



NGS technologies for plant pests diagnostics in Romania: where we are and what we aim?



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Introduction

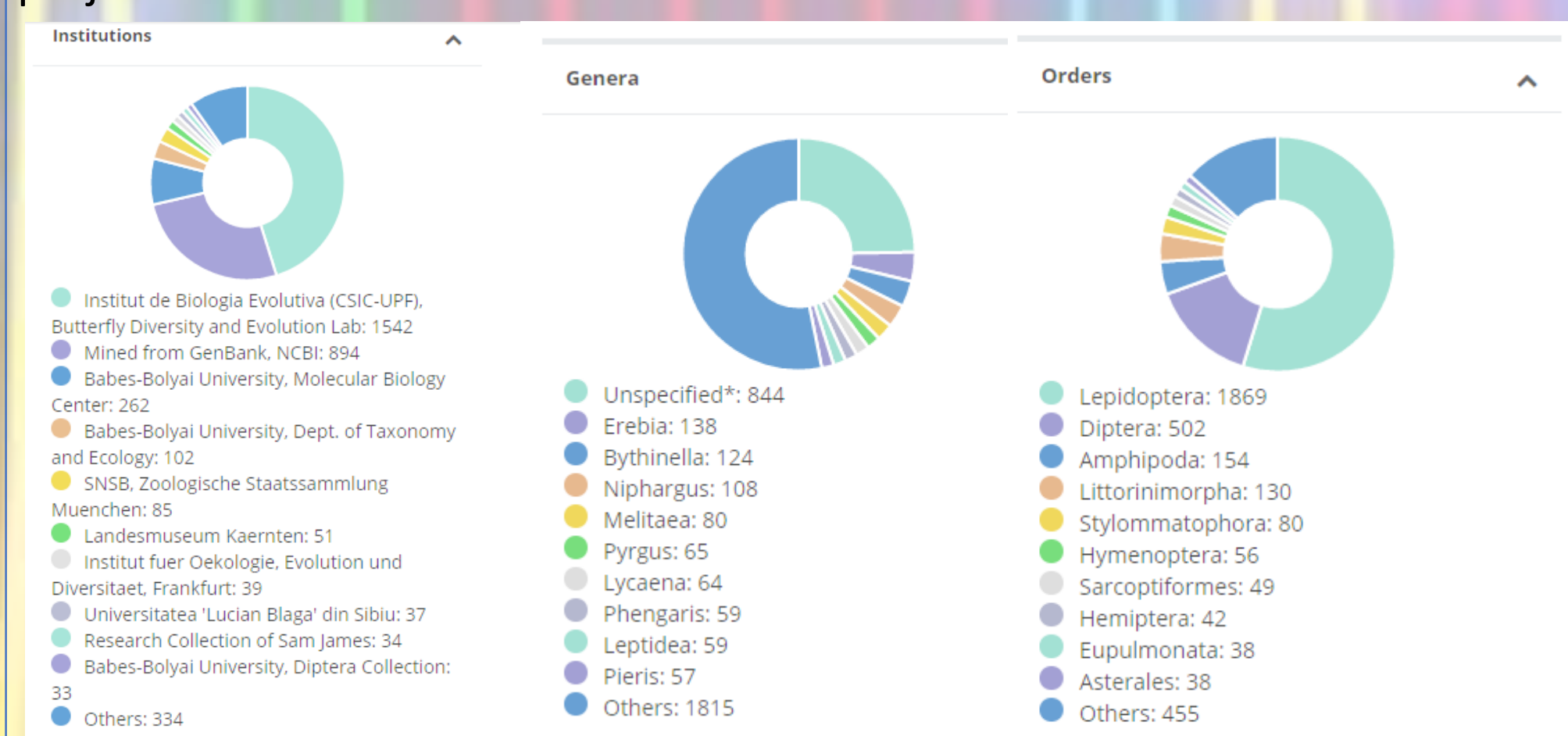
The rapid identification and throughout characterization of different pests and diseases are two decisive steps in the fight for the increasingly difficult struggle of plant protectionists for plant health. Year after year new methods are put in place, however, not all the diagnostic issues have been solved. The available molecular tests are still unable to detect emerging genetic features of the continuously evolving pathogens that spread in the environment, plants, animals and humans, as well as analyze the complex samples which contain multiple organisms. The Next Generation Sequencing technology includes all "non-Sanger-based high-throughput DNA sequencing technologies", by which billions of DNA strains can be sequenced in parallel, with high accuracy, fewer efforts, faster and cheaper. For human health, NGS started to be used as a routine diagnostic tool in the last years and the majority of samples were either outbreak investigations or genotyping of highly resistant micro-organisms.

In the plant protection field, the NGS already has many different applications – diagnosis of known or unknown species on samples with obvious or hidden symptomatology of and other research & diagnostic applications are still expected to arise. Many of the EPPO countries are already using the NGS technology for plant diagnostics as a daily practice, some others, including Romania, are still in the phase of learning about it.

We describe the current situation of NGS utilization in Romania in the field of plant protection, the infrastructure that we could eventually use if we would start using the NGS and we present fears, challenges and question marks related to this subject.

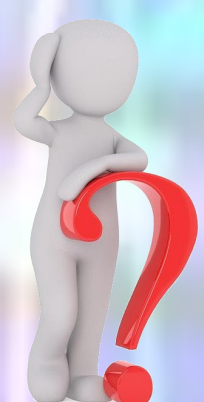
Where we are?

The NGS technology started to be used in Romania first in the medical domain, then in the paleontological research, speology, biodiversity studies and finally in plant protection. To illustrate this, on ScienceDirect there are 2585 references that respond to the quest "next generation sequencing"+ "Romania", but if we look at the authors names and their affiliations, only very few are presenting research performed in Romania. On BOLDSYSTEM, if we just look for sequence records, without adding NGS criteria, we found 3413 published records, deposited in 66 institutions, of which the first one, with 1542 records is an institution from Spain. From Romania, the Babes-Bolyai University from Cluj- Napoca and the University 'Lucian Blaga' from Sibiu seem to be the leading institutions working with molecular biology and sequences. If we look at the analyzed genera, the vast majority are butterflies and few freshwater species, analyzed mainly inside biodiversity projects.



On plant protection detections by phytosanitary institution, NGS is seldom used, even when it comes about quarantine species. When necessary, the samples are sent to other EU partner institutions, to have the sequencing & data analyses performed, mainly with a research & publishing agreement.

For research, there are 4 Romanian researchers involved in COST Action FA1407: "Application of next generation sequencing for the study and diagnosis of plant viral diseases in agriculture", affiliated to Research Institute for Fruit Growing Pitesti, Fruit Research and Development Station Bistrita and Faculty of Biology and Geology of Cluj-Napoca.

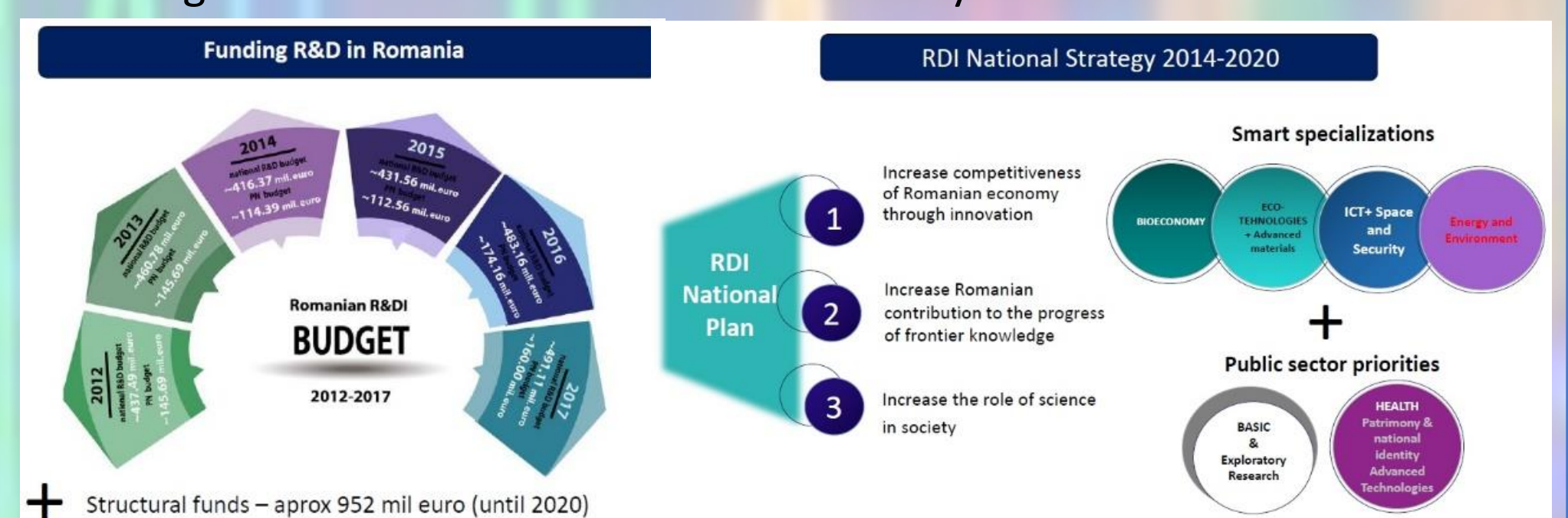


If other examples of NGS activities done by Romanians or in Romania are at your knowledge, please share it with me. Thank you!



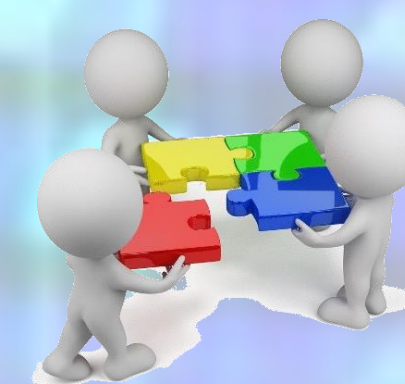
What we aim?

Romania is looking forward filling the gaps from R&D area by increasing the national economy access to innovation, by increasing the rate of participation of Romanian researchers into multinational RDI programs & projects and by increasing the role of science in the citizens daily life.



(Dumitrache Nicoleta, 2017. Romanian Research Landscape, Organic Agriculture in Romania Workshop)

The Research center for studies of food and agricultural products quality is one of the newest research infrastructures of University of Agronomic Sciences and Veterinary Medicine of Bucharest, equipped at European standards (for 2010-2012). It includes four laboratories that deal with molecular analysis: Laboratory of molecular virology, Laboratory of molecular plant biology, Laboratory of molecular plant physiology and Laboratory of diagnosis and plant protection. As infrastructure, we could mention : PCR's Mastercycler Nexus gradient (Eppendorf), automated extractions with innupure C16 (Analytik Jena), homogenizer SpeedMill PLUS (Analytik Jena), preparation of nucleic acids and PCR setup with MagNa Pure LC2.0 Roche, vertical and horizontal electrophoresis (BIORAD & ThermoFischer) including CCD GEL DOC XR BIORAD analyzer, real-time PCR LightCycler® 480 Instrument II, Roche 454 GS Junior sequencer.



=> Romania needs NGS, both for research and phytosanitary reasons.

=> The costs for "in-house" operations for sequencing are much higher than working with a third part sequencing laboratory, but... we should learn it!

Our aims:

=> engage in the NGS usage for plant diagnosis, first as research target, than training and finally implementation in the "state service bodies".

=> Find national & regulatory help

=> Understand & implement the EU protocols, standards and validated methods on NGS technologies in plants health diagnostics, as any other institution working for healthy plants & environment in Europe.

References

Apateanu Tincuta , 2015. Romania Country Report EUFORI Study. Directorate-General for Research and Innovation, EU Commision
Chioncel Mariana, Jana Zifciakova; *RIO Country Report 2016: Romania*; EUR 28490 EN; doi:10.2760/479397

Deurenberg RH, Bathoorn E, Chlebowicz MA, Couto N, Ferdous M, Garcia-Cobos S, Kooistra-Smid AM, Raangs EC, Rosema S, Veloo AC, Zhou K, Friedrich AW, Rossen JW. Application of next generation sequencing in clinical microbiology and infection prevention, J Biotechnol. 2017 Feb 10;243:16-24. doi: 10.1016/j.jbiotec.2016.12.022. Epub 2016 Dec 29