FOLIAR PIGMENTS AND THEYR DYNAMICS DURING DIFFERENT VEGETATION PHENOPHASES IN FIVE CULTIVARS OF *CANNABIS SATIVA* L.

Mihai Alexandru LUCA[1], Constantin GAUCA[2], Liana Doina TOMA[1], Constantin LEONTE[1]
E-mail : luca_mihai_alexandru@yahoo.com

Abstract

The pigments dynamics of leaves were analyzed in five cultivars of *Cannabis sativa* L. during different growth phases. The photosynthetic and flavonoid pigments content were appreciated spectrophotometrically by light absorption of the acetone pigment extract (1%). During intense growth the middle floor leaves play an important role in light absorption and assimilates synthesis, although a certain cultivar from the five studied presents a different behavior. In the dioecious cultivars light absorption and photosynthesis intensity is higher than in the studied monoecious hemp cultivars. The leaves under the inflorescence have an important role in light absorption from the beginning of flowering until fruit formation.

Key words: hemp, dioecious, monoecious, photosynthesis, chlorophyll, flavonoids

Photosynthesis represents the main carbon and energy source available to plants (Toma L.D., et. al., 2002) the light energy being stored as carbohydrates through a series of electron transfers taking place on the thylakoids membranes of chloroplasts (Ustin S.L., et. al., 2009), more than 90% of the dried mass of a plant being derived from the photosynthetic assimilation of CO_2 (Zelitch cited by Chandra S., 2008).

During photosynthesis chlorophylls *a* and *b* are necessary, because they control the quantity of solar radiation that a leaf can absorb (Blackburn B.A., 2007), being indicators of different growth phases. Flavonoids represent another group of foliar pigments. The concentration of flavonoids in leaves offer indices regarding the physiological reaction and plant resistance to stress factors (Toma L.D., et.al., 2002), even though there is not an unifying explanation to their presence and function (Blackburn B.A., 2007).

Hemp cultivation was prohibited in most countries because of it's psychoactive effects (Schumann, E., et. al., 1999) but in recent times it gains a grater importance for Europe (Ranalli P., 2004) because of it's multiple uses (Sandru I.D., et. al.,1996; Small E. and Marcus D., 2002; Zhijian Li, et. al, 2004).

In the present paper we study some indices of the photosynthesis process, represented by the foliar pigments, in order to determine some aspects regarding the physiological reaction of *Cannabis sativa* plants to the climatic condition of the agricultural year 2008-2009.

MATERIAL AND METHOD

Five industrial hemp cultivars were grown in field conditions at the Agricultural Research and Development Station – Secuieni, Neamt in 2008-2009. Fresh leaves were harvested in the morning and transported in cold conditions at the "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine – Iasi for foliar pigment analyses during different vegetation phases: intense growth, beginning of flowering, full flowering and fruit formation. The content of different type of foliar pigment was appreciated based on the light absorption capacity of the acetone extract (1%), analyzed at the computer assisted spectrophotometer (Toma Liana Doina et al., 2002).

The evaluated pigments were: chlorophyll *a* 663, for appreciating the photosynthesis in the reaction center, chlorophyll *a* 430 and chlorophyll *b* 453, components of the absorption center within the photosynthetic systems and the flavonoids with maximum absorption in the close UV radiation zone, pigments that have protective properties to stress factors.

RESULTS AND DISCUSSIONS

The analyses of chlorophyll *a* 663 content, depending on cultivar and leaf floor, during the intense growth period of hemp plants *(fig. 1)*

[1] University of Agricultural Sciences and Veterinary Medicine, Iasi
[2] Agricultural Research and Development Station, Secuieni-Neamt

indicates a modification of this pigment following the uniapical curve model, with a peak that corresponds to the mature leaves situated between the 3rd and 5th floor.

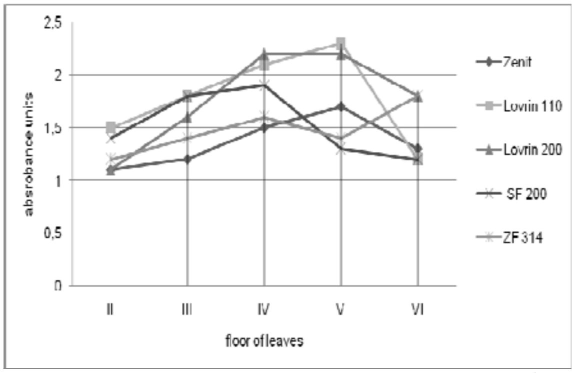

Figure 1 **Light absorption by acetone extract of pigments (1%) in 663 nm wavelenght, dependindg on floor of leaves, in five cultivars of *Cannabis sativa* L.**

The highest values are recorded in the case of Lovrin 110 cultivar on the 5th floor of leaves and Lovrin 200 – the 4th floor. Lower values are recorded for the leaves of the 2nd floor due to senescence (Blackburn B.A., 2007) and the 6th floor where the leaves are young. However the monoecious hemp cultivar ZF 314 shows a different dynamic of chlorophyll *a* 663, presenting a maximum photosynthesis intensity in the reaction center for the the 6th floor of leaves. Although Lovrin 200 hemp cultivar follows the uniapical curve model, it shows a high content of chlorophyll *a* 663 in the young leaves of the 6th floor.

In the case of chlorophyll *a* 430 the pigment content dynamics across the leaves floors register the same uniapical curve allure (data not shown), with maximum values for the 4th and the 5th floor. Stands out the same different behavior for ZF 314 cultivar, a growth of the light absorption capacity in the young leaves.

Another important component of the light absorption center, chlorophyll *b* 453, shows a similar dynamic, depending on cultivar and floor leaves *(fig. 2)*.

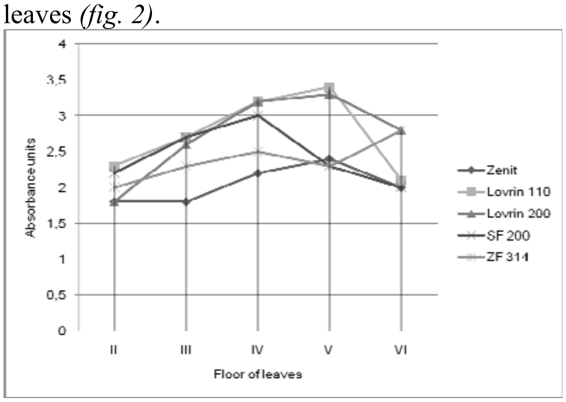

Figure 2 **Light absorption by acetone extract of pigments (1%) in 453 nm wavelenght, dependindg on floor of leaves, in five cultivars of *Cannabis sativa* L.**

The comparative analyses of the different foliar pigments for the five studied cultivars indicates a higher content of chlorophyll *a* 430 and *b* 450, the main components of the light absorption center, and a lower content of chlorophyll *a* 663 *(fig. 3)*.

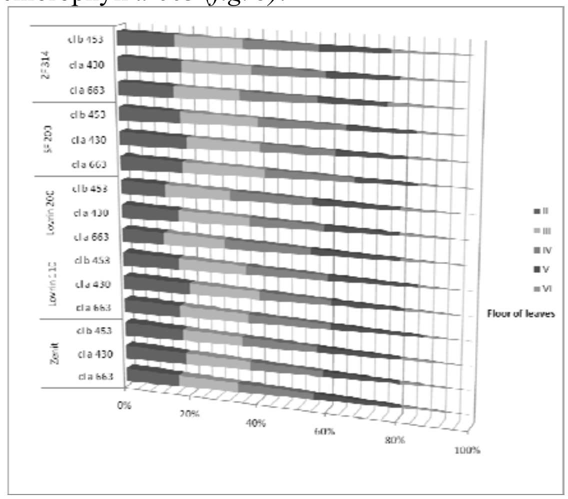

Figure 3 **Foliar pigment content, apreciated by light absorption of the acetone pigments extract (1%) in the visible spectrum and close UV during intense growth phase in five cultivars of *Cannabis sativa* L.**

The presence of a maximum pigment content in the leaves of the middle part of the plant is well known (Gopal N.H., 1973), the growth of the chlorophyll *a* and *b* content in the upper leaves floor in the case of ZF 314 cultivar may be a reaction to favorable climatic condition for this specific cultivar (Parnikoza I.Yu., et. al., 2011).

The comparative analyses of chlorophyll *a* 663 content in leaves from different floors indicates a maximum photosynthetic intensity in the mature leaves of dioecious hemp cultivars Lovrin 110 and Lovrin 200, and much reduced in the case of the studied monoecious hemp cultivars Zenit, ZF 314 and SF 200. The same behavior is manifested also for the main component pigments of the absorption center, indicating a higher light absorption capacity for the dioecious hemp cultivars compared with the monoecious ones, therefore a higher photosynthesis maximum (Chandra S., et.al., 2011) in the case of the dioecious cultivars.

The flavonoid pigments content during intense growth phase depending on the leaves floors shows a similar dynamic with the photosynthetic pigments *(fig. 4)*. The maximum values is registered for the 3rd and 5th floors. This suggests a maximum physiological capacity of the mature leaves in the middle leaves of the plant (Toma L.D., et. al., 2002), and the genetic variability of different cultivars regarding the answer to climatic factors (Daymond A.J., Hadley P., 2004).

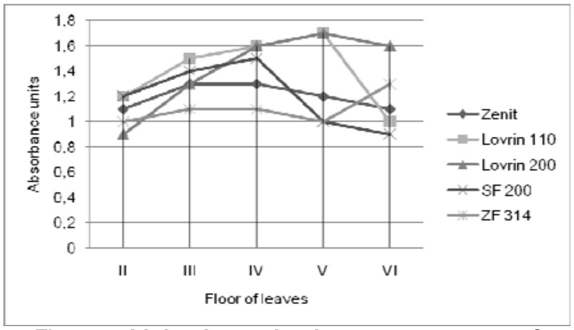

Figure 4 **Light absorption by acetone extract of pigments (1%) in 320 nm wavelenght, dependindg on floor of leaves, in five cultivars of *Cannabis sativa* L.**

The dynamics of chlorophyll *a* 663 from the beginning of flowering until fruit formation, for the five studied cultivars, indicates a maximum content during fruit formation for all cultivars (Gopal N.H., 1973) except the dioecious hemp cultivar Lovrin100.

The middle floor leaves show a superior photosynthetic intensity compared to the leaves under the inflorescence, suggesting their contribution to the synthesis and transport of assimilates.

The light absorption pigments content shows reverse dynamic compared with chlorophyll *a* 663. The chlorophylls *a* 430 and *b* 454 content has maximum values during flowering and minimum during fruit formation, except Lovrin 110 cultivar.

Light absorption, since flowering begins until fruit formation has a greater intensity in the leaves under the inflorescence wile maximum of photosynthesis in the reaction center is identified in the middle floor leaves (data not shown).

The flavonoids content is highest during flowering for Zenit and Lovrin 200 cultivars, while Lovrin 100 and SF 200 register a maximum value during fruit formation.

The foliar pigments dynamic through the whole vegetation period indicates a photosynthetic efficiency in the intense growth period of the studied cultivars, a period that represents half of the entire hemp plant life during which the plants reach 80-90% of the entire length (Gauca C., 1995) *(fig. 5, 6, 7)*.

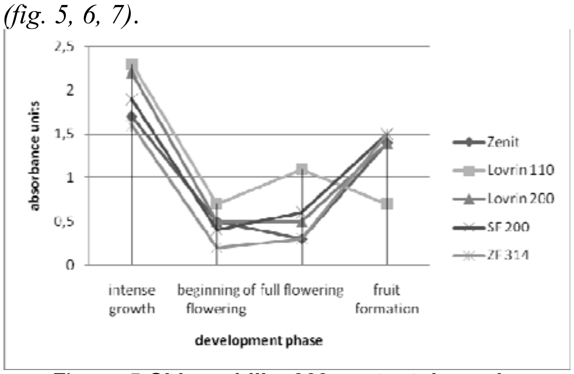

Figure 5 **Chlorophill *a* 663 content dynamic, apreciated by light absorption of the acetone pigments extract (1%), during different development phases in five cultivars of *Cannabis sativa* L.**

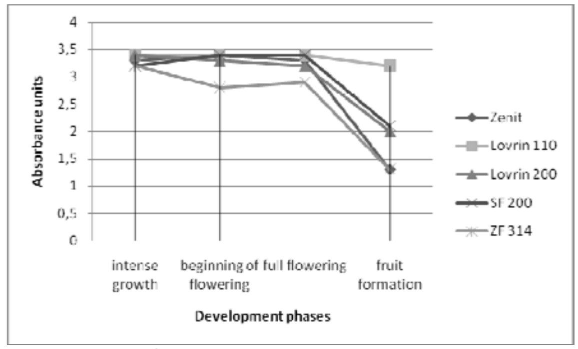

Figure 6 **Chlorophill *a* 430 content dynamic, apreciated by light absorption of the acetone pigments extract (1%), during different development phases in five cultivars of *Cannabis sativa* L.**

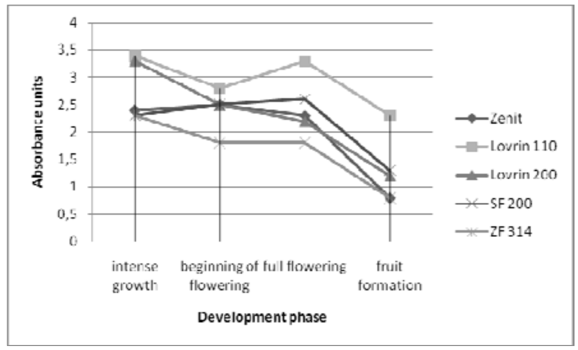

Figure 7 **Chlorophill *b* 453 content dynamic, apreciated by light absorption of the acetone pigments extract (1%), during different development phases in five cultivars of *Cannabis sativa* L.**

CONCLUSIONS

The role of the middle floor leaves in light absorption and assimilates synthesis during intense growth phase in *Cannabis sativa* L. is revealed, being observed the model described in literature (Gopal N.H., 1973). The different behavior of monoecious hemp cultivar ZF 314, namely a higher photosynthetic intensity and light absorption capacity in the young leaves of the superior floor during this phase may be explained as a genotype reaction to some favorable climatic conditions (Parnikoza I.Yu., 2011), but for a better understanding of this behavior further nondestructive experiments over a longer period of time need to be conducted.

The difference between genotypes regarding chlorophyll content is well known (Daymond, A.J., Hadley P., 2004). In the study conducted by us over the five hemp cultivars reveals that the chlorophyll *a* and chlorophyll *b* content is higher in dioecious hemp plants, thus a greater light absorption capacity and photosynthesis intensity.

Photosynthesis efficiency for all studied cultivars reaches it's maximum during intense growth. High values are recorded also during fruit formation, with the exception of Lovrin 110 cultivar.

From beginning of flowering to fruit formation the highest values for photosynthesis intensity in the middle floor leaves, while light absorption is greater in the leaves under the inflorescences, leaves that have an important role also in insuring resistance to stress factors. However, because different explanations exist for the presence and role of the flavonoids (Blackburn B.A., 2007), and also the favorable climatic conditions for hemp in 2009, climatic controlled experiments need to be done in order to determine the role of the flavonoids in counter stress factors in *Cannabis sativa* L.

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