Bulletin UASVM Horticulture, 68(1)/2011
Print ISSN 1843-5254; Electronic ISSN 1843-5394

The Influence of the Gelling Agent and Explant Type upon the *In Vitro* Multiplication *of Pinguicula vulgaris*

Doina CLAPA[1)], Alexandru FIRA[1)], Ioan PACURAR[2)], Anca SOTROPA[2)]

[1)]Fruit Research Station Cluj, 5 Horticultorilor Street, 400457 Cluj-Napoca, Cluj Romania, www.scdpcluj.ro, doinaclapa@yahoo.com
[2)] University of Agricultural Sciences and Veterinary Medicine, 3-5 Manastur Street, 400372, Cluj-Napoca, Romania

Abstract. Our paper presents results regarding *in vitro* multiplication of species *Pinguicula vulgaris* using non-conventional gelling agents and, as explants, whole rosettes as well as rosette fragments. As nutritive medium, Murashige & Skoog 1962 (MS) with 0.5 mg/l 6-Benzylaminopurine (BAP) was used, and the gelling agents were Plant Agar at 6 g/l, Guar gum at 10 and 20 g/l and starch at 70 g/l. The highest number of plantlets/vessel (619) was obtained by using, as explants, rosette fragments and, as gelling agent, 20 g/l Guar gum and the lowest multiplication rate was obtained by using whole rosettes as explants, on the same medium. The explants consisting of rosette fragments generated, on the MS medium gelled with either Guar gum or starch a far larger number of plantlets as compared to the media gelled with agar and the explants consisting of rosette fragments generated a very large number of plantlets as compared to the explants consisting of whole rosettes.

Keywords: starch, guar gum, agar, 6-Benzylaminopurine, Murashige & Skoog 1962

INTRODUCTION

At present, agar is the most widely used gelling agent for plant tissue culture, as it has some important qualities like stability and transparency. The use of this gelling agent implies two major problems: high cost and inhibitory effect in some species.

Starch is an organic substance contained in plant seeds, fruits and tubers and it is used in the food and chemical industries. The starch formula, established by elementary analysis is $(C_6H_{10}O_5)_n$, the same as for cellulose. By acid hydrolysis, starch breaks down to D-glucose, with a certain quantitative efficiency. From the point of view of chemical composition, starch is a mixture consisting of 2 polysaccharides: amylose and amylopectin, which are different from the point of view of structure and reactivity. Starch has an amorphous structure, it is insoluble in water and, in contact with water starch absorbs water and expands.

Guar gum is a substance used as an additive in the food industry and it is obtained from the seeds of locust bean gum (*Cyamopsis tetragonolobus*), a tropical legume cultured especially in Pakistan and India. In the food industry it is used for thickening some products, increasing volume and as a stabilizer in flour. It is labeled E412.

R. Jain and S. Babbar (2005) achieved more spectacular *in vitro* growth in the orchid species *Dendrobium chrysotoxum* on media containing either Isubgol (Psyllium husk) or Guar gum as compared to the media containing agar as gelling agent. Several researchers obtained good results using starch as gelling agent in the nutritive media for various species (Kuria *et al*., 2008; Mbanaso, 2008; Nkere *et al*., 2009**),** which lead us to testing starch in *Pinguicula vulgaris*, also.

Pinguicula vulgaris (fam. *Lentibulariaceae*, ord. *Lamiales*) is a rare carnivorous

species found in humid, swampy areas and the *in vitro* propagation of this species proved to be extremely effective (Clapa *et al,* 2010).

MATERIAL AND METHOD

The plant material used in the experiments originated from *in vitro* cultures on hormone-free MS media and, as explants, either whole rosettes or rosette fragments were used, which resulted from 5 cm long rosettes sliced transversally at 5-7 mm intervals and the leaf as well as stem fragments that resulted were spread on the surface of the media.

As basal medium, Murashige & Skoog 1962 (MS) medium was used and, as gelling agents, Plant Agar from Duchefa (6 g/l), Guar gum (10 or 20 g/l) and starch (75 g/l) were used (Tab. 1).

As culture vessels, 720 ml screw capped jars were used, containing 100 ml of nutritive medium/vessel. All the components were added to the media before autoclavation, the pH of the media was adjusted to 5.8 by using NaOH solution before adding the gelling agents. The media were sterilized by autoclavation for 30 minutes at 121 °C. It is to mention that for adding starch, it should be mixed with 200 ml of cold medium and the suspension should be added to the rest of the medium after the rest of the medium is brought to boiling point on the hot plate/stirrer.

The cultures were incubated in the growth chamber at 24 °C and 2400 Lux.

Tab 1.

The variants of media used for the *in vitro* culture of *Pinguicula vulgaris*

Component	Concentration			
	Variant 1	Variant 2	Variant 3	Variant 4
Săruri	MS*	MS*	MS*	MS*
Myo-inositol	100 mg/l	100 mg/l	100 mg/l	100 mg/l
Vitamin B1	1 mg/l	1 mg/l	1 mg/l	1 mg/l
Vitamin B6	0.5 mg/l	0.5 mg/l	0.5 mg/l	0.5 mg/l
Nicotinic acid	0.5 mg/l	0.5 mg/l	0.5 mg/l	0.5 mg/l
BAP	0.5 mg/l	0.5 mg/l	0.5 mg/l	0.5 mg/l
Sugar	30 g/l	30 g/l	30 g/l	30 g/l
Agar	6 g/l	-	-	-
Guar gum	-	10 g/l	20 g/l	-
Starch				75 g/l
pH adjusted to 5.8				

* Murashige & Skoog 1962

The *in vitro*-rooted rosettes that resulted on these media were transferred *ex vitro* for acclimation in plastic trays containing water with neutral pH, following the acclimation procedure developed at the Fruit Research Station of Cluj (Fira *et al*. 2009) and were cultured for 2 months for acclimation. The acclimated plantlets were transplanted into pots and trays containing a mixture of acid peat and perlite.

RESULTS AND DISCUSSION

After 3 months of *in vitro* culture the plantlets resulted in the 4 experimental variants were counted. In each vessel hundreds of rooted plantlets resulted by direct organogenesis.

Regarding the influence of the gelling agent upon multiplication rate in *Pinguicula*

vulgaris we found that Guar gum as well as starch ensure a multiplication rate far higher than the media with agar. The highest number of plantlets was obtained on the medium gelled with Guar gum at 20 g/l (an average of 505 plantlets/vessel), on the medium gelled with starch 304 plantlets/vessel were obtained, whereas on the medium gelled with agar only 172 plantlets/vessel were obtained (Tab. 2).

Tab. 2

The experimental variants

Variants	Nutritive media	Types of inoculi	Vessel no.	No. of plants resulted/vessel	Average no. of plants/variant
1	MS + 6 g/l agar	1 sliced rosette	1	147	172
			2	197	
2	MS+ 0.5 mg/l BAP+ 10 g/l Guar gum	1 sliced rosette	1	546	415
			2	284	
3	MS+ 0.5 mg/l BAP+ 20 g/l Guar gum	1 sliced rosette	1	391	505
			2	619	
4	MS+ 0.5 mg/l BAP+ 50 g/l starch	1 sliced rosette	1	304	304
5	MS+ 0.5 mg/l BAP+ 20 g/l Guar gum	5 whole rosettes	1	42	27
			2	12	

It is to be mentioned that on the variants with a very large number of regenerated shoots these were long and thin (Fig. 1, Fig. 2), whereas in the variants with low multiplication rates very vigorous rosettes resulted (Fig. 3).

Another aspect considered was the influence of explant type used for the *in vitro* multiplication of this species. Such, in variant 5, on the medium gelled with 20 g/l Guar gum, on which the highest multiplication rates were obtained by using rosette fragments, when 5 whole rosettes were inoculated the average number of plantlets resulted/vessel was only 27.

The results show that cutting or mechanically wounding the plant material used for inoculation leads to the increasing of the multiplication rates, as *Pinguicula* regenerates plantlets from every wounded surface. The plantlets are always regenerated only from the basal pole of the explant.

Fig. 1. *Pinguicula* on the medium MS +0.5 mg/l BAP +10 g/l Guar gum

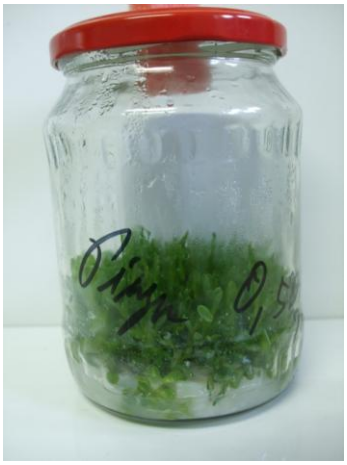

Fig. 2. *Pinguicula vulgaris* on the medium MS+0,5 mg/l BAP + 75 g/l starch

Fig. 3. *Pinguicula vulgaris* – 5 rosettes inoculated/vessel

CONCLUSIONS

For gelling the nutritive media used for *Pinguicula vulgaris* it is recommended to use either Guar gum at 20 g/l or starch at 75 g/l (it can be reduced to 50 g/l, as well), which regenerated an average number of 505 plantlets/vessel, respectively 304 plantlets/vessel, as compared to the medium gelled with agar, where only an average of 172 plantlets/vessel were obtained.

As explant type, it is recommended to inoculate rosette fragments, which regenerate a very large number of plantlets as compared to the variant where whole rosettes were used.

The explants consisting of rosette fragments regenerated very many long, thin plantlets, whereas the explants consisting of whole rosettes regenerated a small number of new rosettes, especially from the wounds, but these plantlets were much larger. This shows that cutting or mechanically wounding the plant material in this species is essential for regenerating new plantlets, regardless of the nutritive medium.

The leaf lamina fragments regenerate plantlets only from the basal pole of the explant.

Acknowledgements. This work was supported by CNCSIS –UEFISCSU, Romania, project number 1089 PNII – IDEI , code CNCSIS 1478/2008

REFERENCES

1. Clapa, D. and Al. Fira (2010). Alternative Gelling Agents for the Micropropagation of Some Horticultural Species. Bulletin UASVM Horticulture, 67(1), 515.

2. Clapa, D., Al. Fira and I. Păcurar (2010). In Vitro Propagation of *Pinguicula vulgaris*. Bulletin UASVM Horticulture, 67(1), 330-336.

3. Clapa, D., Al. Fira, I. Păcurar and A. Sotropa (2010). Possibilities for the Propagation and Re-Population of Peat bogs with Carnivorous Species. Agricultura – Ştiinţă şi practică, nr. 3-4 (75-76) , 42-47.

4. Fira, Al., D. Clapa, (2009). *Ex-Vitro* Acclimation of some Horticultural Species in Hydroculture, Bulletin UASVM, nr. 66 (1): 44-51.

5. Jain, R. and S. B. Babbar (2005). Guar gum and isubgol as cost-effective alternative gelling agents for *in vitro* multiplication of an orchid, *Dendrobium chrysotoxum*. Current Science, Vol. 88, No. 2, 25 January: pp.292-295.

6. Kuria, P., P. Demo, A. B. Nyende and E. M. Kahangi (2008). Cassava starch as an alternative cheap gelling agent for the *in vitro* micro-propagation of potato *(Solanum tuberosum L.)*. African Journal of Biotechnology Vol. 7 (3), pp. 301-307.

7. Mbanaso, E. N. A. (2008). Effect of multiple subcultures on Musa shoots derived from cassava starch-gelled multiplication medium during micropropagation. African Journal of Biotechnology Vol. 7 (24), pp. 4491-4494.

8. Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant 15: 473-497.

9. Nkere, C. K., I. C. Umezurumba and E. N. A. Mbanaso (2009). In-vitro Ginger multiplication: Screening of Starch from Different Cassava Varieties as Gelling Agent in Medium. Plant sciences Research 2 (2): 20-22ISSN 1995-476X.

10. http://www.carnivorousplants.org/IPSG/index.php.

11. http://www.pinguicula.org/A_world_of_Pinguicula_2/Pages/my_experiences_with_tissue_culture.htm.