SfP Summary Report November 2002

SfP-BIOPRODUCTION

SfP: 974453

Title: Alternative Approaches of Bioproduction of Alkaloids and Active Substances from Bulgarian Rare and Threatened Medicinal Plants

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Approval Date: 1 December 2000 Effective Starting Date: 15 February 2001
Duration: 4.5 years; expected completion by mid August 2005

NATO Budget: 371,840 EUR

Information about the SfP Project through Internet:

- home page of the project not yet established
- one page information available in Bulgarian via the on-line version of the journal "Bulgarian soldier": http://bgsoldier.eamci.bg/scripts/isapivwb.dll/doc?THEMEID=12232

Major Objectives

To provide the pharmaceutical industry with alternative technologies for biomass production of *Leucojum aestivum*, a rare and threatened plant species rich in alkaloid active against nervous diseases included AD, thus contributing to preservation of the natural plant populations.

- ✓ To evaluate the current status of the Bulgarian populations of *L. aestivum*
- ✓ To constitute representative germplasm collection from wild populations
- ✓ To evaluate snowflake agronomic and biochemical performances of *L. aestivum* under field conditions
- ✓ To characterize soil composition of diverse natural *L. aestivum* populations
- ✓ To evaluate, select and multiply clones with high contents of biologically active substances
- ✓ To identify suitable biological material for *in vitro* experiments, to develop efficient *in vitro* cell cultures and micropropagation
- ✓ To characterize alkaloid production *in vitro* and *in vivo*
- ✓ To develop lab scale *in vitro* cultures producing biologically active substances

Overview of Achievements since the Start of the Project until 31 October 2002

- ✓ A new Biotechnological Lab of Medicinal Plants, Institute of Botany, Sofia, has been installed, after major renovation funded by both BAS and the end-user and equipment funded by NATO; Field plot and facilities were reorganized;
- ✓ Ecological assessment of 22 Bulgarian natural populations of *L. aestivum* (19 Gal-, 1 Lyc- and 2 Lycorenine-type) has been performed, all information summarized and presented at the OPTIMA meeting (Palermo, September '01); this operation served as the official 2001 assessment of the Bulgarian *L. aestivum* populations by the Ministry of Environment and Waters;
- ✓ Plants and bulbs (3,500), seeds (441 progenies) and 120 kg fresh herbage of the *Leucojum* populations were collected in 2001 & 2002 for the purpose of the Project, in agreement with local authorities in charge of natural population protection; Soil samples were collected from *Leucojum* populations for further analysis;
- ✓ A large Database on current diversity of Bulgarian *L. aestivum* populations and habitats (BLDB) has been further developed; Texts and figures for items of the 1st module have been completed;
- ✓ Germplasm nursery has been constituted; Method for rapid bulb multiplication of *Leucojum* is almost developed;
- ✓ Soil control samples from 8 natural populations were analyzed according to 37 soil parameters;
- ✓ Callus formation was obtained from bulbs or from ovaries, after an intensive study of culture conditions (growth regulators, environment, type of explants); Successful sub-cultivation has ensured formation of more friable callus but needs further improvement;
- ✓ Trial experiments in 3L-Lab Bioreactor have been performed with already available cell suspensions from other plant species; The system is ready for experiments with *in vitro* culture of *L. aestivum;*
- ✓ Shoot clumps have been obtained by *in vitro* micropropagation on bulb, stem, leaf and ovaries segments; First liquid shoot clump culture of *L. aestivum* has been initiated; Rooting of the first larger bulblets is under study;
- ✓ Karyological techniques using root tips are being set up for *L. aestivum*; 11 populations have been investigated for their chromosome number and karyotype structure;

- ✓ Analytical determination of Gal and related alkaloids by TLC has been developed for fast screening purposes; Protocol for Gal determination in dry leaves with HPLC was elaborated; CPC has been adapted for Gal purification but needs final optimization;
- ✓ Comparative assessment of 8 populations according Gal content was carried out (primary by TLC screening in the field, followed by more precise HPLC analyses); They exhibit different patterns which could be interesting to connect with genetic variability;
- ✓ Training of 2 young scientists was performed in France.

Payments through NATO Project Funds: 257,910 EUR

Milestones for the next six Months

- ✓ Completion of the BLDB: data from the investigations within the project;
- ✓ Analysis of several soil samples of interest for the Project will be done;
- ✓ Characterization of *L. aestivum* plants and populations will continue, at karyological and biochemical levels;
- ✓ Study of nutrient media for improved callogenesis will be done; Adaptation of the *L. aestivum* callus culture to submerged cultivation will be analysed; Organogenetic potential will be analysed in correlation with medium composition;
- ✓ Training on HPLC envisaged for participants from Partners 1 at our end-user Sopharma Ltd. will be performed in parallel with the analysis of a number of samples of interest for the end-user;
- ✓ Training of a young scientist from Partner 2 on *in vitro* callogenesis and cell cultures will be performed in the facilities of Partner 4 (France).

Implementation of results

Results are not yet applicable at this stage. Our end-user (Sopharma Ltd., Sofia, Bulgaria) demonstrated their interest in Project with co-funding of setting up of the new BLMP.

NATO Consultant:

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Additional Collaborating institutions

None.

Abbreviations:

AD	:	Alzheimer Dementia
BAS	:	Bulgarian Academy of Sciences
BLDB	:	Bulgarian *Leucojum aestivum* DataBase
BLMP	:	Biotechnological Laboratory of Medicinal Plants
CPC	:	Centrifugal Partition Chromatography
HPLC	:	High Performance Liquid Chromatography
Gal	:	Galanthamine
LBPV	:	Laboratoire de Biologie et Physiologie Végétales
OPTIMA	:	Organization of Phyto Taxonomic Investigations of the Mediterranean Area
TLC	:	Thin-Layer Chromatography