# RELATIONSHIP BETWEEN STOMATAL CONDUCTANCE AND DROUGHT SUSCEPTIBILITY INDEX IN ALFALFA (*Medicago sativa* L.)

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**Abstract**

Global climate change evidentiate an increase in drought frequencies. In this context the strategies for sustainable use of water and drought resistance improvement based on the physiological traits are important and physiological approaches should be integrated in conventional breeding. Research was performed on 16 alfalfa genotypes under vegetation house conditions at two watering levels and field analyses. The objective was to identify the available genetic variation and to establish efficient physiologycall traits for testing which might positively influence alfalfa performance under drought conditions. Our research were focused on stomatal conductance and chlorophyll content. There is a very significant negative correlation between the drought sensitivity index and the stomatal conductance of alfalfa genotypes sown for fodder (r = - 0.70***). This means that genotypes with a higher stomatal conductance were more productive (low drought sensitivity index means better drought adaptability, so higher production) indicating that stomatal resistance has a very significant impact on production under stress conditions. There is a very significant positive correlation between the chlorophyll content and the stomatal conductance of alfalfa genotypes from the comparative culture sown for fodder (r = 0.79***), which shows that stomatal closure is the main factor limiting photosynthetic activity under water limiting conditions.

**Key words**: alfalfa, stomatal conductance, chlorophyll content, drought susceptibility index

Lucerne (*Medicago sativa* L.) is one of the most important fodder plants in Romania and in the world. It is known that alfalfa has the greatest potential for protein production among all legumes and cereals. Together with its high nutritional value for animals, it brings services to ecosystems by fixing atmospheric nitrogen. However, lack of soil moisture and frequent drought limit the establishment, persistence and production of alfalfa crops (Kang Y. *et al*, 2011; Misar C.G. *et al*, 2015). This is predominant in the non-irrigation conditions in the south and south-east of our country.

With a growing demand for water resources due to an increase in human population and industrial water use, plus frequent drought due to climate change, irrigation water has become increasingly scarce and expensive. To sustain high production of alfalfa with limited water resource, alfalfa cultivars with improved water use efficiency is urgently needed. As a first step, physiological evaluation of alfalfa germplasm variability in water stress is required for the development of drought tolerant varieties.

Under conditions of water stress, the conductance of the stomata is affected, the plants trying to avoid excessive water loss by closing the stomata. Stomatal conductance regulates $CO_2$ absorption and water loss from plants. Drought-tolerant plants can regulate their stomatal conductance in drought conditions to limit water loss (Montague T. *et al*, 2008). Relative recent studies have shown that there is a correlation between the stomatal conductance of alfalfa genotypes and production in both optimal water supply and water stress conditions.

The ability of plants to maintain their chlorophyll content in drought conditions is an adaptation of plants to continue to perform photosynthesis, necessary to support growth, especially root growth. Deterioration of chlorophyll content is considered an indicator of early leaf senescence (Kang Y. *et al*, 2011).

The aim of the research was to investigate the effect of stomatal conductance and chlorophyll content related to alfalfa production under hydric stress, useful for the alfalfa breeding program.

## MATERIAL AND METHOD

Thirteen alfalfa genotypes were studied.

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Alfalfa genotypes were sown in 10 l capacity vegetation pots, in soil (cambic chernozem): sand mixture.

The plants were maintained in optimal soil moinsture (70% of the soil water capacity) for three weeks. Then the water stress variant (three pots of each genotype) was watered for 12 days at 40% of the soil water capacity.

Biomass accumulation was determined by gravimetric weighing. The drought sensitivity index (ISS) was calculated based on biomass production. The calculation formula proposed by Fischer and Maurer (1978) was used:

$$ISS = (1-Ys / Ym) / (1-YS / YM),$$

where Ys and Ym represent the production of the genotype in drought respectively optimal conditions and YS and YM represent the average production of all genotypes studied in drought respectively optimal conditions (unstressed plants).

Genotypes with low values of this index are assumed to be tolerant or resistant because they show a decrease of production more less under stress conditions compared to normal conditions compared to the average of all genotypes studied.

In the experimental field of the Lucerne Breeding Laboratory, determinations of stomatal conductance were performed on the genotypes studied in the comparative culture sown as fodder. The determinations were performed after a period of more than 15 days in which no precipitation was recorded.

The AP4 Porometer was used to determine the stomatal conductance. The device determines the stomatal opening based on the stomatal conductance of the leaves, that is, the rate of evaporation of water through the stomata.

The chlorophyll content was determined using the SPAD 502 Minolta portable device.

The values determined by this instrument indicate the relative sum of the amount of chlorophyll present (expressed in SPAD units) in the leaves of the plant, measured by the transmittance of the leaf at two wavelengths, 650 nm (red) and 940 (near infrared - NIR).

## RESULTS AND DISCUSSIONS

Anova analysis for stomatal conductance in comparative culture of alfalfa for fodder shows the significant distinct effect of genotype on this character *(table 1).*

Table 1

**ANOVA analysis for stomatal conductance. Comparative culture for fodder**

| Source of variance | Sum of squares | FD | Mean of squares | F factor |
|---|---|---|---|---|
| Genotyp | 68087.08974 | 12 | 5673.924145 | 2.495** |
| Erorr | 88686.05556 | 39 | 2274.001425 | |

The studied alfalfa genotypes had a different stomatal conductance, from 241 to 463 mmol /m$^2$/s. The mean values for stomatal conductance were 329 mmol / m$^2$/s. Of the studied genotypes F 2610 -17 and F 2611 - 17 presented the lowest values (241, 262 mmol / m$^2$/s) and F 2616 -17 the highest value (400 mmol / m$^2$/s), *(figure 1).*

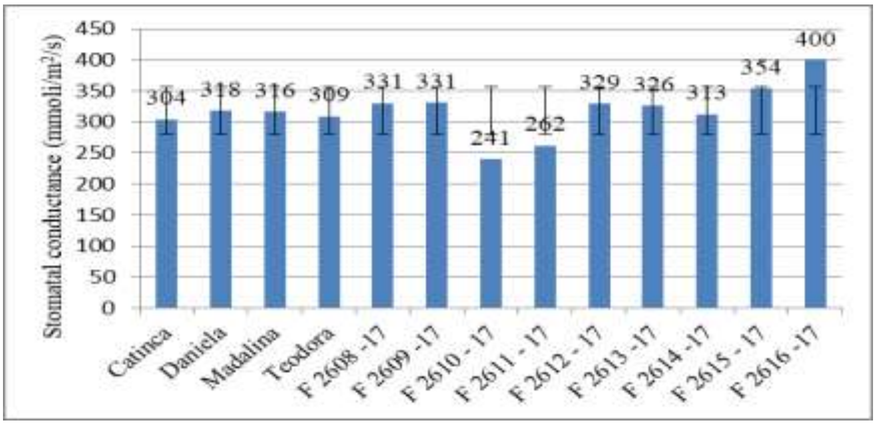


Figure 1 **Stomatal conductance of the studied alfalfa genotypes. Comparative culture for fodder**

The existence of a variability in terms of stomatal conductance shows the different capacity of the genetic material studied in the management of water resources. The genotypes F 2610 -17 and F 2611 - 17 genotypes have a lower stomatal conductance (94-111 mmol / m$^2$/s), which could suggest that they are better adapted to regulate water loss through transpiration, in given environmental conditions. But, on the other hand, it must be borne in mind that alfalfa production is directly related to evapotranspiration. The obtained results show that those genotypes had a higher sensitivity index and between the drought sensitivity index and the stomatal conductance (determined in the field, without water stress) there is a negative correlation *(figure 2).*

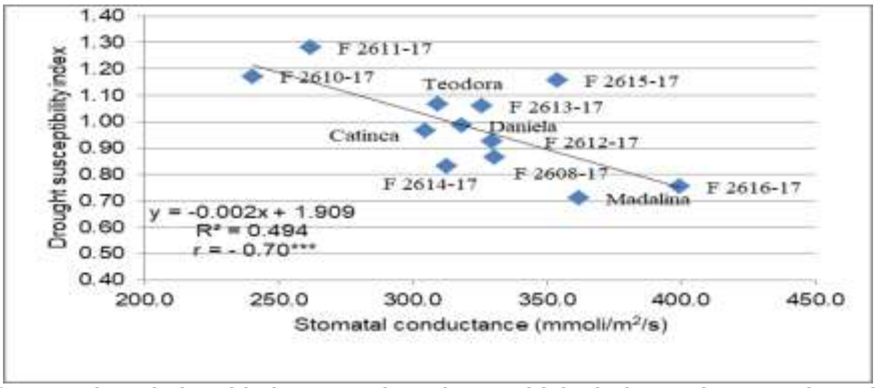

Figure 2 **The relationship between drought sensitivity index and stomatal conductance**

This means that genotypes with a higher stomatal conductance were more productive (low drought sensitivity index means better drought adaptability, so higher production).

Moreover, data from the literature show that high values of stomatal conductance can positively affect the absorption of $CO_2$ and, therefore, plant productivity (Patakas A. *et al*, 2003).

Under the tested conditions, the chlorophyll content had quite high values in almost all the alfalfa genotypes studied, which indicates a delay of leaf senescence and continued photosynthesis *(figure 3).*

Genotype F 2610-17 showed a lower chlorophyll content (30 SPAD units) compared to the Daniela, Madălina, F 2616-17 genotypes (over 35 SPAD units) *(figure 3).*

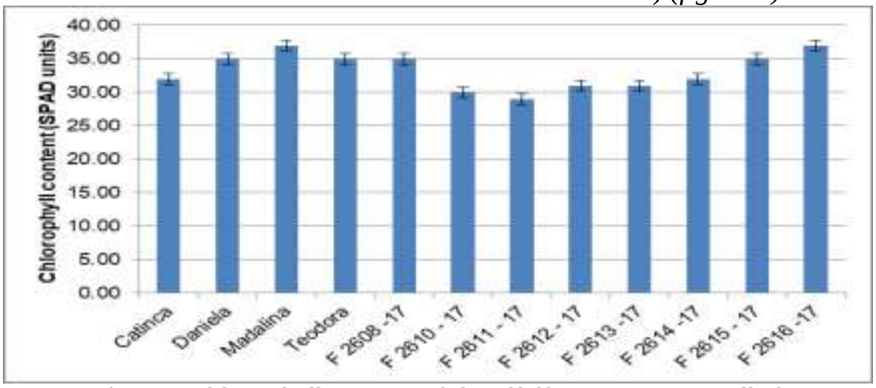

Figure 3 **Chlorophyll content of the alfalfa genotypes studied**

Correlation analysis indicated that there was a very significant positive relationship between the chlorophyll content and the stomatal conductance of alfalfa genotypes in the comparative culture for fodder. (r = 0.79***; *figure 4).*

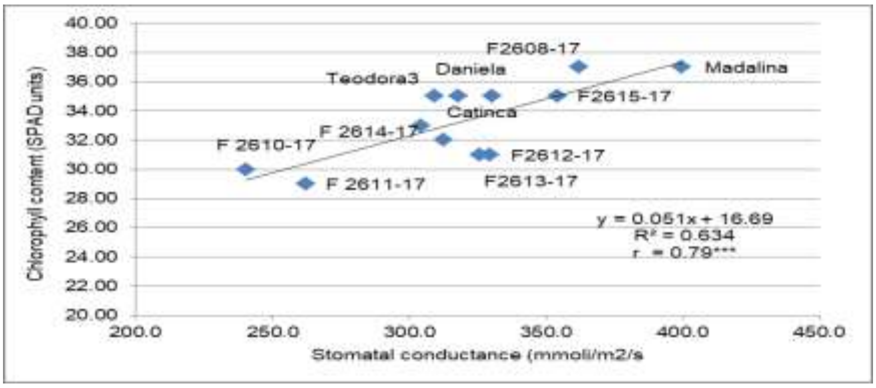

Figure 4 **The relationship between stomatal conductance and chlorophyll content. Comparative culture for fodder**

This result is confirmed by literature data showing that stomatal closure is the main factor limiting photosynthetic activity under moderate water limiting conditions (Chaves M.M. *et al*, 2003).

Decrease of the stomatal conductance will limit photosynthetic assimilation as stomatal conductance is closely related to photosynthesis. Similarly, changes in the chlorophyll content of the leaves will also affect photosynthesis, hence plant growth and plant yield. Thus, the importance of assessing stomatal conductance and chlorophyll content in plant physiological studies.

Overall, it is obvious that drought-resistant genotypes (low susceptibility index, Mădălina, F 26-16-17, F 2608-17, F 2612-17, Catinca and Daniela) had a high stomatal conductance and a higher chlorophyll content compared to sensitive ones (F 2610-17 and F 2611-17) *(table 2).*

When there is a plentiful supply of water it is an advantage to have a small response to stomatal conductance, because this reduces the tendency of the stomata to restrict the supply of $CO_2$ for photosynthesis. However, if water supplies are limited, the plant's priority changes from maximizing assimilation to restricting transpiration while maintaining as much assimilation as possible.

Table 2

**Drought susceptibility index, stomatal conductance and chlorophyll content of studied alfalfa genotypes**

| Genotype | Drought susceptibility index | Stomatal conductance | Chlorophyll content |
|---|---|---|---|
| Madalina | 0.71 | 362.0 | 37.00 |
| F 2616 -17 | 0.76 | 399.6 | 37.00 |
| F 2614 -17 | 0.83 | 312.5 | 32.00 |
| F 2608 -17 | 0.87 | 330.5 | 35.00 |
| F 2612 - 17 | 0.92 | 329.4 | 31.00 |
| Catinca | 0.97 | 304.4 | 33.00 |
| Daniela | 0.99 | 318.1 | 35.00 |
| F 2613 -17 | 1.06 | 325.8 | 31.00 |
| Teodora | 1.07 | 309.3 | 35.00 |
| *F 2615 - 17* | *1.16* | *354.1* | *35.00* |
| F 2610 - 17 | 1.17 | 240.5 | 30.00 |
| F 2611 - 17 | 1.28 | 262.0 | 29.00 |

There is an exception, genotype F 2615-17, which is relatively sensitive to drought but has higher values of stomatal conductance (356) and chlorophyll (35), similar to resistant genotypes *(table 2).* This is a good thing for breeders because deviations from the rule are very valuable.

## CONCLUSIONS

In conclusion, there was a negative relationship between stomatal conductance and drought susceptibility index and positive relationships with chlorophyll content of alfalfa genotypes.

The information and results presented in this study will be useful as baseline information for the constitution of synthetics on base of the physiologycal traits.

## ACKNOWLEGMENTS

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## REFERENCES

**Chaves M.M., Maroco J.P., Pereira J.S., 2003 -** *Understanding plant responses to drought: from genes to the whole plant*. Functional Plant Biology, 30:239-264.

**Fischer R.A., Maurer R., 1978** - *Drought resistance in spring wheat cultivars. I. Grain yield response*. Aust. J. Agric. Res., 29: 897-907.

**Kang Y., Han I., Torres-Jerez M., Wang Y., Tang M., Monteros M., Udvardi M., 2011** - *System responses to long-term drought and re-watering of two contrasting alfalfa varieties.* Plant Journal, 68: 871-889.

**Misar C.G., Xu L., Gates R.N., Boe A., Johnson P.S., 2015** - *Stand Persistence and Forage Yield of 11 Alfalfa (Medicago sativa) Populations in Semiarid Rangeland*. Rangeland Ecology and Management, 68:79-85.

**Montague T., Hellman E., Krawitzky M., 2008 -** *Comparison of greenhouse grown, containerized grapevine stomatal conductance measurements using two differing porometers*. Proceedings of the 2nd Annual National Viticulture Research Conference, July 9-11, University of California Davis.

**Patakas A., Stavrakas D., Fisarakis I., 2003 -** *Relationship between $CO_2$ assimilation and leaf anatomical characteristics of two gravepine cultivars.* Agronomie, 23:93-296.