene analyses including also knockout screening as well as complementation tests may contribute to the functional characterization of QTLs (Borevitz and Nordborg, 2003). Targeted polymorphism between genotypes

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may create so-called “gene-targeted markers” (GTM). However, positional cloning to identify QTL loci is not always feasible depending on the genome of interest (*Neale and Savolainen*, 2004). Additionally, polymorphism and its relationship to gene function is based on investigating one allele only, and the polymorphic sequence motif related to function is not known. This bears again the risk of being lost through recombination. The development of FMs depends on successful screening of polymorphism between allelic sequences. This makes it especially laborious and cost-intensive. Also, only few QTLs have large effects on a defined trait. To make the development of FMs for traits like NE more efficient, elucidation of key processes and components with large effects on the agronomic traits of interest is, therefore, required as the very first step to establish innovative marker strategies in breeding (*Andersen and Lübberstedt*, 2003).

2 Top-down versus bottom-up approaches in systems biology to understand plant behavior

To design feasible approaches towards a better understanding of the most important genetic components for stress-tolerant behavior is highly challenging. Environmental stress occurs as a complex of multiple interacting abiotic and biotic factors. According to the rules of chaos, this makes each stress situation unique and unpredictable. Therefore, in real-life plants as sessile organisms have to respond at any stage of development to dynamically changing complex stress conditions. Virtual experimentation is thought to offer great potential for future research on stress adaptation, because the complexity of plant reactions as well as stress factors could be considered more easily. So, systemic thinking and virtual modeling is promising to advance our knowledge in key reactions underlying adaptive strategies in plants. Since years, systems analysis and crop modeling has successfully been practiced in applied agricultural research by considering functional biochemical or physiological processes (for a review see *Sinclair and Seligman*, 1996). Also, mechanistic simulation of the effect of species-specific morphological traits under environmental constraints, such as water- or phosphorus-limited conditions, is currently used to identify favorable plant traits for yield formation (*Sinclair and Muchow*, 2001; *Dechassa et al.*, 2003). Additionally, a huge amount of data is now available in molecular functional biology. Recent progress in large-scale molecular technologies enables simultaneous analysis of the expression profiles of a high number of genes (*Buckhout and Thimm*, 2003; *Zimmermann et al.*, 2004). In this way, deep insights into the complexity of molecular responses in plants under defined nutritional conditions could be achieved (*Maruyama-Nakashita et al.*, 2003; *Wu et al.*, 2003; *Armengaud et al.*, 2004; *Scheible et al.*, 2004; *Wang et al.*, 2004). The increase in molecular data and progress in mathematics and bioinformatics is now revolutionizing the idea of systems biology. Nevertheless, a hot debate is initiated on the feasibility of bottom-up approaches starting at the molecular level to simulate plant behavior (*Katagiri*, 2003; *Hammer et al.*, 2004). For progress in applied systems biology, linking of top-down approaches, that start at the whole-plant level, to molecular bottom-up

approaches is urgently needed (*Hammer et al.*, 2004). An interesting new logistic approach is proposed by the group of *Tardieu* to integrate ecophysiological models and quantitative genetic analysis to predict tolerance behavior (*Tardieu*, 2003). A linear and robust response of the trait “leaf elongation rate” to temperature and water deficit was found by investigating 100 maize recombinant inbred lines. Applying QTL analysis on the parameters of the ecophysiological model enabled prediction of the elongation rates of new recombinant inbred and parental lines under various temperature and water-availability conditions, that accounted for 74% of the observed variability (*Reymond et al.*, 2003). *Tardieu* (2003) discussed the possibility of considering the activity of major genes of the hormone control system as components in ecophysiological modeling. Furthermore, the author proposed to investigate the influence of single genes on whole-plant behavior by including transgenes in simulation studies under various climatic scenarios, before actually starting wet transformation experiments.

Large-scale analyses are normally performed on bulked tissues respectively organs, such as shoot or root, or even on whole seedlings of *Arabidopsis*. However, programs for gene expression are realized at the level of cells. Adaptation to abiotic stress requires besides coordinated actions at the whole-plant level efficient cell redifferentiation in target tissues (*Arnholdt-Schmitt*, 2004). Sensing of hormonal signals and coordinative initiation of new cell programs may limit the realization of hormone-mediated environmental signals at the cell level. Thus, virtual modeling of adaptive reprogramming in target cells may be the most important key to link systems analysis performed at the whole-plant level and molecular bottom-up approaches. The scientific and technological infrastructure to advance molecular studies and systems analysis at the level of plant cells is currently being established in a very efficient way (e.g., *Girke et al.*, 2003). Defining target cells that are causally related to the phenotypic variation of agronomic traits is, therefore, urgently required and a challenge for plant nutritioners. The need for “dialectic” across all biological levels of organization (*Hammer et al.*, 2004) will implicate recursive rounds to fine-tune and decompose systems into smaller parts and consider sections of diverse robustness at all plant levels (*Katagiri*, 2003).

3 Adaptive cell reprogramming in rhizodermic cells as a tool for breeding on nutrient efficiency

Following *Graham* (1984), nutrient efficiency in agronomy refers to the ability of a genotype to achieve high yield production under low nutrient supply. The trait NE is complex at the organism as well as at the molecular level and requires polygenic control. It depends on plant characteristics for nutrient acquisition as well as for nutrient use. The definition of NE may strongly depend on the crop of interest and its use (e.g., root- or seed-producing cultivars of the same crop, such as in *Daucus carota* L.), the mineral nutrient and its chemistry, and the environment considered (see in *Sattelmacher et al.*, 1994; *Arnholdt-Schmitt*, 1999; *Arnholdt-Schmitt*, 2004).

Nevertheless, in several important agricultural and horticultural crops, the amount of root hairs is highly adaptive in response to nutritional constraints. Under comparable genetic and environmental conditions, the formation and number of root hairs may be highly dependent on the availability of mineral nutrients, such as phosphate, nitrate, and iron. Thus, adaptive root-hair formation is recognized as one of the most prominent candidate traits for high nutrient efficiency (Jungk, 2001). Complex interactions between root characteristics, the availability of water, mineral chemistry, as well as physical and chemical soil properties determine the potentials for nutrient acquisition. Root hairs are more efficient than root cylinders to attract nutrients by diffusion from soil and improve the transfer of nutrients from soil to plants by diverse effects. In recent years, great efforts have been made to increase our understanding of the genetics of root-hair development. Screening of ecotypic mutants of *Arabidopsis* has been especially helpful to identify genes involved. Several genes are now known to contribute to cell-fate specification and initiation of root hairs in the rhizodermis (see Grierson et al., 2001; Müller and Schmidt, 2004). Nutrient-derived changes in rhizodermis patterning seem to be related primarily to differential regulation of those genes involved in the first stages of differentiation. Different environmental factors, such as depletion of phosphorus or of iron, seem to induce different activation cascades of genes involved in root-hair development (Müller and Schmidt, 2004). The effect of nutrient depletion is known to be mediated by hormones, mainly ethylene and auxin (Dolan, 2001; Michael, 2001; Schmidt, 2001; Shi and Zhu, 2002). Experimental support was currently provided for the suggestion, that ethylene is mainly involved in the mediation of environmental effects but may not have *per se* an impact on the genetic control of root-hair formation in development (Cho and Cosgrave, 2002). Endogenous ethylene blockage did not affect normal root-hair formation, however, promotor elements of root-hair-specific expansin genes responded to either ethylene, auxin, and root separation from the agar medium. Thus, the authors conclude that root-hair formation will be determined through two distinct but interacting signaling pathways, related to the developmental program in ontogenesis and induction through environment. These observations are in contrast to earlier expectations based on mutants and have to be confirmed. Although it is currently becoming clear that auxin is essential for the first steps in root-hair development, cross talk between ethylene and auxin makes it difficult to reveal independent effects on root-hair initiation. Polar auxin transport is generally known to be a driving force to mediate the action of auxin (Goldsmith, 1977; Muday and Murphy, 2002). Rahman et al. (2002) focused on the interaction between ethylene and auxin during *Arabidopsis* root-hair development. They suggested that, once cell speciation is achieved, auxin acts as a positive regulator for ethylene-mediated effects and stressed the significance of the auxin-influx modulator CSI on root-hair development. Related to cell-fate redetermination under the influence of environment, Cho and Cosgrave (2002) suggest a role for ethylene rather downstream of auxin-mediated effects. In their experiments, blocking of ethylene perception by a specific ethylene antagonist inhibited auxin-induced root-hair formation. Nevertheless, Zhang et al. (2003) found that impaired auxin efflux diminished positive

effects of low phosphorus supply on trichoblast cell-file numbers as a base for further root-hair initiation. This effect was independent of ethylene. pH-induced transverse cortical microtubule randomization in lettuce was shown to be essential for root-hair initiation and depended on auxin regulation (Takahashi et al., 2003). Also Webb et al. (2002) suggest that microtubules play an important role in cell specification. Their results demonstrate that mutation of a katanin gene changed specification patterns in the rhizodermis of *Arabidopsis*. Katanin proteins are expected to disintegrate microtubules. The results of Knox et al. (2003) revealed control of the timing of root-hair differentiation by the relative abundance of two genes in the auxin signalling-cascade, SHY2 and AXR3. These genes belong to the so-called Aux/IAA family, finally involved in protein degradation through 26S proteasome. Interactions between hormone concentration and sensitivity is still not well understood, but may be elucidated in future approaches based on simultaneous analysis of differential regulation of molecular factors and pathways involved in these traits (e.g., Scheible et al., 2004). Recently, Jones and Grierson (2003) succeeded in the isolation of root-hair cells and could obtain cell-specific cDNA libraries. This success points to rapid future progress in our knowledge on molecular activities linked to hormone sensitivity and reprogramming of rhizodermic cells.

The capability of plants to adapt to low nutrient availability through increased root-hair formation is genetically determined at the species and subspecies level (see in Jungk, 2001; Wissuwa and Ae, 2001). Additionally, basically all rhizodermis cells seem to be inducible to a root-hair fate (Bünning, 1951; Takahashi et al., 2003). This may offer the possibility for breeding on molecular traits related to efficient cell reprogramming in the rhizodermis. Elucidation of important positive and negative regulators in rhizodermis cell-fate determination together with appropriate approaches to calculate the quantitative importance of these factors will be crucial to advance towards identifying potential FMs for breeding on NE. Also, differentiation of transfer cells in the rhizodermis plays an important role in nutrient-stress adaptation and should be considered as target (see Arnholdt-Schmitt, 2004).

Further efforts are required to define candidate target cells of complex agronomic traits. This will have to occur mainly at species-specific levels. For example, carrot plants belong to a species, where root hairs are less important for nutrient acquisition (Itoh and Barber, 1983; Dechassa et al., 2003). In this crop, cytokinin levels in the tap root together with developmentally determined cytokinin sensitivity of the root cells are critical components for yield formation. The cytokinin concentration varies in response to low phosphate and nitrogen. Target cells for both yield formation and environmental responses are the cambial root cells. Strong genetic differences in cytokinin sensitivity of the secondary root phloem cells, that originate directly from the small layer of cambial cells, could be revealed for parental lines and a hybrid of a heterosis system by help of an *in-vitro* test system (Arnholdt-Schmitt, 1999). Recently, microarrays of *Arabidopsis* seedlings revealed differential regulation of genes involved in both cytokinin synthesis and signaling in response to nitrogen (Scheible et al., 2004).

4 Searching for key genes in cell programming as candidates for FMs

Already in the seventies, the concept of single rate-limiting enzymes for metabolic control was substituted by a more quantitative theory (see *Morandini and Salamini, 2003*). Nevertheless, hampered by the enthusiasm for the promising power of gene technology only late in the nineties, insight finally developed that single genes from distinct pathways could not account as unique “bottle-necks” for quantitative traits (e.g., *Stitt, 1997; Morandini and Salamini, 2003*). Also, in various reverse genetic approaches, plant metabolism demonstrated high flexibility through alternative pathways. Large-scale experiments reveal the high complexity in response to environment through down- and up-regulation of large numbers of genes measured at the whole-organ or plant level. These cover genes of various functions. Besides genes of proteins which are directly involved in metabolism, energy supply, and cell structure, differential expression was also shown for regulatory proteins, such as transcription factors, and genes involved in signal-transduction cascades like the mitogen-activated protein-kinase system (MAPK), and genes involved in hormone synthesis or sensitivity, as well as genes related to protein modification and degradation. Additionally, recent technological progress is supposed to enable multi-element analyses at the tissue or cell-type levels (*Salt, 2004*). A simultaneous flux of all these components charac-

terizes cell programs. It seems to be obvious, that directed reprogramming of cells in adaptation to environments needs coordinative regulation. Factors involved in program decisions for cell fate and global coordination are thus especially hot candidates for FMs.

Candidate key genes for agronomic traits may serve as FMs, only if polymorphic motifs will be discovered within the gene sequences and if this polymorphism is related to substantial phenotypic variation. To identify such kind of allelic sequence polymorphisms, extensive candidate gene-based association studies and/or the establishment of isogenic lines are required (*Andersen and Lübberstedt, 2003*). A scheme of the general steps in FM development can be found in Fig. 1. Development of FMs is highly laborious and time-consuming. Focusing on a lower number of functional genes that have large effects on a quantitative trait of interest will substantially increase the efficiency of marker development and marker-assisted selection, reducing overall costs at the same time. This makes the definition of appropriate key genes related to cell programming the most critical task for future success and acceptance of this breeding strategy.

Knowledge about global genome regulation related to environmental plasticity of plants is in its very infancy. General mechanisms and a discussion on its potential role in coordinating stress-induced cell reprogramming can be found

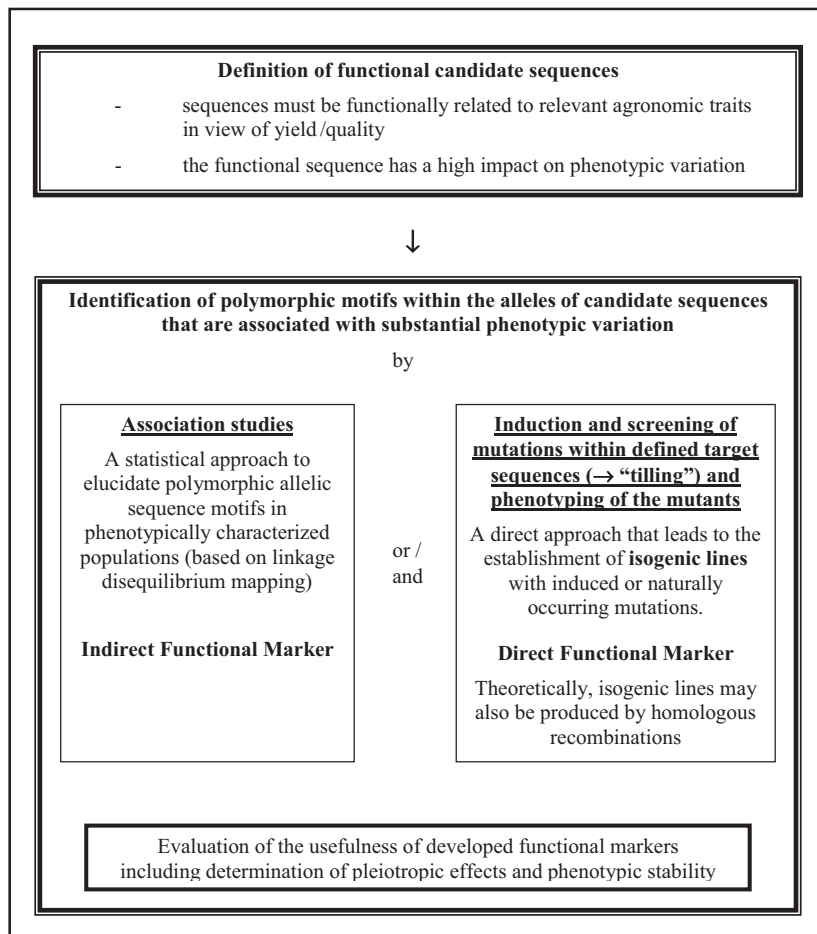


Figure 1: Scheme on general steps in functional-marker development (based on *Andersen and Lübberstedt, 2003*).

Abbildung 1: Schema der generellen Vorgehensweise zur Entwicklung funktionaler Marker (nach *Andersen und Lübberstedt, 2003*).

in a recent update (Arnholdt-Schmitt, 2004). Factors involved in global genome regulation are related to chromatin organization and gene positioning at the nucleus level. Crucial components for the flexibility in genome organization, that should be critically considered in view of environmental plasticity, are chromatin remodeling, the looping and differential attachment of binding sites of chromosomes, DNA methylation as well as DNA arrangements. In Fig. 2, these components are shown in their relationship to the hierarchy of structural genome organization. DNA arrangements change the basic linear organization of the genome and are involved in the second and third higher level of genome organization (see in Arnholdt-Schmitt, 2004). This makes DNA rearrangements especially interesting for research on environmentally induced plasticity in genome coordination. Epigenetic regulation of genes by RNA interference (RNAi) through posttranscriptional gene silencing is a hot topic in recent medicinal and biological research. Current results point to a pivotal role of RNAi also for gene silencing through targeted methylation or formation and maintenance of heterochromatin structures (see Volpe et al., 2002). Small RNAs and heterochromatin may also be involved in programmed elimination of DNA, as could be demonstrated in the ciliate *Tetrahymena* (Mochizuki et al., 2002; Taverna et al., 2002). Nonrandom and reversible loss of repetitive DNA was suggested to be involved in determining the growth potential of cultured carrot-root explants (Arnholdt-Schmitt, 1995). Especially interesting in view of hormone-mediated induction of cell reprogramming is the recent observation that a dsRNA-binding protein, HYL1 of *Arabidopsis*, is involved in the sensitivity to auxin, cytokinin, and abscisic acid (Han et al., 2004). miRNAs were shown to regulate genes involved in the promotion of adaxial identity and meristem maintenance in *Arabidopsis* (Engstrom et al., 2004).

Girke et al (2003) stressed the importance of *in-vitro* cultures for basic studies applying cellular and tissue arrays and systems analysis. They proposed the development of plant-cell cultures that could maintain tissue identity. Additionally, rapid and reproducible reprogramming of differentiated cells can

be studied through primary cultures of defined tissue explants. In this way, DNA analysis of the secondary carrot root phloem could reveal physiologically dependent changes in DNA organization through different mechanisms involved in global genome regulation (Arnholdt-Schmitt, 1995; Arnholdt-Schmitt et al., 1995; Schaefer et al., 2000; Schaffer and Arnholdt-Schmitt, 2001). The role of differential attachment binding sites of chromosomes (Nickerson, 2001) in plant development and plasticity has still to be confirmed. Matrix attachment regions (MAR) are thought to be importantly involved in structural gene regulation through formation of independent transcription units (see Arnholdt-Schmitt, 2004; Rudd et al., 2004). A MAR-binding protein (MFP1) has been associated with development and organ-specificity in plants (Harder et al., 2000). Interestingly, differential expression of a MAR-binding protein (NtMARBP61) was observed in growth-induced tobacco cells (Fujiwara et al., 2002). Rudd et al. (2004) performed a bioinformatic-based study of MAR sequences across the *Arabidopsis* genome. The results revealed a positive correlation between intragenic MARs and transcriptional down-regulation of respective genes. As could be expected from the stabilizing effect of surrounding MARs on transgene expression, the bulk of MARs was found in intergenic positions. Fojtová et al. (2002) proposed an important structural role of MARs also for the early recovering from transient cadmium stress.

5 Concluding remarks

Functional marker-based strategies in plant breeding now offer highly promising perspectives through combining the competencies from plant genetics, plant nutrition/physiology, and molecular biology in favor of crop improvement. Figure 3 summarizes important strategic steps to elucidate molecular-physiological key parameters and components for quantitative agronomic traits, such as abiotic-stress tolerance. The most important bottle-neck for the linkage of whole-plant-based systems analysis and molecular approaches will be the identification of prominent target cells in plant responses

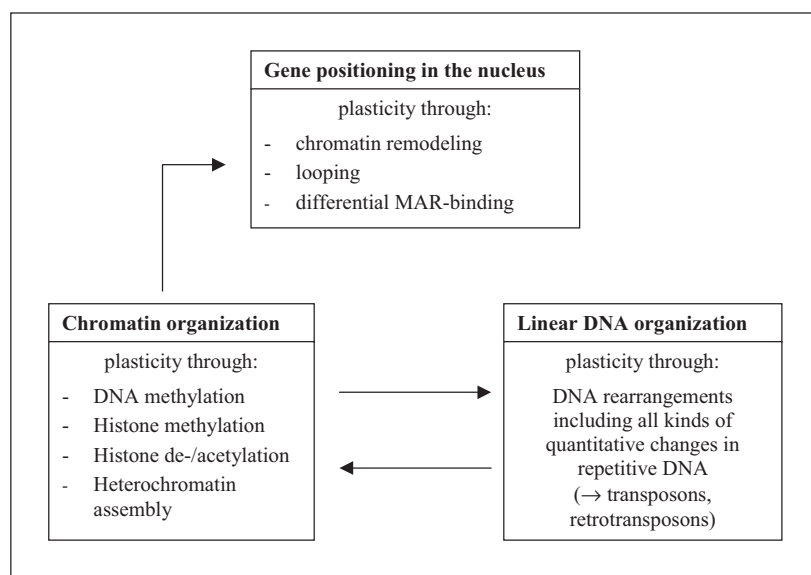


Figure 2: Hierarchical levels of structural genome organization and allocation of mechanisms involved in flexible genome organization during ontogenesis (based on Arnholdt-Schmitt, 2004).

Abbildung 2: Hierarchische Ebenen der strukturellen Genomorganisation und die Zuordnung von Mechanismen, die an der flexiblen Genomorganisation während der Ontogenese beteiligt sind (nach Arnholdt-Schmitt, 2004).

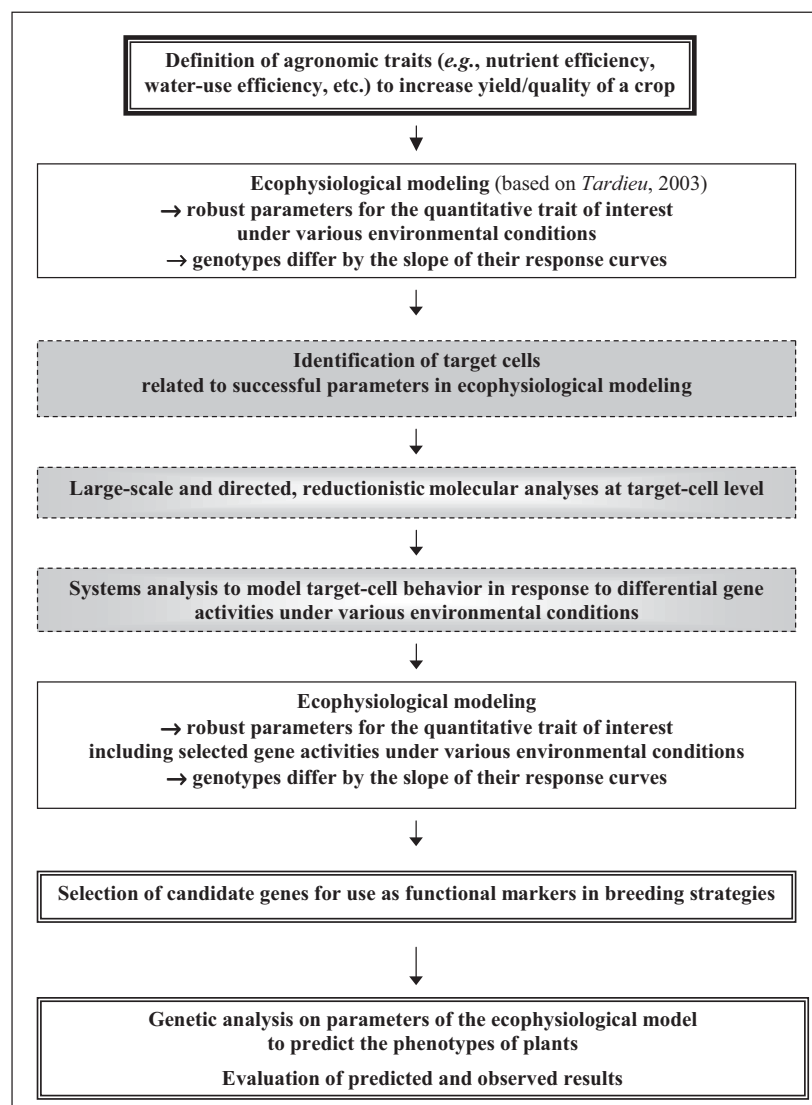


Figure 3: Logistic steps to reveal molecular-physiological key reactions or parameters for abiotic-stress-tolerance behavior—an attempt to link whole-plant analysis and molecular approaches in systems biology.

Abbildung 3: Logistische Schritte zur Aufdeckung molekular-physiologischer Schlüsselreaktionen oder -parameter für Toleranzverhalten gegenüber abiotischem Stress – ein Versuch, Ganzpflanzenanalysen und molekulare Ansätze in der Systembiologie zu verbinden.

to the environment. Whereas the first three tasks in the scheme of Fig. 3, including definition of agronomic traits, ecophysiological modeling, and the identification of target cells, will typically be the responsibilities of plant nutritioners or physiologists, the molecular analyses at plant-cell level and cell modeling is more related to molecular biologists together with systems biologists. Adequate approaches for systems biology at cell level will depend on further progress in plant-cell technologies, mathematics, and bioinformatics. Concerted actions are needed to integrate differential gene expression in ecophysiological models and to select candidate genes. Genetic analyses to predict phenotypes demand cooperative working of plant geneticists and plant nutritioners/physiologists (see in Tardieu, 2003; Reymond et al., 2003) and may directly initiate functional-marker development by revealing polymorphic sequence motifs within candidate genes (see Fig. 1). Overall, a better understanding of the molecular clues of efficient reprogramming in target cells during stress adaptation will bring research in plant nutrition and molecular biology a significant step forward towards application in breeding strategies.

References

- Andersen, J. R., Lübberstedt, T. (2003): Functional markers in plants. *Trends Plant Sci.* 8, 554–560.
- Armengaud, P., Breitling, R., Amtmann, A. (2004): The potassium-dependent transcriptome of *Arabidopsis* reveals a prominent role of jasmonic acid in nutrient signaling. *Plant Physiol.* 136, 2556–2576.
- Arnholdt-Schmitt, B. (1995): Physiological aspects of genome variability in tissue culture. II. Growth phase-dependent quantitative variability of repetitive BstNI fragments of primary cultures of *Daucus carota* L. *Theor. Appl. Genet.* 91, 816–823.
- Arnholdt-Schmitt, B. (1999): On the physiology of yield production in carrots. – Implications for breeding towards nutrient efficiency. *Gartenbauwissenschaft* 64, 26–32.
- Arnholdt-Schmitt, B. (2004): Stress-induced cell reprogramming. A role for global genome regulation? *Plant Physiol.* 136, 2579–2586.
- Arnholdt-Schmitt, B., Herterich, S., Neumann, K.-H. (1995): Physiological aspects of genome variability in tissue culture. I. Growth phase-dependent differential DNA methylation of the carrot genome (*Daucus carota* L.) during primary culture. *TAG* 91, 809–815.

- Borevitz, J. O., Nordborg, M. (2003): The impact of genomics on the study of natural variation in *Arabidopsis*. *Plant Physiol.* 132, 718–725.
- Buckhout, T., Thimm, O. (2003): Insights into metabolism obtained from microarray analysis. *Curr. Op. Plant Biol.* 6, 288–296.
- Bünning, E. (1951): Über die Differenzierungsvorgänge in der Cruciferenwurzel. *Planta* 39, 126–153.
- Cho, H.-T., Cosgrove, D. J. (2002): Regulation of root hair initiation and expansin gene expression in *Arabidopsis*. *The Plant Cell* 14, 3237–3253.
- Dechassa, N., Schenk, M. N., Claassen, N., Steingrobe, B. (2003): Phosphorus efficiency of cabbage (*Brassica oleracea* L. var. capitata), carrot (*Daucus carota* L.) and potato (*Solanum tuberosum* L.). *Plant Soil* 250, 215–224.
- Dolan, L. (2001): The role of ethylene in root hair growth in *Arabidopsis*. *J. Plant Nutr. Soil Sci.* 164, 141–145.
- Engstrom, E. M., Izhaki, A., Bowman, J. L. (2004): Promoter bashing, microRNAs, and Knox genes. New insights, regulators, and target-of-regulation in the establishment of lateral organ polarity in *Arabidopsis*. *Plant Physiol.* 135, 685–694.
- Fojtová, M., Fulnecková, J., Fajkus, J., Kovarik, A. (2002): Recovery of tobacco cells from cadmium stress is accompanied by DNA repair and increased telomerase activity. *J. Exp. Bot.* 53, 2151–2158.
- Fujiwara, S., Matsuda, N., Sato, T., Sonobe, S., Maeshima, M. (2002): Molecular properties of a matrix attachment region-binding protein located in the nucleoli of tobacco cells. *Plant Cell Physiol.* 43, 1558–1567.
- Girke, T., Ozkan, M., Carter, M., Raikhel, N. V. (2003): Towards a modeling infrastructure for studying plant cells. *Plant Physiol.* 132, 410–414.
- Goldsmith, M. H. M. (1977): The polar transport of auxin. *Annu. Rev. Plant Physiol.* 28, 439–478.
- Graham, R. D. (1984): Breeding for nutritional characteristics in cereals, in Tinker, P. B., Läuchli, A.: *Advances in Plant Nutrition*, Vol. 1. Praeger Publ., New York, pp. 57–102.
- Grierson, C. S., Parker, J. S., Kemp, A. C. (2001): *Arabidopsis* genes with roles in root hair development. *J. Plant Nutr. Soil Sci.* 164, 131–140.
- Hammer, G. L., Sinclair, T. R., Chapman, S. C., van Oosterom, E. (2004): On systems thinking, systems biology, and the in silico plant. *Plant Physiol.* 134, 909–911.
- Han, M.-H., Goud, S., Song, L., Fedoroff, N. (2004): The *Arabidopsis* double-stranded RNA-binding protein HYL1 plays a role in microRNA-mediated gene regulation. *PNAS* 101, 1093–1098.
- Harder, P. A., Silverstein, R. A., Meier, I. (2000): Conservation of matrix attachment region-binding filament-like protein 1 among higher plants. *Plant Physiol.* 122, 225–234.
- Itoh, S., Barber, S. A. (1983): Phosphorus uptake by six plant species as related to root hairs. *Agron. J.* 75, 457–461.
- Jones, M. A., Grierson, C. S. (2003): A simple method for obtaining cell-specific cDNA from small numbers of growing root-hair cells in *Arabidopsis thaliana*. *J. Exp. Bot.* 54, 1373–1378.
- Jungk, A. (2001): Root hair and the acquisition of plant nutrients from soil. *J. Plant Nutr. Soil Sci.* 164, 121–129.
- Katagiri, F. (2003): Attacking complex problems with the power of systems biology. *Plant Physiol.* 132, 417–419.
- Knox, K., Grierson, C. S., Leyser, O. (2003): AXR3 and SHY2 interact to regulate root hair development. *Development* 130, 5769–5777.
- Maruyama-Nakashita, A., Inoue, E., Watanabe-Takahashi, A., Yamaya, T., Takahashi, H. (2003): Transcriptome profiling of sulfur-responsive genes in *Arabidopsis* reveals global effects of sulfur nutrition on multiple metabolic pathways. *Plant Physiol.* 132, 597–605.
- Michael, G. (2001): The control of root hair formation: suggested mechanisms. *J. Plant Nutr. Soil Sci.* 164, 111–119.
- Mochizuki, K., Fine, N. A., Fujisawa, T., Gorovsky, M. A. (2002): Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in *Tetrahymena*. *Cell* 110, 689–699.
- Morandini, P., Salamini, F. (2003): Plant biotechnology and breeding: allied for years to come. *Trends Plant Sci.* 8, 70–75.
- Muday, G. K., Murphy, A. S. (2002): An emerging model of auxin transport regulation. *Plant Cell* 14, 293–299.
- Müller, M., Schmidt, W. (2004): Environmentally induced plasticity of root hair development in *Arabidopsis*. *Plant Physiol.* 134, 409–419.
- Neale, D. B., Savolainen, O. (2004): Association genetics of complex traits in conifers. *Trends Plant Sci.* 9, 325–330.
- Nickerson, J. A. (2001): Experimental observations of a nuclear matrix. *J. Cell Sci.* 114, 463–474.
- Rahman, A., Hosokawa, S., Oono, Y., Amakawa, T., Nobuharu, G., Tsurumi, S. (2002): Auxin and ethylene response interactions during *Arabidopsis* root hair development dissected by auxin influx modulators. *Plant Physiol.* 130, 1908–1917.
- Reymond, M., Muller, B., Leonardi, A., Charcosset, A., Tardieu, F. (2003): Combining quantitative trait loci analysis and an ecophysiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. *Plant Physiol.* 131, 664–675.
- Rudd, S., Frisch, M., Grote, K., Meyers, B. C., Mayer, K., Werner, T. (2004): Genome-wide in silico mapping of scaffold/matrix attachment regions in *Arabidopsis* suggests correlation of intragenic scaffold/matrix attachment regions with gene expression. *Plant Physiol.* 135, 715–722.
- Salt, D. E. (2004): Update on plant ionomics. *Plant Physiol.* 136, 2451–2456.
- Sattelmacher, R., Horst, W. J., Becker, H. D. (1994): Factors that contribute to genetic variation for nutrient efficiency of crop plants. *Z. Pflanzenernähr. Bodenkd.* 157, 215–224.
- Schaefer, C., Schaffer, S., Arnholdt-Schmitt, B. (2000): Differential RAPD fingerprints in carrot tissues. *Acta Hort.* 530, 437–445.
- Schaffer, S., Arnholdt-Schmitt, B. (2001): Characterization of genome variation in tissue cultures by RAPD fingerprinting – a methodical comment. *Plant Biosystems* 135, 115–120.
- Scheible, W.-R., Morcuende, R., Czechowski, T., Fritz, C., Osuna, D., Palacios-Rojas, N., Schindelasch, D., Thimm, O., Udvardi, M. K., Stitt, M. (2004): Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of *Arabidopsis* in response to nitrogen. *Plant Physiol.* 136, 2483–2499.
- Schmidt, W. (2001): From faith to fate: Ethylene signaling in morphogenic responses to P and Fe deficiency. *J. Plant Nutr. Soil Sci.* 164, 147–154.
- Shi, H., Zhu, J.-K. (2002): SOS4, a pyridoxal kinase gene, is required for root hair development in *Arabidopsis*. *Plant Physiol.* 129, 585–593.
- Sinclair, T. R., Muchow, R. C. (2001): System analysis of plant traits to increase grain yield on limited water supplies. *Agron. J.* 93, 263–270.
- Sinclair, T. R., Seligman, N. G. (1996): Crop modelling: From infancy to maturity. *Agron. J.* 88, 698–704.

- Stitt, M. (1997): Biochemical principles underlying genetic manipulation of physiological "bottle-necks". *Vort. Pflanzenzüchtg.* 41, 5–20.
- Takahashi, H., Kawahara, A., Inoue, Y. (2003): Ethylene promotes the induction by auxin of the cortical microtubule randomization required for low-pH-induced root hair initiation in lettuce (*Lactuca sativa* L.) seedlings. *Plant Cell Physiol.* 44, 932–940.
- Tardieu, F. (2003): Virtual plant: modelling as a tool for the genomics of tolerance to water deficit. *Trends Plant Sci.* 8, 9–14.
- Taverna, S. D., Coyne, R. S., Allis, C. D. (2002): Methylation of histone H3 at lysine 9 targets programmed DNA elimination in *Tetrahymena*. *Cell* 110, 701–711.
- Volpe, T. A., Kidner, C., Hall, I. M., Teng, G., Grewal, S. I. S., Martienssen, R. A. (2002): Regulation of heterochromatic silencing and histone H3 Lysine-9 methylation by RNAi. *Science* 297, 1833–1837.
- Wang, R., Tischner, R., Gutiérrez, R. A., Hoffman, M., Xing, X., Chen, M., Coruzzi, G., Crawford, N. M. (2004): Genomic analysis of the nitrate response using a nitrate reductase-null mutant of *Arabidopsis*. *Plant Physiol.* 136, 2512–2522.
- Webb, M., Jouannic, S., Foreman, J., Linstead, P., Dolan, L. (2002): Cell specification in the *Arabidopsis* root epidermis requires the activity of ECTOPIC ROOT HAIR 5 – a katanin-p60 protein. *Development* 129, 123–131.
- Wissuwa, M., Ae, N. (2001): Genotypic differences in the presence of hairs on roots and gynophores of peanuts (*Arachis hypogaea* L.) and their significance for phosphorus uptake. *J. Exp. Bot.* 52, 1703–1710.
- Wu, P., Ma, L., Hou, X., Wang, M., Wu, Y., Liu, F., Deng, X. W. (2003): Phosphate starvation triggers distinct alterations of genome expression in *Arabidopsis* roots and leaves. *Plant Physiol.* 132, 1260–1271.
- Zhang, Y. J., Lynch, J. P., Brown, K. M. (2003): Ethylene and phosphorus availability have interacting yet distinct effects on root hair development. *J. Exp. Bot.* 54, 2351–2361.
- Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L., Gruissem, W. (2004): GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. *Plant Physiol.* 136, 2621–2632.