T1992PHY13

# Changes in RuBisCO activity and net photosynthesis under several crop managements applied to sunflower (Helianthus annuus L., cv Topflor).

*Martinez E., Bouniols A.*, Cavalié G.; Blanchet R., Piquemal G.*

* *INRA - Station d'Agronomie, B.P. 27, 31326 - Castanet-Tolosan cedex, France,and Université Paul Sabatier - Centre de Physiologie Végétale, 118 route de Narbonne, 31077-Toulouse cedex, France.*

## SUMMARY

As the RuBisCO enzyme plays a main contribution in the carbon fixation and is involved in the primary process of photosynthesis, its activity largely influences the yield performances.

However, no data exist that represent in field conditions the changes in enzyme content or activity, specially when the sunflower crops are conducted through various crop managements by water and nitrogen supplies, herbicides and fungicides treatments. Changes in RuBisCO content and activity were monitored by immuno-electrophoresis and radiochemical determination, respectively. Net photosynthesis was estimated in parallel by CO2 exchange rate method. The determinations concerned leaves of the medium canopy.

In the two different years, amount and activity of RuBisCO generally peaked at the stage of petals fall. Moreover, the minimun values of the enzyme content and activity were found in the low input level. Net photosynthesis measured on same plant canopies was sensitive to the cultural intensification.

High or medium input levels exhibited high photosynthesis potentiality specially in the first stages of reproductive development. The reduction in C assimilation observed under the low input level may be assigned to water and nitrogen deficiencies due to crop management (no irrigation, poor N fertilization).

* author and address to whom correspondence should be sent

## INTRODUCTION

In C3 plant species such as sunflower and soybean, photosynthetic CO2 fixation is exclusively catalyzed by the ribulose 1,5- diphosphate carboxylase (RuBisCO), this enzyme is largely represented in the soluble protein amount of the leaf (generally up to 50 percent ) and therefore plays an important regulatory control in protein pool in photosynthesis and growth or duration of leaves. Rates of photosynthesis in sunflower (Courtiade 1983, Martinez et al. 1987) and soybean ( Hesketh et al. 1981, Torisky et Servaites 1984) have been correlated positively with the amount of leaf RuBisCO. However, it was demonstrated on wheat (Mächler et Nösberger 1980) or rice (Makino et al. 1983) that the amount and activity of the enzyme fluctuate among genotypes and environmental factors (light intensity, CO2 pression, temperature). On sunflower, the kinetic of CO2 assimilation seems to be linked to nitrogen availability which influences leaf expansion and consequently interception capability (Blanchet et al. 1986). Water supply may also induce changes in contents and activities of carboxylasic enzyme and saccharose-phosphate-synthase (Piquemal et al 1990).

The present study is concerned with field experiments in which the effects of several crop managements such as fertilization, irrigation, pests and weed control were observed on the canopy photosynthesis and also on RuBisCO amount and activity along the growth cycle.

## MATERIAL AND METHODS

The experiment took place in a long-term field study in Toulouse Agronomy station. In order to achieve three rising yields, crop rotations (cereals and oil seed crops) are managed at three levels of inputs (low, medium, high ) as presented in table 1A for sunflower crop. The carboxylasic activity of RuBisCO was determined with the radiochemical method proposed by Raaghavendra and Das (1977), and the enzyme amount by immuno-electrophoresis method developed by Courtiade (1983) with antibodies orientated against sunflower RuBisCO (on 3 replicates for each measurement made on leaves of the medium canopy). Net photosynthesis was measured through CO2 exchange rate on adult leaf of the upper canopy with a CO2 differential analysis apparatus (Binos type). Determinations occured at the different growth stages : floral bud, beginning of flowering, petals fall and akens filling (table 1-B). The tests were conducted for two years (table 1-C). In climate sequences I, copious rains have fallen in may, while in climate sequences II cold temperatures on april have delayed crop growth and a severe water stress has occurred on june and july.

## RESULTS

Figure 1 shows the differentiated RuBisCO amounts with increasing input levels during the contrasted climate sequences. On climate sequences I , foliar growth and crop development have taken advantage of large water availability due to spring rains ; so, the content of RuBisCO in leaves of the medium canopy do not significantly vary with the various crop managements. But, the climate sequences II presented a severe water stress early in the growing season and the crop intensification through irrigations (medium and high input levels) was associated with changes in quantities of the pivotal enzyme of photosynthetic productivity. However, maximum value in RuBisCO content was obvserved at the end of flowering (petals fall).

The potential activity of RuBisCO (fig.2) varied with crop management as it was generally higher under medium on high input levels compared to low input. Late reproductive phases (such as petals fall stage) presented an important in vitro carboxylasic activity on leaves.

As RuBisCO enzyme is the CO2 captor of photosynthetic process, it was of peculiar interest to compare its behaviour under several crop managements with net photosynthesis measured on crop canopy. Figure 3 confirms the great photosynthesis activity of sunflower upper leaves, which may reach the potentialities of C4 species. Furthermore, increasing input levels increased this natural tendancy. The photosynthetic capacity depends on the stage of leaf development and on biomass production. For climate sequences II, due to water stress during the earlier stages leaves had not yet acquired their full biomass at beginning flowering and might capture correspondingly less light. And the peak value was located at petals fall stage. While, on climate sequences I, wet spring had induced an increase in leaf growth and the crop exhibited high photosynthetic capacity as soon as the floral buds were developing.

So, if the crop managements had not a strong effect on the RuBisCO content, they introduced repercussions upon carboxylasic activity and photosynthetic activity.

**DISCUSSION, CONCLUSION**

It is generally difficult to draw up a straight relationship between the productivity and the rate of photosynthesic activity. Nevertheless, the approachs studying the behaviour of enzymes involved in C3 species photosynthesis functioning might enlighten on the biochemical jammings of biological conversion of solar radiation into grain yield. In our 2-years experiments, it was assumed that the chief effect of increasing input levels of crop management may be detected at some reproductive stages (end flowering, petals fall). However, the high photosynthetic activity observed along the different contrasted years may not be attributed to the changes in enzyme contents. That is how, the in vitro RuBisCO activity inversely vary with net photosynthesis and it suggests that a regulatory system acts on the in vivo activity of the enzyme.

Table 1 - Differentiation of input levels (A) and physiological stages (B) of sunflower managed in the long term field experiment at Auzeville during 2 climate sequences (C)

A- Differentiated input levels

| | low | medium | high |
|---|---|---|---|
| labour (number of interventions) | 2 | 2 | 3 |
| P-K-fertization (kj/ha) | 40 | 100 | 120 |
| N fertilization (kg/ha) | 50 | 130 | 170 |
| Weed control (number of interventions) | 2 | 3 | 32 |
| irrigation (mm) | 0 | 80 | 215 |

B- Phenological stages

| | abbreviation |
|---|---|
| floral bud | FB |
| beginning flowering | BF |
| petal fall | PF |
| akens filling | AK |

C- Weather conditions

Climate sequences I

| month | 04 | 05 | 06 | 07 | 08 | 09 |
|---|---|---|---|---|---|---|
| min.temps (°C) | 7,3 | 8,8 | 12,9 | 16,1 | 13,3 | 12,5 |
| max.temp (°C) | 17,1 | 18,8 | 22,7 | 27,7 | 25,7 | 27,1 |
| rain (mm) | 65,0 | 109,0 | 64,5 | 37,5 | 10,5 | 0,0 |
| radiation (h) | 220,5 | 200,3 | 209,0 | 248,9 | 272,2 | 273,8 |

Climate sequences II

| month | 04 | 05 | 06 | 07 | 08 | 09 |
|---|---|---|---|---|---|---|
| min.temp (°C) | 4,8 | 10,4 | 13,0 | 14,6 | 14,7 | 12,7 |
| max.temp (°C) | 12,3 | 20,6 | 22,6 | 25,9 | 26,2 | 24,6 |
| rain (mm) | 71,5 | 47,0 | 14,0 | 6,5 | 20,0 | 40,5 |
| radiation (h) | 108,5 | 211,8 | 213,8 | 294,0 | 252,8 | 210,5 |

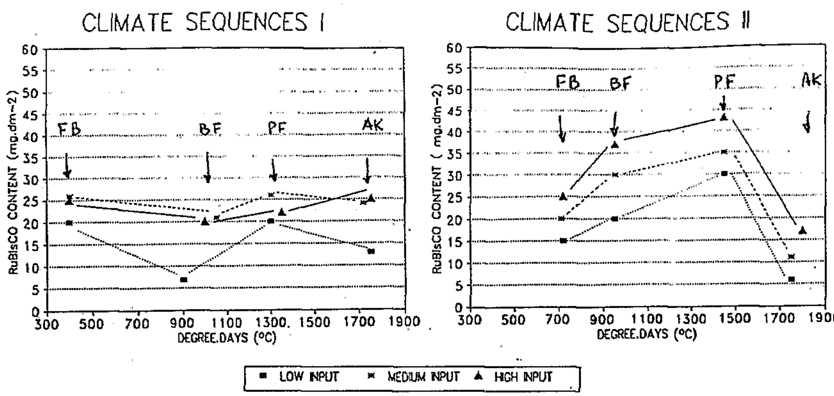


fig. 1

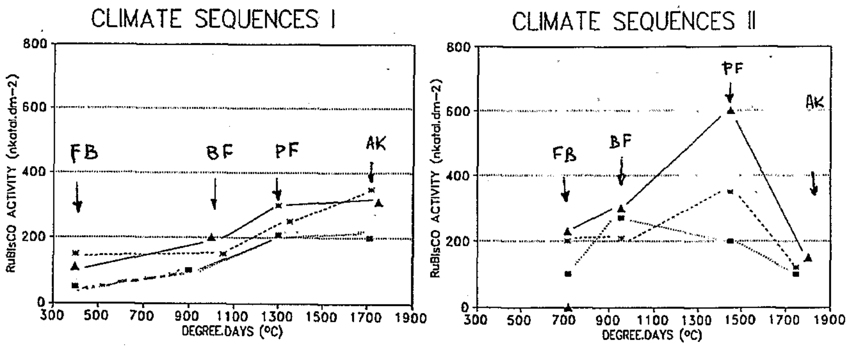


fig. 2

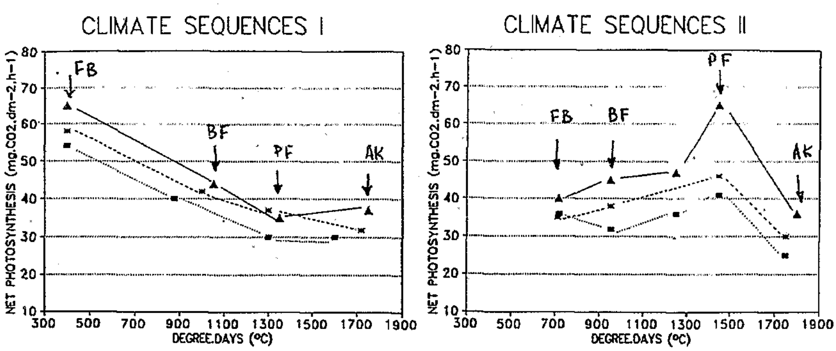


fig. 3

- Figure 1 : RuBisCo Content (mg.dm$^{-2}$ of leaf area) in leaves of medium canopy of sunflower crops managed at several input levels (3 replicates,confidence threshold 0.05).Details of crop management and phenological stages in table 1.
- Figure 2 : RuBisCO activity (nkatal.dm$^{-2}$ in leaves of medium canopy of sunflower crops managed at several input levels.
- Figure 3 - Net photosynthesis ($CO_2$ mg.dm$^{-2}$.h$^{-1}$) in leaves of sunflower crop managed at different input levels (5 replicates, confidence threshold 0.05).

**REFERENCES**

- *Blanchet R., Gelfi N., Piquemal M., Amiel C.,1986. Infuence de l'alimentation azotée sur l'évolution de l'assimilation nette au cours du cycle de développement du tournesol (Helianthus annuus L.) C.R Acad. Sci, Paris, 302, 5, 171-176*

-*Courtiade B. 1983. La ribulose biphosphate carboxylase oxygénase du tournesol; dosage et évolution ontogénique. Thèse Université P Sabatier, Toulouse, 63p.*

-*Hesketh J.D., Ogren W.L., Hageman M.E., Peters D.B., 1981. Correlations among CO2-exchange rates, areas and enzyme activities among soybean cultivars. Photosynthesis Res., 2,21-30.*

-*Mächler F., Nösberger J., 1980. Regulation of Ribulose bisphosphate carboxylase activity in intact wheat leaves by light, CO2 and temperature. J. exp. Bot., 31, 1485-1491*

*Makino A., Mae T., Ohira K. 1983. Photosynthesis and ribulose- Plant physiol., 73, 1002-1007*

-*Martinez E., Gelfi N., Bouniols A., Cavalié G., 1987. Influence de conditions culturales différentes sur l'évolution de la RuBisCO, de la photosynthèse nette et de la production de quelques espèces (blé, colza, féverole, soja, tournesol). Agronomie, 7, 9, 667-676*

-*Piquemal M., Cavalié G., Poeydomenge O., Botella-Brandibas A., 1990. Activité métabolique et translocations chez le tournesol soumis à un stress hydrique. In "Le tournesol et l'eau-adaptation à la sécheresse, réponse à l'irrigation". Points Science du Cetiom, Cetiom edit ISBN : 2-908645-28-9, 32-44*

-*Raaghavendra A. S., Das V.S.,1977. Purification and properties of PEP Case and RuDPCase C4 and C3. Z. Pflanzenphysiol., 82, 315-321*

-*Torisky R. S., Servaites J. C., 1984. Effect of irradiance during growth of Glycine max on photosynthetic capacity and percent activation of ribulose-1,5-bisphosphate. Photosynthesis Res., 5, 251-261.*