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THE INFLUENCE OF SOME FERTILIZERS AND BIOSTIMULANTS UPON THE ANATOMY OF THE FOLIAR LIMB OF *CHRYSANTHEMUM INDICUM* L. (IInd NOTE)

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Abstract. The results presented in this paper belong to the project "Elaborarea de soluţii şi tehnici de cultură neconvenţionale şi nepoluante la plantele ornamentale, în contextul dezvoltării durabile – The elaboration of unconventional and unpollutant solutions and culture techniques, in stable usage context" and presents the testing of three fertilizers and biostimulants (Maxiroot, Dacmarinur Maxi N, Aurora) upon the foliar limb of *Chrysanthemum indicum* L.; they were applied in 3 variants of concentrations (0.2%, 0.4%, 0.6%). The identification of the impact of the fertilizers has been analyzed by identifying the modifications of the foliar limb and middle vein, a comparative analysis of the number of epidermic cells and stomata which belong to both upper and lower epidermis, as well as measuring the dimension of stomata. In all applied products, a few differences appeared in comparison with the blank sample.

Key words: fertilizers and biostimulants, anatomic structure, foliar limb, *Chrysanthemum indicum*

Introduction

Many authors have taken into account the importance of *Chrysanthemum indicum* L. in enrichment of floral assortment [VIDRAŞCU & MITITIUC, 2001; VIDRAŞCU & al., 1986; VIDRAŞCU & al., 1985; BIREESCU & al., 2002; DORNEANU & al., 2001; GAVRILUŢĂ & al., 2005; TOMA, 1975, 1977; TOMA & al., 1985; TOMA & GOSTIN, 2000]. We continue our study regarding the testing of some fertilizers and biostimulants upon the anatomy of *Chrysanthemum indicum* L. In our first note [DELINSCHI & al., 2010], we analyzed the modifications appeared in the stem, while in the present paper we are going to emphasize the structural modification of the foliar limb.

Many papers present information regarding the normal structure of the leaves of *Chrysanthemum indicum* L. [NIŢĂ MIHAELA & al., 2001] or its varieties, but we have not find (in the literature we have) a special study were the variability induced by various substances (foliar fertilizers) could be observed.

The results presented in this paper belong to the project "Elaborarea de soluţii şi tehnici de cultură neconvenţionale şi nepoluante la plantele ornamentale, în contextul dezvoltării durabile – The elaboration of unconventional and unpollutant solutions and culture techniques, in stable usage context" and presents the testing of some fertilizers and biostimulants, with the purpose of identifying the most convenient variants of work which contribute to increasing the ornamental quality of *Chrysanthemum indicum* L. (a flower for all seasons), by using unpollutant products.

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Material and methods

The experimental cultures have been initiated at University of Agricultural Sciences and Veterinary Medicine of Iasi, and have been ordered in randomized blocks, with three repetitions. Four foliar treatments have been applied, at 10 days intervals, on various variants (V_1 – 0.2%, V_2 – 0.4%, V_3 – 0.6%), using the following products: Maxiroot, Dacmarinur Maxi N and Aurora. Maxiroot is a foliar fertilizer with biostimulating effect: N – 2%, K – 4%, Zn – 0.3%, Fe – 0.3%, organic material – 30%, free amino acids (tryptophan, arginine) – 3%, proteins, vitamins. Dacmarinur Maxi N is a n ecologic foliar fertilizer from marine algae (*Ascophyllum nodosum*): N – 216 g/l, K – 96 g/l, P – 38 g/l, cytochinons – 7.5 mg/l, auxines – 11 mg/l, amino acids, vitamins, proteins. Aurora is a Romanian natural extract from plants, with biostimulant effect an contains N – 10%, K – 3.5%, P – 0.5%, B, Ca, Cu, Fe, Mg, Zn – 2% each of them, enzymes, amino acids, vitamins.

The foliar limb has been cross-sectioned, at middle level; the epidermis, in front side view, has also been analyzed and the number of epidermic cells and stomata on surface area have been calculated. In order to identify the modifications appeared after the treatment with the mentioned substances, the length and width of stomata from both epidermis of the foliar limb has been measured.

The sections were coloured with iodine green and carmine red and mounted in gel. The histologic cuttings were analyzed in a Novex (Holland) microscope and photographed by means of a Sony DSC-W5/W7/W15/W17 photo camera.

Results and discussions

Blank sample (the epidermis)

In front side view, the upper epidermis (Pl. II: Fig. 20) consists of cells with polygonal to irregular profile, with curved walls; the stomatic cells, anomocytic type, are quite rare on the surface area. The secretory trichomes and the protective hairs are less numerous. The lower epidermis (Pl. II: Fig. 21) presents cells with moderately curved walls. Unlike the upper epidermis, in the lower epidermis, stomata, protective hairs and secretory trichomes are numerous.

In cross section, the middle vein is strongly prominent at the lower side of the limb and bears a single, big, vascular bundle (Pl. IV: Fig. 32). The protective hairs and the secretory trichomes are present in both epidermis, but more numerous in the lower one.

Judging upon the structure of the mesophyll, the foliar limb has a bifacial-heterofacial structure (normal dorsiventrality); one-layered palisade tissue is present towards the upper epidermis, while a compact lacunary tissue is present towards the lower one (Pl. IV: Fig. 43).

Dacmarinur V_1 (0.2%). The upper epidermis (Pl. I: Fig. 1) consists of cells of irregular shape, bearing weak to waved walls, unlike the lower epidermis (Pl. I: Fig. 2) where the walls are more waved and the number of stomata on surface area is increased. Apart of the anomocytic stomata, there are tetracytic stomata (Pl. I: Fig. 4), too, and, also, clusters of stomata (Pl. I: Fig. 2).

In cross section, a slow increment in the width of the foliar limb in comparison with the blank sample and the other variant could be observed. The mesophyll has a compact structure (Pl. IV: Fig. 40); the middle vein is more prominent, in comparison with the next variant (V_2) (Pl. III: Fig. 26).

Dacmarinur V_2 (0.4%). The action mode of this variant is very interesting; the upper epidermis (Pl. I: Fig. 5) bears polygonal to irregular-shaped cells, with waved walls, in comparison with the upper epidermis of the anterior variant. The lower epidermis (Pl. I: Fig. 3) presents cells of irregular shape, but with moderately waved walls. Stomata of the lower epidermis are more numerous that in the anterior variant.

In cross section, a decrement in the width appears, in comparison with both V_1 and V_3 (Pl. IV: Fig. 41). We can affirm that at this concentration, the modification in the width of the foliar, in comparison with the blank sample, is insignificant. Furthermore, the middle vein is smaller (Pl. III: Fig. 27).

Dacmarinur V_3 (0.6%). The upper epidermis (Pl. I: Fig. 7) presents cells of irregular profile, with moderately waved walls and reduced number of stomata, protective hairs and secretory trichomes. The lower epidermis (Pl. I: Fig. 6) consists of cells with moderately to strongly waved walls and numerous stomata, protective hairs and secretory trichomes.

In cross section, a good development (increment in width) of the foliar limb is displayed, in comparison with V_2, somehow similar to V_1 (Pl. IV: Fig. 42). The mesophyll has more compact structure, while the median vein is similar to that of the leaves used in V_1 (Pl. III: Fig. 28).

Aurora V_1 (0.2%). The upper epidermis (Pl. I: Fig. 10) presents cells of irregular profile with lateral moderately-waved walls in comparison with the lower epidermis which bears cells with strongly-waved walls (Pl. I: Fig. 11). The protective hairs and secretory trichomes from the lower epidermis are more numerous and there is a higher degree of clustering stomata.

In cross section (Pl. IV: Fig. 37), each and there, a second layer of palisade tissue appears and the lacunary tissue is more compact. The middle vein is moderately proemining at the lower epidermis (Pl. III: Fig. 29).

Aurora V_2 (0.4%). The differences between V_1 and V_2 are small (Pl. I: Figs. 12 and 13) and determined by the increment of the dimensions of the cells belonging to both epidermis; there is also an increment in the waving degree of the cellular walls on the lower epidermis (Pl. I: Fig. 13), number of stomata, as well as an increment in the number of protective hairs and secretory trichomes.

In cross section, an increment of the width of the foliar limb could be observed (Pl. IV: Fig. 38), as a consequence of becoming higher the single layer of palissade tissue as well as of the increment of the number of lacunary layers. Numerous regions of stomata with supraepidermic and subepidermic position could be observed, while the sclerenchyma of the middle vein is cellulosed and consists of cells with very thin walls. The middle vein is very good developed, in comparison with the other variants (V_1 and V_3) (Pl. III: Fig. 30).

Aurora V_3 (0.6%). The upper epidermis (Pl. I: Fig. 14) consists of cells with moderately-waved walls in comparison with the lower one (which bears strongly-waved walls) and a little less waved (Pl. I: Fig. 15) in comparison with the epidermis belonging to the leaves used in the anterior variant.

In cross section, there is a visible difference regarding the width of the foliar limb, compared with the other variants; wider than in V_1 but less wide than in V_2 (Pl. IV: Fig. 39). The middle vein is similar as dimension with the one belonging to the leaves of V_1 (Pl. III: Fig. 31).

Maxiroot V_1 (0.2%). The upper epidermis (Pl. II: Fig. 16) bears cells with irregular shape and moderately-waved lateral walls. The protective hairs and secretory trichomes are present in small number; stomata belong to the anomocytic type.

The lower epidermis (Pl. II: Fig. 17) bears cells of irregular profile, but with strongly-waved walls. The protective hairs and secretory trichomes are more numerous that in the upper epidermis; stomata are more numerous, too, and belong to the anomocytic type. Here and there, groups of stomata can be seen, in the axils of the veins (Pl. I: Fig. 9).

In cross section (Pl. IV: Fig. 33), the middle vein is strongly proeminent at the abaxial face of the foliar limb and bears a single, big vascular bundle, with a sheath of periphloemic sclerenchyma which consists of fibers with cellulosed walls. The protective hairs and secretory trichomes are present in both epidermis, but more numerous in the abaxial face.

The mesophyll (Pl. IV: Fig. 44) has a bifacial-heterofacial structure (mornal dorsiventrality); the one-layered palisade tissue towards the upper epidermis, while the lax lacunary tissue is at the lower epidermis; stomata are disposed under the level of the epidermis and presents a common, well developed substomatic chamber.

Maxiroot V_3 (0.6%). In V_3, a strong undulation of the walls of the epidermic cells can be observed (Pl. II: Figs. 18 and 19); the lower epidermis has cells with more curved walls than in the upper epidermis (Pl. II: Fig. 19). The protective hairs and secretory trichomes, as well as stomata are more numerous than in the upper epidermis and have strong tendencies of grouping (Pl. I: Fig. 8).

In cross section, there are no significantly modifications in comparison with the anterior variant; only the foliar limb is wider due to increasing the number of cellular layers in the lacunary tissue (Pl. IV: Fig. 45); the middle vein is a little more developed in comparison with V_1 (Pl. IV: Fig. 34).

In order to understand whether the variants used in the study can be easily identified upon their action in the anatomic structure, beside the mature leaves, we also studied the young leaves in Dacmarinur V_1 and V_3 and Maxiroot V_1 and V_3 (Pl. II: Figs. 22-25, Pl. IV: Figs. 46 and 47).

The analysis of the epidermis, in front side view or in cross section (Figs. 35 and 36) let us can affirm that there are very small differences, hardly visible in the initial stages of development that is why we took into account the anatomy of the mature leaves. The anatomic dates were accompanied by numeric dates referring to the number of epidermic cells and stomata (from the epidermis, on the surface area) in all variants of work (Table I), as well as dates regarding the variation of stomata dimension in both epidermis (μm), related to the substance and concentration used (Table II).

In order to identify the modifications appeared in the epidermis, in front side view, the epidermic cells and stomata were counted and the stomatic index was calculated (Table I).

In the upper epidermis, in comparison with the blank sample, in Maxiroot V_1 and Aurora V_1 and V_2 a higher number of cells on surface area can be observed, while in Dacmarinur V_3, in comparison with the blank sample, the difference is very small. The higher number of stomata appears in Maxiroot V_3 (6 cells), then in Dacmarinur V_1 (4 cells); the other differences are insignificantly (Table I).

In the lower epidermis, the modifications are visible; in comparison with the blank sample (42 cells), Aurora V_1 and V_3 and Maxiroot V_1 and V_3 determine an increment of cell dimensions. There is a large variation in the number of stomata in the lower epidermis (from 3 to 10). The most numerous stomata appear in Maxiroot V_3, then in Dacmarinur V_1 and V_3. In Aurora the differences are insignificantly, in comparison with the blank sample. The comparative analysis of the number of cells (epidermic cells and stomata) in the foliar limb of the young leaves demonstrates large variations which do not let us make an arguable conclusion, but we can affirm that Maxiroot is stronger than Dacmarinur, by its influence upon the cellular division.

Stomata dimension (Table II), in both epidermis, varies in more or less large limits. The biggest length of stomata of the mature upper epidermis appears in Dacmarinur V_1 and V_2 and Aurora V_1, while the biggest width appears in Dacmarinur V_1. In the lower epidermis, the biggest length of stomata appears in Aurora V_2 and Dacmarinur V_3, while the maximal width appears in Aurora V_2 and then in Aurora V_1 and Dacmarinur V_1.

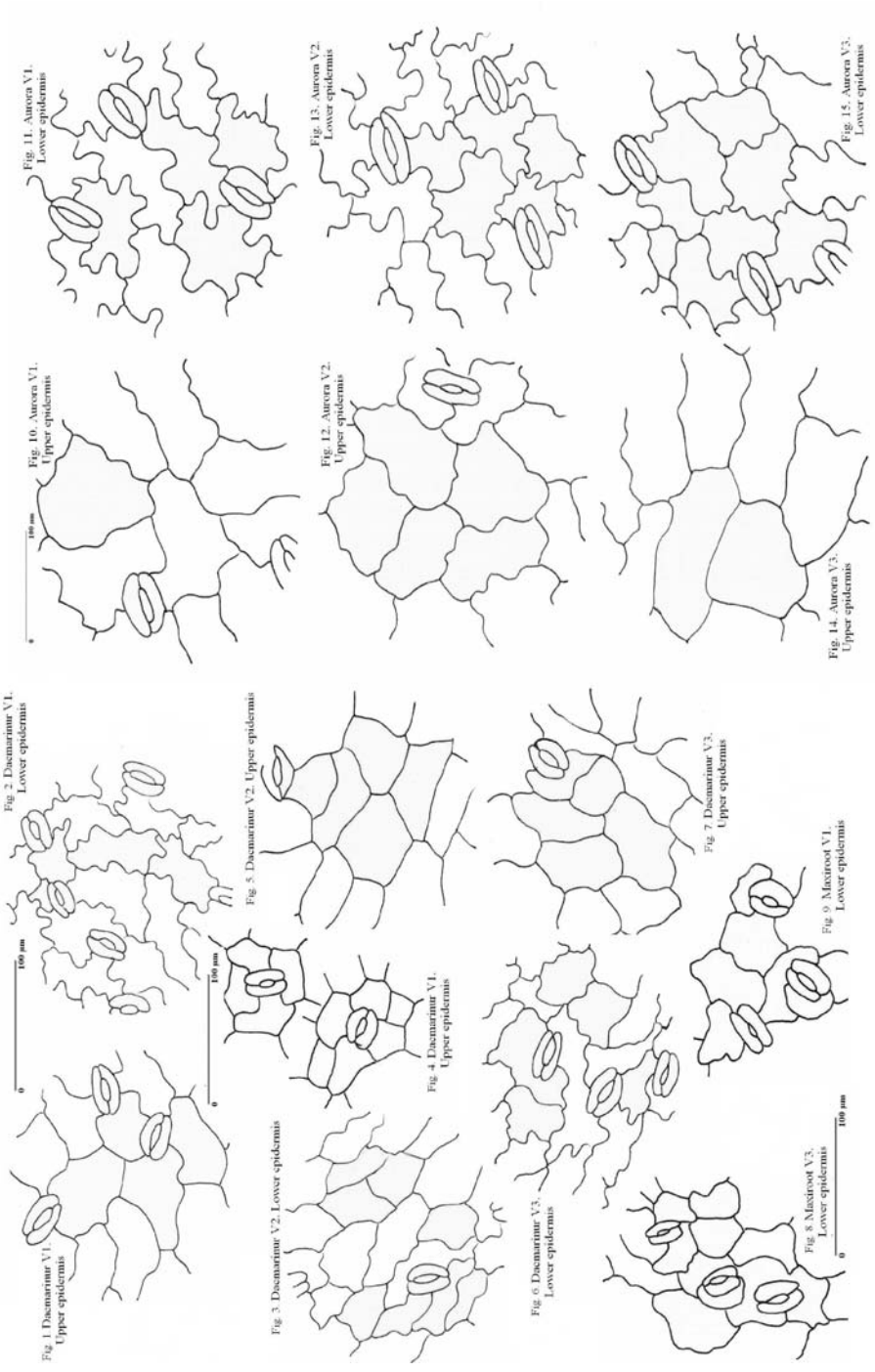
Tab. I. Dates referring to the number of epidermis cells and stomata in the foliar limb, on surface area (Surface=0.0941 mm^2)

Species	Substances	Variants	Upper epidermis			Lower epidermis		
			Number of cells	Number of stomata	Stomatic index	Number of cells	Number of stomata	Stomatic index
Chrysanthemum indicum		Blank sample	41	2	0.0465	42	6	0.1250
	Dacmarinur MAXI N	V_1 – young plant	44	2	0.0434	49	8	0.1403
		V_3 – young plant	35	1	0.0277	32	5	0.1351
		V_1 – mature plant	31	4	0.1142	42	8	0.1600
		V_2 – mature plant	34	2	0.0555	46	6	0.1153
		V_3 – mature plant	38	2	0.0500	40	8	0.1666
	Aurora	V_1	23	1	0.0416	32	5	0.1351
		V_2	26	1	0.0370	41	6	0.1276
		V_3	34	2	0.0555	37	5	0.1190
	Maxiroot	V_1 – young plant	71	1	0.0138	42	7	0.1428
		V_3 – young plant	44	1	0.0222	54	10	0.1562
		V_1 – mature plant	28	2	0.0666	38	10	0,2083
		V_3 – mature plant	30	6	0.1666	37	10	0.2127

Tab. II. Dimension of stomata (μm)

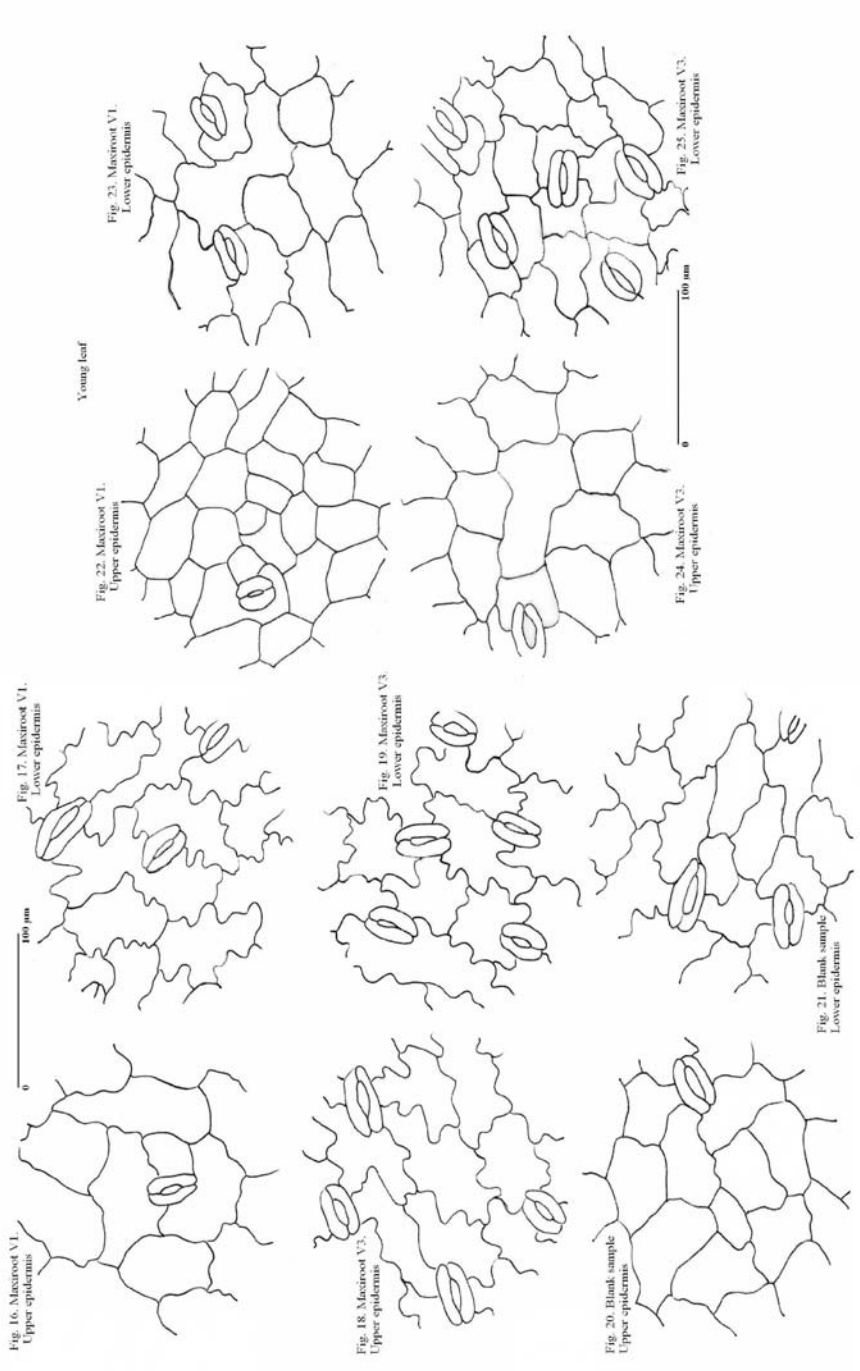
			Upper epidermis		Lower epidermis	
			Length	Width	Length	Width
Blank sample			43.254	30.616	44.144	28.836
Dacmarinur Maxi N	Mature plant	V_1	46.636	32.040	45.212	29.129
		V_2	46.636	28.836	43.788	27.946
		V_3	43.076	29.192	46.636	25.988
	Young plant	V_1	41.652	28.836	49.840	30.794
		V_3	48.060	27.412	48.416	25.988
Aurora		V_1	46.636	31.328	44.500	29.548
		V_2	43.432	30.616	47.348	30.260
		V_3	43.432	27.056	45.390	28.480
Maxiroot	Mature plant	V_1	45.568	30.260	44.856	28.480
		V_3	45.568	28.480	43.076	26.700
	Young plant	V_1	33.820	31.328	40.584	27.768
		V_3	43.289	30.260	45.746	23.140

PLATE I



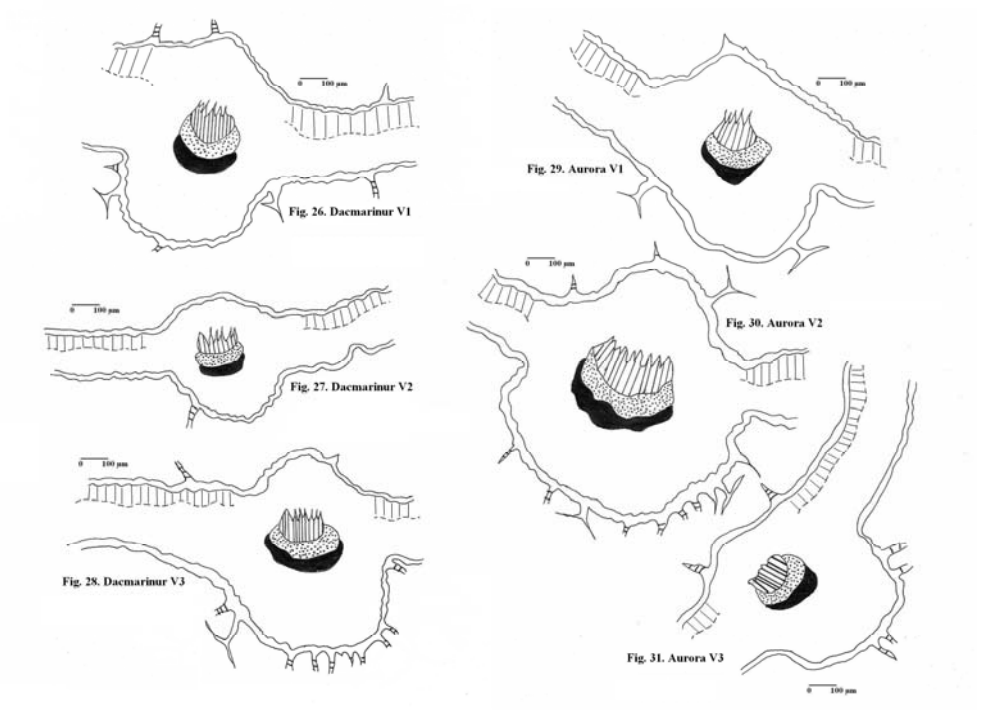
Epidermis in front side view (details)

PLATE II



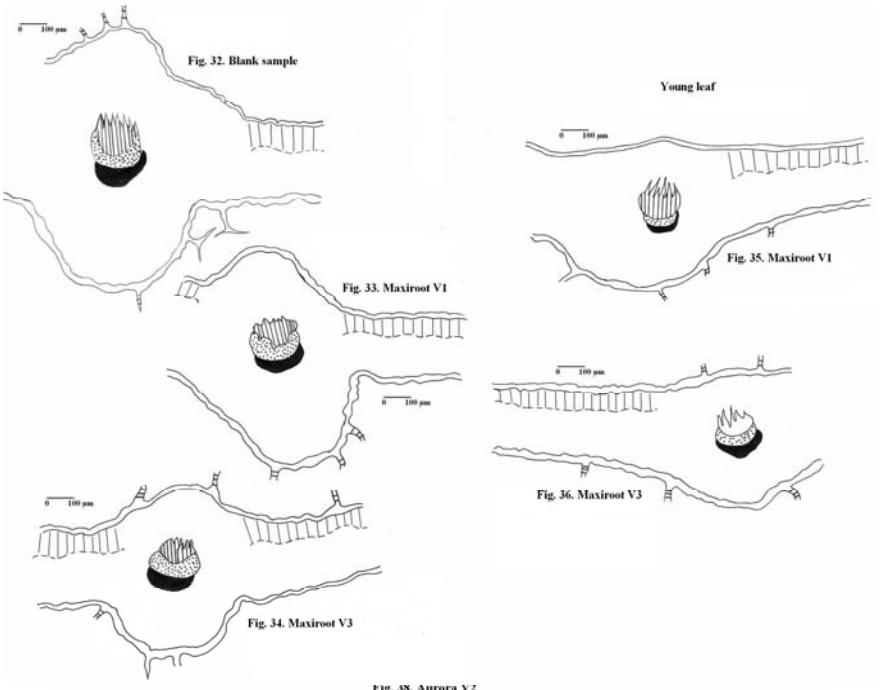
Epidermis in front side view (details)

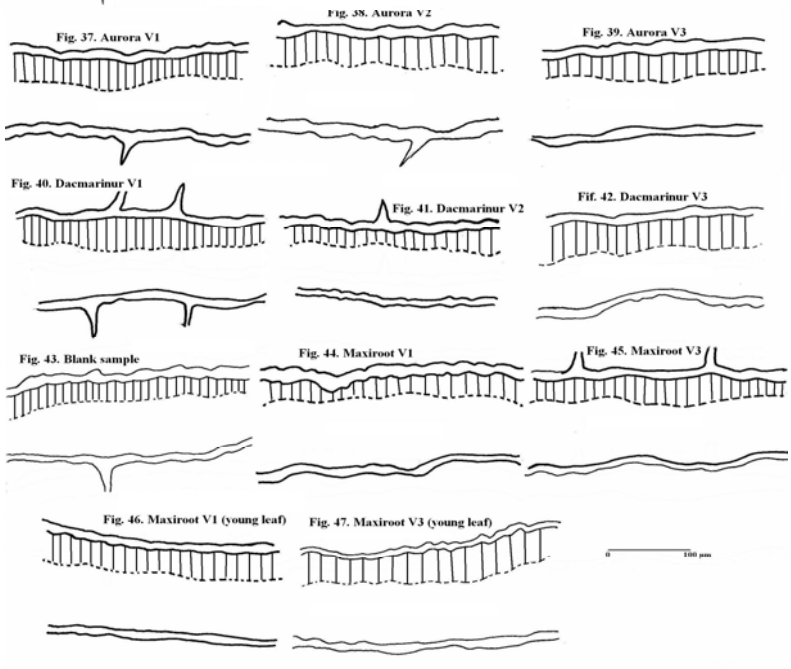
PLATE III



Plan of the cross sections through the foliar limb at middle level: middle vein and its lateral regions

PLATE IV





Plans of the cross sections through the foliar limb middle vein (Figs. 32-36) and its lateral regions (Figs. 37-47)

Conclusions

Judging the results of these products with no pollutant properties, applied in three variant of concentration, some significant differences appeared, in comparison with the blank sample; these differences are notable in various development stages of the foliar limb (young and mature).

A great receptivity of the foliar limb was observed, as a result of the action of the following products, used in various concentrations: Aurora V_2, Dacmarinur V_1 and V_3, Maxiroot V_3 and V_1.

The comparative analysis of cell number (epidermic cells and stomatic cells) of the mature foliar limb demonstrated large variations; Maxiroot is stronger in comparison with Dacmarinur, by influencing the cellular division process.

Stomata of both upper and lower epidermis belong to the following types: anomocytic and tetracytic and have different dimensions; clusters of cells appear in the axile of the veins (when plants are treated with Maxiroot). Although the literature explains that these clusters of stomata appear due to the hybridization phenomenon, in the present case, the foliar fertilizers play an important role in the grouping of stomata.

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