



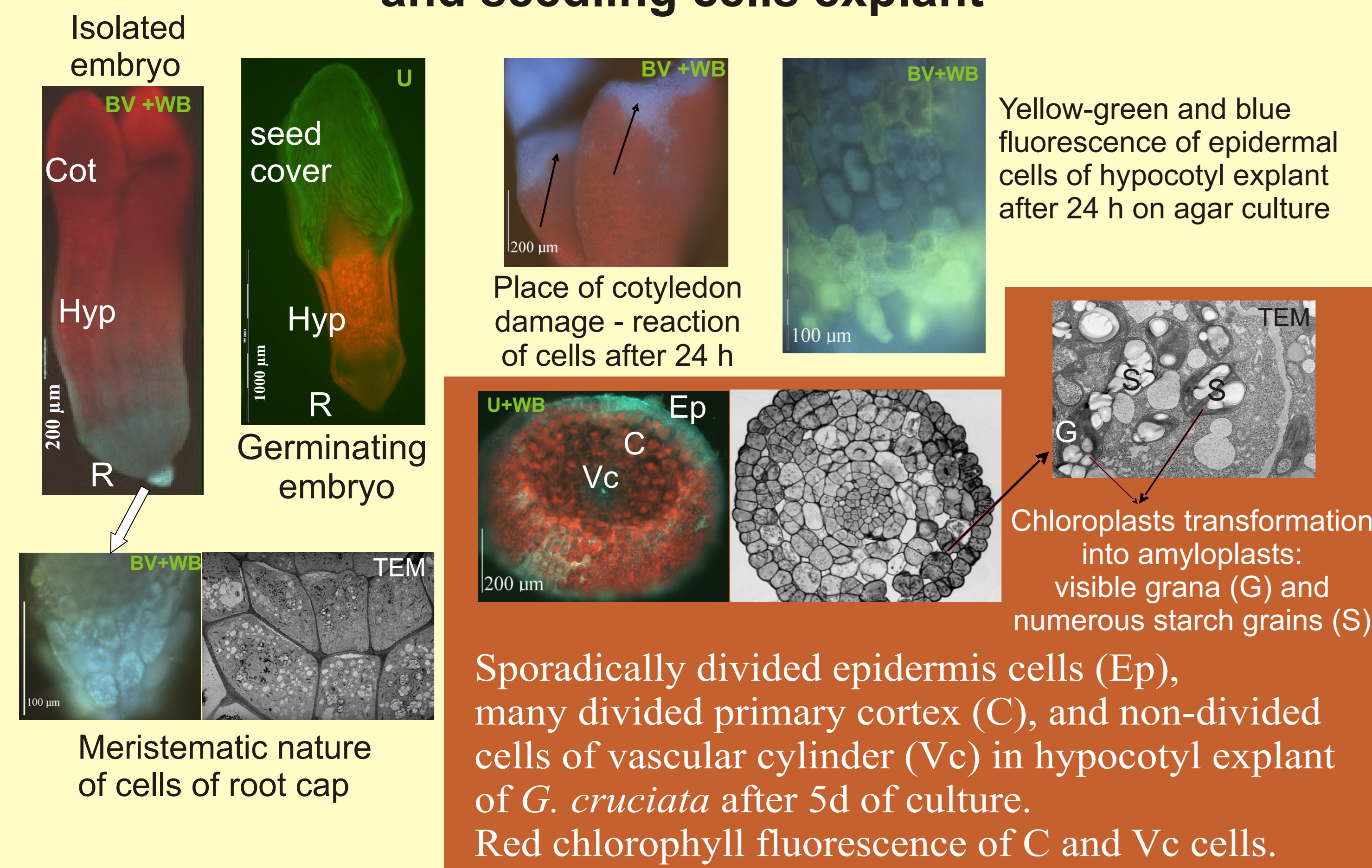
Autofluorescence in *in vitro* cultures of *Gentiana* genus

A. MIKUŁA, M. ŁADYŻYŃSKI and J. J. RYBCZYŃSKI

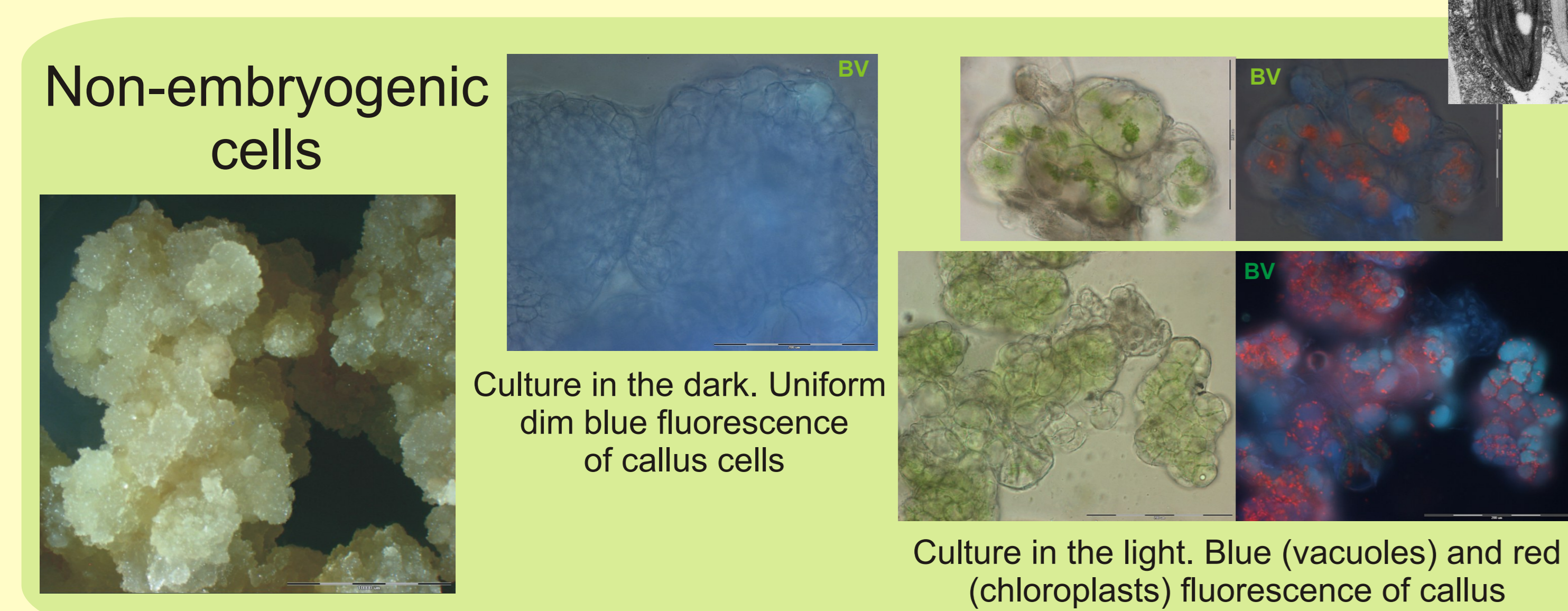
Botanical Garden Center for Biological Diversity Conservation, Polish Academy of Sciences, Prawdziwka 2, 02-073 Warsaw, Poland

e-mail: amikula@ob.neostrada.pl

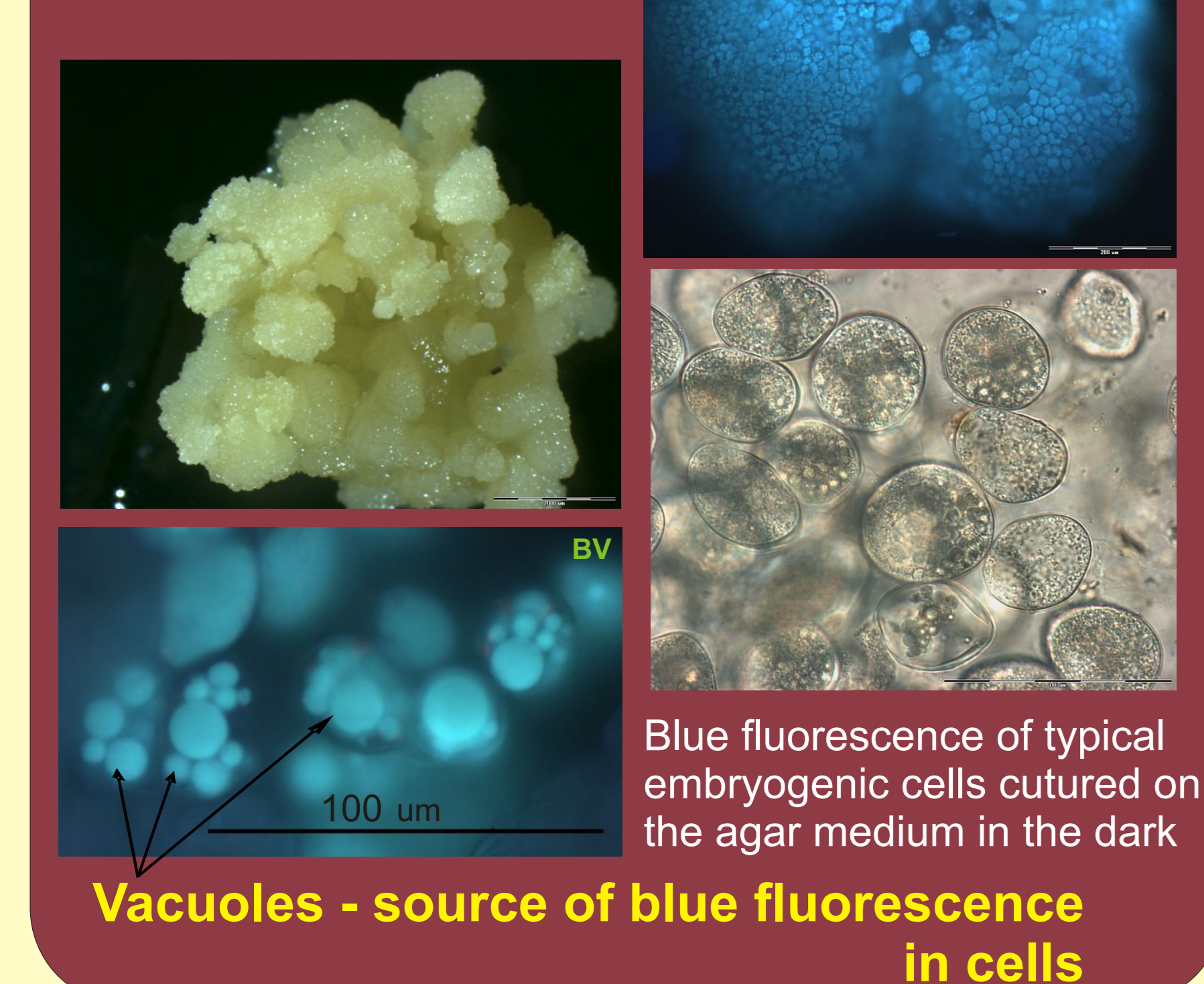
1. The autofluorescence ability of zygotic embryo and seedling cells explant



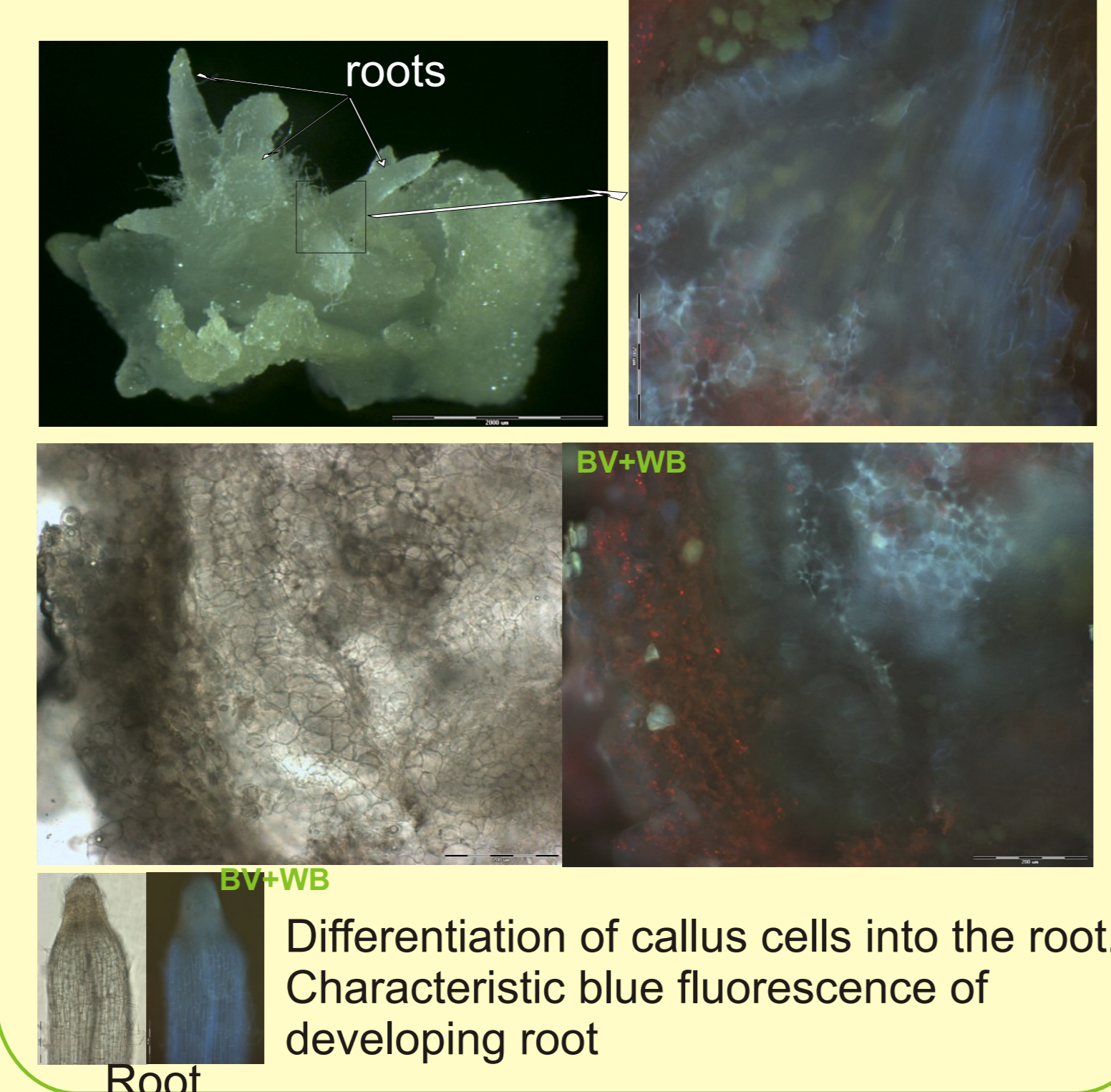
2. The autofluorescence ability of callus tissue induced on agar medium



Embryogenic cells



Rhizogenic callus



Fluorescence microscopy is becoming a popular method for the study of plant structure. Many compounds in plants such as chlorophyll (red), lignin (blue), suberin, cutin (silvery white), and phenolic compounds (from green to blue) can autofluoresce because of their intrinsic properties. Their fluorescence is excited using a short wave-length (UV to blue region of the light spectrum, 330-480 nm) light source. This phenomenon can also be observed in *in vitro* cultures. **This study aimed** to check the autofluorescence ability of various tissue types and organized structures of *Gentiana* spp. in *in vitro* conditions.

MATERIALS AND METHODS

Investigated species: *Gentiana cruciata*, *G. tibetica* and *G. kurroo*.

The experimental material was: (1) zygotic embryo and seedling explants, (2) callus tissue induced on agar medium with and without embryogenic properties, (3) proembryonic mass from suspension cultures, (4) somatic embryos, and (5) protoplasts and their fusion products.

Media { agar: MS + 0.5 mg/l 2,4-D + 1.0 mg/l Kin
liquid: MS + 1.0 mg/l dicamba + 0.1 mg/l NAA + 2.0 mg/l BAP + 80.0 mg/l adenine

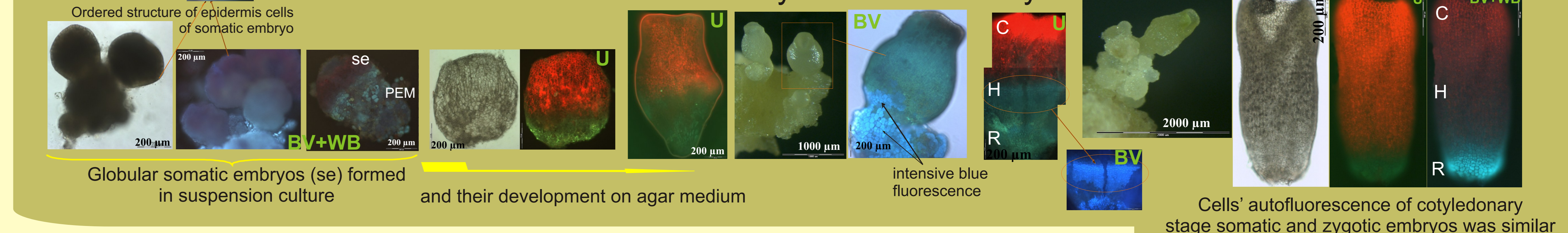
The plant material was observed under Navox-Olympus microscope with the help of computer image analysis system (analySIS program ver. 3.1). Fluorescence was induced by blue-violet light (BV filter: 400-440 nm) or ultraviolet (U filter: 330-385 nm) with or without white balance -WB (analySIS function).

RESULTS

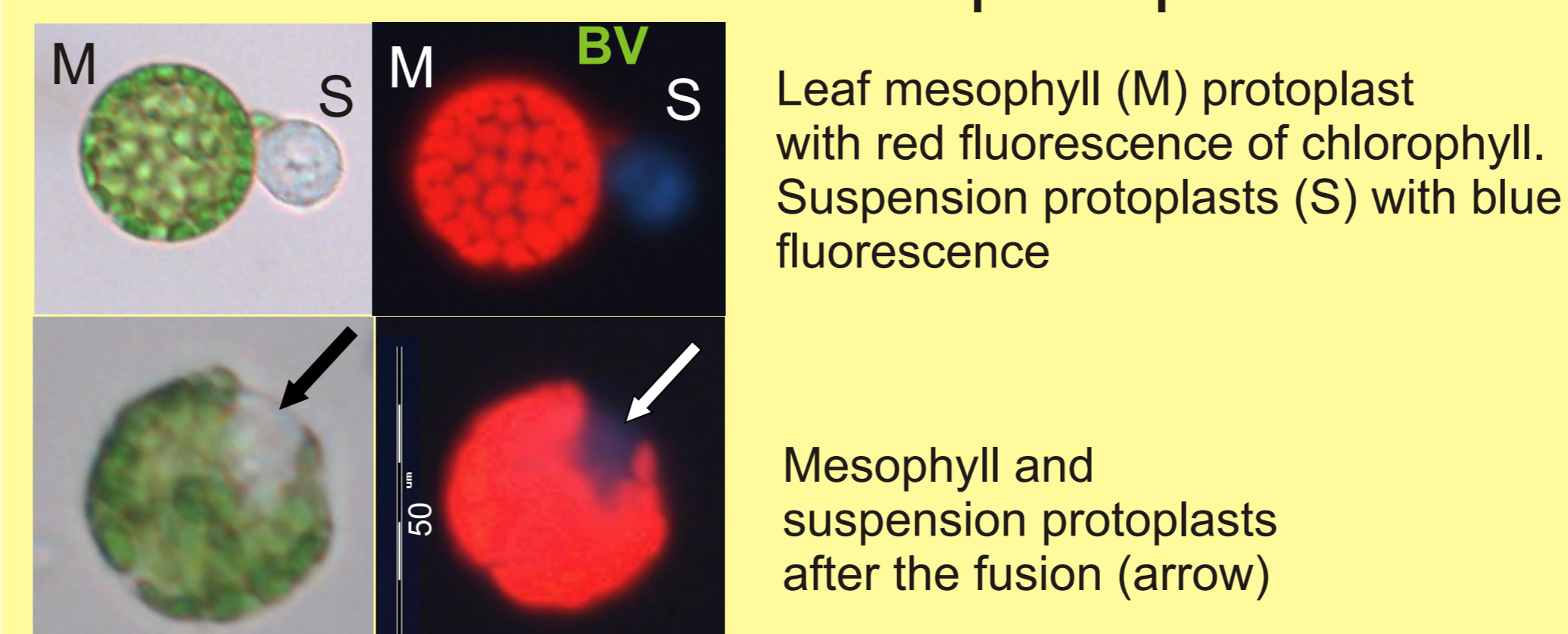
Natural, dim blue fluorescence was observed in all types of tissue (1-3). Chlorophyll red fluorescence in agar culture depended on light presence (2). Intense blue autofluorescence characterized meristematic and embryogenic cells (2-4). In suspension cultures there were three kinds of fluorescence: yellow-green, blue and red (3). During embryogenesis red fluorescence and yellow-blue-green one helped to distinguish embryogenic cells, differentiation of somatic embryos or their dedifferentiation (3), and particular parts of embryo: cotyledons, shortened hypocotyl and radicle (4). The red fluorescence was not observed in the *G. kurroo* suspension (3). This study showed that vacuoles are the source of blue fluorescence (2) and chloroplasts or amyloplasts are the source of the red one (1-3).

Protoplasts isolated from embryogenic cell suspensions of gentians showed, depending on the exciting band used, autofluorescence in different shades of blue and green colour (5). In leaf mesophyll protoplasts there was red chlorophyll fluorescence. Therefore, after the fusion of suspension and mesophyll protoplasts with chemical method (PEG) it was possible to identify the probable heterokaryocytes.

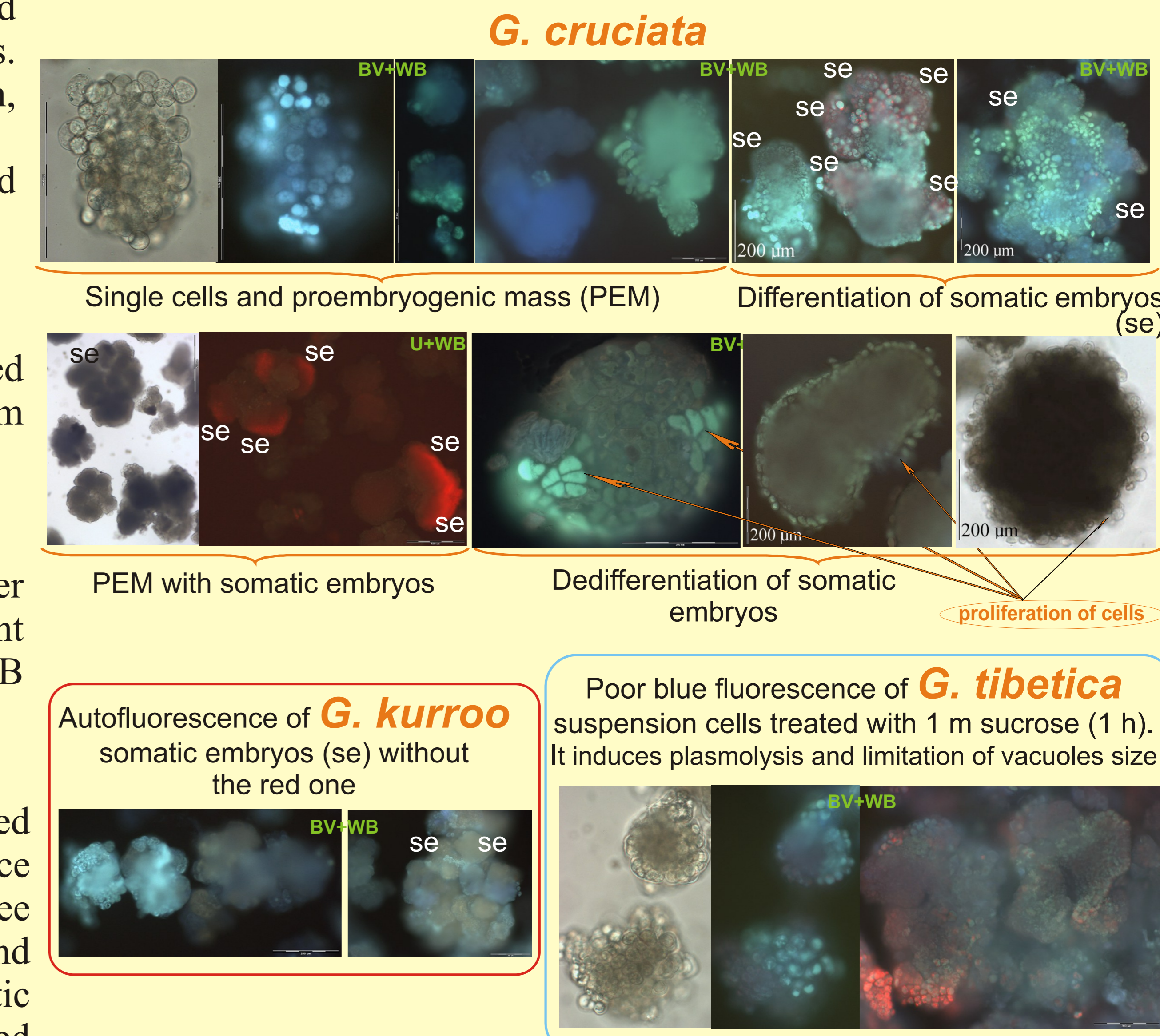
4. The autofluorescence ability of somatic embryos



5. The autofluorescence ability of protoplasts



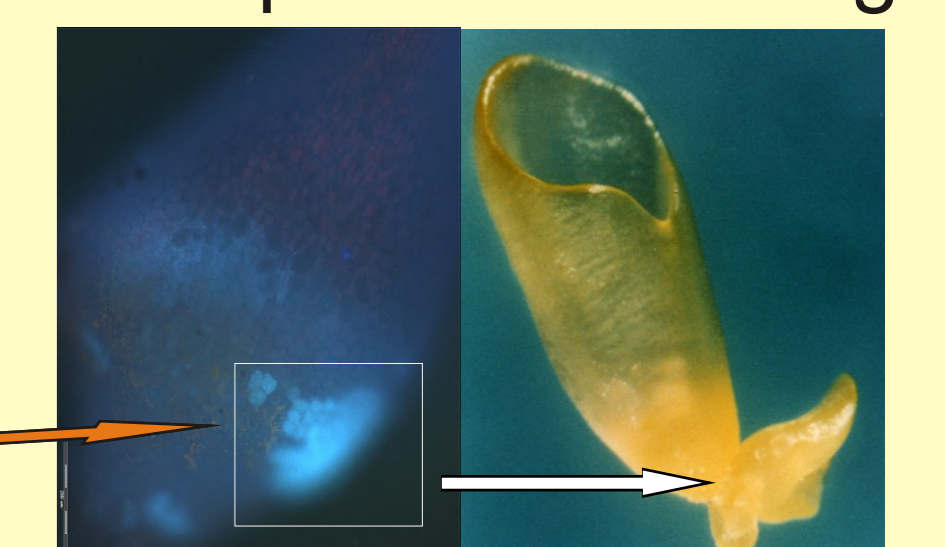
3. The autofluorescence ability of suspension cells



Amyloplasts - source of red fluorescence in suspension cells of *G. cruciata* and *G. tibetica*

Practical application of autofluorescence of gentians cells in *in vitro* cultures:

1. helps to describe various changes which suspension passed: proliferation stage, embryos differentiation and their dedifferentiation;
2. helps to distinguish particular parts of embryo and its maturity;
3. enables fast identification of the heterokaryocytes;
4. shows the cells with embryogenic competence.



This research was founded by Ministry of Sciences and Informatization of Polish Government 3P04C03723.