GRANT AGENCY OF THE ACADEMY OF SCIENCES

Justification of the proposal

(minimum 3 pages, maximum 10 pages)

1. INTRODUCTION

Boron (B) is an essential micronutrient required for plant growth and development. B affects not only yield but also quality of several crops. There is wide variation in B requirement between plant species. Dicots generally require more B than monocots, with graminaceous monocots needing least of all (Goldbach *et al.*, 2001).

Shortly after B was introduced as an essential element for higher plants, structural damage was attributed to B deficiency (for review see Blevins and Lukaszewski, 1998). B deficient plants may exhibit a wide variety of symptoms, depending on the species and the age of the plant (Gupta, 1979; Shelp, 1993). Worldwide, B deficiency is more extensive than deficiency of any other plant micronutrient (Loomis and Durst, 1992).

In the past few years some research developments contributed to better understanding of the role of B in plants. Up to now many roles for B in plants have been proposed, including functions in sugar transport, cell wall synthesis and lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid metabolism, phenol metabolism and membrane transport (Blevins and Lukaszewski, 1998). However, the mechanism of B involvement in each case remains unclear. The physiological role of B in plants is depicted as that of a transducer in several processes initiated by light, gravity, and some plant hormones. It is extremely difficult to disentangle primary from secondary or even tertiary events. B is therefore either involved in many ways in the plant, or it is involved in one or two key areas, impairment of which results in a cascade effect, leading to the multitude of deficiency symptoms observed (Tanada, 1995).

B enters plant roots as undissociated boric acid. Significant differences in B uptake are frequently observed even when plants grow under identical conditions. It has been hypothesized that these differences reflect species differences in permeability coefficient across the plasma membrane (Dordas and Brown, 2000). In vascular plants, B moves from the roots with the transpiration stream and accumulates in growing points of leaves and stems. Once in the leaves, B retranslocation is restricted and it becomes fixed in the apoplast. The phloem movement of B depends on the sugar or polyol transport molecules used by the particular plant (Hu *et al.,* 1997).

The B content of soils is normally relatively constant at approximately 10 $mg.kg^{-1}$ (Loomis and Durst, 1992; Benderdour *et al.,* 1998). In general, there is a small concentration range between deficiency and toxicity; however, toxicity (i.e. excess B) is much less common in the environment than B deficiency. Some soils, especially those associated with mining, may have much higher concentrations and result in plant toxicity and some boronated derivatives are used as herbicides (Howe, 1998).

The mechanisms of B toxicity are not quite clear. In the literature, most of the reports describing B toxicity actually have resulted from the accidental application of excess artificial B (Ghanati *et al.,* 2001). Hayes and Reid (2004) recently found strong evidence that boron tolerance in barley is due to the active efflux from the root. According to Loomis and Durst (1992), ribose, present in abundance in ribonucleotides, is involved in the chemistry of B toxicity. The action of B in root meristems is also associated with ascorbate metabolism (Blevins and Lukaszewski, 1994). Ferrol *et al.,* (1993) observed that B toxicity inhibit ATP-dependent H+ pumping and vanadate-sensitive ATPase activity in sunflower roots and cell suspensions.

There is wide variability in the tolerance of plants to high B, both inter- and intra- specifically. Some more tolerant plants such as wheat and barley accumulate less B, which has been commonly proposed to be due to differences in their membrane transport (Carpena *et al.,* 2000). A study by Mahboobi *et al.,* (2000) in barley, found that the expression of about 20 proteins was affected by transferring plants to high B conditions and that several of these proteins were affected differently in tolerant and sensitive cultivars. Comparisons between B tolerant and sensitive cultivars showed that tolerant plants generally accumulate less B, particularly in their shoots (Nable *et al.,* 1990). Plants with high B requirement, e.g. sunflower, can certainly tolerate higher B levels than wheat, for example, but there have been no reports of differential tolerance between cultivars of sunflower due to higher tissue tolerance.

Molecular investigations of B requirement in plants open new possibilities for improving B deficiency/toxicity stress tolerance of crops. Noguchi *et al.,* (1997) have isolated a novel mutant of *Arabidopsis thaliana* (*bor1-1*) with an altered B requirement. *bor1-1* (high boron requiring) mutant requires a high level of B. Pfeffer *et al.,*(1997) suggest that *bor1-1* contains a mutation in the putative active transport system, dominant at low B concentration. Other *Arabidopsis* mutations with an altered response to B have not been reported yet. Elucidation of the aspects of B nutrition will be a challenging goal for future research.

This project is proposed to study responses to elevated B concentrations of a model plant *Arabidopsis thaliana*. *Arabidopsis* has several advantages, which include a small genome size (125 Mbp distributed on five chromosomes) with known sequence (The *Arabidopsis* Genome Initiative 2000), a rapid life cycle, easy cultivation under limited space and prolific seed production. Many genes isolated from *Arabidopsis* have been used for genetic engineering of crop plants, e.g. to construct herbicide resistant crops (Klee *et al.,* 1987), to clone homologous genes from other plants, or to obtain PCR and RFLP probes for molecular plant breeding (Fabri and Schäffner 1994). International genome projects currently aim at functional analysis of the sequenced genes. Insertional mutagenesis using the T-DNA of *Agrobacterium* is used as an effective technique to generate gene mutations, identify the corresponding genes and characterize their function by genetic analysis (Koncz *et al.,* 1989). Another suitable plant for studying B tolerance are: barley (Tanada 1995), wheat (Furlani *et al.,* 2003), cannola or sunflower or *Chenopodium* (Fleischer *et al.,* 1998).

2. PROJECT DESCRIPTION

2.1 Project objectives

This project will be focused on investigation of the responses of *Arabidopsis thaliana* plants to elevated B concentrations. The results should contribute to better understanding of molecular basis of some roles of B in plant development and possible mechanisms of tolerance to B. Several initial experiments were already started in frame of the post-doc project (No. 521/00/D036, supported by Czech Science Foundation), which was aimed mainly to study of *Arabidopsis* root cells expansion mutants, whereas in this proposal we plan to study response of all plant organs. Similar set of experiments was partially done in Dr. Stephen Rolfe's laboratory from Department of Animal and Plant Sciences of the University of Sheffield (UK). Dr. Stephen Rolfe will be helpful to consult the implementation of proposed procedures. All our existing results led to several hypotheses mentioned below. The aim of this project is to confirm them and further develop prior publishing. We do not propose to study B deficiency symptoms and processes induced under B deficiency, which are more commonly explored around the world. As the most promising research on B deficiency appears using microarray technology in Dr. Blevins laboratory from the Agronomy Department at the University of Missouri.

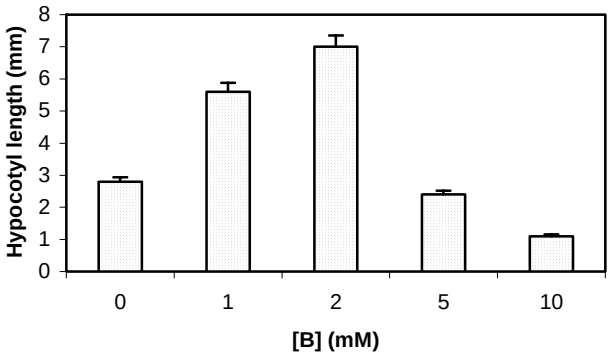
The main objectives of this project include:

2.1.1 Evaluation of growth responses of wild type *Arabidopsis* plants to elevated boron concentrations

Preliminary results:

In our initial experiments, concentration-dependent responses to exogenous B were observed in light-grown seedlings. At concentrations from 0 to 1 mM, B stimulated hypocotyl elongation, whereas at concentrations above 5 mM, B caused toxic effects, independently of the illumination (see graph 1). At the lower B concentrations the seedlings remained green and healthy. In contrast, visible symptoms of the B toxicity were apparent at the higher concentrations. The seedlings were short, stunted and pale. Both, root and hypocotyl growth was impaired.

Graph 1: Effect of elevated B concentrations on *Arabidopsis thaliana* ecotype Columbia plants (irradiance 100 µmol m^{-2} s^{-1}).



Proposed research tasks:

These measurements will be repeated to confirm the reproducibility of the preliminary results. We also want to answer the question how does B stimulate hypocotyl elongation. The stimulation could result from an increase in cell division and/or cell expansion. Thus, the cell length will be determined in the epidermal and cortical layers of the hypocotyls. Also other growth parameters, such as root and petiole length, cotyledon and first leaf area, will be measured and also changes in hypocotyl thickness will be studied. The phenotype of the treated plants (developmental stage, cotyledon and leaf colour density, root branching etc.) will be described. The pattern of the response of wild type plants will be considered as standard response for subsequent studies on *Arabidopsis* mutants (depending on mutant backgrounds).

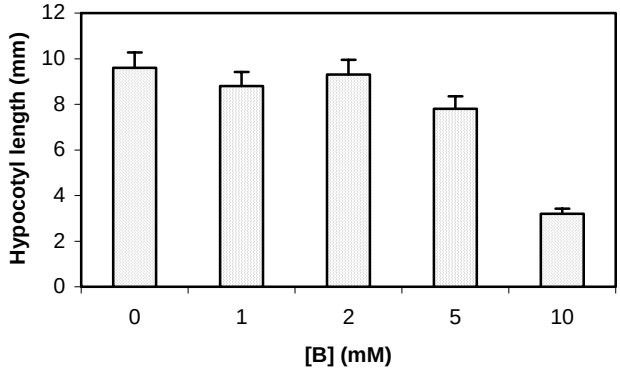
2.1.2 Study of the interactions between boron response and the environment (light quality and quantity)

Hypocotyls are extremely responsive to environmental signals – particulary light; therefore the interaction between B and light on hypocotyls elongation will be investigated.

Preliminary results:

We have observed that the response becomes more marked as the irradiance increases. In darkness there is no significant difference in hypocotyl elongation except an inhibition at the highest concentrations expected. In most cases only inhibition effect was observed (Graph 2). This phenomenon can be connected with the fact that B can act as a transducer in several processes initiated by light. Following absorption of light, photoreceptors interact with other signal transduction elements, which eventually leads to the molecular and morphological responses.

Graph 2: Effect of elevated B concentrations on *Arabidopsis thaliana* ecotype Columbia plants in darkness.



Proposed research tasks:

We will test whether light quality has, along with irradiance, any effect on the response of *Arabidopsis* seedlings to the elevated B. In other words, we will investigate whether specific photopreceptors may be involved in *Arabidopsis* response to B. Preliminary results from the Dr. Rolfe's laboratory show that elevated B stimulate hypocotyls elongation in red, but not under blue light (Rolfe, personal communication).

2.1.3 Study of the interaction between boron and specific mutations

We will study three groups of mutants:

a) **photomorphogenic mutants** based on our previous observation of the influence of light on the B response
b) **cell wall mutants** based on the fact that up to 90% of the cellular B has been localized in the cell wall fraction (Hu and Brown, 1994)
c) **plasma membrane mutants**, with special interest in:
    1. membrane lipids composition based on the fact that boron tolerance correlates with the active efflux from the root (Hayes and Reid, 2004) which is mediated by membrane lipid composition (Zhao *et al.,* 1996)
    2. regulation of osmotic stress because B is known to influence the function of the plasma membrane ATP-ases (Tanada, 1983)

2.1.3.1 Photomorphogenic mutants

Multiple photoreceptors sense light quality and light quantity in *Arabidopsis.* Phytochromes (A, B, C, D, E) sense Red and Far-red light (Chory *et al.,* 1996) and Cryptochromes (1,2) sense Blue light (Lin and Shalitin, 2003). We will test known mutants in specific phytochrome genes to determine which phytochromes are involved in B response.

Preliminary results:

We have already tested five different *Arabidopsis* ecotypes (Col-0, C24, Ws, La-0, No) along with known photoreceptor *hy* mutants *(hy1-1, hy2-1, hy3, hy4)* (Chory *et al.,* 1996). These mutants are characteristic by elongated hypocotyls in light. Interestingly, in comparison with WT, the mutants did not show the B-induced stimulation of hypocotyls growth. The used *hy* mutants were all in Landsberg *erecta* background, so there are two alternatives for this result. Either a photoreceptor mutation or the *erecta* mutation (Yokoyama *et al.,* 1998) in Landsberg background is responsible for the lack of response.

Proposed research tasks:

We will test the B response in other ecotypes including L*er* WT and *erecta* mutant of Columbia together with *hy* and some other photomorphogenic mutants in L*er* or Columbia background.

2.1.3.2 Mutants with defect in cell wall elongation

B plays role in cell wall synthesis and lignification and cell wall structure. Some toxicity symptoms, such as 'leaf cupping' occur in some species (e.g. peppermint) due to inhibition of cell wall expansion (Loomis and Durst, 1992). Cell walls are composed of complex mixtures of polysaccharides. An important component is rhamnogalacturonan II (RG-II), which forms cross-links via boron (Perez *et al.,* 2003).

Proposed research tasks:

To examine the role of cell wall component in B- dependent hypocotyl elongation we will test the response on mutants, which show a phenotype related to the cell wall elongation and structure. We will focus on *mur(1-10)* (Reiter *et al.,* 1997), extensin mutants (Merkouropoulos and Shirsat, 2003), and other possible mutants which will be selected from Arabidopsis TAIR database (http://www.arabidopsis.org) and ordered via ABRC or NASC respectively. In the case of identifying quite different pattern of B response (especially stimulation of hypocotyl elongation) we will select one or two mutants for analysis of expression of relevant genes under elevated B.

2.1.3.3 Plasma membrane mutants

It was concluded that except the cell wall structure, B might play a role in membrane function. Dordas and Brown (2000) found that B accumulation into *Arabidopsis* mutants with differing membrane lipid composition was significantly different.

Proposed research tasks:

In our tests, we will include two other groups of known mutants:

1) Mutants related to membrane lipids composition. These include mainly fatty acid elongation (*fae*) (Millar and Kunst 1997) and fatty acid desaturase deficient (*fad*) mutants (Yadav *et al.,* 1993), and other mutants related to plasma membrane composition, such as diacylglycerol O-acyltransferase *rds1* (Katavic *et al.,* 1995), digalactosyldiacylglycerol synthase *dgd*1 (Härtel *et al.,* 2000), mutants in genes involved in sulfolipid biosynthesis *sqd*1, *sqd*2 (Mulichak *et al.,* 1999), Acyl-CoA:diacylglycerol acyltransferase *tag*1 (Routaboul *et al.,* 1999) and also some sterol content mutants (He *et al.,* 2003).
2) Mutants with changes in regulation of osmotic stress. We will test their response to elevated B concentrations under different light conditions. We have revealed that light can increase the effect of osmotic stress induced by mannitol (Fellner and Sawhney, 2001).

2.1.4 Isolate and characterise mutant with altered responses to elevated boron

Proposed research tasks:

Group of principal investigator owns more than 1500 insertional mutagenesis lines which contain T-DNA insert with tetramer of 35S promoter enhancers constructed for activation tagging approach (Koncz, 1989). It is also possible to order other insertional collections from NASC (The Nottingham Arabidopsis Stock Center - http://nasc.nott.ac.uk). The mutant plants able to develop and growth at the medium with toxic B concentration will be selected as a potentially resistant to B. The basic genetic analysis of selected lines is planned (see chapter 2.2.6). In case of finding promising mutants a new grant application for detailed studies will be proposed.

2.2 Experimental procedures

2.2.1 Growth of plant material

In the first part, a robust set of growth conditions on sterile agar medium will be set up prior mutant screening. B stimulation and B toxicity will be studied separately.

Seeds of *Arabidopsis thaliana* will be surface sterilised using commercial bleach containing 1.6% w/v sodium hypochlorite and 0.1% Tween 20 for 15 minutes and then washed five times with distilled water. Individual seeds will be arranged on MS medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose adjusted to pH 5.8 and solidified with 1% Phytagar. MS medium normally contains 0.1 mM B, which is considered basic for plant growth as it corresponds to approximately 10 ppm B. Also modified MS medium without B in micronutrient mixture will be prepared. The plates with the seeds

will be placed at 4 ^{o}C for 3 days to synchronise germination. Experiments will be performed in polystyrene square Petri dishes (12x12 cm) in vertical position (75^{o}). Seeds will be dispersed onto the solid agar medium in a 6 mm grid pattern to ensure even spacing. Each plate will contain five rows with approximately 20 seeds. All plants will be grown in a growth room under artificial light.

2.2.2 Plant cultivation and light conditions

Arabidopsis plants will develop in growth chambers at 21^{o}C in dark or in continuous white (W), red (R), far red (FR), or blue light. In W, plants will grow either at high red:far-red ratio (R/FR ratio) or low R/FR ratio. Illumination will be provided by a cool white fluorescent tubes and incandescent bulbs. Light intensity will be regulated to provide W of high R/FR ratio (2.9) or W at low R/FR ratio (0.93). Photon fluence rate will be 0.01 to 250 μmol.m^{-2}.s^{-1}. R (maximum irradiance at 660 nm; photon fluence rate from 0.01 to 100 μmol.m^{-2}.s^{-1}) will be provided by white fluorescent tubes wrapped with a red membrane filter (such as Rosco, Hollywood Light Inc.). Blue light (maximum irradiance at 430 nm; photon fluence rate from 0.01 to 100 μmol.m^{-2}.s^{-1}) will be provided by white fluorescent tubes wrapped with a blue membrane filter. FR (spectral irradiance from 720 to 800 nm; photon fluence rate was 0.01 to 50 μmol.m^{-2}.s^{-1}) will be provided by incandescent bulbs (1000W) filtered either through a combination of red and blue membrane filters, or through plexiglass FR filter FRF 700 (Westlake Plastic Company, Lenni, PA). Fluence rates will be measured with a portable spectroradiometer (model LI-1800; Li-Cor; Lincoln, NE). A white mesh will be used as a neutral screen to provide dim light. In the greenhouse plants will be grown in soil under natural W light conditions supplemented with high-pressure sodium lamps.

2.2.3 Evaluation of the response of seedlings to various concentrations of boron

Arabidopsis seedlings will be grown for 10 days prior to measurements. The hypocotyls will be straightened with a forceps if necessary and the plates were placed in a photographic enlarger and projected. The junction between the hypocotyl and the root and the top of the seedlings will be marked. Magnified images of the hypocotyls will be then measured to the nearest 0.1 mm with a ruler. Root lengths were measured to the nearest 0.5 mm by placing the seedlings directly onto a ruler.

Accurate measurements of cotyledons or leaves area will be made using an computational image analysis system (CCD camera, Lucia software from Laboratory Imaging™).

Statistical analyses of measured data, including standard analysis of variance (ANOVA), were performed using Microsoft Excel 98 software.

To ensure if B stimulates hypocotyls cell expansion or division, the size and number of epidermal and cortical layer cells will be measured on confocal microscope (Zeiss LSM 510) according to Bougourd *et al.,* (2000).

2.2.4 Evaluation of the interactions between boron and the light quality and quantity

For this purpose will be the plants grown in the growth chambers with the controlled irradiance as mentioned in the chapter 2.2.2. We plan to test the response under many levels of irradiance in the range between 0 and 250 μmol.m^{-2}.s^{-1}. To grow plants in darkness the plates with the seeds will be covered by aluminium foil. For the analysis of the response under different light quality we will focus on differences between red, far-red and blue light.

2.2.5 Testing of various photomorphogenic, cell wall or plasma membrane mutants for their responses

to the elevated boron

In this part of experimental procedures we will focus on B stimulation of hypocotyl elongation. The seedlings will be grown on media with 0.1 mM B (MS medium without H_3BO_3 addition) and 1 mM B. The mutants mentioned in the project objectives will be obtained from NASC (http://nasc.nott.ac.uk/) or ABRC (http://www.arabidopsis.org/abrc/) or directly from authors. The mutants will be also subjected to the confocal microscope analysis according to Bougourd *et al.,* (2000).

Expression of selected and the most affected genes will be studied. For Northern blot analysis total RNA will be extracted from WT and mutant tissues as described by Audran *et al.,* (1998). The RNA will be fractioned on a 1.2% agarose gel containing formaldehyde in MOPS buffer and then transferred onto membranes. The blot will be hybridized with a ^{32}P-radio-labelled probe made with a PCR fragment of cDNA. RT-PCR will be carried out using a one step RT-PCR kit according the manufacture's instructions. Total RNA will be extracted from different tissues using an RNA extraction kit supplemented with RNase-free DNase during extraction. cDNA will be synthesized using oligo $(dT)_{20}$ primer and using ThermoScript RT-PCR System. The cDNA will be amplified with primers that specifically amplify mutant genes. Control will be carried out with primers that amplify a constitutively expressed elongation factor "EF-1α" cDNA (Liboz *et al.,* 1990).

2.2.6 Screening for boron tolerant mutants

For the screening for mutants tolerant or resistant to elevated B, the seeds will be plated on square Petri dishes (12x12 cm) with MS medium supplemented with B at concentration 7.5 mM. WT plants developing at such level of B are markedly inhibited in their growth, form minimal roots, short hypocotyls and tiny cotyledons. We plan to grow six rows per plate, about 20 seeds in each row, which will represent one line with random T-DNA insertion. The insertional lines from Salk Institute for Biological Studies La Jolla (USA) or other collections ordered via NASC can also be used for the mutant selection. These lines are usually ordered into pools and thus, the individual plants with no or weaker symptoms of B toxicity in comparison to WT will be selected and the progeny tested again. The selected resistant or at least tolerant lines will be subjected to basic genetics analyses (heritability character, back-crossing, SSLP or CAPS markers gene mapping according to Bell and Ecker (1994) or Konieczny and Ausubel (1993), determining of the number of T-DNA inserts, detection of the linkage of the T-DNA insert with the mutated gene or crossing with other mutants with similar phenotype). The identification of mutant gene will depend on the results of the co-segregation analysis between T-DNA insertion site and mutated gene. In case of confirming the co-segregation, iPCR (Ochman et al., 1993) or TAIL-PCR (Liu et al. 1995) approach will be used for determining of the gene tagged by T-DNA. The position of the T-DNA insertion will be determined by BLAST homology search (Altschul et al. 1990). The detailed analysis of the selected mutants could be a subject of following grant proposal.

2.3 Time table of the project

The project is proposed for three years. This period should be sufficient for performing all of the planned tasks.

year	*tasks*	
2005	Evaluation of seedling responses to different concentrations of B	Kocábek
	Testing the influence of irradiance and the light quality on stimulatory or inhibitory effects of B on *Arabidopsis* plants	Fellner
	Evaluation of the responses of various WT ecotypes of *Arabidopsis* to B	Kocábek
	Collecting of the seeds of mutants of interest	Fellner, Kocábek
2006	Analysis of photomorphogenic mutants from the stock center for responsiveness to B.	Fellner
	Analysis of mutants with altered cell wall compositions for responsiveness to B.	Kocábek
	Analysis of plasma membrane mutants for responsiveness to B.	Fellner
	Expression studies of the genes influenced with B	Kocábek, Fellner
2007	Analysis of mutants selected from the České Budějovice collection of *Arabidopsis* insertion lines and mutants from the stock center for responsiveness to B.	Kocábek (home collection) Fellner (other collections)
	Basic genetic analyses of the selected lines – genetic character, T-DNA linkage, number of the inserts, gene mapping using SSLP or CAPS markers, in case of the co-segregation also gene determination.	Kocábek, Fellner

3. CONDITIONS FOR REALIZING OF THE PROJECT

3.1 Readiness of the principal investigator

The principal investigator is a member of Department of Plant Genomics with more than eight years experience in work with *Arabidopsis thaliana,* especially with transformation and genetic or molecular analysis of the mutants. Laboratory has good equipment for molecular biology, tissue culture and greenhouse experiments. T. Kocábek and his co-workers have created their own collection of *Arabidopsis* insertion lines (more than 1500) in the frame of previous projects. The lines were phenotypically characterized and seeds are stored for future experiments. Also, an image analysis system (CCD camera, Leica stereo-microscope, image analysis software Lucia 4.7 from Laboratory Imaging Company™) has been purchased five years ago. T. Kocábek is constantly in touch with Dr. Stephen Rolfe from the University of Sheffield who also partially worked on similar experiments.

3.2 Readiness of the co-investigator

The co-investigator has valuable experiences from abroad where he worked on various *Arabidopsis* projects in plant physiology, genetics, biochemistry, cell and molecular biology (see applicants CVs). The applicant has also live and friendly connections with several laboratories in France, Canada, USA, Germany, and the Netherlands that may be supportive in this project. In August 2003, the co-investigator has returned to the Czech Republic from long-term stay in Canada and USA. During the first five months after his return he built a new lab at the Biological center in Olomouc-Holice.

Following the 2000 completion of the *Arabidopsis* genome sequence by the *Arabidopsis* Genome Initiative, the international community of scientists has developed a long-range plan for the Multinational Coordinated *Arabidopsis thaliana* Functional Genomics Project

(http://www.nsf.gov/pubsys/ods/getpub.cfm?bio0202). *Arabidopsis* researchers representing 14 countries around the world involved in the project have established the Multinational *Arabidopsis* Steering Committee (MASC; http://www.Arabidopsis.org/info/2010_projects/MASC_Info.html). The MASC coordinates programmatic aspects of the *Arabidopsis* research world-wide. One of the scientific objectives of *Arabidopsis* Functional Genomics Project is also to promote international cooperation. In correspondence with Dr. Rebecca Joy (rejoy@biotech.wisc.edu), coordinator for the MASC, we agreed that Middle and East European countries represent potentially important research capacity in the future. The result of our discussion was the idea to faster communication and cooperation among Eastern European research laboratories and the research community involved in the *Arabidopsis* Functional Genomics Project. In December 2002, M. Fellner organized an *Arabidopsis* initiative. In the first step of this activity, he contacted research groups and laboratories in more than 17 Eastern European countries and, in five of them he located laboratories that are involved somehow in *Arabidopsis* research. The second step is to connect these laboratories via a multinational *Arabidopsis* project, "Eastern Europe *Arabidopsis* Activity or EEAA".

3.3 Equipment and facilities

Laminar and vertical flow boxes with tissue culture equipment; cultivation rooms, greenhouse; microclima growth chamber with regulated light conditions; PCR cyclers; Fast Prep homogenizer; analytical and preparative centrifuges; electrophoresis systems; cross-linking device; microtome; light microscopes. The laboratory of principal investigator can also use electron or confocal microscope and sequencing service of other institutes of Academy of Sciences located in České Budějovice.

3.2 Participation of the students

Departments of both applicants for the grant co-operate tightly with the Faculty of Biological Sciences at the University of South Bohemia (IPMB, T. Kocábek) and the Faculty of Life Sciences at the Palacký University (IEB, M. Fellner). The applicants will support involvement of the undergraduate students on the realising of the tasks resulting from the project which could serve as topic of their bachelor or master thesis.

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