REGARDS ON CHRYSANTHEMUM *(CHRYSANTHEMUM MORIFOLIUM RAMAT* VAR. *LAMET)*, LEAFS EPIDERMIS WHICH WERE SPRINKLE, DURING "EX VITRO" ACCLIMATIZATION PERIOD, WITH PI WATER AND DEUTERIUM DEPLETED WATER

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ABSTRACT. These experiment consist in a study of epidermal formations (stoma and tector hairs) of *Chrysanthemum morifolium* Ramat var. *Lamet* exvitroplantlets (after 30 days exvitroplantlets transferring in septic medium), sprinkled with Pi water (PiW) or deuterium depleted water (DDW), after their epidermis was imprinted with collodion pellicle. The study of these morphological aspects is important for control of vitroplantlets acclimatization process. The results can help to improve of acclimatization methods. We concluded that *foliar applying of deuterium depleted water*, for *30 days*, leads to „ex vitro" regeneration of the leaflets with a smaller number of stomata at the superior epidermis level of the exvitroplantlets.

Keywords: stomata, tector hairs, exvitroplantlets, Pi water (PiW), deuterium depleted water (DDW)

INTRODUCTION

In the modern physiological vegetal literature there are researches focused on studying the influence made by different kinds of water, on plants growth and development, fact reflected in numerous seminars and symposiums focused on the thematic (Petruş – Vancea, 2007).

Pi water is produced on the basis of Bio Control System technologies, after a Japanese license. It is obtained through purifying and energizing the drinkable water with the aid of the Life Energy unit, in this way removing the noxious substances from the water, and therefore receiving special physical and chemical qualities (the technical book for Life Energy unit, 2003). The effect of the Pi water started to be more often mentioned in the botanic researches (Godeanu et al., 1999*;* Fülőp, 2003; Petruş and Petruş – Vancea, 2004; Petruş et al., 2004 b; Radoveţ – Salinschi and Cachiţă, 2005 a; Blidar and Cachiţă, 2006; Petruş and Cachiţă, 2008), but it still little known in its effect over the cultures of vegetal tissues and cells.

Researches in the field of using *deuterium depleted water* – produced by the National Institute of Research – Development for the Cryogenic Technologies from Râmnicul Vâlcea – have been made on plants in order to test the possible phyto-physiologic effects of this kind of water in the vegetal biotechnology (Cachiţă et al., 2002; Petruş - Vancea et al, 2003; Blidar et al., 2004; Petruş et al., 2004 a; Radoveţ – Salinschi and Cachiţă, 2005 b; Beleş and Cachiţă, 2006).

Watering the exvitroculture substratum, during the chrysanthemum exvitroplantlets acclimatization period to the conditions of the septic medium, with deuterium depleted water was made by Petruş – Vancea and the contributors (2003). At the end of the acclimatization period (after 30 days form its beginning) it has been noticed an increase of the caulogenesis process and a diminishing of the rizogenesis, at the level of the plantlets corresponding to the batch whose acclimatization substratum has been treated with deuterium depleted water, comparatively to the plantlets corresponding to the control batch, whose substratum has been treated with *distillate water*.

MATERIALS AND METHODS

In order to make the acclimatization, there has been chosen equable chrysanthemum vitroplanlets – at *35 days* of vitroculture in a Murashige – Skoog (1962) mineral medium, without growth regulators – with the rootlet and stalk size of approximate 3 – 4 cm, having a number of 4 – 6 rootlets, a stalk on each plantlet, which presented 5 – 6 knots, without ramifications, exvitroplantlets that have been biometrisated and then placed into incubators, in a *perlite* substratum, humidified each with 250 ml *distillate water (DW)* (control batch), respectively with *Pi water (PiW)* or with *deuterium depleted water (DDW)* (having 25 ppm D), following that, during the acclimatisation period, these batches will be watered according to the bellow mentioned proceedings:

V_0 – exvitroplantlets sprinkled foliar and watered at their base with *distillate water* (control)*;*

V_1 - exvitroplantlets sprinkled foliar with *Pi water* and watered at their base with *distillate water*;
V_2 - exvitroplantlets sprinkled foliar with *distillate water* and watered at their base with *Pi water;*

V_3 - exvitroplantlets sprinkled foliar with *deuterium depleted water, with 25 ppm D* and watered at their base with *distillate water*;
V_4 - exvitroplantlets sprinkled foliar with *distillate water* and watered at their base with *deuterium depleted water, with 25 ppm D*.

During the exvitroculture period, the cultures were kept at a 23±1°C temperature, being illuminated with white fluorescent light, with an intensity of 1700 lux and a photoperiod of 16 hours of light/24 hours, the substratum temperature being with approximate 2 degrees higher, than the air one.

Each experimental option had autonomy in matters of organizing the treatments, using an incubator for each option. The rooting substratum was covered with aluminium foil, and the exvitroplantlets stalks have been put on top of the foil through small openings into it (Figure 1), with a diameter of 0.5 cm, so that the water foliar sparkled does not get into the substratum.

In the first 7 days of acclimatization, the watering of the cultures, with different kinds of water, was made either through sparkling, 10 ml water/batch, or through watering at each exvitroplantlet stalk's basis (5 ml of water/plant), at a period of 2 days. In the next 21 days, in order to avoid the excessive humectation of the substratum, it's watering, with different kinds of water, in accordance with the type of experimental option, was made once a week, through sparkling, also with 10 ml/option plus an additional 10 ml, at the basis of each plantlet; during the entire acclimatization experiment each option benefited of 5 ml of water applied foliar, the total amount of water administrated at the basis of each exvitroplantlet, was of 65 ml. The sparkling was made with a sparkler purchased from a department store.

Figure 1 The covering with aluminium foil of the ground surface for the chrysanthemum exvitroplantlets (Chrysanthemum orifolium Ramat var. Lamet) acclimatized in perlite and sparkled with water [distilled water (DW), Pi water (PiW), or depleted deuterium water (DDW)], through pulverizing the foliar mass.

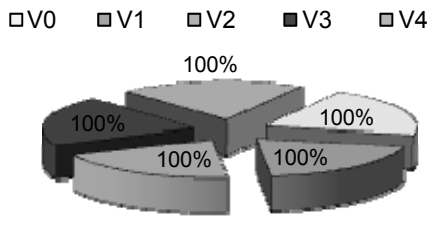
After *30 days* from their „ex vitro" transfer the post-acclimatization survival percent was established, and for obtaining the settled aim, the one of reducing the water losses at the exvitroplantlets level through foliar sparkling of their superior system with different kinds of water, during this experiment we made a density and dimension (width and length) analysis of the stomata, but also of their osteols openings, and we also determined the number of tector hairs that can be found on the superior epidermis surface of the leaf.

In order to study the epidermis formations (stomata and tector hairs) there were made replicas of the foliar epidermis surface, through taking their print and creating a casting on 2% collodium blisters (dissolved in a mixture, in equal shares, of ethyl alcohol and ether); the collodium solution was applied on the dry surface of the leaflets, in a thin layer. After 3 minutes from applying the collodium layer on the epidermis, it solidified, proceeding to its skinning and placing it between two microscopy plates (Andrei and Paraschivoiu, 2003). The moulding represents the epidermis surface's negative. The drawings were made form the superior epidermis of the foliar limb of the newest leaflet, regenerated in the acclimatization period.

The obtained preparations were examined at the optic microscope Leitz, Webster M. label. The evaluation of the stomata number, per microscopic field, was made by a 40X lens and with a 10X (400X) ocular; the osteols opening was measured by an ocular micrometer, with an amplification capacity of 7X and a 40X lens. The hairs have been examined by a 10X resolution lens and a 10X ocular, because they were better emphasized only being magnified by 100X. The micrometric value was determined after the Andrei and Paraschivoiu (2003) method. The stomata cells dimension was determined by measuring their length and width. The photos were taken by a digital camera with a 640/480/300 resolution, with a 10X lens.

RESULTS AND DISCUSSIONS

a. The exvitroplantlets survival percent – after 30 days of acclimatization – was of 100%, at all five experimental options (Graphic 1).



Graphic 1 The survival percent of the chrysanthemum exvitroplantlets (Chrysanthemum morifolium Ramat var. Lamet), after 30 days from their „ex vitro" transfer, where: V0 – distillate water (DW) foliar and basal

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sprinkle; V1 – lot sprinkled with Pi water (PiW) by foliar application; V2 – sprinkled with Pi water (PiW) by basal application; V3 – lot sprinkled with deuterium depleted water (DDW) by foliar application; V4 – lot sprinkled with deuterium depleted water (DDW) by basal application.

b. Aspects of the superior epidermis stomata of the chrysanthemum exvitroplantlets apical leaflets watered with different kinds of water

The chrysanthemum leafs are *amphystomatic*. Both on the inferior and the superior epidermis, their stomata are of amarilidaceu anomocitic kind, each having about three or four annex cells, the later ones, and also the epidermis ones have sinuous cellular walls (Figure 2 A).

The chrysanthemum leaf epidermis has *tector hairs*, pluricellular, ramified to the upper side in a „T" shape (Figure 2 B).

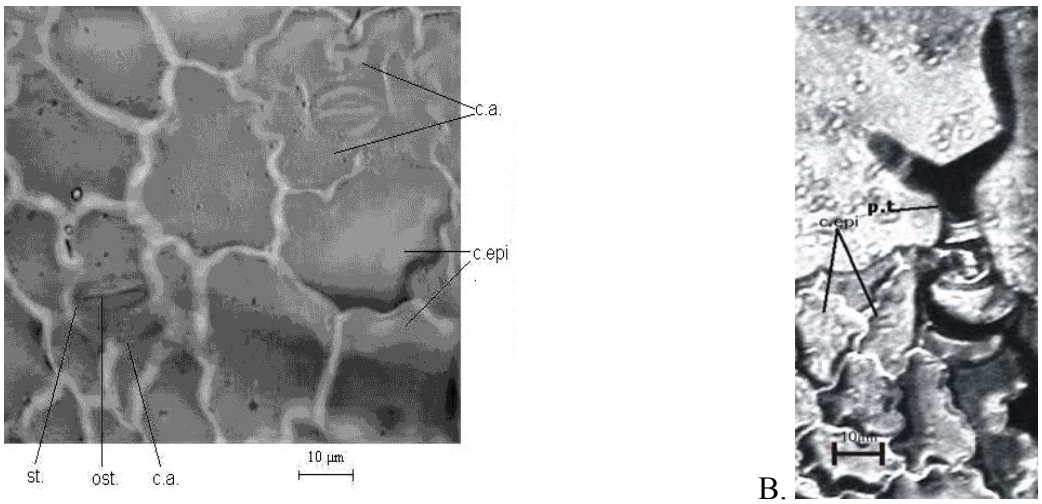


Figure 2 Stomata and tector hairs in the superior epidermis of the foliar limbs of the chrysanthemum leafs (*Chrysanthemum morifolium* Ramat var. *Lamet)*: A. – Stomatal system; B. – Tector hair, view from above (st. – stomata; ost. – osteol; c.a. – guard cells; c.epi. – epidermal cells; t.h. – tector hair).

Table 1

Biometric data about dimension and density of the stomata located on the superior epidermis of the apical leafs of the chrysanthemum exvitroplantlets *(Chrysanthemum morifolium* Ramat var*. Lamet)*, at *30 days* after the plants transfer „ex vitro", being treated with different kinds of water, according to the following variants: V_0 – *distillate water (DW)* foliar and basal sprinkle; V_1 – lot sprinkled with *Pi water (PiW)* by foliar application; V_2 – sprinkled with *Pi water (PiW)* by basal application; V_3 – lot sprinkled with *deuterium depleted water (DDW)* by foliar application; V_4 – lot sprinkled with *deuterium depleted water (DDW)* by basal application

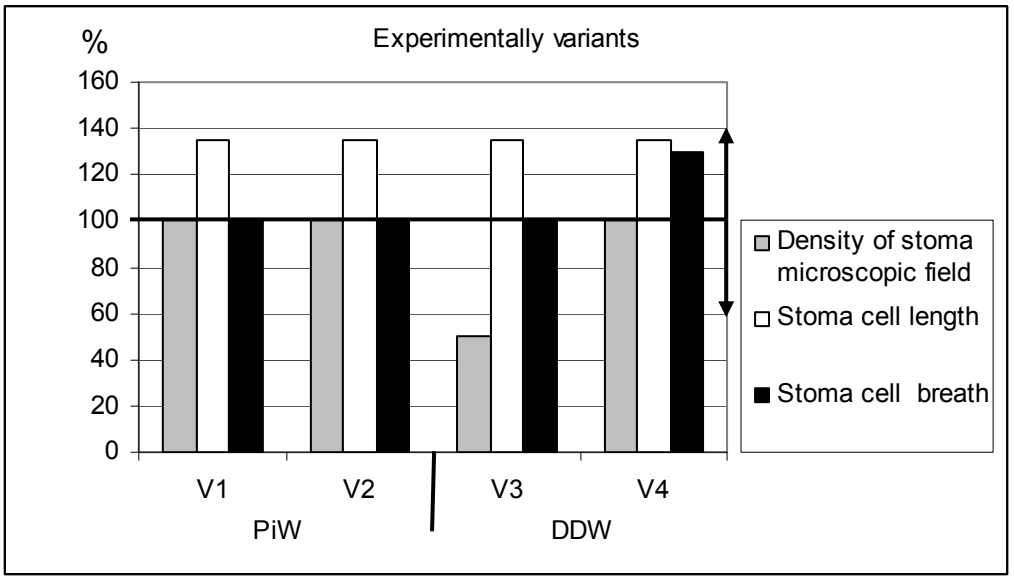
Biometrics / Experimental variants	*Density st/microscopic field* (no)	*Length st. cell* (µm)	*Breadth st. cell* (µm)
V_0	4	28.3	18.9
V_1	4	37.7	18.9
V_2	4	37.7	18.9
V_3	2	37.7	18.9
V_4	4	37.7	24.5

At 30 days after the beginning of the acclimatization process, the stomata (st.) form the superior epidermis (which came in direct contact with the *water,* both the *Pi* and the *deuterium depleted one,* applied foliar) of the apical leaflets (neoformed „ex vitro"), were closed in the measurement moment; their frequencies were of 4 st./field, excepting the situation in which the leafs were sparkled with *ASD* (V_3), where there were seen in an average of 2 st./field (Table 1), the minuses towards the control (the exvitroplantlets of the batch treated both foliar and basal with *distillate water*– V_0) being of 50% (Graphic 2).

The stomata density (width and length) of the exvitroplantlets leafs that have suffered treatments with different kinds of water, have shown similar values, of 37.7/18.9 µm, except the ones of the control, which had smaller stomata, with dimensions of 28.3/18.9 µm, in this way underlining a growth of 133%, at all four experimental variants (V_1 – V_4) and also at the basal treated exvitroplantlets with *ASD* (V_4), which have been slightly larger, in height, of 37.7/24.5 µm (Table 1), the plus at this parameter being of 30% (Table 1 and Graphic 2).

Guard cells of the stomata, three at number have shown sinuous cellular walls; their average dimension was of 66.5/37.7 µm, in the case of the control (V_0) and of 56.7/56.7 µm, in the case of the experimental variants where the exvitroplantlets were treated foliar or basal with *PiW*, respectively *ASD* (V_1 – V_4).

We mention that there were not observed any changes in the tector hairs density of the *Chrysanthemum* exvitroplantlets according to the administered treatment.



Graphic 2 Biometrical data regarding the dimensions and density of the stomata located in the superior epidermis of the apical leaflets of the chrysanthemum exvitroplantlets *(Chrysanthemum morifolium* Ramat var*. Lamet), 30 days* after their „ex vitro" transfer and their sprinkled with different kinds of water, according to the following variants: V_1 – batch sprinkled with *Pi water (PiW)* by foliar application; V_2 – sprinkled with *Pi water (PiW)* by basal application; V_3 – lot sprinkled with *deuterium depleted water (DDW)* by foliar application; V_4 – lot sprinkled with *deuterium depleted water (DDW)* by basal application, whose values have been reported toward the ones registered at the control exvitroplantlets V_0 –*distillate water (AD)* applied both foliar and basal, these being considered as 100%.

CONCLUSIONS

The reducing of the stomata number present at the *Chrysanthemum* exvitroplantlets foliar limb level, *30 days* after the exvitroplantlets transfer in a septic medium was made by *foliar applying of deuterium depleted water*, while the substratum was being sprinkled with *distillate water*, batch where one could observe the presence of exvitroplantlets with a stomata number 50% lower, comparatively with the one registered at the control exvitroplantlets leaflets (sprinkled foliar and basal with distillate water), which implies a lower level of exvitroperspiration at these plantlets.

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