Improving the procedure for the culture of the ornamental plant *Pinguicula* cv. Sethos *in vitro*

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Abstract

Use of silica sand (grain size 0.1 to 1 mm, for glassmaking purposes) plus a liquid medium *in vitro* was tested on *Pinguicula* cv. Sethos, and compared to culture on agar substrates. This minor alteration of the conventional laboratory preparation, that is use of a chemically stable granulose support instead of agar gel, makes the micropropagation much easier. According to statistical data both methods (using silica sand or agar) are equally productive.

Key words: *Pinguicula*; agar; liquid medium; granulose support

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Introduction

The carnivorous plant *Pinguicula* cv. Sethos is a cultivar validly published in 1986 in the USA (Slack 1986). It is a hybrid of two Mexican species, which may only be propagated vegetatively (Fig. 1). It is more resistant than other cultivars and species of this genus, and therefore is now commercially used as an ornamental plant for interiors. The Arboretum Kostelec laboratory of the Czech University of Life Sciences in Prague keeps an *in vitro* culture on agar substrates (Fig. 3).

Agar is commonly part of formulas for laboratory cultures, although despite the fact that it is almost universally used, this substance has a number of disadvantages. These include high cost, varying quality depending on the manufacturer, unpredictable changes in properties as the result of autoclaving, poor diffusion of dissolved substances and gases in agar gel compared to liquid media, and little contact between larger plants in organ cultures with reinforced substrate. The greatest disadvantage is clearly the danger of working with a very hot yet viscous solution when pouring the medium into culture vessels. We investigated the most suitable culture in a liquid medium using silica sand as a support (Fig. 2).

Materials and methods

The use of silica sand (grain size 0.1 to 1 mm, for glassmaking purposes) was tested on *Pinguicula* cv. Sethos. Individual rosettes of approximately equal size (10 mm) were selected as the inoculum. They were placed on a fresh medium (tab. 1). The control series of 50 specimens had this medium reinforced with agar (6 g/l); the tested series used sand as the carrier and the medium was liquid (25 cm3 of sand and 20 ml of solution). 200 ml jars commonly used in the food industry (e.g. for baby food) were used for the cultures. The incubation jars were sterilised normally in an autoclave. This took 30 min under increased pressure at 115 °C.

The specimens were incubated at a temperature of 22°C under fluorescent lamps with 16 hours of light and 8 hours of dark. The experiment was assessed after six weeks. The product was taken out of each jar and the remnants of the medium were thoroughly rinsed off (Fig. 4). After drying in the air, the next day each product was weighed on digital scales accurate to 1 mg and the number of leaves larger than 4 mm was also counted (these can theoretically be used as an inoculum for a new subculture). Contaminated cultures were discarded. The data were recorded (tab. 2) and statistically assessed by testing the null hypothesis of the conformity of the sample averages for the liquid and solid media (F-test of variance conformity and t-test using MS Excel tools).

Results

With the sample sets in Table 2 the F-test found that the t-test to determine the significance of difference between two sample averages with equal variance could be used. The null hypothesis that the sample sets do not differ at the confidence level of 0.01 computed t1 = 0.9448 (for weight) and t2 = 0.8818 (for the number of leaves). The critical value (from the statistical tables) is 2.39. Both calculations, t1 and t2, are lower. We accept the null hypothesis. Cultures created using both methods can be considered to grow equally well (Fig. 2 and 3). For the resulting product of cultures using sand after acclimatization in a normal gardening culture in a greenhouse, see Fig. 5.

Discussion

The disadvantages of agar can be bypassed using an apparatus for cultivation in liquid media. At other times, there have been attempts to replace agar with another support. Fibrous, foamy or powdered (granular) materials have proven to be a success: coconut fibre, cellulose wadding, polyurethane foam, perlite, vermiculite, barley, oatmeal, etc. (Deb and Pongerer 2010; Sharifi et al. 2010; Aggarwal and Nirmala 2012; Oh et al. 2012). The properties of these alternatives are uncertainly defined, may be chemically unstable and may be the source of admixtures which change the properties of the culture medium (Kukułczanka et al. 1987).

Pure glass sand is inert and has proven to work as a support using a liquid MS-medium in the culture of species of the genus *Sorbus* (Prknová 2007; Prknová and Kobliha 2008).

The advantages of using sand, although they might seem trivial, are as follows:

a) Quick and easy to handle (volumetric measurement into vials using a measuring cup, easy to remove after the cultures have finished).

b) It is possible to additionally form any inclined surface in the jar, which is ideal for establishing the inoculum. This area is capillary saturated by the medium.

c) Good contact between the plants (inocula) and the media, partly as they often sink into the sand.

d) Does not affect the pH of the medium when sterilising at high pressure in the autoclave.

e) Does not absorb or adsorb organic substances. Therefore, for example, this ensures perfect diffusion and dilution of growth inhibitors, if produced by the aging inoculum.

f) Sand is absolutely chemically stable and does not contain admixtures of phytotoxic sulphates.

g) Similar material is very cheap and easily available.

This study provides proof that sand can also be used for other types of plants, such as commercially viable herbs. The protocol shows that an *in vitro* culture would be more practical and easier.

Conclusion

Both types of cultivation are suitable and equally good for this cultivar, as they do not differ greatly in statistical terms. Nevertheless, the use of a liquid medium and sand is simpler, faster and technically safer than the preparation of the agar nutrient gel used up to now. This result also confirms the findings made earlier with two species of the genus *Sorbus* (Prknová 2007; Prknová and Kobliha 2008). For the commercial use of micropropagation a change of procedure can be recommended for *Pinguicula* cv. Sethos, involving the use of a liquid medium instead of an agar gel and an inert carrier, i.e. pure silica sand.

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