A HOLISTIC APPROACH TO RESURRECTION PLANTS. *HABERLEA RHODOPENSIS* – A CASE STUDY

D. Djilianov[1], S. Ivanov[2], T. Georgieva[1], D. Moyankova[1], S. Berkov[1], G. Petrova[1], P. Mladenov[1], N. Christov[1], N. Hristozova[3], D. Peshev[3], M. Tchorbadjieva[3], V. Alexieva[4], A. Tosheva[3], M. Nikolova[5], I. Ionkova[6], W. van den Ende[7]
[1]AgroBioInstitute, Sofia, Bulgaria
[2]Centre of Food Biology, Sofia, Bulgaria
[3]Sofia University, Faculty of Biology, Sofia, Bulgaria
[4]Institute of Plant Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[5]Institute of Botany, Bulgarian Academy of Sciences, Sofia, Bulgaria
[6]Medical University of Sofia, Faculty of Pharmacy, Sofia, Bulgaria
[7]Laboratory for Molecular Plant Physiology, Leuven, Belgium
Correspondence to: Dimitar Djilianov
E-mail: d_djilianov@abi.bg

ABSTRACT

Recent environmental changes challenge world agriculture and reconfirm the importance of wild flora as useful source of valuable traits. Due to their extreme desiccation tolerance, the so called "Resurrection plants" are extensively studied and characterized. The Bulgarian endemic species Haberlea rhodopensis, apart from its typical resurrection capacity is very interesting also as a potential source of bioactive compounds with putative application in pharmacology, veterinary medicine and cosmetics. Here we discuss our approaches to Haberlea in the frames of the NSF funded project DO02-105 "Centre for sustainable development of plant and animal genomics".

Keywords: resurrection plants, *Haberlea rhodopensis,* molecular analysis

Biotechnol. & Biotechnol. Eq. 2009, **23**(4), 1414-1416

Background

The recent climatic changes increase the percentage of arid, abandoned regions which until recently were known as crop lands. Global warming and deterioration of agricultural areas are results of many factors including human errors and improper crop management. This worst scenario challenges plant scientists in their efforts to improve the reaction of crop plants to unfavourable environments and to secure sustainable development. Wild flora with its broad biodiversity has always been a source of new ideas and genetic material. On the other hand, the paradigm of sustainable development demands a complex, holistic approach to wild plant species. The search is not only for traits to be transferred to the crops but also for characteristics, if available, that will make the wild plant an attractive producer of valuable bioactive compounds.

In this respect, the so-called resurrection plants can be considered as excellent model systems because of their unique desiccation tolerance (7, 21, 24). Their mature leaves can lose more than 90% of relative water content and survive in such conditions long periods (months, years) of dryness. Upon re-watering, they recover very fast and restore their photosynthetic activity within several hours. These plant species belong to different botanical families; they live in different habitats and under various environmental challenges. Their only common feature that is under investigation so far is the ability of their vegetative tissues to withstand long periods of full desiccation and to recover rapidly upon re-watering (2, 30).

Investigations with various resurrection plants became particularly intensive in the recent 10 years and were extensively reviewed elsewhere (18, 19, 30). Among others, *Craterostigma plantagineum, Myrothamus flabellifolia* and *Xerophyta viscosa* are used not only as models of desiccation tolerance but already for system biology oriented directly to improve the reaction of valuable crops like maize and grapevine to unfavorable environments (19).

The aim of the present mini-review is to highlight the current state of the art in the studies with *Haberlea rhodopensis* – resurrection plant species, endemic for Bulgaria and to outline the investigations planned in the frames of the NSF funded DO02-105 project "Centre for sustainable development of plant and animal genomics".

Object of Studies

Bulgaria is among the few countries in Europe where resurrection plants live in natural habitats. *Haberlea rhodopensis* Friv was documented in the middle of 19th century and was among the first plants recognized as a genuine resurrection plant (8). Both with its relative *Ramonda serbica* Pancic they belong to Gesneriaceae and prefer shady slopes and limestone rocks. It is considered a homoiochlorophyllous plant species, as far as it preserves most of its chlorophyll content during dehydration. Parameters of the reaction to desiccation and recovery have been extensively studied with main target photosynthetic complex and transpiration (9, 10, 11, 13, 14, 22, 23), dynamics of lipids and sterols (28) and some compatible solutes (20). Recently, superoxide dismutase and peroxidase activity during dehydration and subsequent rehydration were followed (32).

All studies on *Haberlea* so far are performed with samples taken from natural habitats or botanical gardens. The availability of procedure for routine *in vitro* propagation is of crucial importance for endangered and rare plants such as *Haberlea* where the populations are often in restricted areas and the plants are of unknown age, stage of growth and development (5). In this respect, all our further studies will be performed with *in vitro* material propagated by us. This will enable us to work under controlled conditions and with uniform materials of known size and stage of growth. Samples from the existing natural localities will be taken when necessary for comparison or for biodiversity studies.

Aim of Studies

Based on the information available so far for other resurrection plant species and for *Haberlea rhodopensis* in particular our aim will be to apply complex approach to our endemite.

Directions of Studies

Our research will be held in several parallel fields:

Desiccation tolerance

Physiological and biochemical studies, proteoimics and metabolomics

Because of the limited molecular data so far, proteomic and mteabolomic studies of *Haberlea*'s response to dehydration and recovery could significantly complement the accumulation of knowledge in the area. Our targets will be the systems involved in the oxidative stress response, free radical scavengers, sugars and glucosides, membrane proteins related to energy supply and solute transport, dehydrins. The sub-cellular components will be of particular interest to evaluate their role in keeping the energy balance of the plant at stress and recovery. Isolation and characterization of compounds with putative protective role will be also a priority.

Molecular studies

There are very limited molecular data of *Haberlea* so far – several nucleotides and protein sequences have been listed in the world databases. Some of them are related to superoxide dismurase genes (submitted before putative publication) and other used in phylogenetic studies (3, 15, 16).

We will apply cDNA-AFLP analysis (1) trying to find transcripts that are differentially expressed in response to dehydration and at recovery. The expression profiles of some Transcript-derived fragments (TDFs) (4) will be verified by Northern blot or RT-PCR analysis. We expect to to identify genes and transcripts already isolated and characterized in other plants, icluding resurrection species but they will be first for our model *Haberlea*. On the other hand, we hope to find also genes and transcripts with unnown function or no match in the databases, making them very interesting for further studies. In addition, the research will provide important information for engineering stress tolerant crops.

Plant transformation

In order to confirm the putative involvement of genes of interest in stress tolerance the most frequently used approach is genetic transformation. So far, successful protocols for gene transfer have been established for only 3 resurrection species. (6, 27, 29, 31). They are based on callus induction and subsequent regeneration of putative transformants. We will test our system for regeneration and micropropagation (5) as an alternative model for direct transformation without a callus stage.

Bioactive compounds

There is limited information about the accumulation of bioactive compounds with putative pharmaceutical action by resurrection plants. In this respect, *Haberlea rhodopensis* is of interest since its habitats are in regions of ancient cultures. There are no strong ethnobotanical data, although there are myths and legends about the application of *Haberlea* in ancient rituals or as a cure or cosmetics.

Metabolomics

Despite the fact that modern science relatively recently realized that resurrection plants are extremely desiccation tolerant it could be speculated that the ancient inhabitants of the regions where the plant species grow were aware of their resurrection capacity. In this respect, the species became involved in ancient myths, legends and rituals (17, 19).

Haberlea rhodopensis is among the most interesting and rare representatives of Bulgarian flora. It is widely accepted that the ancient inhabitants of our region knew about its ability to recover after long-lasting drought. They named it the *Flower of Orpheus*. The legends tell the story of Orpheus' tragic death and how every drop of blood, falling on earth gave the origin of a flower with wonder-working capabilities. There are rumors that the ancient Thracians knew about some putative healing strength of the plant. Unfortunately, till now there are no strong historical and etnobotanical data confirming the ancients' knowledge and use of *Haberlea* as medicinal, cosmetic or ritual attribute. However, one of the local plant names in the Rhodopi mountains is "shap" (food and mouth disease) which is considered as confirmation that the local people were using the plant against animal diseases.

Modern studies on the secondary metabolism and the putative application of *Haberlea* as source of bioactive compounds have just started (12, 25). Our goal will be to examine the availability and to isolate bioactive compounds or bioactive complexes. This will be the first step of a possible application of such compounds in pharmacology, veterinary medicine and cosmetics.

An *in vitro* collection of the species will be developed, based on representatives of the main *Haberlea* localities in the country. Further *in vitro* propagation will allow us to establish plantation under controlled growing conditions and to supply material for metabolite studies. The *in vitro* propagated plants will be compared with representatives of the natural localities for bioactive compounds production. If needed, the propagation and growing protocols will be manipulated to increase the bioactive compounds production and the best producers will be chosen. This will protect the natural populations of this rare and endangered species.

Comprehensive chromatography and spectroscopy studies will be performed using GC-MS and NIRS. Total extracts and purified complexes and compounds will be tested for antioxidant activity and cytotoxicity.

Our findings will allow the establishment of further large-scale production of bioactive compounds with useful application. This production will be under controlled conditions and environment friendly. The possibility to isolate and produce bioactive compounds from cultured *Haberlea* plants will ensure a leading role for Bulgaria in the area, since the plant species is endemic.

Isolation and characterisation of bioactive compounds will bring us to further study the molecular background of their metabolism. Since, it is obvious that there is a real cross talk between gene complexes coding for different traits in the plant or these complxes are multifunctunional, the idea to focus on some genes, involved in bioactive compounds network is very attractive.

Population diversity studies

Apart from isolation of bioactive compounds, the collection of representatives from main *Haberlea* localities will be used also for phylogenetic studies. This will enable us to test modern techniques such as flow cytometry (**26) and population metabolomics, and to compare them with the existing molecular approaches (**15). The information obtained could be very useful in determining the status and potential relationships between the existing localities and even to elucidate the past distribution patterns of the species on both the Balkan and the Rhodopi mountains.

The Research Centre

The recognition of AgroBioInstitute (ABI) as a Centre of Excellence in plant and animal genomics by the National Science Fund in Bulgaria - Project DO02-105 "Centre for Sustainable Development of Plant and Animal Genomics" will allow us to use the updated infrastructure for genomic and metabolomics studies with *Haberlea rhodopensis*. This will reconfirm the leading role of ABI in plant biotechnology studies and will contribute for the establishment of *Haberlea* platform with the involvement of colleagues from other national and international scientific centers.

REFERENCES

1. **Bachem C.W.B., Oomen R.J.F.J., Visser G.** (1998) Plant Mol. Biol. Rep., **16**, 157-173.
2. **Bartels D.** (2005) Integr. Comp. Biol. **45**, 696-701.
3. **Citerne H., Möller M., Cronk Q.C.B.** (2000) Ann. Bot., **86**(1), 167-176.
4. **Creste S., Neto A.T., Figueira A.** (2001) Plant Mol. Biol. Rep. **19**, 299-306.
5. **Djilianov D., Genova G., Parvanova D., Zapryanova N., Konstantinova T., Atanasov A.** (2005) Plant Cell Tis. Org. Cult., **80**, 115-118.
6. **Furini A., Koncz C., Salamini F., Bartels D.** (1994) Plant Cell Rep., **14**, 102-106.
7. **Gaff D.F.** (1971) Science, **174**,1033-1034.
8. **Ganchev I.** (1950) Rep. Inst. Bot. Bulg. Acad. Sci., **1**(1): 191-214.
9. **Georgieva K., Maslenkova L., Peeva V., Markovska Y., Stefanov D., Tuba Z.** (2005) Photosynth. Res., **85**(2), 191-203.
10. **Georgieva K., Lenk S., Buschmann C.** (2008) Photosynth., **46**(2), 208-215.
11. **Georgieva K., Szigeti Z., Sarvari E., Gaspar L., Maslenkova L., Peeva V., Peli E., Tuba Z.** (2007) Planta, **225**(4) 955-964.
12. **Ionkova I., Ninov S., Antonova I., Moyankova D., Georgieva T., Djilianov D.** (2009) Pharmazija, **1**(4), 22-25.
13. **Markovska Y.K., Tsonev T.D., Kimenov G.P., Tutekova A.A.** (1994) J. Plant Phys., **144**, 100-108.
14. **Markovska Y.K., Tutekova A.A., Kimenov G.P.** (1995) Photosynth., **31**, 613-620.
15. **Mayer V., Moller M., Perret M., Weber A.** (2003) Am. J. Bot., **90**(2), 319-327.
16. **Moller M. and Cronk Q.C.** (2001) Evolution, **55**(5), 918-929.
17. **Moore J., Farrant J., Lindsey G., Brandt W.** (2005) J. Chem. Ecol., **3**, 2823-2834.
18. **Moore J., Vicre-Gibouinb M., Farrant J., Driouichb A.** (2008) Phys. Plant., **134**, 237-245.
19. **Moore J., Le N.T., Brandt W., Driouich A., Farrant J.** (2009) Trends in Plant Science **14**(2), 112-117.
20. **Muller J., Sprenger N., Bortlik K., Boller T., Wiemken A.** (1997) Phys. Plant., **100**, 153-158.
21. **Oliver M.J., Tuba Z., Mishler B.D.** (2000) Plant Ecol., **151**, 85-100.
22. **Peeva V. and Cornic G.** (2009) Env. Exp. Bot., **63**(2-3), 310-318.
23. **Peeva V. and Maslenkova L.** (2004) Plant Biol., **61**(3), 319-324.
24. **Proctor M.C.F. and Tuba Z.** (2002) New Phytol., **156**(3), 327-349.
25. **Radev R., Lazarova G., Nedialkov P., Sokolova K., Rukanova D., Tsokeva Z.** (2009) Trakia J. Sci. **7**, 34.
26. **Siljak-Yakovlev S., Stevanovic V., Tomasevic M., Brown S., Stevanovic B. (2008) Env. Exp. Bot. 62(2), 101-112.**
27. **Smith-Espinoza C., Bartels D., Phillips J.** (2007) Plant Cell Rep., **26**, 1681-1688.
28. **Stefanov K., Markovska Y., Kimenov G., Popov S.** (1992) Phytochem., **31**, 2309-2314.
29. **Toldi O., Toth S., Pornyi T., Scott P.** (2002) Plant Cell Rep., **21**, 63-69.
30. **Toldi O., Tuba Z., Scott P.** (2009) Plant Sci., **176**, 187-199.
31. **Toth S., Kiss C., Scott P., Kovacs G., Sorvari S., Toldi O.** (2006) Plant Cell Rep., **25**, 442-449.
32. **Yahubyan G., Gozmanova M., Denev I., Toneva V., Minkov I.** (2009) Plant Growth Regul., **57**, 49-56.