ANATOMICAL LEAF INVESTIGATIONS ON VARIOUS SUNFLOWER HYBRIDS GROWN UNDER DIFFERENTIATED FERTILIZATION CONDITIONS

STUDII ANATOMICE ALE FRUNZEI LA DIFERIȚI HIBRIZI DE FLOAREA SOARELUI CULTIVAȚI ÎN CONDIȚII DE FERTILIZARE DIFERENȚIATĂ

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Abstract: *Various studies pointed out the effect of NPK mineral supply on the biology of sunflower leaf apparatus: form, structure, and activity of photosynthetic apparatus. Investigations have been conducted on five sunflower hybrids (P.I. 2001, Select, P.I. 2002, P.I. 2004, and Performer) with various degrees of precociousness, fertilized with different NPK rates ($N_0P_0K_0$; $N_{60}P_{80}K_{60}$; $N_{80}P_{80}K_{80}$), under pedoclimatic conditions of the Moldavian Plain. Leaf morphogenesis was analysed by determining the number of active leaves and leaf area per plant, as well as the anatomical structure of leaf, at limb and petiole level. The obtained results have demonstrated that leaf morphogenesis was differentiated according to studied hybrid and dose of applied fertilizers. Analyses concerning the anatomical structure of leaf have shown differences between experienced variants, which were found especially at the leading tissue and stomata apparatus. The differences were quantitatively: number of collenchyme layers, number of stomata per area unit, number of leading fascicles, and qualitatively: presence of concentric fascicles, presence of the cap of lignified cells in the phloem parenchyma, and the presence of two stomata types.*

***Rezumat*:** *Diferite cercetări au studiat efectul nutriției minerale cu NPK asupra biologiei aparatului foliar la floarea soarelu: forma, structura şi activitatea aparatului fotosintetic. Experiențele au fost efectuate la 5 hibrizi de floarea soarelu (P.I. 2001, Select, P.I. 2002, P.I. 2004 şi Performer), cu diferite grade de precocitate, cu trei niveluri de fertilizare: NPK, $N_{60}P_{80}K_{60}$ şi $N_{80}P_{80}K_{80}$. în condițiile Câmpiei Moldovei. Morfogeneza frunzei a fost analizată prin determinarea numărului de frunze active şi a suprafeței foliare pe plantă, precum şi prin stabilirea structurii anatomice a frunzei la nivelul limbului şi al pețiolului. Rezultatele obținute au demonstrat că morfogeneza frunzei s-a diferențiat în funcție de hibrizii studiați şi doza de fertilizare aplicată. Cercetările privind structura anatomică la nivel foliar prezintă diferențe între variante vizibile mai ales la nivelul țesutului conducător şi al aparatului stomatic. Diferențele sunt atât de natură cantitativă: numărul straturilor de colenchim, numărul de stomate pe unitatea de suprafață, numărul fasciculelor conducătoare, cât şi calitativă: prezența fasciculelor concentrice, prezența calotei de celule lignificate în parenchimul liberian, existența celor două tipuri de stomate.*

Key words: *anatomical leaf, sunflower, hybrids, fertiliyation*
Cuvinte cheie: *anatomia frunzei, flaorea soarelui, hibrizi, fertilizare*

. INTODUCTION

In the accumulation of sunflower yield, a special part is plaid by plant nutrition, which is not very well known, because the harvesting index is relatively low – 0.40. Different investigations have studied the effect of NPK mineral nutrition on the biology of leaf apparatus in sunflower. It was found that nitrogen fertilization has influenced shape, structure and activity of photosynthetic apparatus, and *a* chlorophyll content has increased simultaneously to nitrogen rate increase. Phosphorus stimulates the transportation of assimilates, and potassium stimulates photophosphorilation and activity of Rubisco enzyme. Potassium is also essential for the synthesis in plants of proteins, glucide, fats, and contributes to the movement of

metabolites between organs and tissues. Due to the positive influence on osmotic pressure and turgescence of cells, by diminishing transpiration, potassium favour plant resistance to draught. In all growing areas, it was found that sunflower preferred residual nitrogen forms, fact that must be taken into account. This paper has investigated the effect of mineral nutrition on some biology elements of leaf apparatus, such as morphogenesis, growth, and anatomical structure in some sunflower hybrids.

MATERIAL AND METHOD

Experiments were conducted on five sunflower hybrids: P.I. 2001, Select, P.I. 2002, P.I. 2004 and Performer, grown at the Agricultural Research and Development Station of Podu-Iloaiei, Iaşi County in 2006, with three fertilization levels: NPK, $N_{60}P_{80}K_{60}$ and $N_{80}P_{80}K_{80}$.

The effect of genotype and mineral fertilization on the biology of leaf apparatus has been studied in growth phenological phase of plants. The number of active leaves and weight of leaf limb/plant (g) has estimated the morphogenesis of the leaf apparatus. The influence of NPK fertilization on leaf area was estimated according to the values of leaf length /width and average area of a leaf (cm^2).

The leaf anatomical structure was analysed by transversal sections, which allowed us to point out different anatomical formations (collenchymas, lignified cells in protoxyllem ligneous parenchyma, phloem cells present in ligneous parenchyma, hadrocentric concentric beams in phloem parenchyma , secretory channel situated at xylem pole) in petiole and (upper epidermis, lower epidermis, leading beams with various dispositions in median rib, leptocentric concentric beams, solitary cell with lignified walls, and secondary rib) in the limb.

RESULTS AND DISCUTIONS

Morphogenesis of leaf apparatus

Data from table 1 have shown that the morphogenesis of leaf apparatus, estimated by the number of leaves/plant at the unfertilized variant was more reduced in hybrids Select, P.I. 2001 and Performer, and more intense in hybrids P.I. 2002 and P.I. 2004. $N_{60}P_{80}K_{60}$ fertilization has greatly stimulated leaf morphogenesis in hybrids Select and Performer, while $N_{80}P_{80}K_{80}$ fertilization has diminished the intensity of leaf morphogenesis in hybrids P.I. 2001, Select and Performer.

The weight of limb/plant at unfertilized variants was minimal in hybrids P.I. 2001 and P.I. 2002, while hybrids Performer, P.I. 2004 and, especially, Select have shown much higher values. $N_{60}P_{80}K_{60}$ fertilization has highly increased the weight of leaf limb/plant in hybrids P.I. 2004 and Performer, while $N_{80}P_{80}K_{80}$ fertilization has diminished this parameter in comparison with the unfertilized variant in hybrids P.I. 2001 and P.I 2002.

Table 1

Influence of NPK fertilization on the morphogenesis of leaf apparatus

Hybrid	$N_0P_0K_0$		$N_{60}P_{80}K_{60}$		$N_{80}P_{80}K_{80}$	
	No leaves/ plant	Weight of limb/ plant (g)	No. leaves/ plant	Weight of limb/ plant (g)	No. leaves/ plant	Weight of limb/ plant (g)
P.I. 2001	16	44	16	45	15	33
Select	15	58	20	56	17	38
P.I. 2002	18	42	19	42	16	44
P.I. 2004	18	54	20	68	19	56
Performer	16	51	23	69	19	34

The limb area, estimated according to values leaf length /width , has classified the

Select hybrid (27/28 cm) on the first place , followed by hybrids Performer, P.I. - 2001, P.I. – 2004, and P.I. – 2002, on the last place, in case of unfertilized variants. The mean fertilization level ($N_60P_{80}K_{60}$) has positively influenced the limb area in hybrids P.I 2001, P.I.2002 and P.I. 2004, while the semi-late hybrids Select and Performer have registered a diminution in this trait. The third fertilization rate had the same negative effect on the increase in values of limb area, being under the level of unfertilized variants in hybrids Select and Performer (table 2).

The mean area of a leaf (cm^2) has registered values between 381.5 in P.I. - 2002 and 776.5 in Select for unfertilized variants, and between 495.3 in P.I. 2002 and 589.3 in Select, at the second fertilization rate. The increase in fertilizer rate has diminished the values of mean leaf area in hybrids Select and Performer, but increased these values in native hybrids P.I 2001, P.I. 2002 and P.I 2004.

Table 2

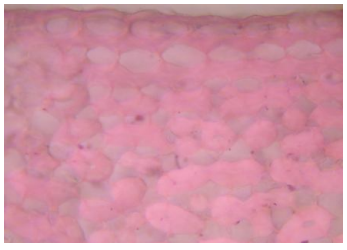
Influence of NPK fertilization on leaf area

Hybrid	$N_0P_0K_0$		$N_{60}P_{80}K_{60}$		$N_{80}P_{80}K_{80}$	
	Length/ width of leaf	Mean area of leaf (cm^2)	Length/ width of leaf	Mean area of leaf (cm^2)	Length/ width of leaf	Mean area of leaf (cm^2)
P.I. 2001	21/24	502.5	23/25	588.5	22/25	591.3
Select	27/28	776.5	26/26	589.3	17/18	328.3
P.I. 2002	18/20	381.5	21/23	495.3	22/23	534.1
P.I. 2004	20/22	450.6	24/26	561.8	25/27	712.3
Performer	25/28	757.2	22/23	522.5	19/18	358.9

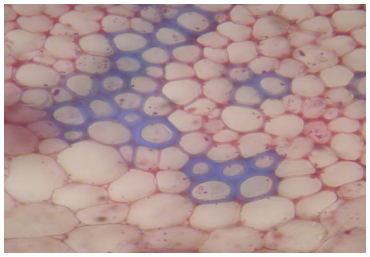
Investigations conducted on the limb anatomical structure have shown differences between variants, which were visible especially at the level of guiding tissue and of stomata apparatus. These differences were both quantitatively: number of collenchymas layers, number of stomata/ area unit, number of leading beams , and qualitatively: presence of concentric beams, presence of the cap of lignified cells in phloem parenchyma and of the two types of stomata.

Anatomical structure of the petiole

At all the studied variants, the outline of the petiole is cordiform, with a ditch on the adaxial face. The number of collenchyma layers and the degree of its development (coverage of lumen cells) varied according to hybrid and fertilization type , being lower in P.I. 2002 hybrid, at unfertilized variants, and in Select hybrid , fertilized with $N_{60}P_{80}K_{60}$ and, respectively, in hybrids P.I.2002 and Performer, fertilized with $N_{80}P_{80}K_{80}$. In most of cases, collenchyma is angular; sometimes is tabular, in the first 3-4 cell layers, situated under the epidermis. An extreme case was found in P.I.2004 hybrid, fertilized with $N_{80}P_{80}K_{80}$ (picture 1), where the number of collenchyma layers was 12-14, and in some cells, the lumen was hardly visible.



Picture 1 – Transversal section through petiole (collenchyma)



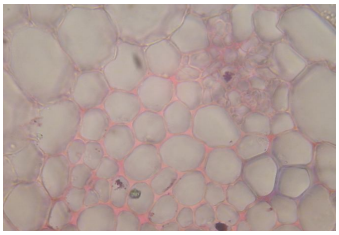
Picture 2 – Transversal section through petiole (lignified cells in protoxylem wooden parenchyma)

In most of studied variants, we have found out in parenchyma mass, the presence of beams, made of phloem elements; rarely, they were accompanied by one or two vessels with slightly thickened and lignified walls. In Performer hybrid, fertilized with $N_{60}P_{80}K_{60}$ the number of these beams was very high.

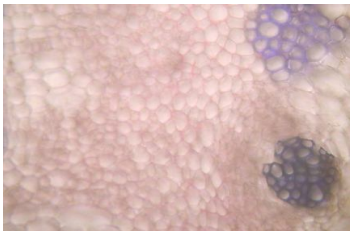
In phloem parenchyma, at the level of high size leading beams, found in hybrids P.I. 2001, 2002 and 2004, unfertilized or fertilized with $N_{60}P_{80}K_{60}$, and in hybrids P.I. 2004 and Performer, fertilized with $N_{80}P_{80}K_{80}$, we have noticed the presence of isolated or grouped cells, with slightly thickened and lignified walls, forming a cap (picture 2).

In protoxylem wooden parenchyma, we have found phloem elements in P.I.2001 hybrid, fertilized with $N_{60}P_{80}K_{60}$ (picture 3), and cells with thickened and lignified walls in Performer hybrid, fertilized with the same rate.

A special case was signaled in P.I.2004 hybrid, fertilized with $N_{80}P_{80}K_{80}$, where a hadrocentric concentric beam was found in the phloem parenchyma of a great size beam (picture 4).



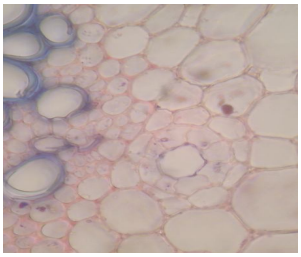
Picture 3 – Transversal section through petiole (phloem cells present in wooden parenchyma)



Picture 4 – Transversal section through petiole (hadrocentric concentric beam in the phloem parenchyma)

In case of P.I. 2002 hybrid, at both fertilizing variants we have noticed beams dispersed in cellulosic parenchyma, with 3 or 4 xylem poles, which were similar to leptocentric concentric ones, the external wooden circle being incomplete.

We have noticed the presence of secretory channels, usually disposed next to the phloem pole of beams. In P.I, 2001 hybrid, fertilized with $N_{60}P_{80}K_{60}$, this channel is disposed at xylem pole (picture 5).



Picture 5 – Transversal section through petiole (secretory channel disposed at the xylem pole)

Limb anatomical structure

The leaf limb has shown stomata on both sides (pictures 6, 7), being amistomatic. The number of stomata/area unit was higher on lower epidermis. On both epidermises, we have

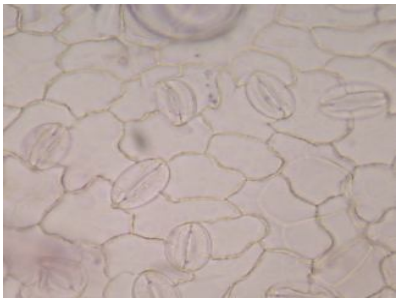
noticed two stomata types: anisocitic (which prevailes) and anomocitic, as well as a relatively great number of tector and secretory hairs.

At the level of median rib, we have noticed the presence of phloem- ligneous leading beams with aleatory disposal (picture 8) and of many beams, made only of phloem elements.

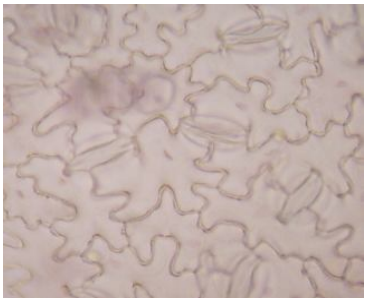
As in the case of the petiole, we have noticed in the phloem parenchyma the presence of thickened and lignified walls and of leptocentric concentric beams (picture 9).

In P.I. 2004 hybrid, fertilized with $N_{60}P_{80}K_{60}$, we have found solitary ligneous vessels in parenchyma (picture 10). In case of greater beams , in phloem parenchyma, we have noticed cells which walls presented a starting point for collenchymatization.

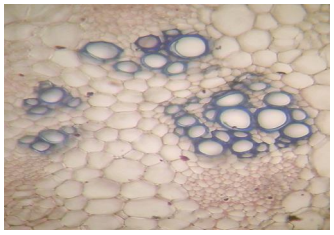
Secondary ribs were made up of one leading beam (picture 11), which was leaned upon the two epidermises through cell pillars with higher size than the pallisadic cells.



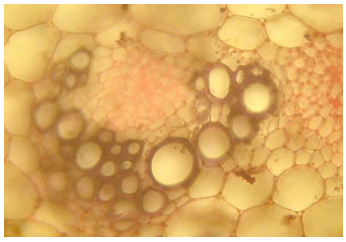
Picture 6 – Superficial section through limb (upper epidermis)



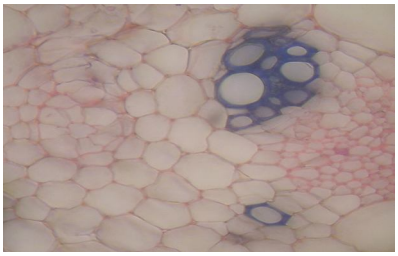
Picture 7 – Superficial section through limb (lower epidermis)



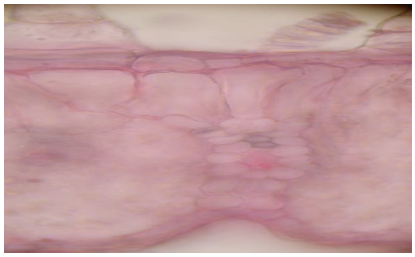
Picture 8 – Transversal section through limb (leading beams with various disposals in the median rib)



Picture 9 – Transversal section through limb in the direction of median rib (leptocentric concentric beams)



Picture 10 - Transversal section through limb (solitary cell with lignified walls)



Picture 11- Transversal section through limb (secondary rib)

Mesophyll was made up of palisade and lacuna parenchyma. In unfertilized P.I.2002 hybrid, the palisade tissue was made of two short cell layers, and in P.I.2001 hybrid, fertilized with $N_{60}P_{80}K_{60}$, it was made of one layer of lenghten cells. In case of P.I.2002 hybrid, fertilized with $N_{60}P_{80}K_{60,}$ the lacunous tissue had lengthen cells, similar to the palisade ones. In case of unfertilized P.I.2004 hybrid, the structure of mesophyll was disorganized, some areas presenting only lengthen lacunous cells.

CONCLUSIONS

1. The moderate fertilization rate $N_{60}P_{80}K_{60}$ stimulates the leaf morphogenesis in hybrids Select and Performer and the limb growth in hybrids P.I. 2004 and Porformer.

2. The increase in fertilizer rate resulted in the augmentation of the leaf mean area in native hybrids P.I. 2001, and P.I. 2004, and in the great diminution of this indicator in hybrids Select and Performer.

3. The mineral fertilization causes changes in the anatomical structure of the petiole, which is expressed by the increase in the number of collenchymas and phloem beams, or by the appearance of some cells with thickened or lignified walls.

4. The fertilization with different NPK rates determines modifications in limb anatomical structure, as concerns the number of stomata/ area unit, number of collenchymas layers and leading beams, as well as by the presence of solitary ligneous vessels or modifications in the structure of palisade or ligneous parenchyma.

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