# THE EFFECT OF HYDRIC STRESS ON SOME CHARACTERISTICS OF SUNFLOWER PLANTS

**Elena Petcu, Adrian Arsintescu, Danil Stanciu**[*)]

## ABSTRACT

**The evolution of leaf area, root volume, chlorophyll content and peroxidase activity for some Romanian sunflower hybrids, under optimal and drought conditions was established. Five Romanian sunflower hybrids was grown in the greenhouse under two watering regimes for each genotype*:* control variant – in which plants were maintained at 70% from TSWC (total soil water capacity)] and *stress treatment* in which sunflower seedlings were irrigated no more than 40% from TSWC. The results showed that hydric stress induced the decrease of leaf area, shoot size, root volume, chlorophyll content and a significant increase of peroxidase activity. Some differences between the tested sunflower hybrids were registered. Also, a significant effect of hydric stress on fatty acid composition of seeds of sunflower plants was registered. In the normal sunflower hybrids, like the hybrids of this study, in addition to the reduction of oleic acid concentration, a simultaneous increase in the content of linoleic acid under drought conditions was registered.**

**Key words**: chlorophyll content, drought, fatty acid composition, leaf area, peroxidase activity, root volume sunflower.

## INTRODUCTION

Drought is probably the most important factor limiting crop yields worldwide and, in Romania, too. Because of its complexity, drought tolerance is probably the most difficult trait to improve through conventional plant breeding. The challenge is even greater for developing drought tolerant cultivars for Romanian environment where the occurrence, timing and severity of drought may fluctuate from year to year.

R.I.C.I.C. Fundulea has devoted considerable effort during the past ten years to improve drought tolerance in wheat, maize and sunflower. Extensive research has been conducted in the area of breeding, agronomy, and most recently, physiology.

The physiology work has focused on morpho-physiologycal traits induced by drought and associated with drought tolerance of plants (I), and the elaboration of screening methods for rapidly measuring of drought to-lerance using plants in earlier stage of vegetation (II).

Sunflower is a well adapted to drought crop, essentially because of the powerful water uptake due to its efficient root system (Belhassen, 1995).

The present paper reports the reactions of five Romanian sunflower genotypes to hydric stress. The aim was to identify morpho-physiologycal traits that could be used as screening criteria in a breeding programme for drought tolerance and which could be rapidly measured using plants in an earlier stage of vegetation.

## MATERIALS AND METHODS

### Experiment 1

Seeds of five sunflower hybrids: Alex, Favorit, Justin, Romina and Splendor were germinated and then planted at a depth of 3–4 cm in PVC tubes (35 cm long and 11 cm diameter) filled with a soil-sand mixture (1:1).

Each genotype was tested in five replicates, at four leaves stage and two watering regimes for each genotype: control variant – [in which plants were maintained at 70% from TSWC (total soil water capacity)] and stress treatment (where sunflower seedlings were irrigated no more than 40% from TSWC).

The biomass of the above and below-ground parts was measured after drying them to the constant weight.

The chlorophyll concentration was assessed using a SPAD-502 chlorophyll meter (Minolta, Japan).

Leaf area was calculated with the formula: L x l x 0.66 where: L = leaf length; l = leaf width and 0.66 = correction coefficient for sunflower. The root volume was measured by water displacement from a filled beaker.

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For peroxidase determination the vertical electrophoresis in polyacrilamide native gel was used.

**Experiment 2**

Five standard sunflower hybrids provided by Fundulea Research Institute were used in this experiment: Alex, Favorit, Justin, Romina and Splendor.

The seeds were sown in pots (27 kg capacity) in soil: sand (4:1) mixture, in vegetation house.

The experimental variants were:

a) control – the sunflower plants were kept up to physiological maturity at optimal soil humidity conditions, 70% soil water capacity respectively;

b) drought – was induced before flowering and 12 days after flowering the sunflower plants were kept at 40% soil water capacity;

The fatty acids were analysed by gas chromatography (GS) according to the conventional method: the transformation of triglycerides to fatty acid methyl esters was performed with trimethylsulfoniumhydroxid (TMSH). The capillary column (BP x 70) by 25 m length on a DELSI gas chromatograph with flame ionization detector (FID) was used. Injector and detector temperature were kept at 270 and 280°C. The carrier gas was helium, with a flow rate of 20 ml/min. The total area of the peaks and the area of each fatty acid peak was expressed as a percentage of the total area.

## RESULTS AND DISCUSSION

As first response of sunflower seedlings grown under hydric stress conditions the reduction of leaf area and height of plants were registered. Leaf area was insignificantly reduced in sunflower grown one week under drought conditions (from 0.8% for Favorit up to 15% for Justin hybrid) and significantly reduced in all sunflower genotypes grown two weeks under drought conditions (up to 50%) (Table 1).

In all sunflower hybrids, the effect of drought treatment consisted in a significant decrease of height of plants, less in hybrid Alex and more in hybrid Justin (Table 2).

*Table 1*. The effect of hydric stress on leaf area of sunflower seedlings

| Hybrids | Variants | Leaf area | | | |
|---|---|---|---|---|---|
| | | Hydric stress (1 week) | | Hydric stress (2 weeks) | |
| | | mm² | % | mm² | % |
| Alex | Control | 4.77 | 100 | 55.52 | 100 |
| | Drought | 4.36 | 91.5 | 24.85 | 44.8 |
| Favorit | Control | 6.23 | 100 | 62.95 | 100 |
| | Drought | 6.18 | 99.2 | 29.83 | 47.4 |
| Justin | Control | 5.23 | 100 | 49.42 | 100 |
| | Drought | 4.44 | 85.0 | 28.46 | 57.6 |
| Romina | Control | 5.55 | 100 | 54.92 | 100 |
| | Drought | 5.32 | 99.6 | 28.68 | 52.2 |
| Splendor | Control | 5.15 | 100 | 56.77 | 100 |
| | Drought | 4.93 | 95.7 | 28.38 | 50.0 |

*Table 2*. The effect of hydric stress on shoot size of sunflower seedlings

| Hybrids | Variants | Height of plants | |
|---|---|---|---|
| | | mm | % |
| Alex | Control | 621 | 100 |
| | Hydric stress (2 weeks) | 457 | 73.6 |
| Favorit | Control | 641 | 100 |
| | Hydric stress (2 weeks) | 459 | 71.6 |
| Justin | Control | 600 | 100 |
| | Hydric stress (2 weeks) | 385 | 64.2 |
| Romina | Control | 659 | 100 |
| | Hydric stress (2 weeks) | 450 | 68.3 |
| Splendor | Control | 571 | 100 |
| | Hydric stress (2 weeks) | 413 | 72.3 |

The significant positive correlation between leaf area and plant height under hydric stress condition is obvious (r = 0.953***, Figure 1).

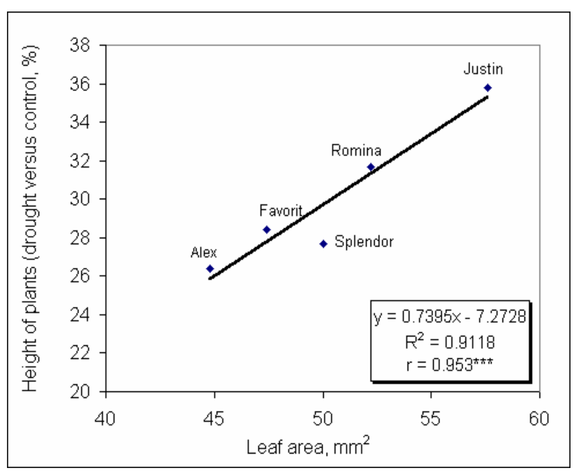


*Figure 1*. The correlation between leaf area and height of sunflower seedlings under hydric stress

During the different stages of vegetation the hybrid Favorit has registered a higher value of leaf area both under control and hydric stress variants as compared with the hybrid Justin (Figure 2). This response could be considered as an usual reaction of sunflower plants in order to reduce water use.

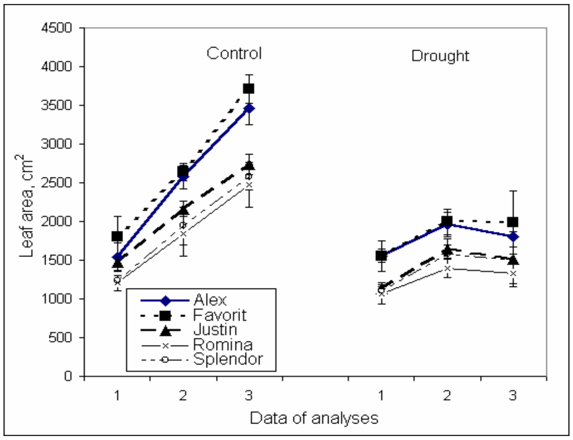


*Figure 2*. The effect of hydric stress on leaf area of sunflower plants at different data of analyses (1 = before flowering; 2 = at 1 week after flowering; 3 = at 2 weeks a fter flowering)

The chlorophyll content is one of the most important indicators of vegetation stage and its degradation is normally considered a measure of drought resistance (Beard, 1973; Kim et al., 1989). The total chlorophyll content (expressed as SPAD units) was reduced under drought conditions, except for Favorit and Justin which presented after two weeks under hydric stress conditions an insignificant increase of chlorophyll content as compared with control (Figure 3).

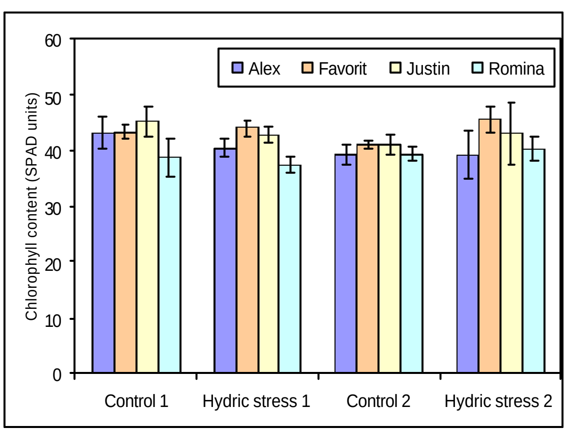


*Figure 3*. Effect of hydri c stress on chlorophyll content in sunflower leaves

Dry matter production of shoots, leaves and roots was significantly reduced under hydric stress conditions for all tested genotypes. Beside the genetic variability of tested sunflower hybrids, differences were registered between the analysed organs, too. It is obvious that leaves and shoots were more influenced by hydric stress than roots.

Thus, dry matter accumulation in roots of Favorit hybrid under drought is higher than under control. Also, the values of the rest of the tested sunflower hybrids were up to 70%. Under hydric stress conditions, the root/shoot ratio increases. The increase in the Favorit hybrid was obvious (72%) and from 23 to 45,8% in Romina and respectively Justin. These results show that the total root mass increases with drought stress (Table 3).

The shoot/root mass ratios consistently decrease under drought stress, which is a universal expression of adaptation (Blum, 1988). The increase of root/shoot ratio is mentioned in literature (Sharp and Davies, 1985; Sharp and Boyer, 1986). Previous reports underlined the genetic diversity of hybrid sunflower roots and the influence of soil environmental conditions on the rooting system (Þerbea et al., 1995; Petcu et al., 1997; Aguera et al., 1997).

Our results show that during the first days of hydric stress the nutritive reserves of sunflower seedlings were conducted to develop the roots, in order to facilitate deep soil moisture extraction. This happens in detriment of shoot development and in this case a drift is occurring in the main sink for surviving. Concerning the root/shoot ratio, the response of mature plants to hydric stress is different than seedling response as the sink is different.

The root/shoot ratio of mature plants decreases under drought stress in Favorit and Splendor but increases in Alex, Justin and Romina hybrid. So, some differences between the tested genotypes in response of drought were noticed (Table 4).

The head/shoot ratio increased under drought in all genotypes, this proving the different sinks at mature plants (Figure 4).

It is well known that water stress has a profound effect on sunflower yield (Murriel and

Downes, 1974; Talha and Osman, 1975) and productive hybrids under drought conditions were Favorit and Justin.

*Table 3.* The effect of hydric stress on biomass accumulation in seedling sunflower hybrids

| Hybrids | Experimental variants | Biomass accumulation (g dry matter) | | | | | | Roots/shoot | |
|---|---|---|---|---|---|---|---|---|---|
| | | Leaves | | Shoots | | Roots | | | |
| | | g | % | g | % | g | % | ratio | % |
| Alex | Control | 2.87 | 100 | 4.37 | 100 | 2.35 | 100 | 0.32 | 100 |
| | Hydric stress (2 weeks) | 1.48 | 51.5 | 1.93 | 44.1 | 1.42 | 60.42 | 0.42 | 131.2 |
| Favorit | Control | 3.24 | 100 | 4.60 | 100 | 1.52 | 100 | 0.19 | 100 |
| | Hydric stress (2 weeks) | 1.86 | 57.4 | 2.87 | 62.2 | 1.58 | 103.9 | 0.33 | 173.6 |
| Justin | Control | 2.72 | 100 | 4.97 | 100 | 1.86 | 100 | 0.24 | 100 |
| | Hydric stress (2 weeks) | 1.70 | 62.5 | 2.91 | 58.5 | 1.60 | 86.02 | 0.35 | 145.8 |
| Romina | Control | 3.22 | 100 | 5.29 | 100 | 1.83 | 100 | 0.22 | 100 |
| | Hydric stress (2 weeks) | 2.22 | 68.9 | 2.5 | 47.2 | 1.29 | 70.49 | 0.27 | 123 |
| Splendor | Control | 3.12 | 100 | 4.88 | 100 | 1.87 | 100 | 0.23 | 100 |
| | Hydric stress (2 weeks) | 1.81 | 58.0 | 3.51 | 71.9 | 1.60 | 85.56 | 0.30 | 130.4 |

*Table 4.* The effect of hydric stress on biomass accumulation in mature sunflower plants

| Hybrids | Experimental variants | Biomass accumulation (g dry matter) | | | | | | Roots/shoot | |
|---|---|---|---|---|---|---|---|---|---|
| | | Shoots | | Leaves | | Roots | | | |
| | | g | % | g | % | g | % | ratio | % |
| Alex | Control | 36.8 | 100 | 20.4 | 100 | 10.8 | 100 | 0.19 | 100 |
| | Hydric stress (2 weeks) | 21.6 | 58.70 | 18.6 | 91.18 | 9.4 | 87.04 | 0.23 | 123.8 |
| Favorit | Control | 36.8 | 100 | 38 | 100 | 21.2 | 100 | 0.28 | 100 |
| | Hydric stress (2 weeks) | 35.6 | 96.74 | 21.4 | 56.32 | 6.4 | 30.19 | 0.11 | 39.6 |
| Justin | Control | 34.4 | 100 | 29.2 | 100 | 9.4 | 100 | 0.15 | 100 |
| | Hydric stress (2 weeks) | 17.4 | 50.58 | 18 | 61.64 | 6.6 | 70.21 | 0.19 | 126.1 |
| Romina | Control | 28.8 | 100 | 18.8 | 100 | 7.6 | 100 | 0.16 | 100 |
| | Hydric stress (2 weeks) | 15.6 | 54.17 | 18.2 | 96.81 | 7.2 | 94.74 | 0.21 | 133.4 |
| Splendor | Control | 29.4 | 100 | 31.2 | 100 | 22.4 | 100 | 0.37 | 100 |
| | Hydric stress (2 weeks) | 21.4 | 72.79 | 23.4 | 75.00 | 10.4 | 46.43 | 0.23 | 62.8 |

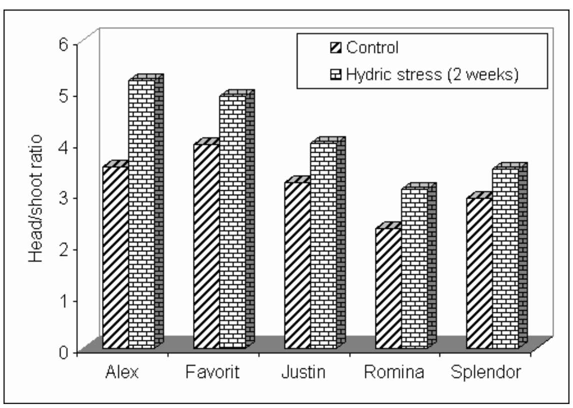


*Figure 4.* The effect of hydric stress on head weight/ shoot weight ratio of the tested sunflower hybrids

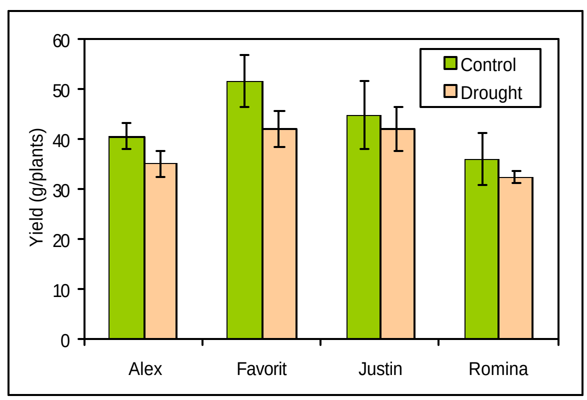


*Figure 5.* The effect of hydric stress on yield of the tested sunflower hybrids

seeds composition in fatty acids is also affected by water stress (Baldini et al., 2000).

Grain yield was affected by hydric stress with the low status treatment yielding 10-13% less than the control (Figure 5). The highest

This suggests that although in Justin hybrid most part of the nutritive reserve is conducted to root development, the yield is not influenced.

The thousand seed weight (TKW) of Justin decreases under drought conditions as compared with Favorit. The TKW of Alex hybrid is diminished under drought conditions, too (Figure 6).

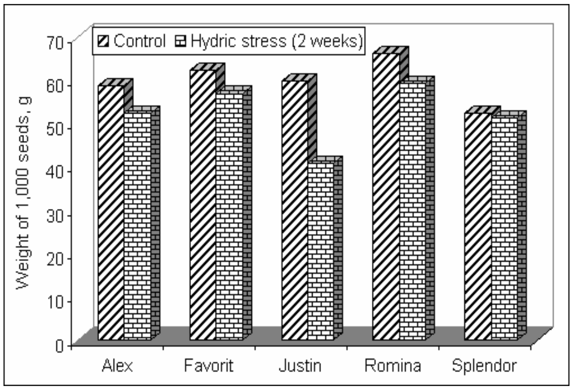


*Figure 6*. The effect of hydric stress on TSW of sunflower hybrids

A significant correlation has been found between diameter of head and root/shoot ratio (Figure 7).

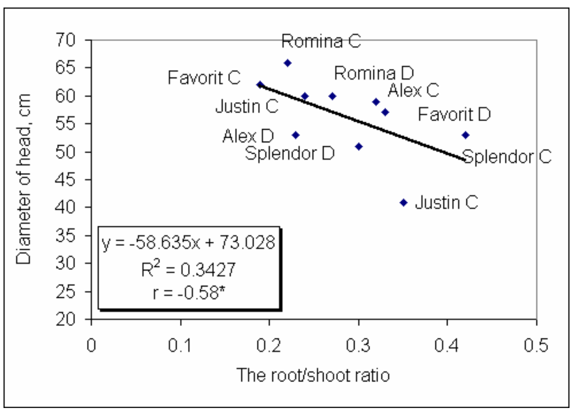


*Figure 7.* The relationships between diameter of head and root/shoot ratio of sunflower seedlings

The peroxidase activity increases in sunflower plants under hydric stress (for peroxidase determination the supplementary hydric stress was induced with 15% PEG for six hours) (Table 5) and some modifications in isoenzime pattern were registered (Figure 8).

*Table 5*. Peroxidase activity (U guaiacol oxidized/min/mg proteins/g dry matter)

| Genotypes | Control | Drought |
|---|---|---|
| Alex | 10,50 | 14,15 |
| Favorit | 12,50 | 16,85 |
| Justin | 9,50 | 12,25 |
| Select | 6,35 | 14,35 |
| Performer | 7,45 | 12,50 |

The adverse environmental growth conditions are reported to induce a degrading process in plants as a consequence of a generating partially reduced oxygen (activated oxygen). It is now well documented that drought conditions, pathogen attack or atmospheric pollution lead to perturbation of the redox state towards an oxidative metabolism within plant cell (Vanacker et al., 1988; Ranieri et al., 1999).

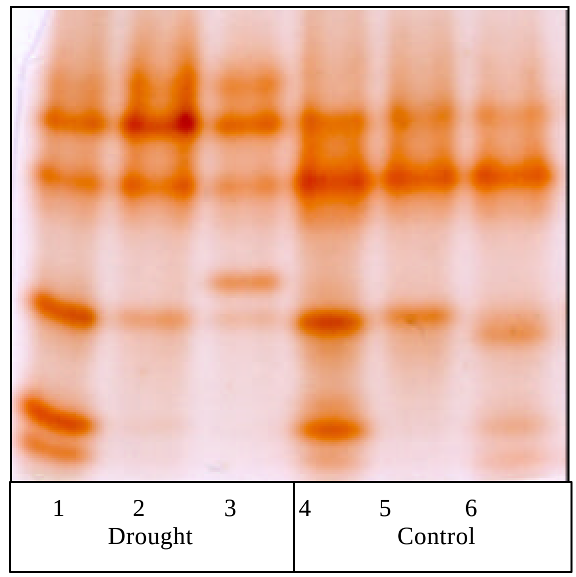


*Figure 8*. Isoenzyme of peroxidase for three sunflower hybrids: Favorit (1), Justin (2), Select (3)

Under stress conditions the enhanced of peroxidase activity stimulated cell wall stiffening, probably reducing cell growth which might represent a mechanical adaptation to adverse conditions (Castillo et al., 1984, 1987).

The saturated fatty acid contents (palmitic and stearic acids) were insignificantly affected by water stress. The palmitic acid concentration increases under two weeks hydric stress conditions (from 6% for Favorit to 12% for Alex hybrid) and stearic acid concentration decreased under the same conditions (from 2 % for Alex to 29% for Favorit hybrid respectively) (Table 6).

There was a significant negative effect of drought on the oleic acid concentration in all tested sunflower hybrids. The decrease was more obvious after two weeks of hydric stress for Alex and Justin (37 and 30% respectively) than for Favorit hybrid (13%) (Tables 6 and 7).

reduction of about 15% in the concentration of oleic acid in standard hybrids.

*Table 6.* The effect of hydric stress (1 week) on fatty acid composition in several Romanian sunflower hybrids

| Fatty acids | Experimental variants | Alex | | Favorit | | Justin | | Romina | | Splendor | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % from total area | diffe-rences % | % from total area | diffe-rences % | % from total area | diffe-rences % | % from total area | diffe-rences % | % from total area | diffe-rences % |
| Palmitic acid | Control | 6.58 | 100 | 5.82 | 100 | 6.38 | 100 | 7.14 | 100 | 6.09 | 100 |
| | Hydric stress | 6.72 | 102 | 6.3 | 108 | 6.28 | 98 | 6.94 | 97 | 6.09 | 100 |
| Stearic acid | Control | 6.57 | 100 | 4.32 | 100 | 3.6 | 100 | 4.41 | 100 | 6.41 | 100 |
| | Hydric stress | 5.63 | 86 | 3.89 | 90 | 4.05 | 113 | 5.37 | 122 | 5.02 | 78 |
| Oleic acid | Control | 36.91 | 100 | 52.09 | 100 | 44.92 | 100 | 29.78 | 100 | 52.03 | 100 |
| | Hydric stress | 33.28 | 90 | 35.27 | 68 | 35.54 | 79 | 29.32 | 98 | 38.59 | 74 |
| Linoleic acid | Control | 46.45 | 100 | 34.89 | 100 | 43.44 | 100 | 56.1 | 100 | 32.89 | 100 |
| | Hydric stress | 51.57 | 111 | 52.11 | 149 | 50.92 | 117 | 55.7 | 99 | 47.38 | 144 |

*Table 7.* The effect of hydric stress (2 weeks) on fatty acid composition in several Romanian sunflower hybrids

| Fatty acids | Experimental variants | Alex | | Favorit | | Justin | | Romina | | Splendor | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % from total area | differ-ences % | % from total area | differ-ences % | % from total area | differ-ences % | % from total area | differ-ences % | % from total area | differ-ences % |
| Palmitic acid | Control | 6.36 | 100 | 6.12 | 100 | 6.03 | 100 | 7.1 | 100 | 5.47 | 100 |
| | Hydric stress | 7.1 | 112 | 6.51 | 106 | 6.66 | 110 | 7.79 | 110 | 6.06 | 111 |
| Stearic acid | Control | 6.24 | 100 | 4.52 | 100 | 4.05 | 100 | 4.23 | 100 | 5.07 | 100 |
| | Hydric stress | 6.1 | 98 | 3.19 | 71 | 3.85 | 95 | 3.23 | 76 | 4.29 | 85 |
| Oleic acid | Control | 38.09 | 100 | 31.47 | 100 | 33.59 | 100 | 28.15 | 100 | 35.95 | 100 |
| | Hydric stress | 23.84 | 63 | 27.35 | 87 | 23.59 | 70 | 20.34 | 72 | 27.94 | 78 |
| Linoleic acid | Control | 46.45 | 100 | 60.18 | 100 | 54.64 | 100 | 57 | 100 | 51.72 | 100 |
| | Hydric stress | 60.53 | 130 | 61.54 | 102 | 64.11 | 117 | 68.09 | 119 | 59.02 | 114 |

In contrast, the linoleic acid concentration increases in drought variants. The proportion of linoleic acid in fatty acid composition ranges between 59.02–64.11%.

Because the late sunflower hybrids are more sensitive to hydric stress, it is possible to accelerate the maturation of seed process, so under these conditions the linoleic acid concentration increases under hydric stress conditions. The Justin hybrid (the latest sunflower hybrid of this study) presents the most obvious differences concerning oleic acid: linoleic acid ratio (the normal value of this ratio in mature seeds is 1:2). But, in this case is difficult to explain the severe modification of oleic acid : linoleic acid ratio to Alex hybrid (early hybrid).

The research work of Baldini et al. (2000) revealed that water stress causes a significant

## CONCLUSIONS

The reduction of leaf area, shoot size and biomass accumulation of sunflower seedlings under hydric stress conditions determined the increase of root/shoot ratio. This suggests that for young plants the main sink is the survival. In late stage of vegetation, the root/shoot ratio decreases under drought stress in some hybrids but increases in other hybrids, this suggesting that for mature plants the main sink is the yield.

The hydric stress has induced changes in peroxidase activities and perhaps the hydric stress accelerates the maturation of seeds, the another enzyme leading to the modifications of fatty acid composition of seeds.

## Acknowledgements

***This research was sponsored by the Romanian Education and Research Ministry (Grant 6172/2000) and DAAD Research Fund (No. A/01/18349). We gratefully acknowledge Prof. F. Marx (Institute for Food Science, Bonn) and Prof. Schnabl's team for technical assistance (Institute of Agricultural Botany, Bonn).***

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Table 6. The effect of hydric stress (1 week) on fatty acid composition in several Romanian sunflower hybrids

| Fatty acids | Experi-mental variants | Alex | | Favorit | | Justin | | Romina | | Splendor | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % from total area | diffe-r-ences % | % from total area | diffe-rences % | % from total area | diffe-rences % | % from total area | diffe-rences % | % from total area | diffe-rences % |
| Palmitic acid | Control | 6.58 | 100 | 5.82 | 100 | 6.38 | 100 | 7.14 | 100 | 6.09 | 100 |
| | Hydric stress | 6.72 | 102 | 6.3 | 108 | 6.28 | 98 | 6.94 | 97 | 6.09 | 100 |
| Stearic acid | Control | 6.57 | 100 | 4.32 | 100 | 3.6 | 100 | 4.41 | 100 | 6.41 | 100 |
| | Hydric stress | 5.63 | 86 | 3.89 | 90 | 4.05 | 113 | 5.37 | 122 | 5.02 | 78 |
| Oleic acid | Control | 36.91 | 100 | 52.09 | 100 | 44.92 | 100 | 29.78 | 100 | 52.03 | 100 |
| | Hydric stress | 33.28 | 90 | 35.27 | 68 | 35.54 | 79 | 29.32 | 98 | 38.59 | 74 |
| Linoleic acid | Control | 46.45 | 100 | 34.89 | 100 | 43.44 | 100 | 56.1 | 100 | 32.89 | 100 |
| | Hydric stress | 51.57 | 111 | 52.11 | 149 | 50.92 | 117 | 55.7 | 99 | 47.38 | 144 |

*Table 7.* The effect of hydric stress (2 weeks) on fatty acid composition in several Romanian sunflower hybrids

| Fatty acids | Experi-mental variants | Alex | | Favorit | | Justin | | Romina | | Splendor | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % from total area | Differences % | % from total area | Differ-ences % | % from total area | Differ-ences % | % from total area | Differ-ences % | % from total area | Differ-ences % |
| Palmitic acid | Control | 6.36 | 100 | 6.12 | 100 | 6.03 | 100 | 7.1 | 100 | 5.47 | 100 |
| | Hydric stress | 7.1 | 112 | 6.51 | 106 | 6.66 | 110 | 7.79 | 110 | 6.06 | 111 |
| Stearic acid | Control | 6.24 | 100 | 4.52 | 100 | 4.05 | 100 | 4.23 | 100 | 5.07 | 100 |
| | Hydric stress | 6.1 | 98 | 3.19 | 71 | 3.85 | 95 | 3.23 | 76 | 4.29 | 85 |
| Oleic acid | Control | 38.09 | 100 | 31.47 | 100 | 33.59 | 100 | 28.15 | 100 | 35.95 | 100 |
| | Hydric stress | 23.84 | 63 | 27.35 | 87 | 23.59 | 70 | 20.34 | 72 | 27.94 | 78 |
| Linoleic acid | Control | 46.45 | 100 | 60.18 | 100 | 54.64 | 100 | 57 | 100 | 51.72 | 100 |
| | Hydric stress | 60.53 | 130 | 61.54 | 102 | 64.11 | 117 | 68.09 | 119 | 59.02 | 114 |