

07-03: From *Arnica montana* to *Taraxacum koksaghyz* NMR-based metabolite profiling supporting breeders

Roland Geyer, Christoph Dotzer, Michael Rettig, Steffen Heelemann, Andreas Krumpel, Renate Kirchhöfer, Fritz Huber

lifespın GmbH, Regensburg, Germany

E-mail: roland.geyer@lifespın.de

Modern nuclear magnetic resonance (NMR)-techniques combined with software solutions for data analyses and comprehensive databases provide powerful tools for plant sciences and breeding. Lifespın realized NMR-based systems perfectly suited for analytical accompaniment of breeding programs – providing both, quantitative metabolite data in sufficient quality and deepness and affordability for breeders. Therefore, the advantages of NMR, to be quantitative by nature, a universal and unbiased detection principle and the broad dynamic range, are enhanced with high-throughput amenability, reproducibility and easy-to use handling.

Three examples will be presented, from uni- to multivariate quantitative metabolite profiles and their respective use in breeding projects.

Based on Arnica and Russian Dandelion is demonstrated that, starting from the wild material, high breeding progress can be achieved in the first selection cycles when breeders and analytics team up. This is necessary when facing the dilemma of an increasing demand for medicinal and herbal plants material from controlled, documented, verifiable, domestic cultivation and the fact that the cultivated product is compared in price with the price of the product from wild collections. Thus, the quality of the material to be grown needs to be clearly differentiated from the wild collection.

An important precondition to succeed in this ambitious environment is, that there is sufficient capacity available to analyze output traits and lead ingredients. As mentioned above, such can be realized for example by means of NMR with appropriate software-based data analysis.

Both projects were supported over several years and more than 10.000 analyzed single plant samples. Optimized sample preparation processes, without any separation or derivatization, and measurement times of less than 5 minutes per sample enable the analysis of more than 100 samples per day. The NMR itself provides a walk-away capacity of even more than 500 samples. Subsequently, the resulting spectra are automatically processed (referencing, phase correction, base line correction) and cover a dynamic range of more than six orders of magnitude. Finally, the target compounds (dandelion: *cis*-polyisoprene, arnica: total and specific sum of helenalines and dihydrohelenalines) are quantified and verified by inter- and intra-serial quality control routines.

Beside targeted quantification, comprehensive metabolite profiles can be recorded and used, as demonstrated by the third example, the project "DRYeGRASS". Perennial ryegrass as one of the most important forage grass species in Europe will be particularly affected by global climate change. Current varieties have no distinct drought tolerance, therefore competitiveness as well as yield will decline in areas impacted by summer drought. Aim of the project DRYeGRASS is to improve tolerance to temporary drought in the sense of good recovery after drought in an efficient way assisted by a combination of innovative selection methods. One of these methods is NMR-based metabolite profiling, resulting in quantitative data on more than 100 metabolites (e.g. known drought stress related amino acids like proline or several carbohydrates). Furthermore, the metabolite profiles are correlated with

data from observations under rain-out shelter conditions. First results and putative biomarkers/metabolite profiles for drought tolerance will be presented.

References

- [1] GEYER, R., DOTZER, C., RETTIG, M., WANDERNOTH, S., PFAHLERT, V., and F. HUBER, 2015: Automated qNMR in high-throughput Quantification of rubber, inulin and determination of inulin degree of polymerization in dandelion roots. PLANTS2030 Statusseminar, Potsdam.
- [2] STOLZE, A., WANKE, A., VAN DEENEN, N., GEYER, R., PRÜFER, D., and C. SCHULZE, 2017: Gronover, Development of rubber-enriched dandelion varieties by metabolic engineering of the inulin pathway. *Plant Biotechnol J.*, **15**, 740-753.
- [3] GEYER, R., EICKMEYER, F., RETTIG, M., HEELEMANN, S., and R. KIRCHHÖFER, 2018: Bedeutung einer effizienten Charakterisierung pflanzlicher Extrakte für die Züchtung und den Übergang von der Wildsammlung zum kontrollierten Anbau. 9. Tagung Arznei- und Gewürzpflanzenforschung des Deutschen Fachausschusses für Arznei-, Gewürz- und Aromapflanzen; Bonn, 2018. Published in: *Julius-Kühn-Archiv*, **460**, 72-75
- [4] WESTERMEIER, P., GEYER, R., WILLNER, E., FEUERSTEIN, U., SCHULZE, S., BÖHM, C., LÜTKE ENTRUP, S. and S. HARTMANN, 2018: Genetische Analyse der Trockenstresstoleranz bei Deutschem Weidelgras (*Lolium perenne* L.) mittels phänologischer, physiologischer und molekularer Differenzierungsmethoden (DRYeGRASS). Innovationstage der BLE; Bonn.