EPIDERMAL FORMATIONS OF *CYMBIDIUM* VITRO- AND EXVITROPLANTLETS

Adriana PETRUŞ - VANCEA[*], Gheorghe Emil BANDICI[**], Anca BACIU[***], Cristian - Felix BLIDAR[*], Liviu POP[*]

[*] *University of Oradea, Faculty of Science, Department of Biology, Romania*
[**] *University of Oradea, Faculty of Environment Protection, Department of Agricultural, Romania*
[***] *Potato Research & Development Station from Târgul –Secuiesc, Romania*
adrianavan@yahoo.com

Abstract. Adaptation to septic medium is dependent to gas change between exvitroplantlets and outside medium, this is why we decided to study the *Cymbidium* and modifications which appear in vitroculture at stoma density, dimension and closing system, and also the capacity of this vitroplantlets to adaptation its antideshydratation defence system, during acclimatization period, using as standard measure those plants which were cultivated to septic medium. After results analyze we observed that the *Cymbidium hybridum* species have amphystomatical leaf only "in vitro" medium conditions, and to greenhouse plants and exvitroplantlets the leafs are hypostomatic. This situation is generated from his special nutrition which exists in vitrocultures. This fact was explained by Salisbury (1927) [8] and Schoch (1978) [9], who demonstrated that the stoma frequency may significant vary to some as ambient factors which modify the morphology and composition of leaf.

Keywords: stoma, vitroplantlets, exvitroplantlets, acclimatization, Cymbidium

INTRODUCTION

Stoma and tector hairs are formed in vitroculture conditions but the specificity of this ecological system – high humidity (85%), constant temperature, moderate illumination, heterotrophic or partial mixotrophic nutrition – give that the stoma is permanently opened and the osteols opening and closing system is non-functional [2, 3, 4]. In vitroculture condition the utility of stoma and tector hairs is significant modified reported to the normal condition [5, 10].

Petruş-Vancea and Cachiţă [6] showed that the *Chrysanthemum* leafs are amphystomatic and those from African violets, are hypostomatic same in vitroculture and "ex vitro" conditions. To both species vitroplantlets showed almost a double number of stomata, comparatively to the greenhouse plants. At the end of acclimatization period, it was not observed a diminution of stoma number at the *Chrysanthemum*, but at the African violets it was observed a reduction of the differences given to the control as regarding this parameter. To the *Chrysanthemum* leafs, on the both sides, the higher density of tector hairs was recorded at the greenhouse plants, and the lowest number at the vitroplantlets. At the African violets it was in reverse order, respectively vitroplantlets leafs were numerous tector hairs [4].

MATERIALS AND METHODS

Vegetal material consisted in: *Cymbidium* vitroplantlets leafs (first placing them „ex vitro", were impression) at *450 days* of vitroculture, exvitroleaflets (studied at 30 days of initiation at septic medium) and greenhouse plants (two years of being). Vitroplantlets were regenerated from protocorms inoculated in aseptic conditions on mineral base medium (M.B) Murashige - Skoog (MS) [7] (modified by us [12]), solidly, without growth regulator, with controlled pH at 5.7. Generally we wished that the vegetal material used in acclimatization proceeds, to be as uniform as possible. "In vitro" inoculation and cultivation was realized in noncolor glass recipients with: 200 ml capacity, 12 cm high and 7 cm diameter, in which the medium culture was 50 ml. The sterilization of culture medium recipients was realized by autoclaving at 121ºC, for 25 minutes. The vitrocultures were exposed as white fluorescent light with an 1700 lx intensity and 16/24 h photoperiod; the temperature oscillated between $23^oC \pm 2^oC$, in light period and $20^oC \pm 2^oC$, in darkness period. With 24 h before microscopic examination of epidermal formations, the vitroculture recipients were passed though darkness.

After 450 days from protocorms inoculation on solid medium – period when were released organogenesis processes – we proceeded transferring the regenerated vitroplantlets on septic medium in special incubators [11]; the plantation of exvitroplantlets was made in a substratum obtained from a mixture of „Top soil" (made in a biobase by a worms culture) with perlite, 3:1 proportion [12].

Epidermal amprentation was realized with colodium solution [1], the epidermal modelling (negative) was drawled from median area of foliar limbs transversal half. It was analyzed both superior epidermal structure and inferior epidermis. Epidermal amprentation in colodium pellicle was realized on undetached leafs from plants, the leafs being detached only when the colodium was solidified.

The preparation was examined at Leitz, Webster M optic microscope. The evaluation of stoma number/microscopic field was made with 40X lens and 10X ocular; the osteols aperture was measured with an ocular micrometer with a 7X optic zoom. The photos were realized with a digital camera, with 640/480/300 resolution, through a 10X microscopic lens. The micrometric index was calculated from Andrei and Paraschivoiu (2003) method [1]. The stomatic cell dimensions were established by measuring their length and high. It were realized three reading and the dates were mathematical processed.

RESULTS AND DISCUSSIONS

Cymbidium leaflets are *hypostomatic*, except the vitroplantlets ("in vitro" regenerated plants) which have *amphystomatic* leaflets, the stoma being disposed in rows and does not shows tector or secretor hairs. The guard cell couldn't be delimitated and superior epidermal cell were shorter those from inferior epidermal were longer and thinner with well contrasted cell wall (**Figure 1**).

A. Aspects regarding stomas of control leaf epidermis, harvested in greenhouse

Greenhouse plants (control) which are an advanced growing stage, had - in case of leafs from basal stem - at the level of inferior epidermis an higher foliar limbs number, with 16 st./microscopic field, than 11 st./field, in case of leaf from the upper stem (**Table 1**). We mention that the stoma from the basal stem was 24.5/24.5 μm dimension (length/breadth), than those from upper stem, all the osteols being almost closed in the harvest moment. Superior epidermis cells was 9.4/47.2 μm dimensions and inferior epidermis cells was 9.4/103.9 μm dimensions.

B. Aspects regarding stomas from vitroplantlets leaflets (Figure 2 A)

Unlike those greenhouse plants leafs, the vitroplantlet leaflets were *amphystomatic*, having a stoma medium density higher at the level of inferior epidermis that those from superior epidermis, especially to those from the top of the leaflet plant (the younger leaflets) having values of 16 st/microscopic field. These were located in parallels with entombs. The stoma dimensions were greater, 30.2/24.5 μm, in case of those located in superior epidermis of foliar limb from apical area, dimension witch were greater because of the larger opening of the osteols (13.2 μm) (**Table 1**). At the basal leaflets, on the superior area, stomas were almost closed and at the leaflets from top and middle of stem, the stomas were greater and wide open.

Two guard cells covered the stoma and those dimensions 28.3 μm length and 9.4 μm breadth. Epidermal cell were similar as appearance and dimensions with those from greenhouse plant.

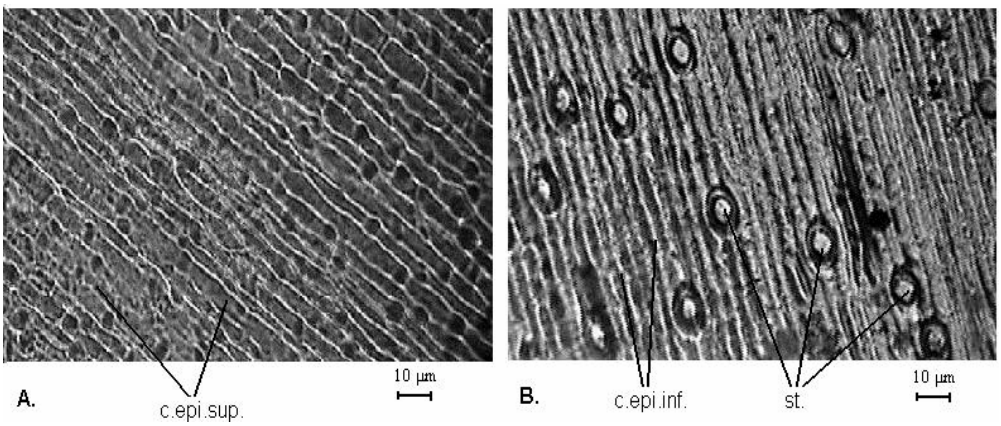

Figure 1. Epidermal appearance of *Cymbidium hybridum* leafs: A. – superior epidermis, without stoma; B. – inferior epidermis (c.epi.sup. – superior epidermis cell, c.epi.inf. – inferior epidermis cell, st. – stoma).

C) Aspects regarding stomas from vitroplantlets leaflets (Figure 2 B)

At the exvitroplantlets level, the leaflets were hypostomatic. *Cymbidium* exvitroplantlets leafs didn't presenting stomas on the superior face of leafs and their density on the inferior face was approximate the same, 12 – 13 st/microscopic field (**Table 1**). The shape of those leafs, located at the basal plant, was elongated, with 28.3/13.2 μm dimensions, and circular to 22.68/18.9 μm, respectively 28.3/20.7 μm, those leafs located at the middle and the top of the plant. The stoma belonging to those leafs from the top of the plant had greater dimensions, 28.3/20.7 μm and were half – opened with an 1.8 – 3.7 μm osteol aperture. The guard cell present same characteristics as those plants from greenhouse.

CONCLUSIONS

- *Cymbidium hybridum* had amphystomatic leafs in "in vitro" conditions and hypostomatic leafs in normal life conditions. In *Cymbidium* foliar limbs the stomas are disposed in rows and do not shows tector or secretor hairs. Generally, vitroleaflet and exvitroleaflet stomas density was smaller comparatively to the control (stomas of foliar limb of greenhouse plants), with an exception namely apical vitro – and exvitroleaflets, which had a superior number of stomas. If the vitroleaflets stoma dimensions are bigger comparatively to the control, the exvitroleaflets had smaller stomas, excepting basal area.
- The presence of the stomas on both vitroplantlets sides, the wide opening of the osteols, without closing possibility, the absence of tector hairs are disadvantages of this species in "ex vitro" transfer moment, this fact being evidenced in the difficulty of their acclimatization to natural medium, comparatively to *Chrysanthemum and African violets* [5].

Table 1. Biometric dates registered at stomas of foliar limb (from basal, middle and apical area) of *Cymbidium hybridum* greenhouse plants, vitro- and exvitroplantlet leaflets.

The type of plants (growth conditions)	The location of foliar limb from stem	**Epidermis type**	**Stoma density/field** (no.)(400X)	**Stoma cell length (µm)(280X)**	**Stoma cell breadth (µm) (280X)**	**Osteols aperture diameter (µm) (280X)**
Greenhouse plants (*control*)	basal	**Superior**	**-**	**-**	**-**	**-**
		Inferior	**16**	**24.5**	**24.5**	**11.8**
	middle	**Superior**	**-**	**-**	**-**	**-**
		Inferior	**19**	**22.6**	**18.9**	**1.8**
	apical	**Superior**	**-**	**-**	**-**	**-**
		Inferior	**11**	**22.6**	**18.9**	**1.8**
Vitroplantlets	basal	**Superior**	2	**18.9**	**9.4**	**11.8**
		Inferior	6	**28.3**	**18.9**	**3.7**
	middle	**Superior**	4	**28.3**	**15.1**	**11.3**
		Inferior	7	**22.6**	**18.9**	**5.6**
	apical	**Superior**	**2**	**30.2**	**24.5**	**13.2**
		Inferior	**16**	**28.3**	**13.2**	**1.8**
Exvitroplantlets	basal	**Superior**	**-**	**-**	**-**	**-**
		Inferior	**13**	**28.3**	**13.2**	**3.7**
	middle	**Superior**	**-**	**-**	**-**	**-**
		Inferior	**12**	**22.6**	**18.9**	**3.7**
	apical	**Superior**	**-**	**-**	**-**	**-**
		Inferior	**13**	**28.3**	**20.7**	**1.8**

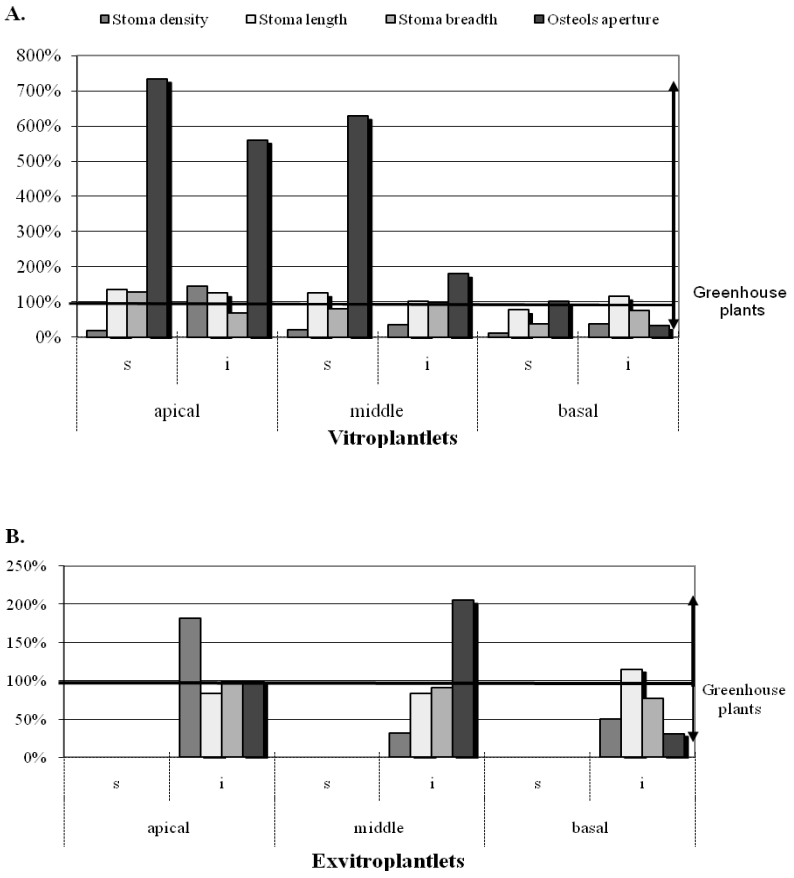

Figure 2. Biometrical values regarding stomas at the epidermal level (s – superior and i – inferior) of foliar limb (from apical, middle and basal area) of *Cymbidium hybridum* plantlets, provided from „in vitro" or „ex vitro" conditions, which was reported to greenhouse plants dates, values considering 100%.

REFERENCES

[1] Andrei, M. and Paraschivoiu, R.M. (2003). *Microtehnică botanică*, Ed. Niculescu, Bucureşti.

[2] Cachiță, C.D. and Crăciun, C. (1991). Vitrification and stress in carnation plantlets transferred from "vitro", "ex vitro", ultrastructural aspects. In: "*In vitro" explant cultures - present and perspective*", The IV-th National Symposium on Plant Cell and Tissue Culture, Cluj-Napoca, pp 129-137.

[3] Cachiță, C.D., Deliu, C., Rakosy – Tican, L. and Ardelean, A. (2004). *Tratat de biotehnologie vegetală,* Vol. 1, Editura Dacia, Cluj – Napoca, 433 pp.

[4] Dogniaux, R. and Nissen, A. (1975). Traite de l'eclarage naturel des serres at abris pour vegetaux. Inst. Royal Meteorologique de Belgique, Bruxelle, pp 25–31.

[5] Petruş – Vancea, A. (2007). Cercetări privind procesele morfofiziologice şi biochimice care au loc în decursul aclimatizării plantulelor generate „in vitro", la viaţa în mediul septic. Doctoral thesis, 357 pp.

[6] Petruş – Vancea, A. and Cachiță, C.D. (2004), Formaţiuni epidermice la vitro- şi exvitroplantulele de crizanteme şi violete africane. *Analele SNBC*, Vol. IX, Nr. 1., CAP. III – Biologie celulară vegetală, pp 396 - 404.

[7] Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth bioassays with tobacco tissue cultures, *Physiol. Plant*., 15, pp 473-497.

[8] Salisbury, L.J. (1927). On the causes and ecological significance of stomatal frequency with special reference to woodland flora, *Phil. Trans. Roy. Soc. Lond*., Ser B, 216, pp 1–65.

[9] Schoch, P.-G., Zinsou, C. and Sibi, M. (1980). Dependence of the stomatal index on environmental factors during stomatal differention in leaves of *Vigna sinensis* L., *J. Exp. Bot*., 31, pp 1211–1216.

[10] Taji, A. M. and Williams, R.R. (1996). Overview of plant tissue culture. In: *Tissue culture of Australian plants* – Chapter 1. Taji, A.M. and Williams, R.R. (eds.), University of England Armidale NSW, 2351, pp 1-2.

[11] Vancea, A. and Cachiță, C.D. (2002). Aclimatizarea vitroplantulelor de *Saintpaulia ionantha*, prin plantarea acestora pe substraturi neconvenţionale. In: *Lucrările celui de al X-lea Simpozion Naţional de Culturi de Ţesuturi şi Celule Vegetale*, Cachiță, C.D., Rakosy, T.L. and Ardelean, A. (coord.), Editura Risoprint, Cluj-Napoca, pp 310–315.

[12] Vancea, A., Cachiță, C.D., Floriş, C. and Blidar, F.C. (2000). Studii preliminare privind aclimatizarea vitroplantulelor de *Chrysanthemum* la mediul septic de viaţă, *Analele Univ. Oradea, Fasc. Biologie*, Tom. VII, pp 283-294.