FOLIAR AND RADICULAR SPRINKLING OF *TRADESCANTIA* CUTTINGS, WITH DIFFERENT TYPES OF WATER, AND THEIR EFFECT TO ORGANOGENESIS AND THE EPIDERMAL FORMATIONS OF FOLIAR LIMBS

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Abstract. We proposed in this experiment to study the spraying effect – during 30 days –on the foliar limbs of *Tradescantia* sp. cuttings leafs, and on the rooting substratum, made with Pi water (PiW) or deuterium depleted water (DDW) (with 25 ppm D), concerning rhisogenesis and plantlets growth index, or about stomata apparatus, identified at the level of inferior epidermis of foliar limb. In the end we concluded that Pi water proved to have a stimulator effect on Tradescantia cuttings organogenesis, by foliar or radicular application. The deuterium depleted water increased only the rhisogenesis, by foliar spraying, comparatively to the control lot (foliar limb and basal cuttings sprinkled with distilled water - DW), which stomas were numerous at the level of foliar limbs of the plantlets sprinkled, to the base, with Pi water and foliar with distillate water.

Keywords: Pi water, deuterium depleted water, *Tradescantia*, organogenesis, stomata

INTRODUCTION

Pi water (PiW*)*, became important in the few last years for Romanian consumers, implicitly for producers who use a Bio Control System technology to produce and is commercialized in a franchise system. Producing technologies were elaborated and developed 40 years ago by Japan and other countries Pi water being obtained by purification and bioenergizing of drinking water, using Life Energy installation. Using this installation the harmful substances are removed from water getting extremely good physical and chemical proprieties.

The interest of Romanian botanic researchers for Pi water has increased in the few last years, their studies being materialized by numerous articles. Every year take place International Symposium „Water a miracle", organized under the patronage of Romanian president, were has been communicated many articles regarding the effects of the Pi water on the plants (cucumbers), in tree growing, rooting on different types of unconventional substratum watered with this type of water [17] and other researches in botanic and vegetal biotechnology [3, 7, 8, 9, 14].

Deuterium depleted water (DDW), produced by the Romanian National Research and Development for Cryogenic Technologies Institute, located in Râmnicul Vâlcea, and is protected by Romanian copywriter laws. This water has a composition similar to distillate water but with a lower deuterium content. The percentage of obtaining the DDW to the desired isotopic concentration, 10 - 120 ppm D/(D+H), and the producer principle consist in continuous water distillation in vacuum on separate high performance columns.

If the effect of Pi water on pants, located in natural life conditions, was studied by different researchers, especially Japanese and Hungarians [6], as early as 60's, the influence of deuterium depleted water on vegetal cell was less known but also was noticed intense researches on positive effects of this water on animal cell especially his role in treating human cancer.

Ours researches regarding Pi water and deuterium depleted water utilization in botanic field started in 2001 at recommendation and was guided by prof. dr. Dorina Cachiţă – Cosma, which results was first published in 2002 [5]. We started these researches studying the effect of these two types of water on the germination of three plant species namely wheat, corn and radish [5] and [9], on *Cymbidium* protocorms [10], [4] and [3], on *Coleus* vitroplantlets [15] and on *Pistia* exvitroplantlets [2].

Sprinkling *Tradescantia* cuttings with Pi water in rooting period was realized by Petruş and collaborators [8] without foliar limb sprinkling only to the cuttings base, with positive results, especially on rhizogenesis. This was the reason why we decided to continue the studies in this direction to discover how this water act on every organogenesis process and in what conditions.

Sprinkling *Chrysanthemum* or African violets exvitroplantlets foliar or basal, in the acclimatization period to the septic life medium conditions, with Pi water or deuterium depleted water was realized [13], [11], authors concluded that at the *Chrysanthemum*, the decrease of stomas number from the level of exvitroplantlets foliar limb, after 30 days from the transfer of exvitroplantlets to the septic life medium, was realized by foliar application of deuterium depleted water in the mean time with the moisten of substratum with distillate water. To this species the number of roots and stemlets was stimulated by applying Pi water to the base of plantlets as well as foliar applying the deuterium depleted water. African violets exvitroplantlets treated foliar as well as basal with Pi water or deuterium depleted water (with 25 ppm D), comparatively with the same parameter as to the control, presented to the inferior foliar epidermis (these are hypostomatic) [12], 20% less stomas, and to that lot, which was foliar sprayed deuterium depleted water (25 ppm D), the stomas numbers was reduced to 40%. In case of this species, Pi water established the organogenesis stimulation to that place where was applied (foliar or basal), meaning that was aroused both caulogenesis and organogenesis. On the other hand those treatments with deuterium depleted water (25

ppm D), spaying the exvitroplantlets caused a rhisogenesis intensification and applying basal this water caused an extremely inhibition of new forming roots.

MATERIALS AND METHODS

Noticing that Pi water and deuterium deleted water (25 ppm) hat benefic effects to the germination process of wheat, corn and radish beans, we proposed to study their effects to the rooting of the cuttings. This is why we choose as vegetal study material apical *Tradescantia* sp. cuttings obtained from our university greenhouse, these having the following dimensions 3 cm length, 5 nods an 3-4 leaflet, placing them in *perlite* only with basal node in substratum the second one being out of this. The perlite was placed in plastic material tray with the following dimensions 5/22/35 cm, over the tray being placed a transparent lid with two arched and slide walls with a 12 cm high, in the central area [16]. To avoid the mixing of both water types used to sprinkle the cuttings leafs and the rooting substratum we covered the perlite with an thin sheet of aluminum which was pierced to get out trough them the *Tradescantia* cuttings stems, the small orifices having 1 cm diameter (**Figure 1**).

Figure 1. Aspects regarding the rooting method of *Tradescantia* sp. cuttings.

The cuttings were planted in the perlite, watered first with 250 ml *distillate water*, or *Pi water*, or *deuterium depleted water* (25 ppm D) varying with the experimental variant. During 30 days we sprinkled the experimental lots following this scheme:

V_0 - cuttings foliar sprinkled and watered to the base with *distillate water* (control);

V_1 - cuttings foliar sprinkled with *Pi water* and watered to the base with *distillate water*;

V_2 - cuttings foliar sprinkled with *distillate water* and watered to the base with *Pi water;*

V_3 - cuttings foliar sprinkled with *deuterium depleted water, with 25 ppm D* and watered to the base with *distillate water*;

V_4 - cuttings foliar sprinkled with *distillate water* and watered to the base with *deuterium depleted water, with 25 ppm D*.

The sprinkling of cuttings lots was realized by foliar spraying or by watering to the base of every cutting with 5 ml of water/variant, respectively per cutting at 2 days lapse of time.

The cultures were exposed to a 22 ± 2°C temperature at day and 20 ± 2 °C temperature at night an 16/24 h photoperiod, illuminating them with fluorescent tubes which give out white light with an 1700 lux intensity.

After 30 days from placing the cuttings to root we made biometrisations, to determine: the adventives roots number which as generated to the basal node level placed in substratum, the ramifications and nodes number located on the main stem, and by summing the longest root length with the length of stem resulted the waist of the plant. The drying weight of stems and leafs – after removing those roots new formed in perlite – after maintaining the vegetal material in drying stove at 115 ºC, during 3 days we weighted this material. Before getting out from perlite the *Tradescantia* rooted plantlets, to make measurements, we proceeded to examine the stomas to the microscope. To reveal this stomas used the imprinting method of superior and inferior epidermis of foliar limbs applied on the youngest leaflets, generated in the rooting period by applying to this level a colloidal draw [1]. To the level of inferior epidermis we determined - at the optic microscope – the numbers of stomas/microscopic field and their dimensions (length/breadth) with 10X object lens and 7X ocular; at the same time we measured the osteols aperture with 40X object lens and 7X ocular. The photos were realized using a digital camera with 640/480/300 resolution and optic zoom 10X.

Biometrical dates were mathematical processed, the values recorded at 30 days from placing the cuttings to root, concordant to the experimental variants $V_1 – V_4$, were related to the homologues dates, biometrisated to the level of plantlets proceeded from cuttings of control lot (V_0), rooted in aerial part humidification conditions or in substratum watered with distillate water conditions, the control statistic calculation dates was considered 100%. All biometric dates were statistical ensured (**Table 1**).

RESULTS AND DISCUSSIONS

Applying foliar the Pi water and radicular the distillate water, according to the experimental variant V_1, give us gain of 125% (**Figure 2**) given to the control which was sprinkled both foliar and radicular with distillate water, only in case of roots length, respectively 102% in case of plantlets waist. Generally the caulogenesis was inhibited by spraying the leafs with Pi water, paradoxically (knowing the stimulator effect of Pi water on the caulogenesis) [8], registering deficits till 11% as regarding the stem knots given to control which leafs were treated with distillate water.

Pluses of root length, even with 132%, were same recorded to those cuttings which rooted in base sprinkling conditions with Pi water and foliar sprinkling with distillate water, in accordance with V_2 variant, but in this case the caulogenesis was at least as the control level, the stem length presenting only 106% gains, but in the other cases were recorded 200% plus

of stem ramifications, this variant presenting the numerous ramifications plantlets.

Although the deuterium depleted water is well known through his inhibitor effects on cells, in the case of applying this water on *Tradescantia* cuttings (placed to rooting process on perlite sprinkled with distillate water - V_3), by spraying, we noticed 132% and 111% gains of roots length respectively of stem length and 118% plus in case of plantlet waist. To those plantlets belonging to this variant we did not noticed minuses to neither of biometrisated growing index (Figure 2).

On the other hand the radicular application of the deuterium depleted water lidded to minuses of growing indexes, especially to those expressing the rhisogenesis, meaning the length and number of roots, this parameters registering inferior values of 79% respectively 65%, comparatively to the control.

Table 1. Statistic processing of biometric measurements done at the level of Tradescantia plants, at 30 days of their rooting proceeding from follow experimental type: V0 - cuttings foliar sprinkled and watered to the base with distillate water (control); V1 - cuttings foliar sprinkled with Pi water and watered to the base with distillate water; V2 - cuttings foliar sprinkled with distillate water and watered to the base with Pi water; V3 - cuttings foliar sprinkled with deuterium depleted water, with 25 ppm D and watered to the base with distillate water; V4 - cuttings foliar sprinkled with distillate water and watered to the base with deuterium depleted water, with 25 ppm D.

Biometrics / Statistics evaluation	Roots length	Roots number	Stems length	Ramifications number	Knots number	Plants height
Type V_0						
$\bar{x} \pm S_{\bar{x}}$	5,00 ± 0,12	11,50 ± 0,11	11,08 ± 0,33	0,15 ± 0,08	6,00 ± 0,00	16,08 ± 0,38
s	0,55	0,50	1,49	0,36	0,00	1,69
S%	10,95%	4,35%	13,41%	238,05%	0,00%	10,51%
Type V_1						
$\bar{x} \pm S_{\bar{x}}$	6,25 ± 0,21	9,85 ± 0,18	10,20 ± 0,36	-	5,35 ± 0,11	16,45 ± 0,46
s	0,94	0,79	1,60	-	0,48	2,04
S%	15,07%	8,04%	15,69%	-	8,92%	12,38%
Type V_2						
$\bar{x} \pm S_{\bar{x}}$	6,60 ± 0,13	11,35 ± 0,20	11,70 ± 0,38	0,30 ± 0,14	5,95 ± 0,09	18,30 ± 0,41
s	0,58	0,91	1,71	0,64	0,38	1,82
S%	8,83%	8,01%	14,58%	213,44%	6,45%	9,94%
Type V_3						
$\bar{x} \pm S_{\bar{x}}$	6,60 ± 0,11	11,45 ± 0,19	12,30 ± 0,46	-	6,00 ± 0,07	18,90 ± 0,51
s	0,49	0,86	2,08	-	0,32	2,28
S%	7,42%	7,55%	16,88%	-	5,27%	12,05%
Type V_4						
$\bar{x} \pm S_{\bar{x}}$	3,95 ± 0,05	7,70 ± 0,10	9,75 ± 0,17	-	5,85 ± 0,08	13,70 ± 0,17
s	0,22	0,46	0,77	-	0,36	0,78
S%	5,52%	5,95%	7,86%	-	6,10%	5,70%

Note: $\bar{x} \pm S_{\bar{x}}$ (average ± standard deviation of the average), s (standard deviation), S% (variability coefficient).

As regarding the aspect of epidermis of *Tradescantia* foliar limb we noticed that leafs are hypostomatic, without hairs. The stomas are amaryllidaceous tetracithic types (**Figure 3**).

Superior epidermis of *Tradescantia* leafs is formed by polygonal cells (Figure 3 A), three times larger than the inferior epidermis, respectively 122.8/137.9 µm (**Table 3**).

Inferior epidermis show polyhedric cells with similar forms as superior epidermis, but smaller than those, which are elongated at entombs level.

Treatments made with different types of water did not influence the stomas density, which was 5 – 7 st/field (Table 3). At the same time we din not registered modifications of stomas length, the value of this parameter was around 47.2 µm, with one exception marked in case of basal applying the *Pi water*, this treatment causing an shortened of stomas length from 47.2 µm, to 41.4 µm. In case of cuttings leafs sprinkled only with *distillate water* and those sprinkled with *deuterium depleted water* the stomas medium breadth was 37.7 µm and to those variants with both foliar and basal applying the breadth was 28.3 µm. The smallest stomas were recorded to those cuttings leafs which were basal watered with *Pi water*. At the time of imprinting, in day light time period, all stomas were closed except those from control cuttings leafs which had an aperture greater, 9.45 µm (**Table 3**).

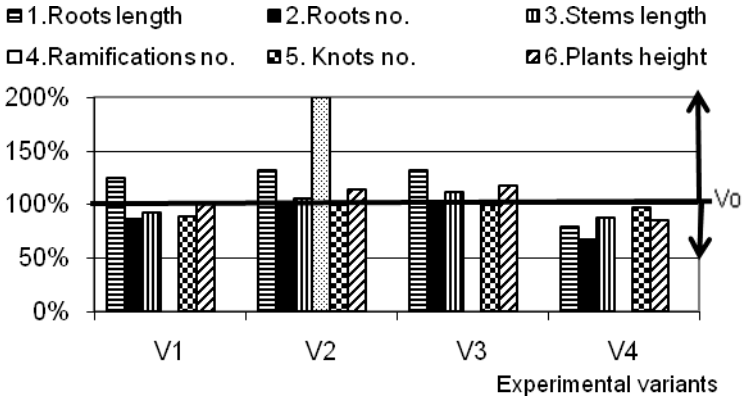

Figure 2. Biometrical data regarding the growth index of *Tradescantia* plants, at *30 days* of their rooting proceeding from follow experimental type: V_1 - cuttings foliar sprinkled with *Pi water* and watered to the base with *distillate water*; V_2 - cuttings foliar sprinkled with *distillate water* and watered to the base with *Pi water;* V_3 - cuttings foliar sprinkled with *deuterium depleted water, with 25 ppm D* and watered to the base with *distillate water*; V_4 - cuttings foliar sprinkled with *distillate water* and watered to the base with *deuterium depleted water, with 25 ppm D*, whose values have been reported toward the ones registered at the control plants (V_0 - cuttings foliar sprinkled and watered to the base with *distillate water)*, these being considered as 100%.

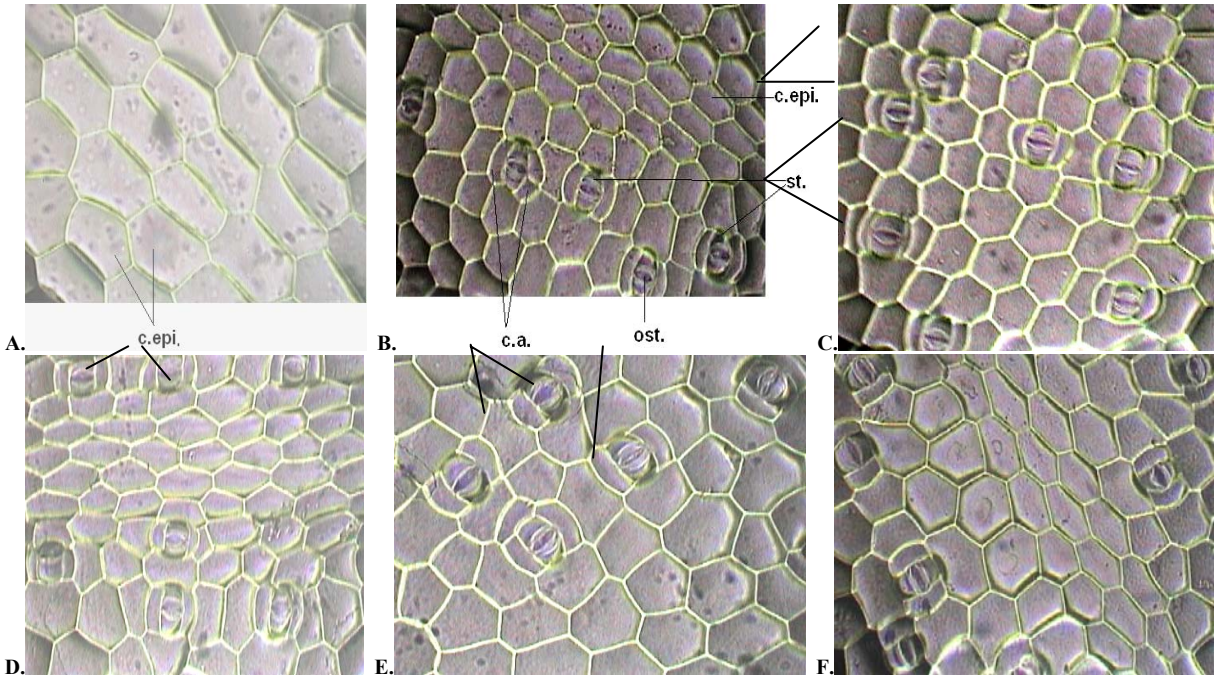

Figure 3. Epidermal formations at the level of *Tradescantia* leafs: A – *superior* epidermis of leafs proceeded from cuttings witch were foliar and basal sprinkled with *distillate water*; B – *inferior* epidermis of leafs proceeded from cuttings witch were foliar and basal sprinkled with *distillate water*; C – *inferior* epidermis of leafs proceeded from cuttings witch were foliar sprinkled with *Pi water* and basal with *distillate water*; D – *inferior* epidermis of leafs proceeded from cuttings witch were basal sprinkled with *Pi water* and foliar with *distillate water*; E – *inferior* epidermis of leafs proceeded from cuttings witch were foliar sprinkled with *deuterium depleted water* and foliar with *distillate water*; F – *inferior* epidermis of leafs proceeded from cuttings witch were basal sprinkled with *deuterium depleted water* and foliar with *distillate water* (c. epi – epidermal cells, ost – osteol, c.a. – guard cells, st. – stomas) (A – D 100X; E and F 200X).

There are four ***guard cells*** of *Tradescantia stomas*, two on sides, with smaller dimensions and placed one by one to the opposite poles, located parallel with osteol aperture, with greater dimensions (**Figure 3**).

The greatest guard cells with greater dimensions, 56,7/18,9 µm (the sides one) and 75, 6/37,7 µm (the poles one), were those from stomas proceeded from cuttings leafs sprinkled foliar with deuterium depleted water and smallest one were biometrisated to those cuttings treated with *Pi water*, especially to the base, were marked values of 37,7/24,5 µm to the lateral guard cells and 66,1/28,3 µm to the polar guard cells (**Table 4**).

Table 3. Stoma aspects at 30 days from placing the *Tradescantia* cuttings to root and sprinkled them with different types of water: V_0 – *distillate water* applied both foliar and radicular; V_1 – *Pi water* applied foliar; V_2 – *Pi water* applied radicular; V_3 – *deuterium depleted water* applied foliar; V_4 – *deuterium depleted water* applied radicular.

Epidermis types	**Stomas density/microscopic field (no.)**					Stoma cells length (µm)				
	V_0	V_1	V_2	V_3	V_4	V_0	V_1	V_2	V_3	V_4
Superior epidermis	-	-	-	-	-	-	-	-	-	-
Inferior epidermis	**6**	**6**	**7**	**5**	**6**	**47,2**	**47,2**	**41,4**	**47,2**	**47,2**
Epidermis types	Stoma cell breadth (µm)					Osteols aperture diameter (µm)				
	V_0	V_1	V_2	V_3	V_4	V_0	V_1	V_2	V_3	V_4
Superior epidermis	-	-	-	-	-	-	-	-	-	-
Inferior epidermis	**37,7**	**28,3**	**28,3**	**37,7**	**37,7**	**9,4**	**0**	**0**	**0**	**0**

Table 4. Guard cells dimensions of *Tradescantia* stomas, according to the following variants V_0 – *distillate water* foliar and radicular applied; V_1 – *Pi water* foliar applied; V_2 – *Pi water* radicular applied; V_3 – *deuterium depleted water* foliar applied; V_4 – *deuterium depleted water* radicular applied.

Lateral guard cells length/breadth (µm)					**Polar guard cells length/breadth (µm)**				
V0	V1	V2	V3	V4	V0	V1	V2	V3	V4
47,2/18,9	37,7/26,4	37,7/24,5	56,7/18,9	56,7/28,3	72/28,3	66,1/28,3	56,7/18,9	75,6/37,7	72/37,7

CONCLUSIONS

- *Pi water* was proved to have a stimulating effect to the *Tradescantia* cuttings rhisogenesis, both foliar and radicular applying, by spraying leafs and sprinkling substratum. In the case of radicular applying this type of water stimulated the caulogenesis process, especially by ramifications appearance, which were numerous.
- *Deuterium depleted water* (25 ppm D) applied foliar caused a stimulation of cuttings rhisogenesis, in the mean time the caulogenesis registered similar values to the control. Applying radicular, this type of water had a different effect to the previous one, by the presence of deficits to all growing parameters, especially to the rhisogenic indexes. The plantlets of the same experimental variants were the smallest from the all tested variants.
- Stomas were smaller, but numerously at the foliar limbs level of those leafs which were sprinkled with Pi water and at the foliar level with distillate water. Only opened stomas were distinguished to the control plants, sprinkled both basal and foliar with distillate water, although the amprentation was realized in identical conditions to all five experimental lots.

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