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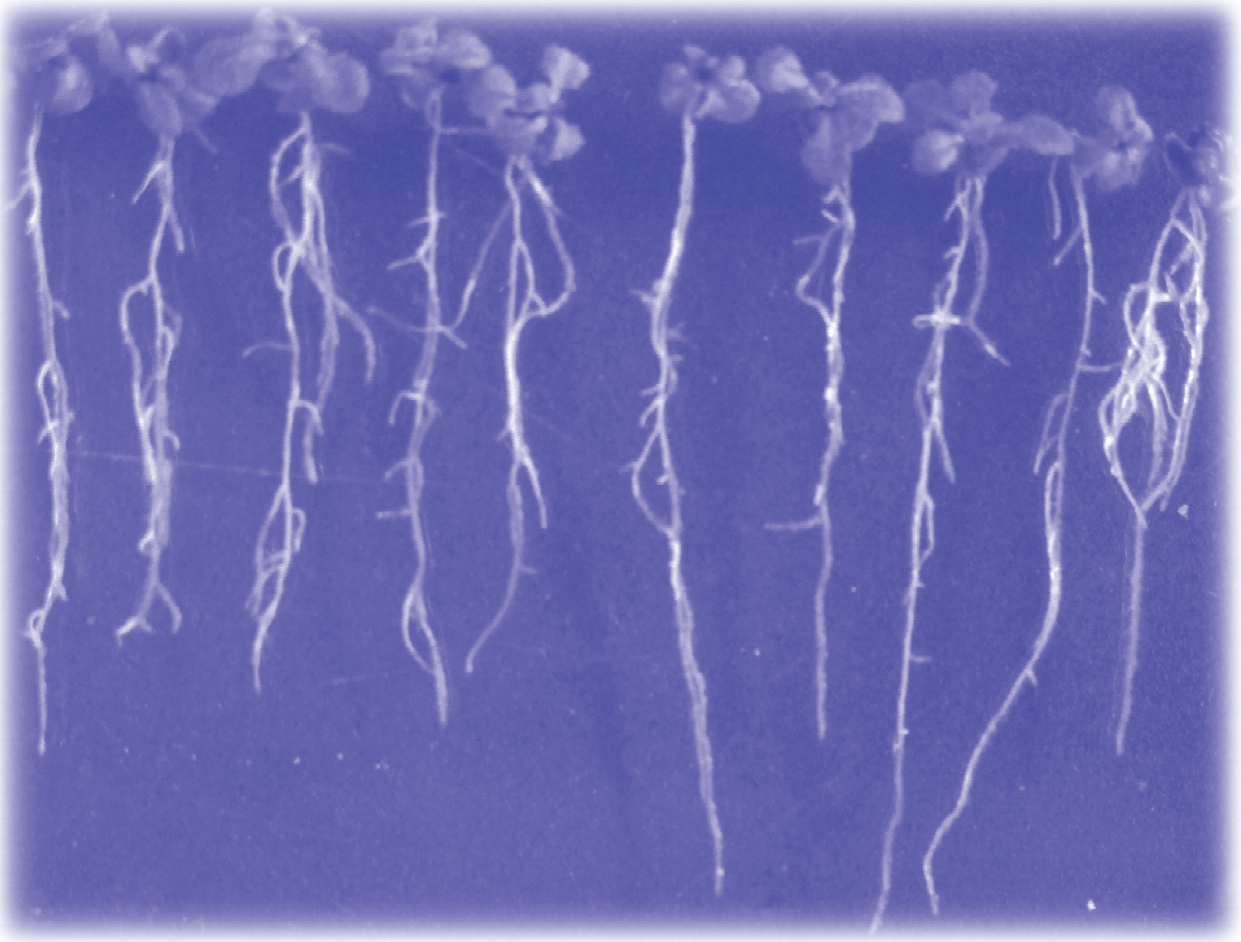

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All authors are responsible for submitting manuscripts in comprehensible US or UK English and ensuring scientific accuracy.

Original picture on front cover:
Plantlets of *Arabidopsis thaliana* cultivated *in vitro*

FIRST PART OF THIS ISSUE IS DEDICATED TO

"Plants for the future" Conference

Plant biotechnology for the future of agriculture in the Central European region conference

30th September – 2nd October 2013, Cluj-Napoca, Romania

Organized by:

Romanian Biotechnology Association
Pannonian Plant Biotechnology Associat
Zoltán Barabás Biotechnology Association

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=== ORAL PRESENTATION ABSTRACTS ===

”PLANTING FOR FUTURE”, THE EASAC DOCUMENT PREPARED BY AN EASAC EXPERT PANEL

ERVIN BALÁZS[1,✉]

Agriculture faces major challenges to deliver food security at a time of increasing pressures from climate change, social and economic inequity and instability, and the continuing need to avoid further loss in ecosystem biodiversity. The introduction of new EU legislation requiring farmers to reduce reliance on crop protection chemicals creates additional challenges for maintaining levels of crop productivity.

Previous EU agricultural policy had focused on constraining food production but there is a new realisation that the EU should now increase its biomass production for food, livestock feed and other uses, including renewable materials to support the bioeconomy. The production of more food, more sustainably, requires the development of crops that can make better use of limited resources. Agricultural innovation can capitalise on the rapid pace of advance in functional genomics research and it is unwise to exclude any technology a priori for ideological reasons. Sustainable agricultural production and food security must harness the potential of biotechnology in all its facets.

In previous work, EASAC has described the opportunities and challenges in using plant genetic resources in improved breeding approaches, for example by using marker-assisted selection of desired traits. In the presented report, EASAC explores some of the issues associated with the genetic modification of crops, where the EU has fallen behind in its adoption of the technology, compared to many other regions of the world. Concerns have been expressed that a time-consuming and expensive regulatory framework in the EU, compounded by politicisation of decision-making by Member States and coupled with other policy inconsistencies, has tended to act as an impediment to agricultural innovation. Controversies about the impact of genetically modified (GM) crops have too often been based on contested science or have confounded effects of the technology with the impact of agriculture per se or changes in agronomic practice. It is vital to address the policy disconnects because there is a wide range

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of opportunities in prospect for improving agricultural productivity and efficiency, environmental quality and human health, by using all available technologies where appropriate.

Previous work by member academies of EASAC has documented where there is excellent, relevant science to be nurtured and utilised, and where problems have arisen because of the failure to use science to inform the modernisation of regulatory approaches to benefit-risk assessment. The goal of the presented report is to clarify the implications for policy-makers of alternative strategic choices in utilising the tools, collectively termed crop genetic improvement technologies, for delivering sustainable agriculture.

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=== ORAL PRESENTATION ABSTRACTS ===

ALLELE DISCOVERY SUPPORTED BY PHENOTYPING OF DROUGHT RESPONSE OF CEREAL PLANTS

ANDRÁS CSERI[1], LÁSZLÓ SASS[1], OTTÓ TÖRJÉK[1], JÁNOS PAUK[2], IMRE VASS[1] and DÉNES DUDITS[1,✉]

Genetic improvement of complex traits, such as drought adaptation can be advanced by the combination of genomic and phenomic approaches. Semi-robotic phenotyping platform was used for computer-controlled watering, digital and thermal imaging of barley plants grown in greenhouse. In soil with 20% water capacity the reduction in green pixel-based shoot surface area of tested barley variants ranged from 0% to 76% as compared to control plants grown with 60% water capacity. The EcoTILLING and the subsequent DNA sequencing have defined four (A-D) haplotypes of the HvA1 gene encoding the group 3 LEA protein. The green pixel mean value of genotypes with haplotype D was higher than the mean value of the remaining haplotypes under drought. Thermal images indicated genotype-dependent variation in elevation of the canopy temperature of drought-exposed plants. The drought-induced changes in leaf temperature showed low correlation with the water use efficiency (r2 = 0.431). The haplotype/trait association analysis based on the t-test has revesaled a positive effect of the haplotype B of the gene encoding the barley fungal pathogen induced mRNA for pathogen-related protein (HvPPRPX) on harvest index, thousand grain weight, water use efficiency and grain yield. The presented pilot study establishes basic methodology for the integrated use of phenotyping and haplotyping data in characterization of genotype-dependent drought responses in barley.

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=== ORAL PRESENTATION ABSTRACTS ===

COMBINING DIFFERENT BIOTECHNOLOGICAL TOOLS FOR BETTER INTROGRESSION OF RESISTANCE TRAITS IN POTATO

ELENA RAKOSY-TICAN[1,✉], IMOLA MOLNAR[1], RALUCA MUSTATA[1], ABDELMOUMEN TAOUTAOU[1] and RAMONA THIEME[2]

The new tools of plant genetic enhancement, molecular biology, marker assisted selection (MAS), haploidization or other *in vitro* techniques, such as stress selection as well as the data from functional genomics and metabolomics should be used together towards a common goal, the transfer of resistance genes into crops. Today challenges such as climate change and exponential increase of human population impose new strategies to increase crop yield. The majority of the crops have got wild relatives, which developed during a co-evolutionary race between the host plant and disease or pest, different mechanisms of resistances, some controlled by resistance genes (R genes). It appears that many of those genes are clustered on few chromosomes and have some common motifs like nucleotide binding sites, leucine rich repeats (NBS-LRR). Although it was thought that resistance could be transferred into crops by one gene, it occurred soon that one R gene could only confer a short-lived resistance. In order to achieve the goal of durable, sustainable resistance the only way is to transfer more quantitative trait loci (QTLs) and to apply more biotechnological tools for the introgression and expression of resistance traits. Creating diversity by using more wild genetic resources and mechanisms and combining more biotechnological tools i.e. combinatorial biotechnology would eventually allow us to obtain a new generation of crops with both good quality and resistance to biotic or abiotic stress.

This strategy is going to be exemplified in the case of potato crop. Potato ranks third in the global crop production but suffers great loses because of diseases and pests.

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The goal of this presentation is to discuss the strategy of combining different biotechnological tools with the ambitious aim to deliver resistance genes into potato gene pool. In the case of potato such a combination includes: genetic transformation of *Solanum chacoense* (chc resistant to Colorado potato beetle – CPB), for MMR deficiency to increase homeologous recombination, somatic hybridization of potato cultivars with chc by protoplast electrofusion, selection of resistant hybrids by using molecular markers (RAPD) linked to leptines biosynthesis, repellents for CPB, testing of resistance by the use of laboratory bioassay and choice test, the analysis of trichomes and foliar metabolites and cytogenetic analysis. Other examples for multiple resistance traits or resistance genes stalking as for instance the *Rpi-blb1* and *Rpi-blb3* genes from the wild species *S. bulbocastanum* to induce resistance to late blight, or the combination of gene transfer for PVY resistance with stress selection for drought will be also presented.

Acknowledgements. The project CNCS PNII-ID-PCE-2011-3-0586 is kindly acknowledged for funding part of this research.

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=== ORAL PRESENTATION ABSTRACTS ===

BIOTECHNOLOGY OF BIOMASS SUPPLY CHAIN: CENTRAL EUROPEAN PERSPECTIVE

M.G. FÁRI[1,2,✉], G. ANTAL[1], T. ALSHAAL[1,3], N. ELHAWAT[3,4], H. EL RAMADY[3], E. KURUCZ [1] and É. DOMOKOS-SZABOLCSY[1]

By applying advanced plant breeding and biotechnology to dedicated energy crops, we should deliver sustainable energy solutions that (1) displace vast amounts of fossil fuels and provide greater energy security; (2) create new economic opportunities for farmers and rural communities; (3) protect the land, air and water; (4) meet our commitments to stakeholders. Due to the increased demand for growing dedicated biomass crops some previous and new molecular breeding methods as well as *in vitro* somatic techniques have to be integrated into the creation of specially designed „biogeneration" crops. In order to elaborate new biological solutions and growing techniques, our Biogeneration Crops Working Group is conducting some R&D activities since 2010 on perennial rhizomatous grasses (PRG, *Miscanthus gigantheus* sp., giant reed, Hungarian powergrass), as well as on interesting perennial herbaceous subshrubs (PHS, Virginia mallow, giant energy mallows, hemp mallow) which can probably be grown under marginal field condition. As a new candidate of the biogeneration energy plants, energy mallow species are able to serve considerably the Biomass Supply Chain (BSC) strategies in our region. Biotechnological and environmental research of these crops is necessary before we take them into cultivation for industrial biomass production chain. The aim of our biotech-assisted breeding program is to develop new methods for energy mallows propagation and industrial-scale nursery operations. There is substantial variation in water use efficiency (WUE) both within and across biomass crops. Therefore we investigate the role of different watering regimes on growth dynamics and biomass production of PRG and PHS crops. We also investigate the ability of different PRG and PSS ecotypes and/or cultivars for

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tolerate and removal of heavy metals under *in vitro* and hydroponic culture conditions. Our team conducts basic research on induced polyembryony, vegetative embryoidogeny, hemmorhizogeny in order to develop industrial-scale somatic seedling technology (SST) for the future biogeneration crops. To prognosticate the effect of climate changes on growing techniques, we are studying natural habitats as well as biotechnologically propagated growing fields of SST populations (giant reed and *Miscanthus* sp.). We believe in creating new opportunities for growers, sharing the value of our innovations, and collaborating broadly with scientists and industry groups in Pannonian regions.

Acknowledgements. This work is partly supported by the TÁMOP-4.2.2.A-11/1/KONV-2012-0041 project and co-financed by the European Union and the European Social Fund. Additional financial support is also gratefully acknowledged for the MOP Biotech Co Ltd. (Nyíregyháza, Hungary) and Ereky Foundation (Debrecen, Hungary).

=== ORAL PRESENTATION ABSTRACTS ===

COMPARATIVE ANALYSIS OF THE SEQUENCES OF GENE FAMILY *psbA* IN CYANOBACTERIA

IULIANA SIMEONI[1], CIPRIAN CHIŞ[1] and COSMIN SICORA[1,✉]

The D1 protein of Photosystem II (PSII), encoded by the *psbA* genes, is an indispensable component of oxygenic photosynthesis. Due to strongly oxidative chemistry of PSII water splitting, the D1 protein is prone to constant damage and requires its replacement by a new copy every 5h under low light conditions and every 20 minutes under intense illumination, whereas most of the other PSII subunits remain ordinarily undamaged. In Cyanobacteria the D1 protein is encoded by a *psbA* gene family, ranging from 1 to 6 members. The presence of multiple *psbA* genes encoding different D1 isoforms is an indication of their importance in regulatory mechanisms responsible for maintaining a functional PSII upon changing environmental conditions in natural habitats of cyanobacteria.

Here we present a comparative analysis of the sequences of gene family *psbA* in cyanobacteria, with characteristically features of all *psbA* genes and the D1 protein isoforms that they encode.

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=== ORAL PRESENTATION ABSTRACTS ===

ROOT ARCHITECTURE OF *BRACHYPODIUM* – ROOT ARCHITECTURE OF *BRACHYPODIUM* – MORPHOLOGICAL, MOLECULAR AND GENETIC APPROACHES

MÁRIA SZÉCSÉNYI[1,✉], ZOLTÁN ZOMBORI[1], MAGDOLNA GOMBOS[1], GYÖRGYI SÁNDOR[1] and JÁNOS GYÖRGYEY[1]

Root is the main plant organ that is responsible for water and nutrient uptake. Hence, its architecture determines strongly how it can fulfill the above role. On the other hand, the characteristics of root architecture are affected significantly by the underground environment, such as water and nutrient availability in the soil.

In our group, characterization of various *Brachypodium distachyon* (L.) P. Beauv. ecotypes grown in pots or rhizotrons under either well-watered or reduced watering conditions is conducted. Comparison of plants grown in pots focuses mainly on the length and thickness of primary and nodal roots; however their photosynthetic and other shoot parameters are measured, too. Thus, the evaluation of different groups involving distinct ecotypes with similar shoot or root parameters can inform us about the importance of the presence/absence, thickness or length of different root types as well as their correlation with shoot parameters under optimal and adverse circumstances. Moreover, growing plants in rhizotrons allow us to follow the daily growth rate of primary, nodal and even lateral roots. Hence, the ecotypes can be distinguished on the basis of their root morphological plasticity in response to water-deficit stress.

The plant-specific LBD (Lateral Organ Boundaries Domain) gene family is essential in the regulation of plant lateral organ development. Several LBD genes are related to almost all aspects of plant development, including embryo, root, leaf, and inflorescence development. Our group studies the transcript pattern of various LBD genes in different plant parts, selecting especially the root-specific ones that are probably involved in the lateral root formation in *Brachypodium*.

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Reverse genetic approaches of the above-selected LBD genes can inform us about their spatial and temporal expression. Therefore, transformants containing constructs with self-promoter and reporter gene together with transformants with ectopic over-expression of the selected LBD genes are being analyzed. Silencing of the selected LBD genes is also planned in the future.

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=== ORAL PRESENTATION ABSTRACTS ===

THE INVOLVEMENT OF CHROMATIN PROTEINS IN *AGROBACTERIUM*-MEDIATED PLANT GENETIC TRANSFORMATION

GABRIELA N. ŢENEA[1,2,✉], JOERG SPANTZEL[2], LAN-YING LEE[2], HEIKO OLTMANNS[2] and STANTON B. GELVIN[2]

Agrobacterium-mediated genetic transformation is the dominant technology used for many years in production of genetically modified transgenic plants. Recent study has demonstrated the implication of plant genes and proteins in Agrobacterium mediated transformation. We investigated the role of several histone proteins and an anti-silencing factor A (*SGA1*) in plant transformation. Transgenic *Arabidopsis* plants containing additional copies of cDNAs encoding histone H2A (*HTA*), histone H4 (*HFO*), or *SGA1* displayed increased susceptibility to transformation. Over-expression of all tested histone H2B (HTB) and most histone H3 (HTR) cDNAs did not increase transformation. A parallel increase in transient gene expression was observed when the histone *HTA* or HFO cDNAs were co-transfected, together with a plant active *gusA* gene, into tobacco protoplasts. An increase in *gusA* transcripts when the histone *HTA1* cDNA was over-expressed in protoplasts was also detected. No such increase in gusA activity was seen when a *SGA1* cDNA was co-transfected with a gusA gene into BY-2 protoplasts. Over-expression of histone or *SGA1* cDNAs does not increase expression of a previously integrated transgene, nor could *HTA1* reverse silencing. These data suggests that histones may increase transgene expression by working directly on the promoter of incoming DNA, or that histones may play a role in stabilizing transgene DNA (and thereby transgene expression) during the initial stages of transformation and *SGA1* might lead to enhanced plant transformation by allowing T-DNA and complexed proteins greater access to plant target DNA, thus facilitating T-DNA integration.

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=== ORAL PRESENTATION ABSTRACTS ===

APPLICATION OF TISSUE SPECIFIC GENE EXPRESSION TO IMPROVE FUNGUS RESISTANCE IN WHEAT

MILÁN IVANICS[1], ANDRÁS KISS[1], GÁBOR TÓTH[1,2], KRISZTINA TAKÁCS[3], ANDREA BALOGH[1], JÓZSEF FODOR[4], KLÁRA MANNINGER[4], LÁSZLÓ TAMÁS[2] and BARNABÁS JENES[1,✉]

The rust disease (*Puccinia* spp.) belongs to the group of most important fungal diseases in wheat (*Triticum aestivum* L.). The leaf rust (Puccinia recondita f. sp. tritici) and the stem rust (*Puccinia graminis* f. sp. *tritici*) cause – depending on the year effect – very severe loss of quality and quantity in cultivated wheat year by year in Hungary. Wheat is one of the major agricultural crops in Hungary as well as in many countries all over the world. The conventional breeding methods were not able to produce an outbreak in providing considerably tolerant genotypes. Based on this observation it seems to be useful to take new approaches to develop the resistance of wheat against rust diseases and to combine the genetic transformation and the classical plant breeding. We expect stabilization of yield quality and yield safety by the increased resistance against upper mentioned rust fungus diseases with the use of available tools and processes of biotechnology.

When plants are attackted by fungi part of their response is producing PR (pathogenesis related) proteins as chitinases, glucanases. But the induced self-defense mechanism does not provide enough protection in most of the cases, because either they are not effective or they are activated too late because there are many different processes of metabolic pathways.

Our goal was to build the *cmg1* gene, coding for a 83.2 kDa exoglucanase enzyme of *Coniothyrium minitans* into wheat with direct genetransfer.

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Considering the constitutive gene regulation, the mentioned resistant plants produce the protein of hydrolase activity in all parts of the plants, what means a certain risk of GM technology in today's public opinion. According to the upper mentioned facts we inserted the transgene under tissue specific regulation, so that the protecting recombinant protein is present only in the green tissues of the transgenic plants and we cannot detect it in the starchy endosperm of the wheat kernel.

To achieve this goal *cmg1* gene had to be inserted into a gene construction where it was under regulation of the wheat"s own ribulose-1,5-bisphosphate carboxilate-oxigenase gene's (*rubisco*) promoter.

We developed a modified biotest system, after the standard methods, so that we could be able to test the biological efficiency of this „general" resistance. After the molecular genetics proofs – PCR, RT-PCR, Western Blot, etc. – the transgenic plants underwent a biotest, where all the available races of leaf rust in Hungary was used for provoked infection. The results of biotests indicated that many of the GM wheat lines showed considerably high-level resistance against *P. recondita*.

=== ORAL PRESENTATION ABSTRACTS ===

SMALL RNA BASED VIRAL METAGENOMICS IN CULTIVATED GRAPEVINE

VITANTONIO PANTALEO[1], GYÖRGY SZITTYA[1], EMESE SZABÓ[1], GÁBOR TÓTH[1], SHOU WEI DING[1] and JÓZSEF BURGYÁN[1,✉]

In plants, microRNAs and siRNAs are small RNAs regulating gene expression at the post-transcriptional level. Here, we use deep-sequencing, computational and molecular methods to identify, profile, and describe conserved and non-conserved miRNAs. We also identified and characterized vsiRNAs derived from grapevine field plants naturally infected with different viruses belonging to the genera Foveavirus, Maculavirus, Maravirus and Nepovirus. These vsiRNAs were mainly of 21 and 22 nucleotides (nt) in size and were discontinuously distributed throughout viral genome. In addition we have identified host and viral genes targeted by small RNAs.

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=== ORAL PRESENTATION ABSTRACTS ===

IN VITRO MICROPROPAGATION OF *LYCIUM BARBARUM*

ALEXANDRU FIRA[1,2], DOINA CLAPA[1,✉] and ELENA RÁKOSY-TICAN[2]

In vitro culture was successfully initiated by using seeds from cultivar Ningxia 1 as inocula cultured on Murashige and Skoog (1962) hormone-free medium, solidified with agar. The highest proliferation rates were obtained on the MS media with either 0.3 or 0.5 mg/l benzyl adenine, gelled with wheat starch as an agar alternative. The experimental treatments with 0.5 mg/l benzyl adenine ensured proliferation rates superior to the ones with 0.3 mg/l benzyl adenine, but the shoots obtained on MS + 0.3 mg/l benzyl adenine were longer and more robust. Also, the inoculation of large microcuttings onto the multiplication media ensured superior results regarding *in vitro* survival rates, the number of shoots regenerated / plantlet and the vigor of the plantlets. The microcuttings inserted vertically into the media yielded superior growth and multiplication as compared to the microcuttings placed horizontally on the surface of the media. The shoots regenerated in the multiplication stage could be used for cyclic *in vitro* multiplication. Explants either two centimetres or four centimetres in length proved to be effective. The non-rooted shoots resulting from the treatment with 0.3 mg/l benzyl adenine were either rooted *in vitro* on hormone-free MS medium gelled with starch or used for tests of *ex vitro* rooting and acclimatization. The optimal number of microcuttings in the *in vitro* rooting stage proved to be 40 explants/jar and the rooted plantlets were efficiently acclimatized *ex vitro* by three methods: float hydroculture in floating cell trays, floating perlite as well as Jiffy7 pellets. A successful alternative to *in vitro* rooting and subsequent *ex vitro* acclimatization was the direct *ex vitro* rooting and acclimatization in Jiffy7 pellets, in the same stage, using shoots excised from plantlets cultured in the multiplication stage. For direct *ex vitro* rooting and acclimatization, all other substrates we tested except for Jiffy7

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pellets failed. *Ex vitro* rooting in floating perlite was stimulated by IBA but the technique was difficult and the results were not conclusive. After ex vitro acclimatization the resulting plants grew rapidly and vigorously.

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=== ORAL PRESENTATION ABSTRACTS ===

CHARACTERIZATION OF TWO SER/THR TYPE *ARABIDOPSIS THALIANA* PROTEIN KINASES: THEIR POSSIBLE ROLES IN REGULATION OF ABIOTIC STRESS RESPONSES

GÁBOR RIGÓ[1], FERHAN AYAYDIN[1], GYÖNGYI SZÉKELY[2a], DORINA PODAR[2b], LAURA ZSIGMOND[1], HAJNALKA KOVÁCS[1], ANNAMARIA KIRÁLY[1], OLAF TIETZ[3], KLAUS PALME[3], LÁSZLÓ SZABADOS[1], CSABA KONCZ[1,4] and ÁGNES CSÉPLŐ[1,✉]

Due to global warming of overall climate, it is an increasing demand to breed plant cultivars tolerant to high salt concentration in order to improve their chance to survive deleterious effects of abiotic stress conditions. Our aim is - within the frame of a Hungarian-Romanian TET (TET_12_RO_1-2013-0010) - to characterize and compare abiotic stress response regulatory genes arised from *Arabidopsis* and extremely high salt tolerant picoalgae. Identification of a set of cDNAs conferring dominant stress tolerance phenotypes was described in *Arabidopsis* (Papdi *et al.,* 2008). Two Ser/Thr protein kinases (from this set line N022 and the CRK5) were chosen for abiotic stress response investigations. Preliminarly, we functionally characterized the CRK5 protein kinase which is involved in regulation of gravitropic responses of *A. thaliana* roots (Rigo *et al.*, 2013). CRK5 is a PM-associated kinase that forms U-shaped patterns facing outer lateral walls of root epidermis cells. CRK5 phosphorylates hydrophilic loop of PIN2 *in vitro*, and PIN2 shows accelerated accumulation in brefeldin bodies in crk5 mutant. Delayed gravitropic response of *crk5* mutant thus likely reflects defective phosphorylation of PIN2 and deceleration of its brefeldin-sensitive membrane recycling. In future, we would like to investigate role of CRK5

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protein kinase in NO signaling also. We expect to find new information about role of NO molecule in auxin signaling and oxidative stress. Additionally, for comparative studies, we plan to investigate the osmotic and oxidative responses of the other protein kinase, line N022, which has unknown function yet.

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=== ORAL PRESENTATION ABSTRACTS ===

APPLICATION OF RECOMBINASE TECHNOLOGY TO PRODUCE MARKER FREE TRANSGENIC CROPS

LÁSZLÓ TAMÁS[1,✉], CSABA ÉVA[1], ESZTER KISS[1], FLÓRA TÉGLÁS[1], KLÁRA MÉSZÁROS[2] and LÁSZLÓ LÁNG[2]

One of the main concerns regarding GM plants is the presence of the selection marker gene in their genome. Although several non-antibiotic and non-herbicide based selection systems exist, but they are the most frequently used, which raised the fear in the public. The biggest problems addressed by the green movements are the so called "escape of these selection marker genes into the environment causing negative effects on it. The other problem with the most widely used methods modifying the plant genome is the random insertion of the "foreign" gene into it and as a consequence it may generate unpredictable expression patterns. To reduce some of the concerns and make the gene technology approach acceptable by the society scientists have to address all these issues. Selection marker genes have to be chosen carefully or have to be removed when they are no longer necessary in the genome. The genes for modifying the physiological characteristics of the plant or the property of the products made of plant tissue have to be chosen prudentially introducing genes from the same species or from the same family. To avoid random insertion for example site-specific genome editing is powerful and highly desirable methodology. Both site-specific recombination and restriction enzyme mediated gene targeting are feasible approaches in genome modification of plant cells.

A method based on the widely used Cre/lox recombination system has been developed in or laboratory to produce selection marker free transgenic barley plants. The inducible recombination system is under the control of a cold responsible promoter. It has been demonstrated that the system works properly cutting of the unnecessary DNA fragment from the barley genome. The remaining mutated loxP site provide an option for site specific insertion of any desirable DNS fragments to engineer specific properties of either the plants or plant based products.

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=== ORAL PRESENTATION ABSTRACTS ===

EFFECT OF UV-B STRESS ON THE PHOTOSYNTHETIC FUNCTION OF *ERUCA SATIVA* MILLER

LIA MLADIN[1,2,✉], NORA PRODAN[1] and COSMIN IONEL SICORA[1]

The global warming can determine the alteration of normal functionality of the ecosystems, threatening with extinction a significant number of species. Most important environmental factors inducing different types of stresses on plant photosynthesis are: temperature, excessive light, CO_2 concentration. Destruction of the ozone layer that absorbing short wavelength UV radiation, increases the amount of UV-B radiation reaching the Earth's surface. These radiations can cause damage of photosystem I and II, carbon fixation, low levels of chlorophyll and sucrose in plants.

The study of photosynthesis utilizing chlorophyll fluorescence measurements is one of the most modern techniques used by plant physiologists and ecophysiologists. The modern fluorometers can measure the chlorophyll fluorescence in the laboratory and in the field. For the study of UV-B stress on *Eruca sativa* we have utilized the MONI PAM fluorometer that monitors the efficiency of PS II in greenhouse conditions under normal and enhanced UV-B irradiation. The monitored parameters are: PAR (Photosynthetic Active Radiation), basic fluorescence level (F) maximal fluorescence level (FM´), ETR (Electron Transport Rate), YII (efficiency of Photosystem II). Measurements were done on control samples, plants treated with low UV-B radiation and high UV-B radiation producing a wide range of adaptative responses including irreversible damages of photosynthetic processes.

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=== ORAL PRESENTATION ABSTRACTS ===

THE EFFECT OF THE P19 SILENCING SUPPRESSOR ON ANTIVIRAL siRNA BIOGENESIS

LEVENTE KONTRA[1,✉], EMESE SZABÓ XÓCHITL[1] and JÓZSEF BURGYÁN[1]

Here we showed that in CymRSV – *Nicotiana benthamiana* interaction DCL2 dependent 22nt small RNAs are generated in the greatest number. However this is masked by the 1 nucleotide shortening of the siRNA induced by P19.

DCL2 and DCL4 drived small RNAs are crucial in mediating RNA silencing against RNA viruses. DCL2 and DCL 4 are capable of cleaving double stranded viral RNA into 22 and 21 nucleotide (nt) long viral small interfering RNAs (vsiRNA) respectably. And both can program RISC complexes to cleave the complementary viral RNA. P19, a viral protein capable of suppressing silencing, can invert the size ratio of vsiRNA in Cymbidium Ring Spot Virus (CymRSV) infected *Nicotiana benthamiana* (N.b.) plants. We know that a wild type CymRSV infected N.b. the amount of 21 nt long vsiRNAs is grater than 22nt vsiRNA's, however in N.b. infected with P19 deficient CymRSV (Cym19S) it alters and 22 nt vsiRNA becomes the most common. We set our goal to shed some light on the under laying mechanism of the ratio change.

We have proved that this can not be explained by the CymRSV's ability to replicate in tissues where the Cym19S becomes silenced. By showing that DCL2 and DCL4 are both present in the assessed tissues and there is no significant difference in expression levels even when the viruses are present. We also investigated the accumulation of small RNAs in these tissues, with next generation sequencing and find that there is a small divergence of 21 and 22 nt vsiRNA ratio but the presence or absence of P19 distinguishes the samples the most.

We discarded the possibility that the ratio sift is due to the P19's putative ability of conserving the 21 nt based on the fact that P19 bind 21 and 22 nt siRNA in vitro with roughly the same affinity.

We hypostatized that P19 is capable of inducing the shortening of the bound siRNSs by one nt. We had reported that when P19 was co-agroinfiltrated with GFP and

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its hairpin into N.b. we found that 22 nt siRNS could not been detected, and a grate number of 20 nt appeared on the northern blot, though none of the P19 independent small RNA (24nt siRNA, miRNA) shown any change after interaction with plant endogenous RNase. This hypothesis can also explain the enrichment of 20nt siRNA in our and in previously published small RNA sequencing. *In silico* maping of vsiRNA reads also supports this theory.

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=== POSTER ABSTRACTS ===

GROWTH DYNAMICS AND BIOMASS PRODUCTION OF GIANT REED UNDER DIFFERENT WATERING REGIMES

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In recent years many national and international initiatives have started in order to identify new sources of renewable energy. This growing interest in renewable energy is driven by two main reasons. Firstly, fossil fuels such as oil, coal, and natural gas are limited resources on our planet and if the level of our consumption does not change, the estimated times of depletion of these energy sources will be approximately 50 years for oil, 70 years for natural gas, and 170 years for coal (International Energy Outlook, 2006). Secondly, the combustion of fossil fuels emits large amounts of gas into the atmosphere, increasing the natural greenhouse effect. Carbon dioxide (CO_2) and methane (CH_4) are the main components of greenhouse gases (GHGs). The new policies for sustainable energy production in recent years have endorsed for the use of non-food, perennial grasses for biomass plantation for the next generations of bio-fuels production. To maximize the limit of carbon dioxide emissions (recognized as the main source of the greenhouse effect), in January of 2007, the European Commission has to recommend an obligatory minimum biomass participation in the energy balance of 20% by 2020. So, the generation of biomass for energy production is becoming a real business opportunity for farmers all over the world, even thought the use of grains and other food is giving rise to ethical issues. Tall perennial grasses, such as giant reed (*Arundo donax* L.), have been evaluated as potential lignocellulosic bioenergy crops. C3 grasses like giant reed have been evaluated for bioenergy use

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in the region. High dry matter yields of about 30 attrotons/ha^{-1} have been reported in temperate climates for giant reed. Thus, there is an ever increasing need to achieve greater crop production with less water use and/or more efficient water use. This is important for all crops, but it is especially needed for bioenergy crops to allow for production on marginal lands and to minimize competition with food crops. There is substantial variation in water use efficiency (WUE; g biomass produced kg^{-1} of water transpired) both within and across crops. The objective of our study is to investigate three different watering regimes (75, 50 and 30 % of saturation percent) on growth dynamics and biomass production of three different ecotypes (S: Hungarian ecotype, B: North-American ecotype, E: Spanish ecotype). The preliminary data for number of new buds, plant height and number of leaves after one month from transplanting showed no big differences among the ecotypes and among treatments indicating that giant reed could consider as drought tolerant plant; not only to stay survive under limited water supply circumstances but also produce significant biomass feedstock for energy and paper purposes. As well as Fv/Fm ratio for ecotypes under different watering regime after one month also showed no big differences among ecotypes.

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=== POSTER ABSTRACTS ===

COMPARATIVE PERFORMANCE OF COPPER UPTAKE BY TWO ECOTYPES OF GIANT REED (*ARUNDO DONAX* L.) GROWN ON HYDROPONIC CULTURE

N. ELHAWAT[1,2,✉], T. ALSHAAL[1,3], É. DOMOKOS-SZABOLCSY[1], J. KÁTAI[4], L. MÁRTON[5], M. CZAKÓ[5], M. MOLNÁR[1] and M.G. FÁRI[1]

Contamination of water bodies such as streams, drains and ground water resources with trace elements represents a potential threat to humans, animals and plants, and thus removal of these metals from contaminated waters has received increasing attention. Increase of man's needs requires development in life activities, progress industrialization, transportation tools, enhancement of agriculture and exploitation of natural resources. Soil and water resources are extremely exposed to pollution from different aspects. Agrochemicals in particular, have created severe problems, since they release thousands of chemicals to the environment. Studies on the effect of environmental pollutants on agro-ecosystem have been carried out. On the other hand, the importance of heavy metals as environmental pollutants is well known and well documented in literature. Copper toxicity is a problem of both agricultural and environmental significance. Sources of Cu contamination include mining and smelting, urban, industrial and agricultural wastes, and the use of agrochemicals. Despite its environmental and agricultural importance, the concentration, distribution and fractionation of anthropogenic, and naturally occurring Cu in soils is poorly known. Although the total Cu content in soils is a useful indicator of soil deficiency and/or contamination, it does not provide enough information about its environmental impact. Copper availability to biota (as a nutrient or toxin) and its mobility are the most

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important factors to be considered when assessing its effect on the soil environment. Copper must be absorbed in small amounts on a daily basis to maintain good health. A daily dietary intake of 1–2 milligrams is required. However, high levels of copper can be harmful to health. Inhaling high levels can cause irritation to the nasal passages, mouth, eyes and throat, and ingesting high copper concentrations can lead to nausea, vomiting and diarrhoea. Exposure to very high levels can damage the liver and kidneys and may lead to death. Copper is classified as a hazardous substance. Giant reed (*Arundo donax* L.) is widely employed as raw material for the production of paper. It is also a good candidate for soil phytoremediation as it is a high plant with deep roots and fast growth. Giant reed's ability to tolerate and accumulate heavy metals such as nickel, cadmium, zinc and lead has been well documented. The aim of this study is to investigate the ability of two different ecotypes ("ESP" and "08") of giant reed for tolerance and removal of copper under hydroponic culture using different concentrations of copper as follow; 0, 1. 2, 3, 5, 10 and 26.8 mgL^{-1}. To determine the effects of copper on giant reed chlorophyll a and b, activities of catalase (CAT), peroxidase (POD), superoxidase dismutase (SOD), and photosynthetic activities were measured. Our result confirmed that giant reed showed a potential of phytoremediation of contaminated soil charged with low concentrations of Cu.

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=== POSTER ABSTRACTS ===

BREEDING STRATEGIES OF *SIDA HERMAPHRODITA* FOR BIOENERGY

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Virginia mallow (Sida, *Sida hermaphrodita* Rushby) is a perspective perennial semishrub plant species originating from North-America. This endemic and endangered species will probably receive much more attention in Central Europe as one of the best dedicated candidate among temperate climate biomass crops. Based on former investigations, Sida can cultivate under marginal land conditions and it can tolerate the oscillations of climate changes and drought with a biomass output around 10-20 attroton ha^{-1}. Besides this one other important economical character is that the water-content of Sida stems decrease bellow 40% until November, preceded the perennial grasses and woody energy species. As a new candidate of the second generation energy plants, *Sida* is able to serve considerably the Biomass Supply Chain (BSC) strategies in our region. The breeding of biomass plants can be economical and sustainable, if the cultivated plants are adaptable, and it possible to grow calculable and CO_2-positive field cultivation way. Biotechnological and environmental research of *Sida* is necessary before we take it into cultivation for industrial biomass purposes. The aim of our biotech-assisted breeding program is to develop new methods for *Sida* propagation and industrial-scale nursery operations. The directions of our investigations are the followings:

Improvement of seed germination: *Sida* is a semi-domesticated species therefore it has some field propagation difficulties. Freshly collected unimproved maturated seeds have a very low germination rate which is varied between 5 to 15%. Therefore, one of our main seed physiological researches was to increase the seed germination percentage of our Sida populations up to 70-80%. By means of special seed-priming methods such high germination capacity now is possible and we are able to manage large-scale propagation in industrial plantlet factories.

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Polyploidization program: Another important area of mallow breeding and research is neodomestication and crossing of mallow species with 2n=28 chromosomes. In 2010 the *Sida* polyploidization program has been initiated, both from the seeds originated from our former collection (under the name of *Napaea dioica*) as well as from seeds originated from wild populations (USA).

Investigation of *Sida* stress-tolerance: We started to study the *Sida* dry-stress tolerance in the point of view of the functional changes / disorders of the photosynthetic system.

Discover of Sida plant pathological background: Because of deficient knowledge of *Sida* phytopathological background, the farmers who plant *Sida* from root cuttings confronted considerable risks. We think that it has to give preference to propagation from seeds that from root cuttings. The most serious disease of *Sida* was *Sclerotinia* blight, moreover we observed other soilborne stem and root infections as well as airborne seed infections which molecular identifications and characterizations are in progress.

Breeding of ornamental *Sida*: In 2011 different stem colour variations have been identified in our populations. The discovered stem colours are linked closely to the colour of the pistillum, indicating genetic marker of clonal stability of the colour variations. Due to their lovely purple, yellowish and other stem colour characters, Sida can also be appropriate species for ornamental purposes.

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=== POSTER ABSTRACTS ===

SPONTANEOUS VIVIPARIA AND NODAL SHOOT FORMATION AFTER WINTER SEASON IN GIANT REED (*ARUNDO DONAX* L.)

G. ANTAL[1,✉] and M.G. FÁRI[1]

Due to the increased demand for growing dedicated biomass crops some previous and new molecular breeding methods as well as *in vitro* somatic techniques have to be integrated into the creation of specially designed „biogeneration" crops. Such as perennial rhizomatous grasses (PRG), like bamboo species, *Miscanthus* sp., giant reed, switchgrass, sugarcane and energy cane hybrids etc. can be cultivated under marginal fields. In order to prognosticate the effect of climate changes on biological and growing techniques, the Working Group of Biogeneration Crops conducted some R&D activities about natural habitats as well as on biotechnologically propagated synplant populations of giant reed and miscanthus since 2010. In the case of giant reed there were observed some surprising phenomenon closely linked to the climate changes. We observed spontaneously formed viviparia and sprouting of secondary nodal shoots from two-year-old stems. Among the monocotyledonous plants species it can be found some good examples for viviparia. One of the known examples is *Poa bulbosa*, however similar phenomenon has already described in *Festuca viviparum* and *Deschampsia alpina*. In 2010 we conducted field research in southern-east part of Portugal where asexual viviparia was also observed in some wild (naturalized) giant reed populations. We measured that there were about 2.500 pieces of well developed shoot-buds and micro-shoots of 1-10 cm lengths per fully developed panicle. Thirty shoot-buds were removed and separated randomly by hand and they were placed into tap water where all of them rooted successfully. We think that such modification of generative panicles to vegetative propagules is considered as strong physiological alterations of which exact background is till now unknown. We think that they were probably formed under extreme heat stress conditions. In spring of 2013, we observed another vegetative reproduction ability of giant reed in Hungary. From the nodes of two-year-old stems there were observed spontaneous

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secondary nodal sprouting; this is well known phenomenon across the Mediterranean, subtropical and tropical countries. In June of 2013 we find that this phenomenon was quite usual in some location at Lake Balaton mostly next to the roads and railways, gardens and parks too. In middle of July, 2013 we measured that a medium-size cane of 1 m diameter and 2.5 m high contained in average 20 pieces of secondary sprouting stems and 200 nodal (secondary) shoots of 10 to 40 cm lengths. There were isolated one-node cuttings and they were planted to soil for rooting. From these lateral shoots there were successfully isolated shoot-meristems under *in vitro* conditions.

Based on our observations carried out on giant reed, the following conclusions can be summarised:

1.) To prevent potential invasiveness in our climate it has to be avoiding establishing farms at surrounding areas of water banks and lakes;

2.) The undeveloped young secondary nodal buds of the second-year-old stems are highly useful pathogen-free source for initiation of *in vitro* propagules.

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=== POSTER ABSTRACTS ===

FOLIAR APPLICATION OF PLANT PROTEIN HYDROLYSATES ON VEGETABLE CROPS

É. DOMOKOS-SZABOLCSY[1,✉], B. BLIZMANN[1], A. SZTRIK[2], J. PROKISCH[2] and M.G. FÁRI[1]

Nowadays plant derived protein hydrolysates using as foliar application in crop production is an intention especially in the organic agriculture. Uptake mechanisms are not well described however presumably it is got into primarily through stomas. The main advantage of hydrolisated protein as organic nitrogen fertilizer is that it can provide readymade building blocks for protein synthesis. At the same time some protein-building amino acids are fundamental metabolites in the process of vegetable tissue formation and chlorophyll biosynthesis. Hence the increased chlorophyll concentration can contribute more intensive photosynthesis. In our case the objective was to investigate the effect of different plant (soybean, lupine and pea) derived protein hydrolisates in 2 mg L^{-1} concentration as foliar application for some quantitative, qualitative and physiological parameters of red pepper and tomato in field experiment. As expected on the basis previous experiment no significant difference was shown in the average weight of tomato nor green pepper. However the soy protein hydrolysates increased the photosynthetic pigment content both in tomato and green pepper leaves. The photosynthetic efficiency were higher applied lupin and lupin+pea hydrolysates in the same vegetables however all the treatments were in the normal concentration range (0.75-0.85 mg L^{-1}). As concern the qualitative parameters significant increasing were measured in the ascorbic acid content of pepper samples using all treatment and the total carotene also was significantly higher in case of soy hydrolysate.

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=== POSTER ABSTRACTS ===

IN VITRO ROOTING AND GROWTH OF ANANAS USING DIFFERENT INORGANIC SELENIUM FORMS

É. DOMOKOS-SZABOLCSY[1,✉], R. PINTÉR[1], P. ESZENYI[2], A. SZTRIK[2], J. PROKISCH[2] and M.G. FÁRI[1]

After bananas and mangoes the pineapple is the third most economically important tropical fruits in the world. The pineapple is routinely propagated vegetatively however *in vitro* micropropagation of it has many advantages over conventional methods. For instance, this technique allows an efficient and rapid increase of selected elite pineapple varieties. At the same time *in vitro* propagation can provide opportunity for controlled biofortification. This is important whereas pineapple is excellent source of several vitamins however it contains small amount microelements especially, selenium. Based on these knowledge, the goal of our study was to see if the sodium-selenate and the red elemental selenium nanoparticles (nanoSe) as inorganic selenium forms are able to influence the rooting and growth of pineapple *in vitro* culture combining with plant hormones (NAA or TIBA). The experimental data clearly indicated that the nanoSe is better tolerated selenium form than the selenate. The selenate in 10 mg L^{-1} concentration inhibited the root developing and shoot growth, and the plantlets competely died by 100 mg L^{-1} of it. However, no toxic symptoms were shown in case of nanoSe in the applied concentration range (1-100 mg L^{-1}) regardless of rooting media composition. Both of two selenium forms could uptake in pineapple *in vitro* culture however selenate accumulated in higher amount than nanoSe applied the same concentration in the media.

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=== POSTER ABSTRACTS ===

ESTABLISHING A MULTIFUNCTIONAL SYSTEM FOR FUNCTIONAL EPITOPE SEARCH AND EDIBLE VACCINE EXPRESSION IN PLANT

SÁRA PÓLYA[1], EDINA POCZKODI[1], ANNA POZSONYI[1], GÁBOR TÓTH[1] and LÁSZLÓ TAMÁS[1,✉]

The main aim of the project we have been working on is to produce edible vaccines in selection marker gene free transgenic barley endosperm. To reach these goals a system has to be established to generate proper transgenic barley lines. The most suitable epitopes of a particular antigen can be quickly selected through the expression of several molecules in plant tissue culture, followed by the appropriate functional studies. To build up the above mentioned system transformation cassettes have been assembled and tested. Results are presented here on the development of the barley heterologous protein expression system.

Antigenic proteins which trigger an effective mucosal immune response can also be produced in transgenic plants. This plant derived edible vaccines are not only cheaper and safer than recently used subcutaneous vaccines but they are considered to be more effective. Edible vaccines are particularly effective against those pathogens which enter trough the mucosal membrane.

The cholera toxin B subunit (CTB) as a strong mucosal adjuvant was used to elicit the mucosal immune response, because CTB has an effective adjuvant activity as a carrier protein for genetically fused unrelated proteins. Genes of potential immunogenic proteins or epitopes are able to fuse easily in this cloning system with the gene of the adjuvant protein.

Our intention is to develop a high throughput transient expression system to test and investigate these potential antigenic proteins. Both *Agrobacterium* mediated and biolistic methods are able to use for barley cells transformation. To achieve high expression level in short time the maize ubiquitin promoter, which is a strong constitutive promoter in cereal was used to drive the protein expression in the

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barley tissue. The produced proteins can be used for small scale functional studies in immunology assays because they have desirable biochemical and immunological properties.

For animal feeding experiments and biopharmaceutical industrial applications endosperm specific expression system has to be established. The aim is to create selection marker gene free transgenic barley plant. The vaccine genes are driven by a wheat HMW glutenin promoter, which is strictly endosperm specific. The genes were chosen for preliminary studies can be cloned into the stable transformation vector in one single step.

=== POSTER ABSTRACTS ===

EX SITU CONSERVATION AND MICROPROPAGATION ON *FRITILLARIA MELEAGRIS* L. AT BIOLOGICAL RESEARCH CENTRE JIBOU

ENIKO SELEK[1], LIA MLADIN[1,2], OANA SICORA[1] and COSMIN SICORA[1,✉]

One of the main activities of the Botanical Gardens is *ex situ* conservation of threatened and endangered species. Among the species from the Romanian Red List of Endangered Species is *Fritillaria meleagris* L. known under the popular name "lalea pestriţă", "bibilică". At Biological Research Centre this species is conserved and multiplied *in vitro* for potential repopulation of habitats were *Fritillaria* existed, but now has disappeared (Valea Sălajului, near Cehu Silvaniei). In Sălaj county the species exists, on small habitats, near Chiesd, on the place named Coronzel and in "Poiana Mică" from Poarta Sălajului. Because of the anthropic activities, mainly excessive grazing, the population of *Fritillaria* decreases every year. For this reason, we set up an *in vitro* propagation protocol from bulb scales. After a few passages on MS medium with different concentrations of hormones, we obtained plantlets for going for the next step. After the aclimation the plantlets will be cultivated in the Botanical Garden "Vasile Fati" for *ex situ* conservation. Our goal is to produce enough biological material for possible repopulation of the areas were *Fritillaria* doesn't exist anymore.

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=== POSTER ABSTRACTS ===

PHOTOSYNTHETIC ANALYSIS OF MOUNTAIN FLORA WITH MONI PAM FLUORIMETER

LIA MLADIN[1,✉], OANA SICORA[1] and COSMIN SICORA[1]

Due to a higher exposure of the mountain plants to UV radiation, there is a greater vulnerability of those plants regarding the climate changes. The degradation of the ozone layer causes a specific increasing of the UVB radiation reaching the Earth. The mainly effects of UVB stress in plants are: CO_2 deficiency, stomata closure, diminishing of chlorophyll level, damage of PSI and PSII, sugar decline.

We have studied 3 species from the mountain area: *Vaccinium* sp., *Juniperus* sp. and *Carex* sp. with a MONI PAM fluorimeter. The technique of Pulse Amplitude Modulation (PAM) offers, besides images of chlorophyll fluorescence, images of all relevant photosynthetic parameters: chlorophyll fluorescence, photosynthetic photons flux density (PPFD), PSII efficiency (YII), photosynthetic active radiation (PAR), electron transport rate (ETR) and others. In this way can be detected the photosynthetic activity and its spatio-temporal variations.

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=== POSTER ABSTRACTS ===

EFFECTS OF SALINITY STRESS ON POTATO (*SOLANUM TUBEROSUM* L.) MICROPROPAGATION

ANDREEA NISTOR[1,✉], MIHAELA CIOLOCA[1], NICOLETA CHIRU[1], MONICA POPA[1] and CARMEN BADARAU[1]

The effect of salinity on plantlets growth was determined under saline medium and non-saline with five varities of potato (Christian, Roclas, Marfona, Riviera, Tresor). Plantlets belonging to those varieties, were propagated through single nodal culture. To study the effects of salinity (NaCl) on the growth of single nodal explants, they were cultured on MS media with different concentrations of NaCl including 0, 25, 50, 75 and 100 mmol l^{-1}. Growth of single nodal explants on the media with NaCl indicated that all the characters differed significantly among salinity levels. By increasing salinity level the value for all the parameters decreased.

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=== POSTER ABSTRACTS ===

PRELIMINARY RESULTS REGARDING NBS PROFILING OF POTATO + *SOLANUM BULBOCASTANUM* SOMATIC HYBRIDS

ABDELMOUMEN TAOUTAOU[1,✉], TÜNDE-ÉVA DÉNES[1], CONSTANTIN BOTEZ[2] and ELENA RAKOSY-TICAN[1]

Potato, after cereals, is the most important crop with a production of more than 373 MT and 368 MT in 2011 and 2012, respectively. Late blight caused by the oomycete *Phytophthora infestans* is the most devastating disease of potato. Due to the concerns of consumers about possible adverse effects of GMOs and pathogen resistance to fungicides, resistance breeding is an optimal alternative. Conventional resistance breeding of potato against *P. infestan*s was based on introgression of resistance (R) genes from the wild species *S. demissum*. However, this kind of resistance, based on *S. demissum* R genes, was easily overcome by the pathogen.

Since genetic manipulation by gene transfer is still not accepted by the public and single gene resistance is short lived, somatic hybridization is a good option to overcome the sexual incompatibility. There are 5 families of R genes. All the R genes against *P. infestans* are members of the Nucleotie Binding Site-Leucine Rich Repeat (NBS-LRR) family. NBS domain is a highly conserved domain in the resistance genes toward pathogens. Primers constructed based on NBS domain sequence tag target the R genes and their homologues.

In this study we used NBS primers to reveal the polymorphism between the somatic hybrids of potato in comparison with parental lines. Somatic hybrids between potato cultivars Delikat and Rasant with *S. bulbocastanum*, their backcross progenies (BC1) and parental lines, which carry two known resistance genes *Rpi-blb1* and *Rpi-blb3*, one of those genes or none of them have been comparatively analyzed. The polymorphism of NBS domain will be also evaluated in relation with late blight resistance assays done with detached leaf assay and in the field.

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=== POSTER ABSTRACTS ===

CYTOGENETIC ANALYSIS OF SOMATIC HYBRIDS AND BACKCROSS PROGENIES BETWEEN POTATO AND *SOLANUM BULBOCASTANUM*

TÜNDE-ÉVA DÉNES[1,✉], IMOLA MOLNÁR[1] and ELENA RÁKOSY-TICAN[1]

Potato late blight is the most devastating disease of potato caused by the oomycete pathogen, Phytophthora infestans a very virulent and adaptable pathogen. The wild potato species, *Solanum bulbocastanum* (2n, 2x24), is highly resistant to all known races of *Phytophthora infestans*, even under intense disease pressure. Oomycete infects foliage and tubers alike, resulting in 16% annual yield loss. Because potato varieties and S. bulbocastanum are sexually incompatible somatic hybrids were produced by protoplast electrofusion. Putative somatic hybrid shoots were selected through flow cytometry, only the hexaploid plants being regenerated. The BC1 and BC2 progenies were obtained by crossing hybrids with different potato cultivars. The ploidy level of somatic hybrids and their derived BC progenies maintained *in vitro* is presented here after chromosome counts and analysis. The chromosomes were stained with 4'6 diamidino-2-phenilyndole (DAPI) and numbered under epifluorescent microscopy (Olympus BX-60). Micromeasure 3.3 software was used to measure chromosomal parameters from electronically captured images and assemble ideograms of parents, somatic hybrid clones and BC progenies.

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=== POSTER ABSTRACTS ===

AN INVESTIGATION INTO THE GROWTH POTENTIAL OF DIFFERENT STRAIN OF *RHIZOBIUM* ON DIFFERENT MEDIA

MONICA NISTE[1,✉], ROXANA VIDICAN[1], I. ROTAR[1] and RODICA POP[1]

Rhizobium are a group of bacteria that have the ability to nodulate legumes. In agricultural systems the symbiotic associations between legumes and soil bacteria designated as rhizobia is the most important nitrogen-fixing agents. These bacteria infect the root nodules of leguminous plants, leading to the formation of nodules where the nitrogen fixation takes place. Different envionmental factors like (water deficit, salinity, extreme temperature and mineral nutrients) they affect not only the formation of nodules but also affects the symbiosis. The main objective of the experiment was to identify which medium is more suitable for the development of different strains of rhizobia. In the present study, strains of *Rhizobium* were isolated from root nodules. *Rhizobium* strains are rod shaped to pleomorphic, forming random colonies and mucus producing. The *Rhizobium* strains were isolated using different media Yeast extract mannitol agar (YEMA) with Congo red, a mediu with yeast extract, mannitol and soil extract and a medium for N_2 fixing organisms. The Petri plates were incubated at 28 ºC in the dark. Further characterizations were done by performing morphological traits and also determine what media is more suitable for the growth of rhizobia. Regard to number of colonies formed on all media, *Rhizobium trifolii* wild type showed a larger number of CFU compared to *Rhizobium trifolii* which has a lower number of colonies. The optimum medium for the growth of *Rhizobium* species was seen to be the one with mannitol, yeast extract and agar (YEMA), also appeared to work well medium for N_2 fixing organisms for the wild type. In conclusion rhizobia can grow best on glucose and mannitol and in general are easy to culture.

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=== POSTER ABSTRACTS ===

EFFECTS OF SALINITY ON THE GERMINATION CAPACITY OF SOME *ARABIDOPSIS THALIANA* MUTANT LINES

OANA CIUZAN[1,✉] and DORU PAMFIL[1]

Arabidopsis thaliana, a small spring annual plant belonging to the Brassicaceae family, is used since the 1940s as a model in plant genetics and biotechnology due to some advantages, like small genome, large production of seeds and short life cycle. From the three major ecotypes found in the spontaneous flora (Columbia, Landsberg and Wassilewskija) a series of loss of function and gain of function mutants were created since the 1960s in order to answer questions related to several genes and proteins involved in the physiological mechanisms of plants. The most attention was laid on the genes and proteins involved in plant adaptation to abiotic and biotic stress conditions. The glycine-rich RNA-binding proteins are a major class of proteins found in superior plants that are known to have a big implication in plant adaptation to low temperatures and other osmotic and oxidative stresses. The main focus of this research was to assess the involvement of two glycine-rich RNA-binding proteins (GRP2 and GRP7) in seed germination capacity under salt stress conditions. The plant material was composed out of seed batches obtained from the T-DNA insertion *Arabidopsis thaliana* mutant lines labelled *grp2* (knock-out mutant for the glycine-rich RNA-binding protein 2), *grp7-1* (knock-out mutant for the glycine-rich RNA-binding protein 7), *WS7ox* (overexpressor for the GRP7 protein) and the Col0 ecotype, which was used as control. For the germination assay, three different concentrations (100 mM, 150 mM and 200 mM) of NaCl were used, with whom the control medium was supplemented. The control medium chosen consisted of ½ MS (Murashige & Skoog) vitamin medium without sugar. Fifty seeds were placed on each petri dish and they were assessed for seven days. After this period, the germination rate was estimated. Three independent replicates were used and the statistical analysis was obtained with the help of the trial version from GraphPad Prism software. The results obtained showed that the seeds belonging to the *grp2* mutant were not affected by the high salt concentration, in contrast to the ones obtained from the *grp7-1*

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mutant and *WS7ox* line. Also, in comparison to the wild-type, the germination rate of these seeds was higher. As a conclusion, we can assume that even though the two proteins taken under observation belong to the same class and family, they have different roles regarding the seed germination capacity.

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=== POSTER ABSTRACTS ===

MODIFICATION OF THE PHENYLPROPANOID PATHWAY AFTER DIFFERENT SALICYLIC ACID TREATMENTS IN WHEAT

ORSOLYA KINGA GONDOR[1], TIBOR JANDA[1] and GABRIELLA SZALAI[1]

Salicylic acid (SA) was isolated first from bark of the willow tree (*Salix*) and used as a medicine. Its biosynthesis is part of the phenylpropanoid pathway where its first precursor is the phenylalanine. Two routes from phenylalanine to salicylic acid have been described that differ at the step involving hydroxylation of the aromatic ring. Phenylalanine is converted into cinnamic acid (CA) by phenylalanine ammonia lyase. Cinnamic acid can be hydroxylated to form ortho-hydroxycinnamic acid (oHCA) followed by oxidation of the side chain. Alternatively, the side chain of cinnamic acid is initially oxidized to give benzoic acid (BA), which is then hydroxylated in the ortho position. Flavonoids are important protectants during stresses and their biosynthesis derived from the cinnamic acid via trans-hydroxycinnamic acid. The aim of the work was to investigate physiological/biochemical processes induced by the different exogenous SA treatments.

Mv Emese winter wheat variety was used for the experiments. Plants were grown in hydroponic solution. SA treatment was carried out either by soaking seeds in 0.5 mM SA for overnight before sowing or by addition of 0.5 mM SA to the hydroponics of seven-day-old plants for a day. Leaf and root samples were collected after 1 and 7 days of the hydroponic SA treatment. For detection of the oxidative stress the lipid peroxidation was measured via malondialdehyde (MDA) content spectrophotometricaly. 0.5 g plant material was used for determination of SA, BA, CA, oHCA and flavonoids. Methanol soluble free, methanol soluble bound and methanol insoluble bound fractions were measured. The analysis was carried out using an HPLC equipped with a UV-VIS and fluorescence detector.

It can be seen from the results that the level of SA and its precursors changed after the treatments. The level of BA, CA did not change while the SA and oHCA content increased after 1 day of SA treatment in the leaves. The MDA concentration

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also increased compared to the control which alludes to the increased oxidative stress. oHCA can serve as an antioxidant so its elevated level can be a consequence of the stress. The BA content decreased, SA content increased while the oHCA content did not change in the methanol soluble bound fraction. The level of SA, BA, CA and oHCA did not change in the methanol insoluble bound fraction. Seed soaking did not have any effect on the content of the endogenous SA and its precursors in either of fractions. Free oHCA increased after seven days but there was no change in the bound fractions. The SA level increased in the case of hydroponic SA treated plants in all the fractions while the BA and CA content remained at the initial level. The CA content decreased while the oHCA content increased in the free fraction in the roots of the hydroponic SA treated plants after one day. The MDA concentration also increased as a marker of the oxidative stress. The SA content increased in all the fractions. The level of free SA increased after the seventh day of hydroponic SA treatment while the CA and BA did not change.

Some of the flavonoids were also analysed. Myrecetin (M), kaempferol (K), quercetin (Q) and rutin (R) were measured. The M, Q and R level increased in the leaves after the SA treatments. R and K level decreased in the free fraction in the roots after one day and it still remained at this level for seven days compared to the control values. The content of the methanol soluble bound M and R decreased after one day but increased after seven days. The Q concentration increased only in the free fraction of the leaves after one day and a decrease could be observed in the roots in all the fractions. The level of free rutin slightly increased but it decreased in the roots after one day. An increase could be seen in the free rutin content in the roots after seven days.

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=== POSTER ABSTRACTS ===

EFFECTS OF UV-B RADIATION UNDER DIFFERENT ABIOTIC STRESS CONDITIONS IN WHEAT

VIKTÓRIA KOVÁCS[1,✉], ORSOLYA KINGA GONDOR[1], IMRE MAJLÁTH[1], GABRIELLA SZALAI[1], TIBOR JANDA[1] and MAGDA PÁL[1]

At the continental climate high UV-B radiation, especially in combination with aridity, causes several morphological and physiological changes in plants, leading to the decrease in crops quality and quantity. In the background of diverse plant responses to UV-B there are different processes such as the changes in the enzymatic antioxidant system and numerous other defence mechanisms, which can be linked to the salicylic acid-dependent signal transduction. Salicylic acid is known as a signal molecule which has role in the induction of acclimation processes under abiotic stresses conditions in plants. The aim of the present work is to better understanding of the effects of UV-B radiation, cadmium and drought stress and the cross-talk between these abiotic stresses in wheat. So we studied the effects of these stresses individually and in combination. Seedlings of winter wheat (Triticum aestivum L. Mv Emese) variety from Martonvásár were grown in growth chamber under normal light conditions or at normal light combined with UV-B radiation at the same time. One part of the two weeks old plants was the control, second part of the plants was treated with 50 μM $Cd(NO_3)_2$ for 7 days and the third part of the plants was treated with 15 % polyethylene glycol (PEG-6000) for 5 days. The three different stresses induced changes in the antioxidant system, salicylic acid metabolism and biosynthesis were characterised in leaf and root samples using spectrophotometric methods and HPLC. Seedlings showed reduced growth and induced anthocyanin production under supplemental UV-B radiation. PEG+UV-B treated seedlings showed smaller degree of shrivelling, increased content of bound oHCA and free SA, and induced activity of glutathione reductase and catalase, compared to the seedlings treated only with PEG. Cd+UV-B treatment enhanced the effects of Cd. The leaves of these plants became yellowish, the bound oHCA and SA levels of them significantly increased while the activities of the antioxidant enzymes

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decreased. However, in the roots, the first organ exposed to Cd, the antioxidant system induced. Although UV-B decreased the plant development, it was found that under these conditions UV-B radiation caused successfully hardening against to drought stress, but resulted in stronger damages Cd-treated wheat seedlings.

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=== POSTER ABSTRACTS ===

"GLOBALIZATION" IN THE SEXUAL REPRODUCTION OF CEREALS: WIDER CHOICE, BETTER HYBRIDS

DÁVID POLGÁRI[1], KATALIN JÄGER[1], BEÁTA BARNABÁS[1] and LÁSZLÓ SÁGI[1,✉]

Ultrawide hybridization (UWH), i.e. non-GM trait transfer by sexual crossing between distant species or genera, has long been applied to introduce novel genes into crop plants. Major hurdles prohibiting more widespread and routine use of UWH are the low frequency and viability of hybrid embryos, which makes embryo rescue and nursing plants by tissue culture compulsory steps in the process. Beyond its agronomical importance UWH, however, offers a fascinating model to study early phases of reproductive development. Here we asked whether low success rates of UWH in cereals are caused by a low frequency of fertilization (including egg activation and pollen tube formation) and/or by incomplete or blocked endosperm development.

By using an optimized wheat (♀) x barley (♂) hybridization system and via a comprehensive microscopical study we have found that pollen tube formation and fertilization can successfully be accomplished. Endosperm development, however, was essentially blocked at or before the onset of the cellular phase, which can cause early embryo abortion. Via suppressing this endosperm block we were able to produce normal, endosperm-containing wheat-barley hybrids. As a result, harvested hybrid seeds germinated readily without the aid of tissue culture. The majority of these plants contained the full, 7-chromosome complement of barley.

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STUDIA UBB BIOLOGIA, LVIII, 1, 2013 (p. 57-69)

EX SITU IN VITRO CONSERVATION OF *DIANTHUS SPICULIFOLIUS*, ENDANGERED AND ENDEMIC PLANT SPECIES

VICTORIA CRISTEA[1,✉], MAGDALENA PALADA[2], LILIANA JARDA[1] and ANCA BUTIUC-KEUL[3]

SUMMARY. The preservation of endangered and/or threatened plant species by biotechnological methods as *in vitro* culture is a complementary alternative of in situ conservation. Several individuals of *Dianthus spiculifolius* Schur from two distinct and spatially separated populations, from Hăşmaş and Vlădeasa Mountains (Romania) were preserved by *in vitro* culture. Following several stages of *in vitro* culture as culture establishment, stabilization, stable culture multiplication, *in vitro* rhizogenesis and outdoor acclimatization, an optimal protocol for conservation and acclimatization was achieved. Four culture media variants were used for different culture stages. During the stable culture multiplication stage, after 60 days of culture, a maximum of 104.8 new shoots/uninodal explant for the Vlădeasa Mt. population and 24.9 new shoots/explant for the Hăşmaş Mt. population were obtained. Nodes explants, horiozontally inoculated generated a very high number of shoots (maximum 345.8 shoots). The rhizogenesis ratio was 10 roots/explant in population from Hăşmaş Mt. and 5.3 in population from Vlădeasa Mt. The vitroplants were successfully acclimatized and they were cultivated in a special outdoor rocky area.

Keywords: acclimatization, endangered, endemic plant, explant type, micropropagation.

Introduction

Besides the different advantages of *in vitro* culture, the *ex situ* conservation by *in vitro* collection, also offers the possibility to generate biological material either for in situ repopulation programs, or collections in botanical gardens, international exchanges or fundamental and applied studies. Thus, the pressure of collecting plants

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from the natural populations is avoided. *In vitro* collections also have a social impact, serving to raise awareness among non-scientists about the *ex situ* conservation of plants.

Nowadays, the prioritization of endemic and/or threatened species and their natural habitats is a crucial point of conservation actions, particularly in areas with rich endemic floras (Bacchetta *et al*., 2012). Target 8 of 2011-2020 Global Strategy of Plant Conservation required having "At least 75% of threatened plant species in *ex situ* collections, preferably in the country of origin, and at least 20% available for recovery and restoration programs". The Red Book of Vascular Plants of Romania mentions that among the 3795 existing vascular plants taxa, 548 (14.4%) are endangered (Dihoru and Negrean, 2009). The number of the endangered plant taxa preserved *in vitro* in Romania is only 52 (less than 1.4%) (Cristea *et al*., 2006; Păunescu, 2009; Holobiuc *et al*., 2009; Cristea 2010, Cristea *et al*., 2010). Lately, in this context, an increasing importance is given to *in vitro* conservation of endemic and/or endangered species all over the world (Papafotiou and Stragas, 2009; Radojević *et al*., 2010; Gorgorov *et al*., 2011; Kumaria *et al.*, 2012, etc) including Romania (Holobiuc *et al*., 2009; Cristea *et al*., 2010; Jarda *et. al*, 2011; Coste *et al*., 2012).

D. spiculifolius Schur (*Caryophyllaceae*) is a phytogeographically and sozologically important species. It is endemic for the Eastern Carpathian Mountains (Tutin, 1964; Ciocârlan, 2009) and in Romania it is considered as Rare (Oltean *et al*., 1994; IUCN, 1997), Vulnerable (Dihoru and Dihoru, 1994; Sârbu et al., 2003) or Endangered (Sârbu *et al.*, 2007). It is a caespitose perennial species, 10-30 cm height, with white, rarely pink scented flowers, that appear in June-August. This species is growing in the mountain and alpine belt, on skeletal, calcareous rocks (Ciocârlan, 2009). Because of its caespitose aspect, nice and scented flowers this species has ornamental importance as well.

There are some studies on *D. spiculifolius* that refer to *in vitro* introduction and multiplication (Zăpârţan, 1995; Butiuc-Keul *et al*., 2001; Cristea *et al*., 2002; Pop and Pamfil, 2011) but, some data are unclear or contradictory and do not permit replication of them. When initiating an *in vitro* collection it is very important to introduce and maintain *in vitro* more individuals from different populations, to avoid the genetic uniformity. There are no previous studies about the reaction of different individuals from natural populations to *in vitro* culture.

The aims of this study were to establish an optimal and high-frequency multiple micropropagation protocol, delineating more stages of *in vitro* culture, outdoor acclimatization of individuals from different populations of *D. spiculifolius*.

Materials and methods

Plant material and culture medium

The plant material originates from two distinct and spatially separated in situ *D. spiculifolius* populations: population 1 (encoded Ds 1) with 3 individuals (Ds 1.1; Ds 1.2; Ds 1.3), from ROSCI0027 Natura 2000 site, Hăşmaş Mountains (Romanian

Oriental Carpathian) and population 2 (Ds 2), with 3 individuals (Ds 2.1; Ds 2.2; Ds 2.3), from ROSCI0002 Natura 2000 site, Vlădeasa Mountains (Romanian Occidental Carpathian). All the collected individuals were planted in a special rocky area in the Alexandru Borza Botanical Garden from Cluj-Napoca, dedicated to the *ex situ* outdoor collection of endemic/endangered *Dianthus* taxa from Romania (Cristea *et. al*., 2013). *in vitro* culture was induced from different explants prelevated from these individuals.

Culture media containing MS macro - and microelements and FeEDTA (Murashige and Skoog, 1962), supplemented with thiamine 2.96 μM, pyridoxine HCl 4.86 μM, nicotinic acid 8.12 μM, myo-inozitol 0.55 mM, sucrose 2% and agar 0.7% [w/v] were used. Four different variants of culture media were studied: V1 - initiation medium (with 6-benzyladenine (BA) 4.44 μM and α-naphtaleneacetic acid (NAA) 5.37 μM); V2 - multiplication medium (with BA 4.44 μM and NAA 0.54 μM) and 2 rhizogenesis media: V3 - with reduced PGRs (plant growth regulators) concentration (BA 0.44 μM and NAA 0.05 μM) and V4 without PGRs.

Culture establishment and stabilization

In vitro culture was initiated from explants consisting of apices with 2-3 nodes from young shoots. We used the following variants for sterilization: (a) 20% Domestos (Unilever, United Kingdom) for 10 min; (b) 0.2% mercuric chloride for 10 min and (c) 10% Domestos for 5 min plus 0.2% mercuric chloride for 5 min. Then, the explants were washed stirring well, 5 times with sterile water. The explants were inoculated on V1 medium. All *in vitro* cultures were grown at 22 ± 2°C, under 16 h photoperiod (cool-white fluorescent lights, 50 μmol × s^{-1} × m^{-2} PPFD). The sterilization and survival index and the multiplication of the explants were monitored after 30 days from inoculation. Then, the entire cluster of shoots formed from an explant was transferred on fresh medium V2, two times, at 30 days. After a period of 90 days, the following parameters were scored: the multiplication rate, the length of shoots and the number of internodes. To study the variability during *in vitro* culture, the length variation of the 17 new shoots generated by a single individual (e.g., Ds 1.3) was also recorded.

Stable culture multiplication

For an optimal multiplication and a proper maintenance of a living collection we studied 3 different methods of multiplication, comparatively, using 3 different types of inocula, cultivated in Steri Vent culture containers (Duchefa Biochemicals, Haarlem, The Netherlands): a) binodal apices; b) uninodal fragments, from 2nd and 3rd nodes; c) 5 to 7 nodes fragment (under the apex), horizontally positioned. V2 culture media were used. The number of shoots generated/explant during a 60 days subculture was recorded.

In vitro rhizogenesis and acclimatization

Binodal apices from stable culture were inoculated on V3 and V4 culture media to induce or to improve the roots generation. At the same time, other explants were *in vitro* cultured in sterile wet sand or perlite instead of agar-containing medium. After 60 days from inoculation the plantlets were quantified and then transferred for ex vitro acclimatization on 2 types of substrate: perlite and perlite + sterile soil (1/1). The plantlets were soaked with a MS 1/2 macroelements solution (Murashige and Skoog, 1962) and maintained under high humidity the first week. Then, the atmospheric humidity was reduced progressively. After 3 weeks the plants were transferred in soil, in the greenhouse and then were weaned in shelter for 1 month. In the spring the plants were transferred on a special rocky area created for the vitroplants collection of the Alexandru Borza Botanical Garden from Cluj-Napoca.

Statistical analysis

The *in vitro* experimental design consisted of 12 replicates per each individual and the experiments were repeated twice. The results were expressed as the average of replicates ± standard deviation (SD). Data were subjected to One-Way ANOVA test (for testing 3 or more columns) or to the t tests (for testing 2 columns), with a 95% confidence intervals. In case of significant differences following the ANOVA test, Tukey`s test was used in order to determine the significance of the differences between the average values at $P < 0.05$ [$P < 0.001$ = extremely significant (***); P 0.001 to 0.01 = very significant (**); P 0.01 to 0.05 = significant (*); $P \geq 0.05$ = no statistical significance (ns)]. The statistical analysis was performed using GraphPad Prism (version 5.00 for Windows, GraphPad Software, San Diego, USA).

Results

Culture establishment and stabilization

The best results regarding the explants sterilization were obtained using the $HgCl_2$: 88% sterile inocula for (b) sterilization variant and 82% for variant (c). The viability index after sterilization can be considered as high as: 96% for (c) sterilization variant and 100% for variant (b). After 30 days from inoculation, the explants generated an average of 4 new shoots, with or without roots (Table 1 and Fig. 1). During the second and third subculture the proliferation increased, up to 74.3 shoots/initial explant, after 90 days. The results for the length of the generated shoots by a single individual (e.g., Ds 1.3) are presented in Fig. 2. It was observed that the length of shoots ranged between 9.3 cm and 40.0 cm, the average length being 21.3 cm.

Table 1.

D. spiculifolius explants evolution during the initiation stage (at 30 days of culture) and the stabilization stage (after another 60 days); on V1 culture medium supplemented with BA 4.44 µM and NAA 5.37 µM.

D. spiculifolius population:	Ds 1	Ds 2
Average no. of new shoots/explant after 30 days	4.8 ± 1.6 ^{ns}	3.2 ± 1.8 ^{ns}
Average no. of new shoots/explant after 90 days	25.9 ± 5.1 ^{ns}	74.3 ± 11.1 ^{ns}
Average length of shoots after 90 days (cm)	12.3 ± 3.0 ^{ns}	9.5 ± 3.3 ^{ns}
Average no. of internodes/shoot after 90 days	9.7 ± 2.1 *	8.9 ± 2.5 *

The results are presented as the mean ± SD.

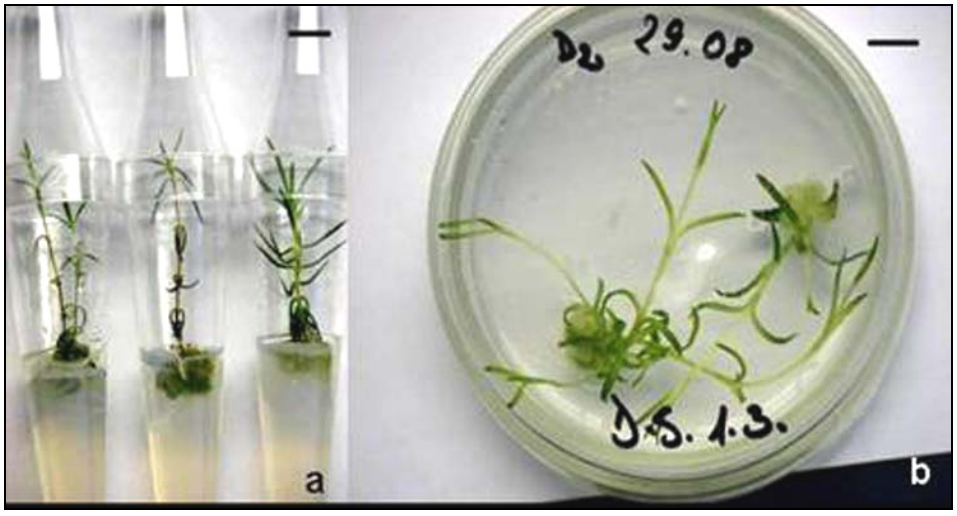

Figure 1. *D. spiculifolius in vitro* culture generated by binodal apices sampled from young shoot, in a: DeVit tubes (Duchefa Biochemicals, Neetherland) and b: Petri dishes. Culture on V1 medium, supplemented with BA 4.44 µM and NAA 5.37 µM. Bars represent 1 cm.

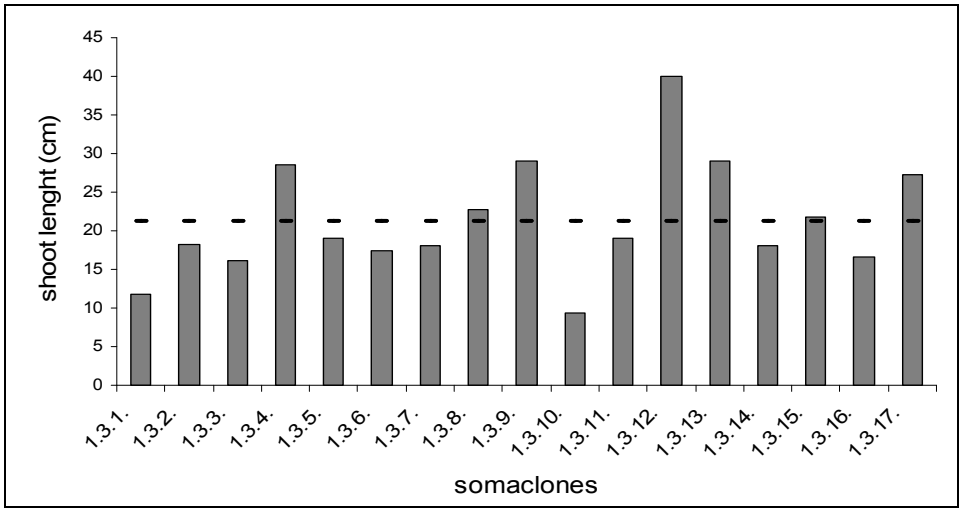

Figure 2. Variation of shoots length to the somaclones of Ds 1.3 individual, on V1 culture medium supplemented with BA 4.44 μM and NAA 5.37 μM. The dashed line represents the average value (21.3 cm) of the somaclones' length. ANOVA test: ns

Stable culture multiplication

After 60 days of culture on medium V2, the multiplication rate obtained for binodal apices and uninodal fragment is comparable (Table 2). The better results obtained for plurinodal horizontal inocula can be explained by the large number (5-7) of nodes/explant. Population Ds 2 shows a higher multiplication rate compared with Ds 1 for all the 3 types of explants: 2.6 times higher multiplication rate at binodal apices, 4.6 times higher multiplication at the uninodal fragments and 4 times higher multiplication at the multinodes fragments horizontally cultured. Different stage of *in vitro* culture of binodal apices and of 5-7 nodes horizontally cultured inocula are presented in Fig. 3.

Tabel 2.

Influence of explant type on the number of regenerated shoots, during the stable culture stage, at different individuals of the two *D. spiculifolius* populations, on V2 medium, supplemented with BA 4.44 μM and NAA 0.54 μM; results after 60 days of culture.

Population	Individuals	No. of regenerated shoots/explant		
		bA	uE	5-7n
Ds 1	Ds 1.1.	34.8 ± 4.3	23.1 ± 5.8	43.8 ± 6.3
	Ds 1.2.	41.7 ± 7.1	24.9 ± 4.4	69.5 ± 8.4
	Ds 1.3.	9.8 ± 1.3	4.9 ± 1.4	34.0 ± 3.6
average Ds 1		28.8 **	17.6 **	49.1 **

Population	Individuals	No. of regenerated shoots/explant		
		bA	uE	5-7n
Ds 2	Ds 2.1.	60.4 ± 5.3	57.1 ± 16.4	112.6 ± 20.9
	Ds 2.2.	98.7 ± 14.3	104.8 ± 15.3	345.8 ± 45.6
	Ds 2.3.	65.8 ± 18.4	78.9 ± 11.8	139 ± 26.8
average Ds 2		74.9 **	80.3 **	199.2 **

bA = binodal apices explant. uE = uninodal explant. 5-7n = 5 - 7 nodes explant horizontally inoculated. Data are presented as the mean ± SD. ANOVA test: between the 3 type of explant ns; t test: interpopulational variability: Ds 1 vs Ds 2 **; intrapopulational variability: Ds 1.1 vs Ds 1.2 and Ds 1.3 ns; Ds 1.2 vs Ds 1.3 *; between Ds 2.1, 2.2 and 2.3 ns.

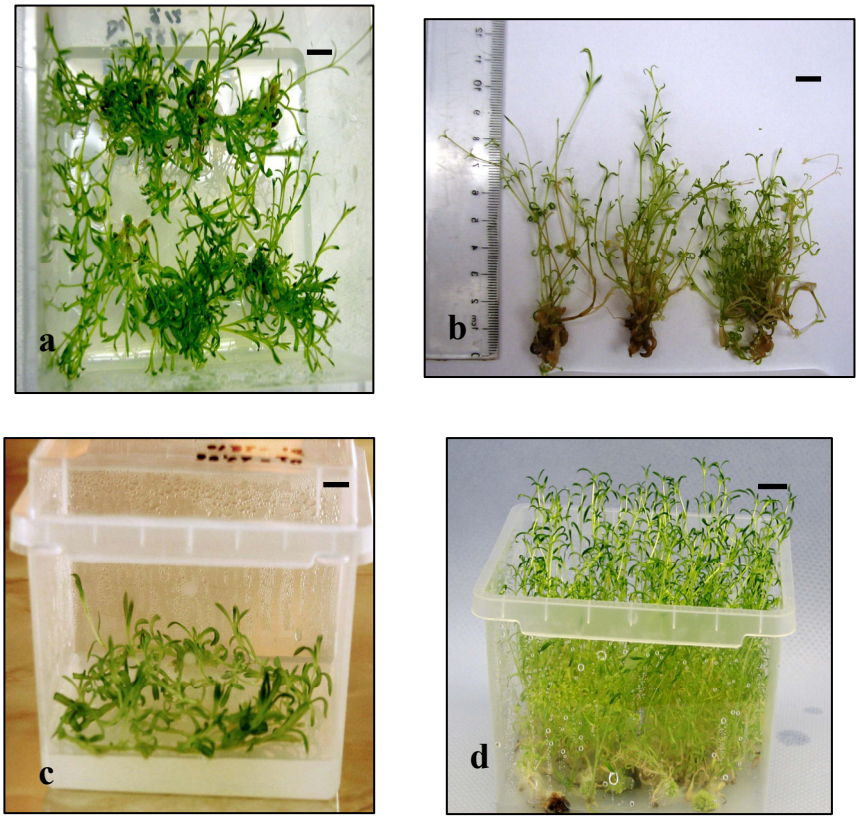

Figure 3. *D. spiculifolius* stable culture multiplication, on V2 medium supplemented with BA 4.44 µM and NAA 0.54 µM. a, b: binodal apices, of individual Ds 1.3, 60 days of culture; c, d: 5-7 nodes horizontally cultured inocula of individual Ds 1.2; c: 10 days; d: 60 days of culture. Bars represent 1 cm.

Rhizogenesis and acclimatization

The main characteristic of *D. spiculifolius* was the weak influence of culture medium composition on its growth parameters (Tables 3). There are no significant differences between the results obtained on V3 - with PGRs - and V4 - without PGRs - culture media. The number of roots/explant (between 4.7 and 15.8), their length (between 1.3 and 5 cm) and their vigor recommending them as suitable for transplanting regenerated plants in *ex situ* conditions. The vitroplants acclimatized on perlite (Fig. 4 a) had the best root system development, comparative to that developed in perlite+sterile soil (1/1). After 3 weeks of ex vitro culture in the laboratory, the root system was well developed. The efficiency of ex vitro survival and development was increased from about 50% to about 70% when the rhizogenesis was induced *in vitro* in sand or in perlite instead of agar-containing culture medium. After the green house and shelter acclimatization (Fig. 4 b), the plants were cultivated (Fig. 4 c) on a second special rocky area dedicated to different micropropagated species (Fig. 4 d).

Table 3.

Influence of the culture media on the rhizogenesis of *D. spiculifolius* binodal apices development. V3 = BA 0.44 μM and NAA 0.05 μM; V4 = without PGRs; after 60 days of culture.

Culture media	V3			V4		
Population individual	LS	NoR	LR	LS	NoR	LR
Ds 1.1.	8.8 ± 0.6	8.1 ± 0.4	3.1 ± 0.2	11.7 ± 0.3	8.2 ± 0.1	4.1 ± 0.1
Ds 1.2.	15.0 ± 0.1	15.8 ± 0.7	5.0 ± 0.2	15.1 ± 0.3	15.7 ± 0.6	4.6 ± 0.2
Ds 1.3.	11.8 ± 0.4	6.2 ± 0.3	2.8 ± 0.1	11.8 ± 0.3	6.3 ± 0.2	2.8 ± 0.1
average Ds 1	11.9 ^{ns}	10.0 ^{ns}	3.6 ^{ns}	12.9 ^{ns}	10.1 ^{ns}	3.8 ^{ns}
Ds 2.1.	11.2 ± 0.4	5.6 ± 0.4	1.7 ± 0.4	10.0 ± 0.1	5.9 ± 0.1	2.4 ± 0.3
Ds 2.2.	10.1 ± 0.9	4.7 ± 0.1	1.3 ± 0.1	10.5 ± 0.2	5.0 ± 0.3	2.8 ± 0.2
Ds 2.3.	12.3 ± 0.1	4.7 ± 0.4	1.5 ± 0.2	11.1 ± 1.0	5.0 ± 0.2	2.8 ± 0.1
average Ds 2	11.2 ^{ns}	5 ^{ns}	1.5 ^{ns}	10.8 ^{ns}	5.3 ^{ns}	2.7 ^{ns}

LS = length of shoots/explant (cm). NoR = no. of roots/explant. LR = length of roots/explant (cm). Data are presented as the mean ± SD. t test: V3 culture medium vs V4 ns; Anova test: intrapopulational variability: only individual 1.2 vs 1.3 *; between the other individuals there are not significantly differences.

Figure 4. a: *D. spiculifolius* acclimatization in perlite soaked with mineral nutrient solution before transplanting plants in soil. b: *D. spiculifolius* plants transferred in soil, after green house and shelter acclimatization; c: *D. spiculifolius* plants obtained by *in vitro* culture, after their outdoor transfer; d: special rocky area of Alexandru Borza Botanical Garden, dedicated to different endemic/endangered micropropagated species, at the time of planting. Bars represent 1 cm.

Discussion

Culture establishment and stabilization

The results regarding *in vitro* culture initiation of D. *spiculifolius* are better, when compared to that obtained for other endemic/endangered Romanian *Dianthus* species (Miclăuş *et al.*, 2003; Cristea *et al.*, 2009; Holobiuc *et al.*, 2009). They can be explained by the lower infestation degree of the explants and by using combinations of different disinfectant agents. Very good results for *in vitro* culture initiation are expected when seeds are used (Cristea *et al.*, 2010).

Considering the multiplication rate during the initiation stage, comparative results were obtain in the case of other Romanian endemic and/or endangered *Dianthus* species: *D. petraeus* ssp. *simonkaianus* (Miclăuş *et al*., 2003), *D. glacialis* ssp. *gelidus* (Cristea *et al*., 2006), *D. henteri* (Cristea *et al*., 2010), *D. giganteus* ssp. *banaticus* (Cristea *et al*., 2006; Jarda *et al*., 2011) and also for other sozologically important European *Dianthus* species, like *D. giganteus* ssp. *croaticus* (Prolic *et al*., 2002), *D. gratianopolitanus* (Fraga *et al*., 2004), *D. pyrenaicus* (Marcu *et al*., 2006), *D. giganteus, D. alpinus, D. ferrugineus* and *D. gallicus* (Cristea *et al.*, 2006). Statistical analysis reveals that in this stage the number of shoots/explants and their length, after 90 days of culture, show no significant differences between the 2 populations (Table 1). However, the number of internodes/shoot reveals differences between the two populations. The average length of internodes was about 1 cm, as well as the initial vegetative shoot fragments used as explants source for *in vitro* culture establishment. This is important because, unlike other species, *in vitro* culture of *D. spiculifolius* does not cause significant morphological differences compared to in situ plants. More or less similar results regarding the regeneration capacity of this species were reported by some authors, but they didn't specify the type of inoculum (nodal or apical, position of nodes on the stem), the number of nodes of the inoculum, or the period of culture (Holobiuc *et al*., 2004-2005; Pop and Pamfil 2011).

This species show a large variability regarding the length of the somaclones generated by one individual (Ds 1.3), as can be observed in Fig. 2. To date, there are no results regarding comparative studies about *in vitro* reaction of more populations or more individuals.

Stable culture multiplication

Analyzing the data presented in Table 2, we conclude that, in this stage, significant differences appear between the responses of the 2 populations. Likewise, there were also differences between the different individuals of the same population. All the 3 explants types have provided a satisfactory multiplication, however rooting was often poor (Fig. 3). Comparing the 3 variants, the advantages and limitations of each one of them emerge: (i) the binodal apices are uniform explants recommended to be used in experiments to test different culture media and growing conditions; (ii) the uninodal fragments offer the possibility to highlight potential somaclonal variation that is phenotypically expressed, as a consequence of sensitivity differences to cytokine; (iii) plurinodal fragments horizontally cultured offer practical advantages, being easy to handle; at each node, a number of shoots occur that are easy to separate and simultaneously develop adventives roots, enabling early transplanting in soil for acclimatization. Previous studies performed on the *in vitro* multiplication of those species, after more transfers, revealed more or less similar results: a multiplication rate of 30-40 new shoots/explant, after 45-115 days of culture, on a medium with 1/10 auxins/cytokinins ratio (Cristea *et al*., 2002; Holobiuc *et al*., 2004-2005).

Rhizogenesis and acclimatization

On both studied culture media the root formation was appropriate for acclimatization. The obtained results lead to the idea that there is no need of PGRs for rhizogenesis induction in case of this species. This is very important, knowing the fact that PGRs are one of the factors that can induce somaclonal variability (Bairu et al. 2011). Even if some authors (Pop and Pamfil, 2011) have obtained only random adventitious roots for the same PGRs and the same PGRs balance, in our experiments, the rhizogenesis was medium or appropriate, not only on the media without PGRs, but even if the PGRs balance was in the detriment of auxins. For other endemic and/or endangered *Dianthus* species (Kovác, 1995; Miclăuş *et al*., 2003; Marcu *et al.*, 2006; Jarda *et al*., 2011) the number of *in vitro* generated roots was similar to that reported in the present paper. The outdoor existence of acclimated vitroplants in a botanical garden is even more important because none of the previous studies on this species was followed by outdoor acclimatization after *in vitro* culture.

Conclusions

A high frequency multiple micropropagation protocol having several stages was obtained for endangered and endemic plant species *Dianthus spiculifolius*. Both multiplication and rhizogenesis were optimal on culture media studied. Vitroplants can be easily acclimatized and grown in outdoors conditions. All three types of explamts could be used for preservation depending on the aim of experiments. There are no significant differences between the reactions of the individuals belonging to the two populations in the initiating stage of *in vitro* culture, but significant differences were observed in the stage of stable culture multiplication. The individual belonging to Vlădeasa Mt. population showed a rate of multiplication of 2.6-4 times higher than the individual from Hăşmaş population. The are no significant differences regarding the reaction to *in vitro* culture of the individuals belonging to the same population.

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THE EFFECT OF DIFFERENT LIGHT INTENSITIES ON PHOTOCHEMICAL ACTIVITY IN *MICROCYSTIS AERUGINOSA* AICB 702 STRAIN (CYANOPHYTA)

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SUMMARY. *Microcystis aeruginosa* AICB 702 was grown at room temperature, on GZ medium, in air-lift conditions, using a medium light intensity of 260 μmol. $m^{-2}.s^{-1}$. The exponential-phase cells were exposed to 800, 1500 and 2100 μmol. $m^{-2}.s^{-1}$ light intensities and the fluorescence measurements were performed after 15, 30, 45, 60, 75, 90, 105 and 120 minutes of light exposure. Dark-adapted probes were analyzed after 120 minutes recovery period. The oxidation state of primary acceptor and the fraction of the reaction centers are reduced (damaged), the photochemical process and the light harvesting are slightly inhibited when an 800 μmol. $m^{-2}.s^{-1}$ light intensity is used. Also, the reduction of the primary acceptor and the reaction centers closure were maximal. The F_v/F_m and Y(II) quantum yield were inhibited. The Fv/Fm values registered toward the end of the experiment represented 46.5% of the theoretical value, which also indicates the ratio of the PS II reaction centers that were photoinhibited. The non-regulated energy dissipation increased, and the high values of qP and qL coefficients showed an enhanced photochemical process with a low fluorescence emission. Increased F_0 and F_m and Fv decreased when 1500 μmol.$m^{-2}.s^{-1}$ was used. The Fv/Fm and Y(II) diminished and the non-regulated energy dissipation increased. When a light intensity of 2100 μmol.$m^{-2}.s^{-1}$was used, the Fo raised during a 90 min period, then decreased to 85%. Fm and Fv decreased, also. The Fv/Fm and Y(II) diminished, while the non-regulated energy dissipation increased. The decrease of F_0, F_m and Fv/Fm yield and that of the Y(II) certified the photoinhibition effect of 2100 μmol.$m^{-2}.s^{-1}$ light intensity on the activities carried out by the photosystems antenna. When an 800 μmol.$m^{-2}.s^{-1}$ light intensity was used the recovery of the photosynthetic activity was faster compared with other high-light intensities treatments.

Keywords: chlorophyll fluorescence, maximal fluorescence Fm, minimal fluorescence F0, maximal PS II quantum yield (Fv/Fm), effective PS II quantum yield (Y_{II}), quantum yield of non-regulated energy dissipation (Y_{NO}), recovery, photoinhibition, coefficient of photochemical quenching (qP, qL).

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Introduction

Cyanobacteria showed different strategies of acclimation and adaptation to light. Numerous cyanobacterial strains possess the ability to change their phycobilisomes composition in response to different wavelengths of light (Everroad *et al*., 2006). This ability, known as *chromatic adaptation* is present under various forms in different species (Kehoe and Guțu, 2006). The cyanobacterial sensory proteins initiate a signal transduction cascade in response to environmental signal, consisting in recognizing and translating the signal, followed by the cell response (Montgomery, 2007). The photoinhibition term describes the reduction of the photosynthetic capacity which is induced by the exposure to visible light (400-700 nm), regardless of the changes in pigments concentrations (Powles, 1984).

In Cyanobacteria, the photosynthetic system is related with the main metabolic pathways, thus the signal of the chlorophyll fluorescence can provide information on photosynthesis and the acclimation state. According to Campbell (1998), the excitation energy can be used in four distinct pathways as it follows:

- the excited electron produced in photochemical reactions which are carried out by the chlorophyll reaction centers is transferred to the electron transport chain.
- the high-energy electron falls back into its ground state and releases its energy as thermal energy dissipation.
- the transfer of the excitation energy to the adjacent pigment by the light-harvesting antenna system.
- the fluorescence is emitted with a lower energy, but a longer wavelength than the absorbed photon.

Although, these processes are in competition with each other, the energy of an excited molecule is used in the pathway that has the highest constant rate. The chlorophyll fluorescence yield is usually low and, *in vivo,* the chlorophyll fluorescence is produced by the PS II. The PS I contribution to the fluorescence value, which is presumed to have a constant level, is 15 to 20% (Roelofs *et al.,* 1992; Trissl *et al.,* 1993).

The photosynthetic organisms have developed multiple protection mechanisms to survive in high-light conditions. Recently, a possible mechanism dissipation of the excess energy absorbed by the phycobilisomes of the extramembrane antenna has been described (Wilson *et al.,* 2006). This mechanism which occurs in phycobilisomes is characterized by the fluorescence quenching under blue light and a carotenoid protein – *orange carotenoid protein* (OCP), encoded by the *srl1963* gene is especially involved in this process, also (Wilson *et al.,* 2006).

This study shows the results of the photochemical activity of PS II photosystem in *Microcystis aeruginosa* AICB 702 cyanobacterium (Chroococcales) under various light intensities.

Material and methods

The cyanobacterium *Microcystis aeruginosa* AICB 702 was cultivated for eleven days on GZ medium in conditions of 260 μmol.m^{-2}.s^{-1} light intensity, at room temperature and in air-lift conditions. The cyanobacterial cells reaching the exponential phase of growth were exposed to high-light intensity treatments of 800, 1500, and 2100 μmol.m-2.s-1 respectively. An incandescent light source (FHI-5000 W) was used and the light intensity measurements were taken with Quantum Sensor QSPAR Hansatech. The effect of light treatment was monitored at 15, 30, 45, 60, 75, 90, 105 and 120 min after the initiation of the exposure. The chlorophyll fluorescence parameters were taken with Walz Dual-100 fluorometer on light exposed probes. After 120 min recovery period when the light exposure was ceased, fluorescence parameters were measured on dark-adapted probes.

The assimilatory pigments (chlorophyll *a,* carotenoids) were extracted in acetone and quantified by spectrophotometric method based on their absorption coefficients (Arnon, 1949; Lichtenthaler and Wellburn, 1983), and their identification was made based on their absorption peak obtained with Jasco V-630 spectrophotometer. Phycobiliproteins were estimated based on Gantt and Lipschultz (1974). The results were expressed as mg/L of cellular suspension.

Results and discussions

The biomass of *Microcystis aeruginosa* AICB 702 strain showed an optical density (OD_{680}) of 0.518. The *in vivo* absorption spectra emphasized the spectral features of the carotenoids in the blue range (448 nm), of the chlorophyll *a* at 436 nm and 680 nm and that of the phycobiliproteins at 621 nm (fig. 1). Phycobilisomes are mainly composed of phycobiliproteins and they are attached to the surface of the thylakoid external membrane. Phycobilisomes function as peripheral light harvesting organelles (Lemasson *et al.,* 1973).

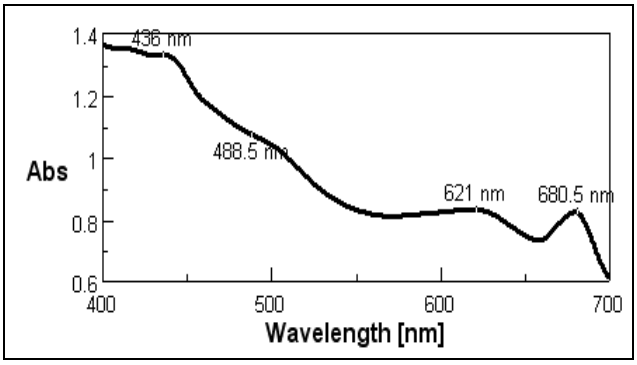

Fig. 1. *In vivo* absorption spectrum of *Microcystis aeruginosa* AICB 702 cell suspension cultivated in normal growth conditions.

The content in assimilatory pigments measured at the starting point of the experiment, prior of exposing the culture to high-light conditions, is listed in Table 1.

Table 1

The quantity of assimilatory pigments at *Microcystis aeruginosa* AICB 702 strain mg/L.

Cyanobacterial strain	Chlorophyll *a*	Carotenoids	*a*/c	Phycobiliproteins		
				Phycocyanin	Allophyco-cyanin	C-phyco-erythrin
Microcystis aeruginosa AICB 702	5.646	1.342	4.20	4.165	4.642	3.450

When the environmental light in changing, the stoichiometric regulation of the photosystems (PS I/PS II ratio) allow an increasing in the efficiency of the photosynthetic electron transport chain, due to the different absorption spectra of the antenna (Sonoike *et al.,* 2001). The content of PS I was decreasing relative to PS II when the cells were exposed to the light which is mostly absorbed by PS I and it increased when the light was mostly absorbed by PS II (Melis *et al.,* 1989; Cunningham *et al.,* 1990).

The evolution of the chlorophyll fluorescence parameters at *Microcystis aeruginosa* AICB 702 exposed to an 800 $\mu mol.m^{-2}.s^{-1}$ light intensity is shown in fig. 2. Generally, the minimal fluorescence, $F_{0,}$ increased up to 110%, relative to control sample. This fact shows that the oxidation of the primary acceptor and the opening of the reaction centers were slowed down (disturbed) and the photochemical process and light energy harvesting were inhibited. The maximal fluorescence F_m increased gradually. By the increasing of fluorescence from F_0 minimal level to F_m maximum level, the PS II primary electron acceptor became totally reduced, the photochemistry was blocked and the reaction centers closed. F_m increasing pointed out that the reduction of the primary electron acceptor and the closure of the reaction centers were maximal. The variable fluorescence reached negative values relative to control probe, except for the first 30 minutes of exposure.

The maximal quantum yield ($F_v/F_{m)}$ and the effective quantum yield Y(II) were inhibited gradually, and decreased by 90% at the end of the experiment (fig. 2B). Fv/Fm value recorded at the end of the experiment was 0.382, which represents 46.5% from the theoretical value. The Fv/Fm theoretical value of 0.82 outlined the maximum fraction of absorbed photons which are used in photochemistry. The values lower than 0.8 indicates the ratio of the PS II reaction centers that are photoinhibited.

The quantum yield of non-regulated energy dissipation, Y(NO), increased up to 106%. In the first 15 minutes of high-light exposure the dissipation lowered below the control sample value. In theory, the quantum yield of non-regulated energy dissipation conveys the sum of the energy dissipation processes which occur at the

level of photosystems antenna. qP and qL photochemical coefficients were stabilized at maximum values (fig. 2 C). The recorded high values confirmed the maximal opening of the reaction centers ratio. The qP coefficient enables the estimation of the fraction of oxidized quinone acceptor from PS II and that of the open PS II centers (Grace and Logan, 1996). The high values of qP and qL coefficients pointed out an enhance photochemical process with a low fluorescence emission.

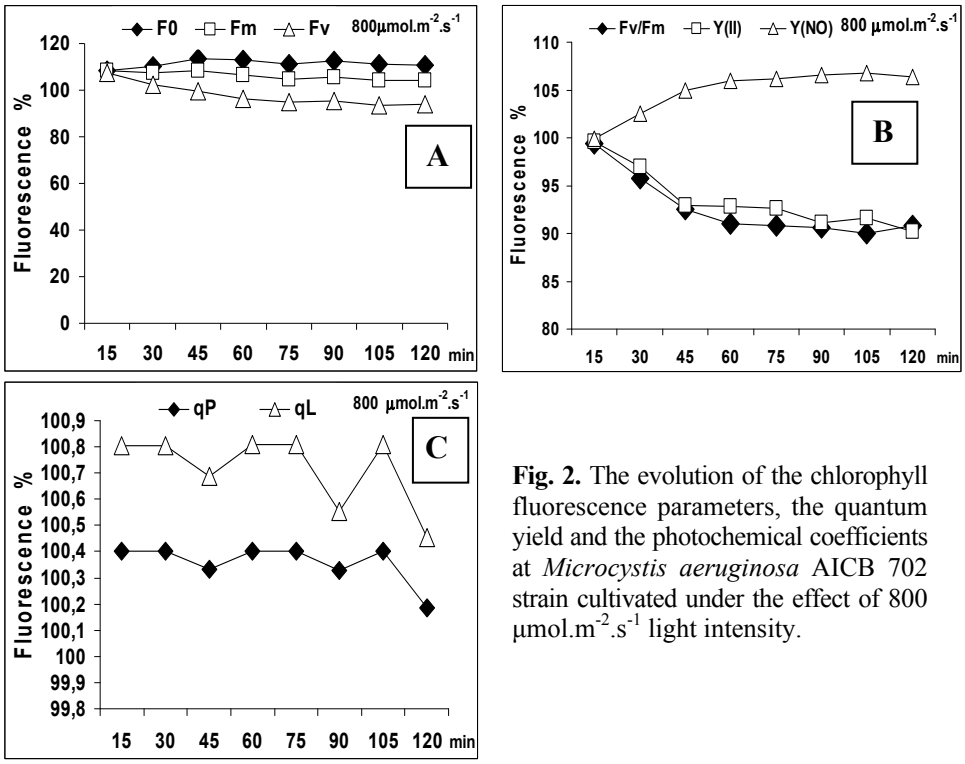

Fig. 2. The evolution of the chlorophyll fluorescence parameters, the quantum yield and the photochemical coefficients at *Microcystis aeruginosa* AICB 702 strain cultivated under the effect of 800 $\mu mol.m^{-2}.s^{-1}$ light intensity.

The fluorescence photochemical quenching is maximal and the fluorescence yield is low, as the PS II reaction centers are open and the photochemistry potential is maximal. The photochemical quenching is null and the fluorescence yield is maximal, while the PS II reaction centers are closed and photochemical processes are ceased. The excitation energy is transferred from the closed to the open reaction centers through the oxidized plastoquinone.

When exposed to high-light conditions, the capacity of cyanobacterial strains to maintain the PS II reaction centers in an open state pointed out a complex and flexible electron transport system (Geerts *et al.,* 1994; Mi *et al.,* 1995; Schubert *et al.,* 1995; Shyam *et al.,* 1993) and a high PS I/PS II ratio, also (Campbell *et al*., 1996; Fujita *et al.,* 1985; Papageorgiou, 1996).

The evolution of chlorophyll fluorescence parameters when exposed to 1500 μmol.m^{-2}.s^{-1} light intensity is shown in fig. 3. F_0 increased up to 114%. Generally, F_m parameter lowered to 87%. Interestingly, F_m value increased in the first 15 minutes of light exposure. The variable fluorescence decreased proportionally to 49%. The oxidation and the reduction of the primary acceptor Q_A and the opening and the closure of the reaction centers were disturbed.

The maximal quantum yield F_v/F_m and the effective quantum yield Y(II) decreased gradually to 57% (fig. 3 B). The quantum yield of non-regulated energy dissipation Y(NO), increased proportionally up to 130%. Generally, the coefficients of the photochemical quenching, qP and qL, recorded maximal values, emphasizing the oxidized state of the primary acceptor Q_A (fig. 3 C).

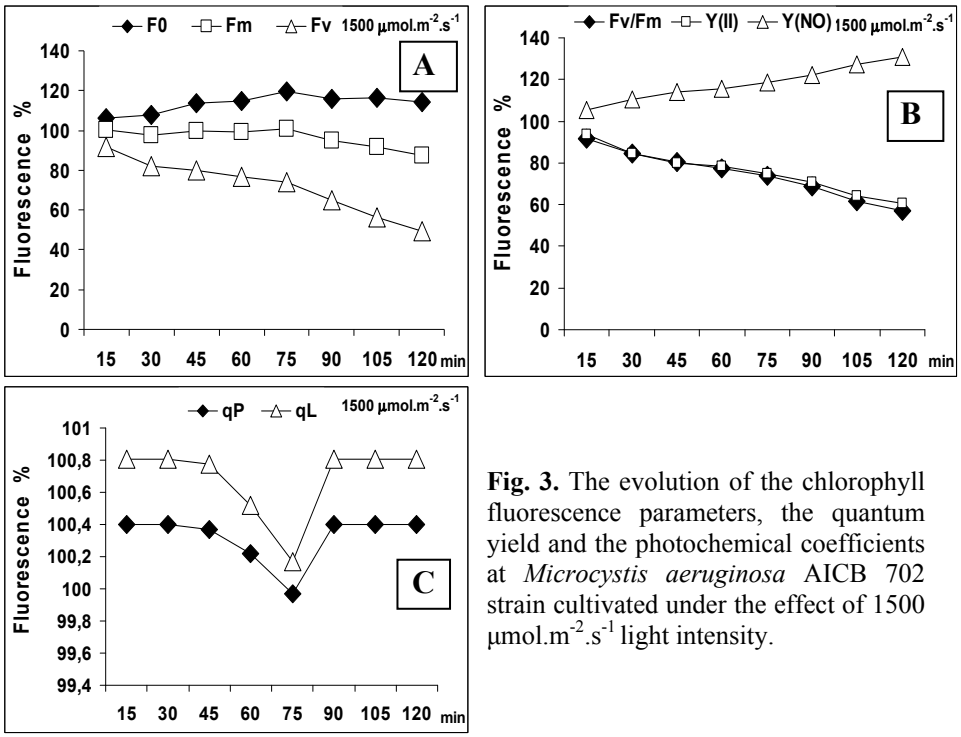

Fig. 3. The evolution of the chlorophyll fluorescence parameters, the quantum yield and the photochemical coefficients at *Microcystis aeruginosa* AICB 702 strain cultivated under the effect of 1500 μmol.m^{-2}.s^{-1} light intensity.

The chlorophyll fluorescence induction kinetics suffered some changes when the cell suspension was exposed to the 2100 μmol.m^{-2}.s^{-1} light intensity (fig. 4). Thus, F_0 registered high values for 90 minutes, than decreased to 85%. After the first 15 minutes, Fm dropped to 59%. The decrease of the maximal fluorescence caused a dropping in the variable fluorescence to 24%.

The maximal quantum yield F_v/F_m and the effective quantum yield Y(II) decreased proportionally to 41%, pointing out the rising of the fraction of photoinhibited reaction centers (fig. 4 B). The quantum yield of the non-regulated energy dissipation Y(NO) increased up to 142%.

The coefficients of photochemical quenching, qP and qL kept high values, which certify the high ratio of the opened reaction centers (fig. 4 C). The high values of the coefficients pointed out the usage of the excitation energy in the photochemistry process in the reaction centers, followed by chlorophyll fluorescence decay.

The initiation of the photoinhibition process occurs at the level of the photosystems antenna, when exposing to 2100 μmol.m^{-2}.s^{-1}, as the F_0, F_m fluorescence parameters decayed and the quantum yields Fv/Fm and Y(II) decreased.

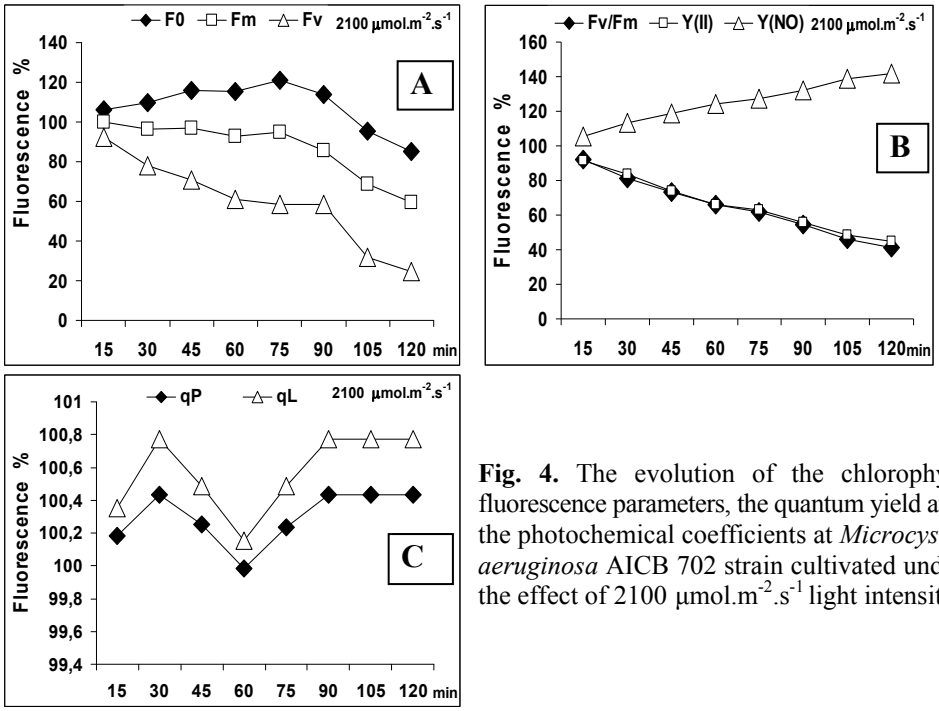

Fig. 4. The evolution of the chlorophyll fluorescence parameters, the quantum yield and the photochemical coefficients at *Microcystis aeruginosa* AICB 702 strain cultivated under the effect of 2100 μmol.m^{-2}.s^{-1} light intensity.

After 120 minutes recovery period of the dark-adapted samples, F_0 reached values close to the control probe value, more significantly in the case of 800 and 1500 μmol.m^{-2}.s^{-1} variants (fig. 5). Fm parameter kept a low value which induced a reduction in the value of the variable fluorescence. The maximal quantum yield and the PS II effective quantum yield remained low, except for the 800 μmol.m^{-2}.s^{-1} variant, when the recovery was faster. The quantum yield of the non-regulated energy dissipation Y(NO) kept high value. When exposing to an 800 μmol.m^{-2}.s^{-1} light intensity, the recovery of the photosynthetic activity was faster compared to the other used high light intensities.

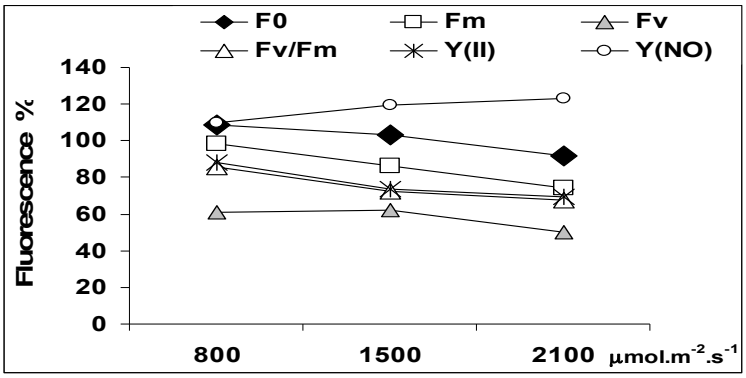

Fig. 5. The evolution of the chlorophyll fluorescence parameters at *Microcystis aeruginosa* AICB 702, in the recovery period that followed the high-light exposure **treatment.**

During the recovery period, the coefficients of the photochemical quenching, qP and qL reached values close to one, which confirm the maximal opening state of the PS II reaction centers (fig. 6).

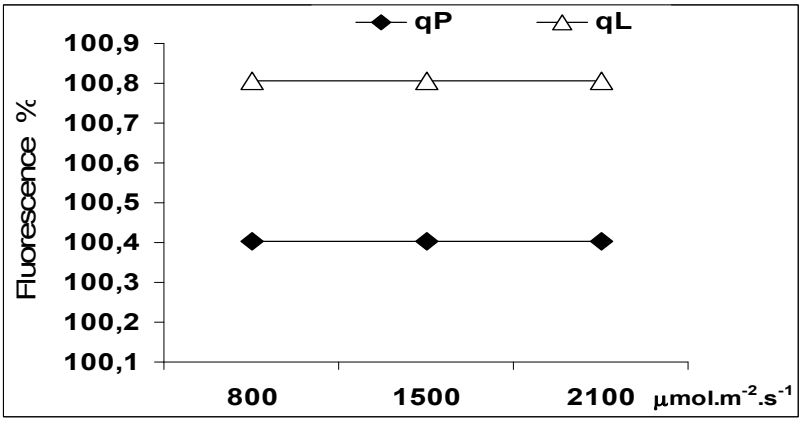

Fig. 6. The evolution of the photochemical coefficients at *Microcystis aeruginosa* AICB 702, in the recovery period that followed the high-light exposure treatment.

Conclusions

Microcystis aeruginosa AICB 702 cells were grown on GZ medium, at room temperature, under air-lift conditions, using a light intensity of 260 µmol.m^{-2}.s^{-1}. The components of the photosynthetic apparatus such as chlorophyll *a,* various types of carotenoids and phycobiliproteins form the light harvesting antenna. The pigment quantity and other components of the photosynthetic apparatus are regulated in response to the light intensity conditions.

The oxidation state of the primary electron acceptor and the opening level of the reaction centers are reduced (deranged), the photochemical process and the light harvesting are slightly inhibited when an 800 μmol. $m^{-2}.s^{-1}$ light intensity is used. Also, the reduction of the primary acceptor and the reaction centers closure were maximal. The maximal PS II quantum yield ($F_v/F_{m)}$ and the effective quantum yield Y(II) were inhibited. The Fv/Fm values registered toward the end of the experiment represented 46.5% of the theoretical value, which also indicates the ratio of the PS II reaction centers that were photoinhibited. The quantum yield of the non-regulated energy dissipation Y(NO) increased at the level of the photosystems antenna. The high values of qP and qL coefficients showed an enhance photochemical process with a low fluorescence emission.

The F_0 parameter increased and F_m and Fv decreased when 1500 $\mu mol.m^{-2}.s^{-1}$ was used. The oxidation and the reduction of the primary electron acceptor Q_A and the opening and the closure of the reaction centers were disturbed. The maximal quantum yield and the effective quantum yield Y(II) diminished and the quantum yield of non-regulated energy dissipation increased. Generally, the photochemical coefficients qP and qL reached higher values relative to control, fact that characterizes the opening state of the reaction centers and the oxidized state of the primary electron acceptor $Q_{A,}$ respectively.

When a light intensity of 2100 $\mu mol.m^{-2}.s^{-1}$was used, the Fo parameter raised during a 90 min period, then decreased to 85%. Fm and Fv decreased. The maximal quantum yield and the effective quantum yield Y(II) decreased, while the quantum yield on non-regulated energy dissipation Y(NO) increased. The photochemical coefficients qP and qL were higher relative to control, which assert the high ratio of the open reaction centers. The decreasing in $F_{0,}$ F_m fluorescence parameters, Fv/Fm yield and that of the effective quantum yield Y(II) certified the photoinhibition effect of 2100 $\mu mol.m^{-2}.s^{-1}$ light intensity over the activities carried out by the photosystems antenna.

During the recovery period, F_0 reached values close to the control, in the case of 800 and 1500 $\mu mol.m^{-2}.s^{-1}$ light intensity treatments. Fm parameter was diminised. The maximal quantum yield and the effective quantum yield remained low, except for the 800 $\mu mol.m^{-2}.s^{-1}$ variant, when the recovery was faster. The quantum yield of the non-regulated energy dissipation was characterized by a high value. When exposing to an 800 $\mu mol.m^{-2}.s^{-1}$ light intensity, the recovery of the photosynthetic activity was faster compared to the other used high-light intensities.

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MICROWAVES IRRADIATION EXPERIMENTS ON BIOLOGICAL SAMPLES

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SUMMARY. The goal of the present study was to investigate the effects of low power microwave irradiation, at 2.45 GHz, on plant samples. We choose the microwave power level used in wireless LAN communications between a wireless LAN Router and a local PC laptop. With this power level plants samples have been irradiated in specific experimental conditions. The irradiated plants were compared with non-treated plants. Reference and irradiated plants were phenotypically similar, but the growth was strongly correlated with microwave irradiation. The protein metabolism was activated under microwave irradiation, demonstrated by higher values of total soluble protein content in irradiated plants.

Keywords: microwave power density; pigments; protein content; izoenzymes.

Introduction

This experiment is part of a research program, which intends to decipher if low power microwave affect biological samples, more precise the growth and development of plants at a power level where the thermal effect of the microwaves is insignificant.

The effects of microwave irradiation on plants have scarcely been studied. However, some aspects of microwave irradiation have been investigated. Among the most interesting for practical applications the following studies can be mentioned: extraction of essential oils using microwaves (Saoud *et al*., 2005), increased seed germination (Aladjadjiyan, 2002), elimination of microorganisms (Bhaskara et al., 1998), increased oil production from rape seeds under microwave irradiation (Valentová *et al*., 2000; Novotná *et al*., 1999), stimulation of antioxidant activity in bean (*Vicia faba*)

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(Randhir and Shetty, 2004) or protection of cells against UV radiation (Chen, 2006). The use of controlled influence of physical factors on biological behavior during development of different cultures is a modern trend in combining plant technologies with the ecological requirements (Aladjadjiyan, 2007). Regarding the effects of microwave irradiation, gene expression in plants was affected (Vian *et al*., 2006) or cytogenetic changes occured (Pavel *et al*., 1998).

In this study, the effects of microwave irradiation on germination of bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) seeds, on growth and development of plants have been investigated. The studied parameters were: a) seed germination, b) growth of shoots and roots, c) total soluble protein content, d) chlorophyll content and e) izoenzyme activities (peroxidase and superoxid-dismutase).

Materials and methods

Microwave irradiation. Experimental setup

The experiments were carried out in two identical anechoic chambers (I- Irradiated chamber, R- Reference 1 chamber) both with controlled environment (Fig. 1). Temperature and humidity sensors (SHT-17) (1) from I and R chambers were connected through a microcontroller system (2) to a personal computer (3) (Fig. 1).

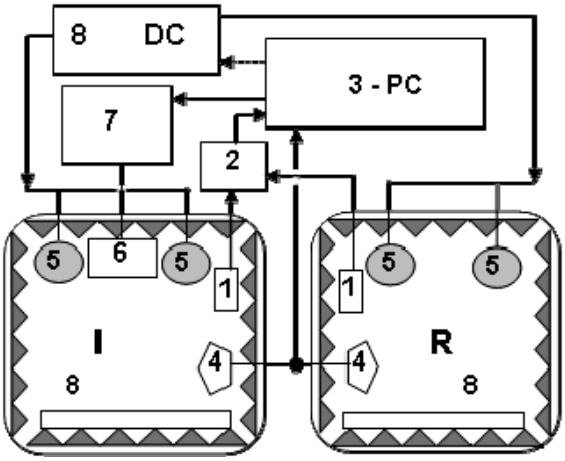

Figure 1. Experimental setup configuration. I and R are identical anechoic chambers, I-chamber for microwaves irradiations, R-chamber as reference without microwaves. (1) humidity and temperature sensors, (2) microcontroller, (3) personal computer, (4) WEB camera, (5) illuminating system, (6) microwave antenna, (7) microwave generator, (8) DC source, (9) ceramic pot.

A high sensitivity camera (SPC 900NC) (4) and multi-LED illuminating system (5) was also used in the experiment. The DC source (8) of the illuminating system was controlling the multi-LED illumination cycle. At the top of the I-st chamber one microwaves antenna (6) connected to a microwave generator (7) was installed. The inner volume of the experimental chambers was 24 cm^3. The plants were placed in a ceramic pot (8) with 18 cm diameter and 3 cm height at the bottom of the chambers.

The microwave generator was a commercial dual band Wireless Router 802.11a+g (TEW-511BRP). The frequencies used ranged between 2.40-2.48 GHz (g-band) at a maximum output power of +17dBm. The connection from microwave generator to antenna was made with a microwave coaxial cable with 1.2dB attenuation. The microwave modulation signal, was the specific protocol of the Wireless Local Area Network (WLAN) connection known as Wireless Fidelity (Wi-Fi), in the "searching network users" mode (IEEE 802.11 Standard, 1997). This mode is characterized by intermittent emission (~1.5ms active, 100ms pause) of a specific modulated data on microwaves carrier and a specific sweep of the frequency in the chosen band. The operating mode and frequency channel are set using the router software via LAN connection, from the PC internet explorer interface. In our experiment the router was set on Channel 9 at 2.452GHz frequency.

Microwaves measurements

First the microwave power at the pot level was characterized and the measurement configuration is presented in Figure 2.

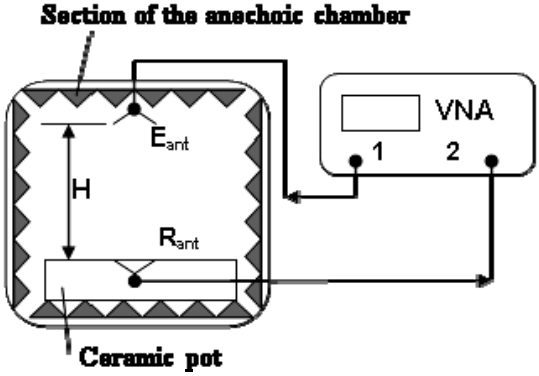

Figure 2. Microwaves power measurement configuration;
VNA is an Vector Network Analyzer, Agilent N5230A with two port "1" and "2", E_{ant} is the emission antenna, R_{ant} is the reception antenna, H is the height of the chamber.

More precisely, the specific attenuation (A) of the anechoic chamber was measured. For this measurement two identical antennas E_{ant} and R_{ant} (with gain G) connected to one Vector Network Analyzer (VNA) were used. In fact this is an emission-reception link characterized by the equation:

$$P_t = P_r + A + 2G \quad (1)$$

Where: P_t is the transmitted power in (dBm) at the output "1" of the VNA, P_r is the received power in (dBm) at the input "2" of the VNA, A is the attenuation in (dB) of the anechoic chamber at the distance H from E_{ant} antenna and G is the gain of the antennas in (dBi).

Considering: $S_{12} = Pt-Pr$, where S_{12} is the transmission parameter measured by the VNA in (dB), the attenuation at the ceramic pot level is:

$$A\ (dB) = S_{12}-2G \quad (2)$$

In the Table 1 are presented the values of the principal parameters measured, for a set of two identical antennas. In these measurements the input power generated by the VNA was $P_t = 0$ dBm and H = 175mm. Considering the antenna maximum dimensions (D), the anechoic chamber link respect the far field condition at the ceramic pot level (Foegelle, 2002):

$$H > R = 2D^2/\lambda$$

Where: D is the maximum antenna dimension, λ is the wavelength at the working frequency (λ=122.3 mm) and R is the minimum distance from emission antenna E_{ant} where we can consider homogeneous microwaves field.

The optimum height of the growing plants, to be in homogeneous microwaves field, is $H_{plant} = H-R$. In our experiment the maximum value for H_{plant} is 15 cm (Table 1).

Table 1.

Principal parameters on microwaves power characterization of the anechoic chamber.

G (dBi)	D (mm)	S_{12} (dB)	A (dB)	A_{cable} (dB)	R (mm)	H_{plant} (mm)	P_t (mW)	P_{pot} (μW)
+2.20	80	-25.64	-29.64	-1.2	105	70	50	69
- 3.68	29	-37.03	-29.67	-1.2	14	151	50	18

G - antenna gain, D – antenna maximum dimension, S_{12} – transmission parameter, A –specific attenuation of the anechoic chamber, A_{cable} – attenuation of the cable between microwave generator and emission antenna, R – distance from emission antenna where microwaves are homogeny distributed, H_{plant} – optimum height of plant to grown in homogeny microwaves, P_t – output generator power, P_{pot} – estimated microwave power at the pot level.

It is necessary to measure the change in attenuation (A) over this distance and a decrease in attenuation with less of $\Delta A_H = 0.2$dB was found. Also, in the circular area of the ceramic pot, the measured variation in attenuation's was less than $\Delta A_{pot} = 0.1$dB. These values prove a good homogeneity of the microwave field in the considered volume.

To calculate the microwave power at the pot level is necessary to know the input power of the microwave generator (Fig. 1) used in experiment P_t (dBm), the gain G (dBi) of the antenna and the attenuation A (dB) at the distance H:

$$P_{pot} = (P_t - |A_{cable}| + G) - |A| = EIRP - |A| \quad (4)$$

Where EIRP is the effective radiated power compared to an isotropic radiator. In Table 1 are presented also the values of input power P_t and the power at ceramic pot P_{pot} for two antennas. If we consider all the measured variations of the chamber attenuation (ΔA_H and ΔA_{pot}) the maximum estimated microwave power at the pot level is $P_{pot} = (70 \pm 3)\mu W$. At this power, considering all microwaves transformed in heat, the temperature rate raising is less than 10^{-6} ^{0}C/s for the initial seeds-water volume, approximated as 40 ml of water. The measured power density at the ceramic pot level was $P_d \approx 0.005 mW/cm^2$, which is 200 times lower than the ICNIRP recommendation ($1 mW/cm^2$) for the environmental microwave safety power level (ICNIRP, 1998). During the experiments the measured difference in temperature of the Irradiated and Reference 1 chamber was less than 0.5 ^{0}C, and the difference in relative humidity was less than 4%.

Plant material

The effects of microwaves were studied on bean (*Phaseolus vulgaris* L., cv. Ardeleana) and corn (*Zea mays* L., cv. Favorit) plants obtained from seeds. Experimental variants were: a) plants grown in a growth chamber at 24°C, under a 16 h light photoperiod with 39.06 $\mu Em^{-2}s^{-1}$ photosynthetically active radiation (PAR) (Reference 0) (R0), b) plants grown in the anechoic chamber with controlled environment without microwaves irradiation (Reference 1) (R1) and c) plants grown in the anechoic chamber with microwaves irradiation (Irradiated) (I). The tested parameters were analyzed on plant material from the three experimental variants.

Seed germination and growth measurement

Seeds were sowed in laboratory vessels (18 cm in diameter and 3 cm in height) on humidified filter paper for germination (50 seeds / vessel). Tap water was added periodically to maintain filter paper humidity. Growth of plants was determined by measurement of hypocotyls, epicotyls and root length at the end of the irradiation period. The irradiation duration was 14 days for bean and 25 days for corn.

Determination of total soluble protein content

Plant material representing leaves (100 mg) was grounded with 1.5 ml phosphate buffer at pH = 6.1 on ice. The extract was centrifuged 15 min at 10000 rpm at 4°C and the supernatant was stored at 4°C until utilization. Total soluble protein content was determined spectrophotometrically at 595 nm wavelength (Bradford, 1976). For the standard curve bovine serum albumine (BSA) (Sigma) was used. Total soluble protein content was determined on fresh weight (FW) basis.

Determination of assimilatory pigment content

The absorbance properties of pigments facilitate the quantitative analysis of them. Total chlorophyll (chlorophyll *a*, chlorophyll *b* and carotenoids) content was determined spectrophotometrically at wavelengths 663 nm, 645 nm and 470 nm and calculated according to the Wellburn (1994 formula. The pigments were extracted in dimetylformamide (DMF) (Moran and Porath, 1980). For extraction leaves (about 50 mg) were incubated in 2.5 ml of DMF at 4°C for 48 h in dark conditions followed by centrifugation of the extract at 5000 rpm for 5 min.

Identification of izoenzymes

Young leaves were used for izoenzymes separation. Fresh leaves (100 mg) were mortared on ice with extraction buffer (w/v) containing 0.1 M Tris-HCl, pH 7.5, 1 mM EDTA, 10 mM $MgCl_2$, 10 mM KCl_2, 14 mM 2-mercaptoethanol, (2-mercaptoethanol was not added in buffer solution when peroxidases were analysed), 10-50 mg/ml polyvinil-pirolidone-PVP-40) (Brinegar and Goundan, 1993). Samples have been centrifuged at 10000 rpm, 10 min at 4ºC. The pellet was discarded and the supernatant was used for electrophoresis. Izoenzymes separation was accomplished by continuous PAGE without SDS by izoelectric focusing (IEF). Gel concentration was 5%. For gel preparation a stock solution of acrylamide/bisacrylamide mixed with ampholine A, pH=3.5-5.0/ampholine B pH=3.5-10.0 ratio 1:1, H_2O, amonium persulphate 10% and Temed. Two buffers were used in cuvettes: 20 mM NaOH/10 mM H_3PO_4. Running was performed at 120 V for 1 h with a Consort device.

Histochemical identification of izoenzymes was performed according to several protocols described by (Pasteur *et al*., 1987; Murphy *et al*., 1990; Acquaah, 1992). Some of them were modified by us. Peroxidases were identified using 0.05 M Na acetat, pH 5,0 in wich we added in order 10 mg $CaCl_2$, 250 µl H_2O_2 3%, 50 mg 3-amino-9-etil-carbazol, dissolved previously in 5 ml dimethylformamide. Peroxidases appear as red bands after 15-30 min. SOD were identified using 0.2 M Tris-HCl, pH 8.0 buffer in wich we added in order 10 mg $MgCl_2$, 20 mg NAD, 10 mg PMS (phenazin-methosulphate), 10 mg MTT (3-(4.5-dimethylthiazolil-2)-5-dipheniltetrazolium bromide). SOD appears as white bands on blue gel.

Statistical analysis of results

All experiments were carried out with three independent repetitions and the results were expressed as the mean values ± standard deviation (SD). Data were analyzed by two-way analysis of variance (ANOVA) using the Tukey test for data comparison.

Results and discussion

Germination of seeds and growth of plants under microwave irradiation

The experiment was started with seeds and continued until the plants achieved approximately 15 cm in height (Fig. 3). In each chamber video cameras were placed for monitoring of growth and miniature sensors for temperature and humidity measurement. The temperature difference was less then 0.5°C between the reference R1 and the irradiated chambers and the humidity difference was less then 3% RH over the entire growing period. During the growth period the temperature variation was between 20°C to 24°C, the artifficial illumination was continuous and the relative humidity was kept between 68 and 88% RH in both chambers. The microwave power at the plants-pot level was maintained at a value of less than -10dBm. The modulation was the WLAN standard protocol.

Germination percentages of seeds in the three mentioned experimental variants were significantly different for the same species (Table 2). Microwave irradiation influenced significantly only the germination of bean seeds in comparison with seed germination under R1 conditions. It is possible that the culture conditions could affect the germination rate. Regarding corn, the germination of irradiated seeds was different in comparison with the other two tested variants.

Table 2.

Germination percentages of bean (*Phaseolus vulgaris*) and corn (*Zea mays*) seeds

Plant material	Seed germination (% ± SD)*		
	Reference 0	Reference 1	Irradiated seeds
bean	99.0 ± 1.41[a]	95.9 ± 2.79[b]	98.9 ± 1.44[a]
corn	95.0 ± 1.41[b]	94.9 ± 1.50[b]	96.9 ± 1.47[a]

* Data represent means ± SD. Values followed by the same letter within a row are not significantly different ($P < 0.05$).

Plant growth and development involves complex morphological, physiological, biochemical and molecular processes. Plants developed under the experimental conditions in the anechoic chambers (with and without irradiation) were phenotypically similar to the plants grown in the growth chamber. It was noted a stimulating effect of the microwaves on plant growing for both species (Fig. 3). Generally the length of bean and corn plants under irradiation conditions was higher than for reference plants. The highest values for growth after the end of the irradiation period (14 days for bean and 25 days for corn) were noted for the epicotyl with 12.5 cm for bean and 8.2 cm for corn (Fig. 4A, B). The stimulation effect of microwaves was observed also for hypocotyles and roots. In this case the mean length of irradiated hypocotyles was 5.7 cm for bean and 2.2 cm for corn. The length of irradiated roots was 12.1 cm for bean and 7.9 cm for corn (Fig. 4A, B).

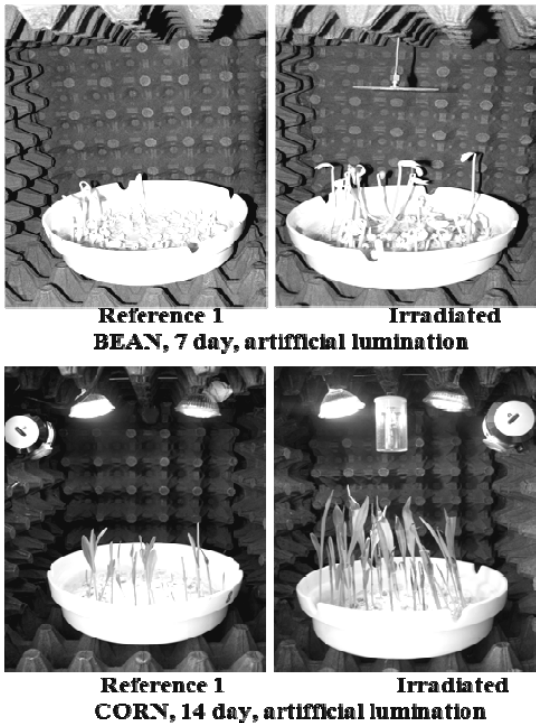

Figure 3. Effects of microwave irradiation on growth of bean (*Phaseolus vulgaris*) and corn (*Zea mays*) plants. A) plants grown in the reference anechoic chamber; B) plants grown under irradiation.

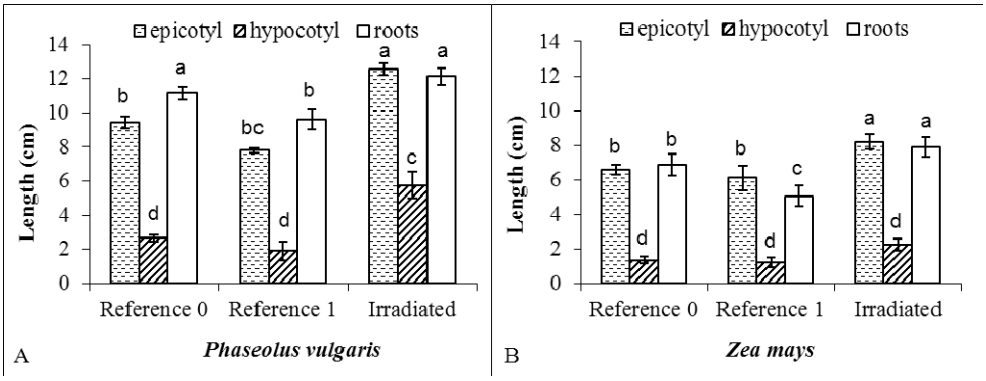

Figure 4. Effects of irradiation on growth of bean (*Phaseolus vulgaris*) (A) and corn (*Zea mays*) (B) plants. Measurement of hypocotyls, epicotyls and root length was made at the end of the irradiation period (14 days for bean and 25 days for corn). Vertical bars represent standard deviations. Different letters are significantly different ($P < 0.05$).

The stimulation effect of cell division was reported also for *Catharanthus roseus* protoplasts following growth in magnetic field (Haneda *et al*., 2005).

Effects of microwave irradiation on totale soluble protein content

The comparative study of totale soluble protein content in the leaves of bean and corn plants showed differences between species and the experimental variants (Fig. 5A, B). It was noted a high value of protein content in the leaves of irradiated plants (Fig. 5A, B). At the end of the irradiation period (14 days for bean and 25 days for corn) the protein content was 0.27 µg/g FW for bean and 0.25 µg/g FW for corn. The values representing the content of total soluble protein content for reference 0 and reference 1 were almost similar. After prolonged irradiation duration the protein content was lower for both species. Changes in protein expression, accumulation and synthesis in tomato plants have been observed in stress conditions (Chen and Tabaeizadeh, 1992). Numerous physiological and biochemical changes occur in response to stress factors in various plant species. The physiological mechanisms involved in cellular response to stress factors generated have been investigated (Turner, 1997; Neuman, 1997).

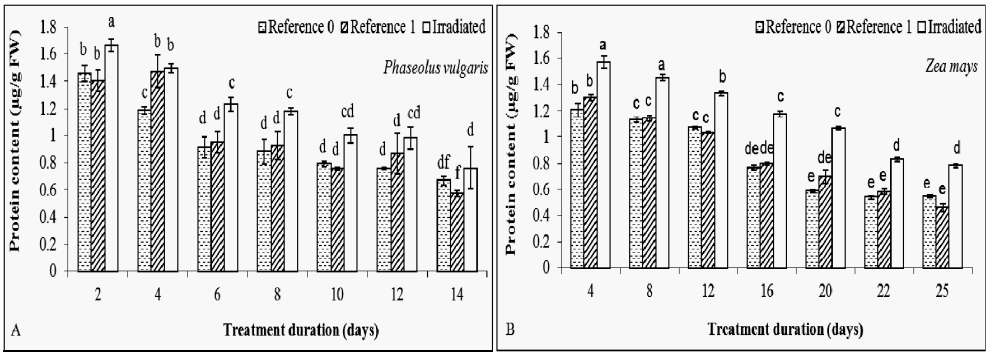

Figure 5. Effects of microwave irradiation on total soluble protein content on fresh weight basis in the leaves of reference and irradiated bean (*Phaseolus vulgaris*) (A) and corn (*Zea mays*) (B) plants. Vertical bars represent standard deviations. Different letters are significantly different ($P < 0.05$).

In accordance with the obtained data we can conclude that the protein metabolism was stimulated and activated under microwave treatment.

Effects of microwave irradiation on assimilatory pigment content

Following 2 weeks of growth of bean plants under microwave irradiation conditions the content in chlorophyll *a*+*b* was 0.54 mg/g FW whereas after the same period the content in chlorophyll *a*+*b* in reference 0 plants was 0.42 mg/g FW and in reference 1 plants was 0.57 mg/g FW (Fig. 6A). The content in carotenoids obtained

for the same period showed differences for the experimental variants with 0.38 mg/g FW for irradiated plants, 0.32 mg/g FW for reference 0 plants and 0.29 mg/g FW for reference 1 plants (Fig. 6B).

In leaves of corn plants following 25 days of irradiation the content in chlorophyll *a*+*b* was 0.29 mg/g FW. In the other experimental variants the content in chlorophyll *a*+*b* was 0.30 mg/g FW for reference 0 and 0.29 mg/g FW for reference 1 (Fig. 6C). The values representing the content in carotenoids were 0.26 mg/g FW for irradiated plants, 0.23 mg/g FW for reference 0 and 0.24 mg/g FW for reference 1 plants (Fig. 6D). For corn the differences in chlorophyll *a*+*b* and carotenoids between categories were not significantly different. The results of the experiment showed that chlorophyll *a*+*b* content in bean is stimulated by microwave irradiation exposure. Higher values of carotenoids could be due to the stress protective function of carotenoids which has been well-established (Eskling *et al*., 1997). Anthocyanin accumulation is known as a characteristic in stress conditions (Eryilmaz, 2006). It is to note that irradiation did not affect negatively the synthesis of chlorophylls in none of the tested species. These results suggests that microwave radiation enhanced plant metabolism. An increased content in chlorophyll *a*, *b* and carotenoids was noted also under growth of potato (*Solanum tuberosum* L.) in vitro cultures in null magnetic field (Rakosy *et al*., 2005). The chlorophylls and carotenoidic pigments content are biochemical indicators for the physiological processes in plants. Analyses of chlorophylls and carotenoids in this case have not proved differences between the microwaves irradiated plants and the references in the anechoic chamber.

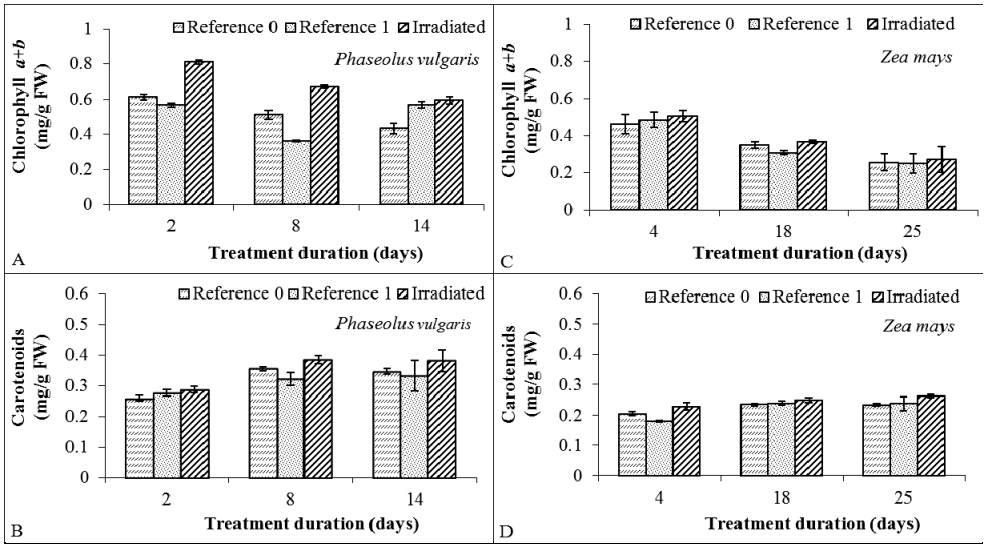

Figure 6. Effects of microwave irradiation and dynamics of pigment contents in the leaves of reference and irradiated bean (*Phaseolus vulgaris*) plants (A, B) and reference and irradiated corn (*Zea mays*) (C, D) plants. Vertical bars represent standard deviations.

Study of izoenzymes

The isoenzymatic profile of peroxidases expressed in leaves of corn plants exposed to microwave irradiation is shown in Fig. 7. The pH gradient created in the gel with ampholines is ranged between 9 (in the upper part of the gel) and 3 (at the lower part of the gel). As it could be seen most of the peroxidases are located in the alkaline region of electrophoresis gel. The izoperoxidase pattern is the same in reference plants and plants exposed to microwaves. Five alkaline izoperoxidases have been revealed in all plants having, izoelectric points between 9 and 6. In the neutral region of pH gradient in the gel, only one izoperoxidase have been expressed, this is also present in all plants, reference and exposed to microwaves as well. In sample 1, an other neutral izoperoxidase was observed. In the acid region of the pH gradient in the gel only one izoperoxidase have been observed. This izoperoxidase was expressed only in sample 1 and 13, and is not correlated with microwave exposure. It is well known that the acid peroxidases are located prevalent in the cell wall being involved in the polymerization of the monomers of lignine. Alkaline peroxidases are usually involved in plant respons to different stress factors (Gaspar *et al*., 1996).

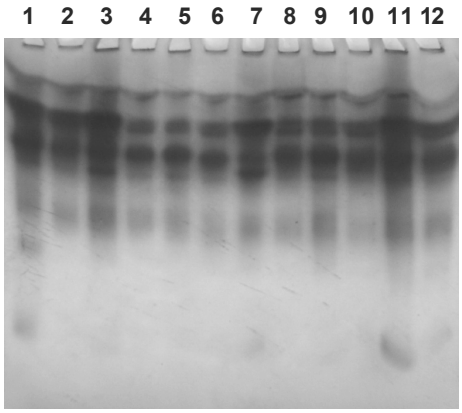

Figure 7. Isoenzymatic profile with IEF of peroxidase expressed in leaves of corn plants exposed to microwaves.
(1) reference 0 – after 2 days; (2) reference 1 – after 12 days; (3) irradiated – after 12 days; (4) reference 1 – after 16 days; (5) irradiated – after 16 days; (6) reference 1 – after 20 days; (7) irradiated – after 20 days; (8) reference 1 – after 22 days; (9) irradiated – after 22 days; (10) reference 1 – after 25 days; (11) irradiated – after 25 days; (12) reference 0 – after 25 days.

Peroxidases in higher plants could be clasified in two major groups, according to their functions and specificity of substrate. The guaiacol peroxidases (GPX) have low specificity of substrate and it seems to have different peroxidative functions in the cell. The other peroxidases as glutation peroxidase (Beeor-Tzahar *et al*., 1995) and ascorbat peroxidase (APX) (Asada, 1992) are crucial for neutralization of H_2O_2, organic hydroperoxides and lipid peroxides. Up to now, GPX have been found in

the vacuoles, cell wall, cytosol, extracelular space and corn mytocondria (Asada, 1992; Prasad *et al*., 1995), whereas APX are present mainly in chloroplats and partially in cytosol and glyoxisomes (Asada, 1992; Asada *et al*., 1993; Mittler and Zillinskas, 1993; Bunkelmann and Trelease, 1996). Extracellular peroxidases play an important role in plant respons to stress factors. Thus, the peroxidases are involved in the neutralization of free radicals produced by oxidation of AIA, monoamines, salycilic acid and kitoolygozaharides (Kawano *et al*., 2000). Peroxidases modulates the redox equilibrium of the cell being involved in plant development (Broin *et al*., 2002) and the signal tranduction mediated by calcium that is necessary for induction of plant response against stress factors (Kawano, 2003). Moreover, it was allready demostrated that APX1, one of the peroxidases from *Arabidopsis* is the central component in the complex of the enzymes involved in the neutralization of free radicals (Davletova *et al*., 2005). It has been demonstrated that APX F, a peroxidase from mitocondria is essential for homeostasis and root growth in *Arabidopsis* under different stress conditions (Finkemeier *et al*., 2005). As it is shown in Fig. 7, there is no izoperoxidase expressed only plants exposed to microwaves. It could be concluded that the irradiation of corn plants with microwaves power density of $P_d = 0.005 mW/cm^2$, is not associated with any changes in the pattern of peroxidases, so in these plants could be not detected izoperoxidases that ussualy are induced in stress conditions. Unlike peroxidases that are extremly inducible with different stress factors and plants development there are other enzymes involved in neutralization of free radicals as superoxid dismutases and esterases (Dobrota *et al*., 2003, 2004). Isoenzymatic profile of SOD izoenzimes is shown in Fig. 8. In the alcaline region of pH gradient in the gel, two izoenzymes have been observed, their expression being similar in reference plants and plants exposed to microwaves.

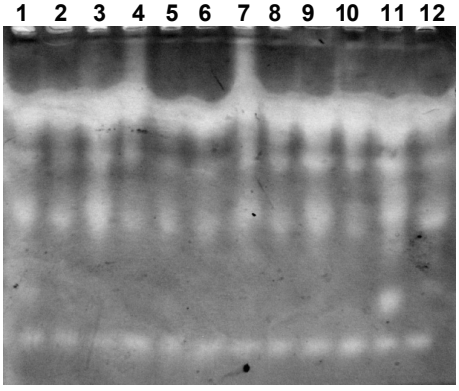

Figure 8. Isoenzymatic profile with IEF of superoxide dismutase expressed in leaves of corn plants exposed to microwaves.

(1) reference 0 – after 2 days; (2) reference 1 – after 12 days; (3) irradiated – after 12 days; (4) reference 1 – after 16 days; (5) irradiated – after 16 days; (6) reference 1 – after 20 days; (7) irradiated – after 20 days; (8) reference 1 – after 22 days; (9) irradiated – after 22 days; (10) reference 1 – after 25 days; (11) irradiated – after 25 days; (12) reference 0 – after 25 days.

In neutral region of pH gradient, only one izoenzyme was expressed in all plants. In acide region of pH gradient, only one izoenzyme was expressed accidentaly, in sample 1 and 13. An other izoenzyme having an izoelectric point arround 3, have been expressed in all plants. Similar to izoperoxidase pattern, the SOD pattern does not reveal any izoenzyme associated with plants exposure to microwaves. Because the enzyme SOD is involved in controlling the superoxide radical, its activity suggests that microwaves power density of $P_d = 0.005 mW/cm^2$ does not induce stress effects on these plants. Reactive oxygen species are generated as by-products of metabolism mainly in chloroplast (Assada, 2000, 2006) but they are also produced in peroxisomes and the cell wall (Dat *et al*., 2000). Oxidative overproduction of ROS may be induced by a wide spectrum of abiotic and biotic stress factors, but in our experiment could be not detected such changes in SOD activity.

Conclusions

Growth was strongly correlated with irradiation of plants. A stimulating effect of cell division under microwave irradiation was noted. Plants grown in reference and irradiated conditions were phenotypically similar. The protein metabolism was activated under microwave irradiation, demonstrated by higher values of total soluble protein content in irradiated plants. Chlorophyll concentration was not strongly affected by microwave irradiation in comparison with chlorophyll content in reference leaves.

The irradiation of corn plants with microwaves is not associated with any changes in the pattern of peroxidases and SOD, wich suggest that this intensity of microwaves does not induce stress effects on these plants. The results presented revealed that microwaves power density of $0.005 mW/cm^2$, did not activate other enzymatic systems besides the usual ones in normal physiological conditions for maintenance of cell homeostazy.

Microwave irradiation as a physical method for increasing the vegetable production could be used for seeds or plant treatment with the goal of accelerating plant growth and development.

Further experiments involving exposure of other plant species and different periods of time and various wavelengths would be necessary to establish other effects of microwaves on plant development.

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=== MINI-REVIEW ===

DIVERSITY OF ENDOLITHIC PROKARYOTES LIVING IN STONE MONUMENTS

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SUMMARY. Stone monuments (statues, fountains, tombs, memorials, buildings, etc.) are apparently indestructible artefacts that were built to last. Depending on the chemical composition and structure, stones may be friendly or hostile to colonization by eukaryotic and/or prokaryotic communities. Among various lithotypes, porous rocks (limestone, sandstone) have highest bioreceptivity. Abiotic factors have a strong impact on the deterioration rate of stone monuments. Biological activity of living colonizers may intensify the weathering of such substrates. Moreover, human activity could enhance the bioerosion by providing more nutrients to epilithic and endolithic microbial communities. Highly diverse lithic communities have been detected in stone monuments, both by culture-dependent and molecular approaches. Epilithic and endolithic prokaryotes are well represented by cyanobacteria that are among the pioneers of colonizing the stone surfaces in partnership with fungi and algae. Active boring algae and cyanobacteria are euendoliths *par excellence*. Chemoheterotrophic actinobacteria and gammaproteobacteria are dominant bacterial species found in biofilm as well as in endolithic niches of stone sampled from various types of monuments. To date, there is little information on endolithic archaea and chemolithoautotrophic bacteria. Further in-depth investigations on the diversity and metabolic roles of lithic communities are needed with respect to the conservation strategies.

Keywords: actinobacteria, biodeterioration, bioreceptivity, cyanobacteria, halotolerant bacteria, lithotypes.

Introduction

Stone monuments from impressive megaliths of Neolithic Age and delicate limestone sculptures of Antiquity to modern marble and concrete buildings are expressions of human sensitivity and need for everlasting proofs of existence. The

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basic materials used for most of stone monuments are either light rocks, such as sandstone and limestone, or hard materials (e.g. granite, dolomite). Various mixtures of basic soft or hard materials could be used to prepare mortar, ceramic or concrete. Porous or light rocks were used by ancient civilizations for their ease to extract and shape. Monuments such as Greek or Roman statues, temples, thombstones or Egyptian pyramids and obelisks were made of such materials. Granite, dolomite, and marble were preferred when long lasting masterpieces were constructed. Weathering by a combination of water, wind, heat and cold contrast is the main destructive cause of such solid man-made structures. Besides these abiotic factors, the biological activity due to the growth of macroscopic or microscopic organisms on the surface or inside the stone is another reason for an even faster deterioration of the stone monuments. When stone degradation is catalyzed by living organisms, the process is called bioerosion or biodeterioration. Bioerosion affects stone artifacts at different rates depending on the type of rock material (lithotype) used for building, on the abiotic factors and not at least, on the degree of environmental pollution as result of human activities. The present short review aims to summarize recent knowledge on the diversity of prokaryotes living inside building stones, their metabolic strategies to overcome the limitation of life in the rock and their involvement in biodeterioration of monuments made of stone.

Ecological niches hosted by rocks and stone artifacts

There are two sorts of habitats that can be found in relation with a solid mineral material: on the surface and inside. Depending on the environmental conditions and chemical composition of the substrate, the surface of rock could be colonized by macroscopic eukaryotes, mostly represented by mosses (class Bryophyta), fungi and lichens (as a symbiotic consortium of fungi, green algae and/or cyanobacteria) that seem to be the first colonizers of stone surfaces (Lisci *et al.*, 2003). When humidity and light reach appropriate values, biofilms form. Biofilms appearing on rock and, correspondingly, on stone monuments surfaces are complex consortia of algae and bacteria that are oftenly grazed by microscopic eukaryotes (protozoa). All forms of life prone to living on surfaces of stones are categorized as *epilths* (a.k.a. *epilithobionts*). *Hypoliths* are organisms that develop just below the surface of rocks. Cyanobacteria seem to form the dominant group (46-90%) of hypolithic prokaryotes in natural environments followed by heterotrophic bacteria (Chan *et al.*, 2012). When surface is broken into crevices or fissures, as well as when porosity of stone material is large enough, water and organic compounds difuse within the body of stone. These are, however, the minimal requirements to support life and rock become colonized by inside. *Endoliths* (a.k.a. *endolithobionts*) are all microscopic eukaryotes (fungi and protozoa) and prokaryotes (bacteria and archaea) inhabiting pores or crevices of hard materials such as rocks, corals or even shells. In these minute ecological niches, light and organic carbon are hardly present, while water become accessible only intermitantly.

Thus, no wonder that many endolithic taxa are considered as extremophiles – organisms that thrive at extreme environmental conditions (Pikuta *et al.*, 2007). In this category several examples of archaea and bacteria that survive within an extremely narrow space in micropores of deep sediments (e.g. igneous rocks), away from water and organic compounds, both at high temperature and pressure, in acidic or alkaline rocks, are known (Jiménez, 1990; Newberry *et al.*, 2004; Fry *et al.*, 2008). The diversity of endolithic prokaryotes in deep sediments is surprisingly high and representatives of Alpha-, Beta- and Gammaproteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes, as well as archaea from Euryarchaeota and Crenarcaheota classes have been identified both by culture-dependent and molecular approaches. Several aerobic and anaerobic deep-subsurface endoliths have been successfuly cultured (e.g. species belonging to *Carnobacterium* spp., *Clostridium* spp., *Marinilactibacillus* spp., *Pseudomonas* spp., *Acetobacterium* spp., *Deinococcus* spp., *Arthrobacter* spp., *Micrococcus* spp., etc.). It seems that the uncultured species are actually the dominant ones (Zlatkin *et al.*, 1996; Parkes *et al.*, 2009). Interesting observations point that the majority of endolithic prokaryotes adopted an anaerobic or microaerophilic heterotrophic way of life, in an environment where the organic substrates could also be generated by thermal activation of organic matter buried in rocks (Balkwill *et al.*, 1989; Fredrickson *et al.*, 1991; Fry *et al.*, 2008). On the other hand, chemolithoautotrophic microbes inhabiting deep sediments gain energy by the reduction of oxidized sulfur, manganese or iron minerals. These prokaryotes belonging to „intraterrestrial" biosphere (or subsurface biome) as well as members of recently characterized microbial communities of extremely arid zones (e.g. Atacama desert) are good models for astrobiologists to imagine similar forms of life that might be found on extraterrestrial grounds (Wierzchos *et al.*, 2011). Moreover, the deep-subsurface biosphere could represent the largest microbial habitat yet to be explored (Edwards *et al.*, 2012).

Stone matrix itself accomodates several types of lithobiontic ecological niches. In this regard, Golubic and his colleagues (1981) have divided endoliths by their strategy of cavity colonization into three categories. The true endoliths or *euendoliths* are actively boring the interior of rock forming tunnels or microcrevices. After vacation by euendoliths, these small cavities could be secondary colonized by *cryptoendoliths*. Cryptoendoliths also dwell the structural pores of rock. *Chasmoendoliths* are rather opportunistic invaders of cracks (or large cavities) formed in stone. The precise characterization of the composition of endolithic communities and their interaction with the mineral surroundings is the key of understanding the role of such organisms in the biodeterioration of stone monuments (de los Rios and Ascaso, 2005). Therefore, a multitude of classic and novel approaches have been employed to study the endolithic prokaryotes inhabiting stone monuments, from microscopic studies (light and scanning electron microscopy) to molecular techniques.

Phototrophic endoliths

Active boring endoliths mainly comprise the phototrophic microflora including algae and cyanobacteria. Phototrophic euendoliths have been found to perforate calcareous substrata and they are responsible for the biological erosion of coastal limestone, calcareous shells, coral skeletons, limestone oolites, and sand grains (Shachak *et al.*, 1987; Salvadori, 2000; Aline, 2008; Zardi *et al.*, 2009). Among euendolithic chlorophyta, *Ostreobium quekettii* is particularly abundant in coral skeletons along with the cyanobacterium *Plectonema terebrans* (Aline, 2008; Verbruggen and Tribollet, 2011). On stone monuments, green algae (Division Chlorophyta) and diatoms (Class Bacillariophyceae) have been frequently identified and studied as inhabitants of epilithic biofilms. Species of genera *Chlorella*, *Stichococcus*, and *Chlorococcum* are found on the surface of many outdoor stone monuments of various lithotypes (limestone, granite, marble, travertine and sandstone) around Mediterranean Basin (Macedo *et al.*, 2009) or in tropical areas (Kumara and Kumar, 1999). Epilithic algae in association with fungi and bacteria form patinas or sheaths on stone surfaces wherever moisture, light, and inorganic nutrients are available at optimal values. However, little is known about endolithic algae living in stone artifacts. Species of *Trentepohlia*, *Chlorella*, *Klebsormidium*, and *Stichococcu*s were observed as colonizers of cryptoendolithic niches on churches in Portugal and Spain (Macedo *et al.*, 2009).

Active burrowing cyanobacteria are found within several morphogenera of uncertain phylogeny: *Hyella*, *Solentia*, *Plectonema*, and *Mastigocoleus*. Boring cyanobacteria possess a unique mechanism of calcium carbonate dissolution while excavating the solid carbonate substrate. Garcia-Pichel *et al.* (2010) have proposed a mechanism by which calcium is extracted from the matrix at the front (apical) pole of the trichome, and carried intracellularly by Ca^{2+}-channels. Calcium ions are further transported along the multicellular cyanobacterial filaments, passing from one cell to another by membrane Ca^{2+}-ATPase. At the distal pole of boring filament, a P-type H^+/Ca^{2+}-ATPase actively excrete calcium ions to external milieu at the exchange with protons ($2H^+/Ca^{2+}$). Simultaneously, at burrowing pole, the bicarbonate (HCO_3^-) results from calcium carbonate (CO_3^{2-}) dissolution under the presence of protons exported by cyanobacterial cells. Bicarbonate is further taken up and used for inorganic carbon fixation during photosynthesis. Besides their damaging effects on natural or anthropogenic carbonate substrate, euendolithic phototrophs (green and red algae, cyanobacteria) are thought to play important role in natural recycling of calcium and carbonate ions.

Cyanobacterium *Hyella fontana* have been identified as member of endolithic community on marble and limestone monuments in Spain (Fountain from Patio de la Lindaraja in Alhambra, Granada) (Bolivar and Sánchez-Castillo, 1997) and marble statues in Rome, Italy (Macedo *et al.*, 2009). *Plectonema battersii* and strains of *Plectonema* spp. have been described in biofilms covering fountains (Trevi Fountain, Rome) (Nugari and Pietrini, 1997) and walls and statues (Medici Fortress and statues in

Boboli Garden, Florence) (Tomaselli *et al.*, 2000). As a result of their boring activity, euendolithic cyanobacteria may form tunnels with diameters ranging from 3 to 25 μm, depending on the size of endolithic filaments (Salvadori, 2000). In this manner, mechanical destruction of monuments at microscopic scale is achieved and niches for other colonizing microbes are created.

Some of the most widespread cryptoendolithic phototrophs are the cyanobacterial species from genera *Gloeocaps*a, *Chroococcus*, *Chroococcidiopsis* (Order Chroococcales), and *Scytonema* (Order Nostocales). They were found to form biofilms on surfaces as well as within the matrix of stone artifacts and natural rocks (Friedmann, 1980; Banerjee *et al.*, 2000; Tomaselli *et al.*, 2000; Pointing and Belnap, 2012). Cryptoendolithic cyanobacteria have a preference for porous transparent rocks (such as marble, limestone, travertine, or sandstone) and do not grow within dark and dense volcanic stone (granite, dolomite) (Macedo *et al.*, 2009). *Chroococcidiopsis* strains are versatile and face harsh environmental conditions including high or low temperatures, high salinity, high UV irradiation and desiccation (Billi *et al.*, 2011; Stivaletta *et al.*, 2012; Baqué *et al.*, 2013). *Gloeocapsa* spp. includes highly adaptable species to changing environmental conditions. It was observed that *Gloeocapsa* strains synthesize intracellular and water-soluble mycosporine-like amino acids (MAAs) that have high UV absorbance, therefore acting as UV sunscreens (Garcia-Pichel *et al.*, 1993). Moreover, scytonemin is another photoprotective compund that is excreted by some cyanobacterial strains (e.g. *Scytonema* spp.) populating biofilms of stone monuments (Keshari and Adhikary, 2013). When dessication impedes the photoprotective mechanisms, cyanobacteria retreat inside rock and form colonies as endolithic biofilms. Because of light limitation, phototrophic endoliths do not grow deep in stone, preferring microniches down to only few milimeters below rock surface (Warscheid and Braams, 2000). Salvadori (2000) reported the abundant presence of cyanobacterial endoliths at a depth of 0.11-0.25 mm below surface of Carrara marble and the colonization of filamentous boring cyanobacteria at a maximum depth of 1.9 mm in limestone scales taken from Neptune fountain (Trento, Italy).

Endolithic Bacteria and Archaea on Stone Monuments

Acting as pioneers in stone colonization, phototrophic epi- and endolithic organisms provide nutrients and assure humidity and light (UV) shelter for heterotrophic communities of bacteria and archaea (Scheerer *et al.*, 2009). Autotrophic bacteria inhabiting natural rocks or stone monuments rely mainly on the water and mineral nutrients brought by external factors (wind, rainfall, or direct human activity) and/or having endogenous origin.

Heterotrophic bacteria that invade and populate the interior of stone-made monuments are highly diverse. Chemotrophic prokaryotes may contaminate stones down to 5 cm deep or even deeper (Waarscheid and Braams, 2000). Considered as one of the first colonizers of rock habitats together with phototrophic microorganisms,

Gram-positive actinomycetes (Class Actinobacteria) are well represented in endolithic communities around the world (Walker and Pace, 2007). In stone artifacts, actinobacteria are consistently represented by species from genera *Arthrobacter* (identified as hypoliths of mural paintings in indoor stone monuments from Spain and Austria) (Heyrman *et al.*, 2005). Several strains of *Streptomyces* have been isolated from ancient Egyptian mural paintings and stone supports (Abdel-Haliem *et al.*, 2013). *Streptomyces* spp. was pointed as one of main agents causing colour change of tomb paintings by producing a range of acids (oxalic, citric, and sulfuric acid), biopigments (melanins) and hydrogen sulfide. Molecular approaches have revealed that the endolithic microbiota of Mayan stone monuments of limestone origin has a high abundance of Actinobacteria, Acidobacteria, and low G+C Firmicutes (McNamara *et al.*, 2006). Full genome sequence of *Blastococcus saxobsidens*, an actinobacterium isolated from calcareous stone in Sardinia (Italy), was recently unveiled (Chouaia *et al.*, 2012).

Chemoheterotrophic bacteria from Phylum Firmicutes are frequently identified on surface as well as inside the stone artifacts. Strains of *Bacillus* spp. with its spore-forming strategy of survival and broad range of nutrients utilized, are some of most oftenly found bacteria within various lithotypes (see review of Scheerer *et al.*, 2009). Surface samples (3 mm depth) samples from wall paintings and building materials allowed Piñar et al (2001) to isolate several strains related to moderately halophilic Gram-positive *Halobacillus litoralis* by enrichment procedure. Interestingly, it was found that *Halobacillus* spp. is able to precipitate carbonate in a process called biomineralization (Rivandeyra *et al.*, 2004). The saline ecological niches related to stone materials seem to be important in several situations such as salt efflorescences that form during weathering of stone. Locally, carbonate, chloride, nitrate, and sulfate salts accumulate and extremely saline microniches appear. In such endolithic saline microhabitats, a predominance of halotolerant actinobacteria (*Kocuria* spp., *Micrococcus* spp., *Arthrobacter* spp.) and bacilli communities (e.g. *Bacillus* spp., *Staphylococcus* spp., *Paenibacillus* spp.) is remarkable (Laiz *et al.*, 2000). Other chemoheterotrophic bacteria detected by means of molecular biology from various monuments belong to Gammaproteobacteria (e.g. *Halomonas* spp., *Pseudomonas* spp.) (Rölleke *et al.*, 1996; Suihko *et al.*, 2007).

The oligotrophic lifestyle is also present in low nutrient stones and it is carried out by methanotrophs and methanogens. Isolates belonging to *Methylobacterium* spp., *Methylocystis* spp. (both from Class Alphaproteobacteria), and to facultative methylotrophic *Methylosinus* spp. (Class Gammaproteobacteria) have been described in stone samples from historical buildings in Germany and Italy. These methane-oxidizing bacteria belong to type II methanotrophs and their presence was well correlated with sandstone and limestone materials. From the deteriorated marble samples of a Kremlin crypt (Moscow, Russia), Doronina *et al.* (2005) have isolated an aerobic and moderately haloalkaliphilic methylotroph, *Methylophaga muralis* (formely *M. murata*). It was suggested that the low atmospheric methane concentration

alone cannot support the presence of methane-utilizing bacteria on stone samples. Anthropogenic emissions of methane and methanol as well as traces of biological methanogenesis present in stone substrate could enhance methanotrophic growth in these ecological niches (Kussmaul *et al.*, 1989). Same author, however has demonstrated the presence of bacterial mini-methane producers (*Clostridium* spp.) and of methanogenic archaea in several stone samples. Their presence would have been possible in the anoxic microsites of stone material and, in this regards, they are endoliths.

Chemolithoautotrophic bacteria present as epi-, hypo- or endolithic microbiota use CO_2 (or HCO_3^-) as carbon source and inorganic compounds as electron donors. Inorganic sources of energy mainly consist of reduced sulfur compounds ($S_2O_3^{-2}$, S^0, H_2S), reduced nitrogen compounds (NO_2^-, NH_3). As a result of their activity, inorganic acids (HNO_3, HNO_2, H_2SO_4) are produced with dissolving effects on hosting non-alkaline material (Waarscheid and Braams, 2000; Scheerer *et al.*, 2009). Populations of chemolithoautotrophic sulfur-oxidizing (*Thiobacillus* spp.) and nitrogen-cycling bacteria (*Nitrobacter* spp., *Nitrosomonas* spp., *Nitrospira* spp.) have been detected in samples of stones originated from various monuments (see review of Scheerer *et al.*, 2009) .

Conclusions

Various types of stone matrices have the potential to accommodate a variety of ecological microniches. When appropriate physical and chemical conditions meet, rock is firstly colonized by mixed populations of phototrophic microbes forming biofilms. Additionally, fungi and macrophytes may find favorable conditions to develop on stone surfaces. Biological activity of epilithobionts overlaps the effects of the abiotic factors, providing further deep microhabitats for cyanobacteria, chemotrophic bacteria and archaea. The endolithic prokaryotic communities inhabiting various stone monuments were found to be surprisingly diverse, many members of these ecological group showing high adaptative versatility. Altough the description of molecular and culturable diversity of endolithobionts is well established, their metabolic interactions and overall effects on the rate of deterioration of stone monuments is less understood.

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=== BOOK REVIEW ===

BATS OF BRITAIN, EUROPE AND NORTHWEST AFRICA.

ENDRE JAKAB[1,2]

Bats of Britain, Europe and Northwest Africa, Christian Dietz, Otto von Helversen, Dietmar Nill, A & C Black, London, 2009, 400 p.
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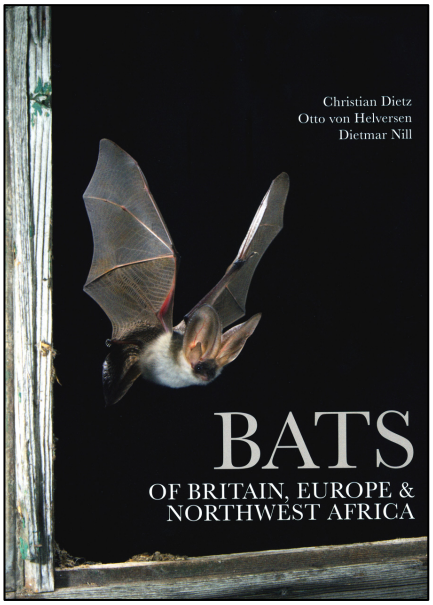

It is a complete handbook which provides useful information not only for specialists but for bat enthusiasts too. This is an English translation of the "Handbuch der Fledermäuse Europas und Nordwestafrikas: Biologie, Kennzeichen, Gefährdung", which appeared in 2007 in Germany.

The book can be divided into two parts.

The ***first part*** of the book presents the body structure, evolution and taxonomy of the bats. The introductory chapters describe the global diversity and the flight characteristics of the bats. A brief chapter is dedicated to echolocation: call 'design', ecological adaptation to habitat and hunting methods. The following chapters discuss the hunting strategies and prey-preferences of the European and Northwest African bats. The next chapters describe the migration and orientation mechanisms, the torpor and hibernation strategies, social behaviour, roost structure and roosting habits developed by the bats. The final chapters of the first part present the population biology and some practical conservation measures of the bats. A short chapter is dedicated to species identification by using echolocation calls.

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The ***second part*** presents all the 51 bat species of Vespertilionidae, Rhinolophidae, Hipposideridae, Rhinopomatidae, Nycteriade, Pteropodidae, Miniopteridae, and Molossidae families that occur across Europe and Northwest Africa. This part also contains the identification keys to bat families preceded by descriptions of standardized measurement methods. Every species-description contains a discussion about identification methods, echolocation calls, habitat, behaviour and migration data. The descriptions are completed with amazing pictures, illustrations and distribution maps.

The last chapter of the book presents the current conservation status of European bats based on IUCN data.

Finally I would like to thank to Vicky Atkins from Bloomsbury Publishing Plc. providing me the inspection copy of this book.