

# CO<sub>2</sub> GAS EXCHANGE PARAMETERS AS THE MEASURE OF BIOMASS PRODUCTION OF THE HUNGARIAN ENERGY GRASS

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## **Abstract**

Leaf CO<sub>2</sub> gas exchange parameters has been reported of the Hungarian energy grass (*Elymus elongatus* (Host.) Runemark subsp. *ponticus* (Podp.) Melderis cv.) at the early stage of its life trait. Plants were grown in experimental field and growing pots, in situ CO<sub>2</sub> gas exchange parameters were measured on the upper fully expanded leaves of significant vegetative phenophases using a portable IRGA system. Net photosynthetic rate ( $A_n$ ), assimilation capacity ( $A_{max}$ ) and stomatal conductance for carbon-dioxide ( $g_s$ ) as the measures of assimilation were calculated. Displaying our results daily courses of the net assimilation to abiotic environmental parameters were constructed. Remarkable values of  $A_n$ -PAR curves (light compensation point, assimilation capacity) of the Hungarian energy grass were calculated and compared to other grasses to characterize its relative biomass potentialities.

According to our results light showed closest correlation with the net photosynthetic rates overall. Compensation points were suitably low for positive assimilation rates in the light periods. Assimilation patterns through the phenophases by  $A_{max}$  slightly differed in the experimental sites owing to different abiotic environmental conditions. There was a correlation between stomatal conductance and photosynthetic rate consequently, so this proved to be a mediately regulatory environmental factor. Progressing with the phenophases illumination had a permanently decreasing, stomatal conductance had an increasing role for the regulation of the assimilation. In comparison with C<sub>3</sub> agricultural grasses and other energy crops, potential assimilation of Hungarian energy grass will surely be appropriate to have good biomass production and be a carbon-dioxide sink for the environmental CO<sub>2</sub> balance.

## **Introduction**

Renewable energy sources play an increasingly important role in securing both the energy supply and sustainable development of the European countries. The specific energy targets in the EU-25 for 2020 are to increase the proportion of renewable energy up to 20% of gross energy consumption. In this process biomass crops will have more and more importance due to their various environmental adaptability. Knowledge based on research and experience leads to the operative action plans on national or international level (Lewandowski et al 2003, Rosillo-Calle et al. 2008).

*Elymus elongatus* (Host.) Runemark (syn.: *Agropyron elongatum* (Host.) Beauv., *Elytrigia elongata* (Host) Nevski, *Lophopyrum elongatum* (Host) Á. Löve) is native to the Eastern Mediterranean region, from southern Europe to Asia Minor and Crimea, occurs in saline meadows and along seashores (Duke 1983, Darbyshire 1997). It is well known in several parts of the world. In the USA it have been widely used for land rehabilitation because of salt resistance, in reuse of saline drainage waters for irrigation (Grieve et al. 2004) and to prevent erosion for a long time (Sykes 2000, Gillen and Berg 2005). In Turkey the plant is successfully grown under dry land conditions to produce hay (Sengul 2003). It is also suitable for reutilization of soil mixtures improved by coal fly-ash and sewage sludge (Wong and Su 1997). According to the phytomass files annual productivity ranges of the tall

wheatgrass is from 2 to 15 MT/ha depending on the cultivation and the environment (Duke 1981).

Tall wheatgrass is classed among plants of C3 photosynthetic pathway with a cool season characteristic, in moderately salt habitats and seasonally different water use features (Carter and Peterson 1962, Johnson 1991, Bleby et al. 1997). Several cultivars have been developed based on adaptability to different environmental conditions. Expanding knowledge about the functioning of the cultivars are essential in using for special purposes. 'Szarvasi-1' cultivar so called 'Hungarian energy grass' originated from Hungarian populations of tall wheatgrass (*Elymus elongatus* subsp. *ponticus*) (Bagi and Székely 2006). It has recently been introduced to cultivation in Hungary as an alternative energy crop. Some preliminary results were presented about histological and molecular taxonomical features (Farkas et al. 2004, Kocsis et al. 2008). Relevant information are available on weed composition and predation survival of ground nesting birds in agricultural stands (Pál and Csete 2008, Purger et al. 2008).

The carboxylation process of plants with C3 assimilation pathway is directly depends on the availability of carbon-dioxide and light. Net photosynthetic capacity ( $A_{max}$ ) is slight to high (20-40  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ ) compared to other crops with C4 assimilation pathway (30-70  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ ) under natural  $\text{CO}_2$  conditions, saturated light intensity, optimal temperature and adequate water supply (Larcher 2003). Maximum value of net photosynthetic rate ( $A_{max}$  as photosynthetic capacity) in cultivated and natural C3 grasses is very diverse. In case of winter wheat (*Triticum aestivum*) 14-22  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  (Morgan and LeCain 1991), rice (*Oryza sativa*) 7,1-8,7  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  (Yeo et al. 1985) and related couch-grass (*Agropyron repens*) 5,82  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  (Engloner et al. 2003).

In our study main  $\text{CO}_2$  leaf gas exchange characteristics of the new Hungarian energy grass are discussed as the potentialities of biomass production or carbon-dioxide consumption, and will be compared to several C3 and C4 grasses and crops.

### **Materials and methods**

Plants were grown in experimental field (monoculture stand near Bóly, EF) and growing pots (in the Botanical Garden of University of Pécs, GP) on cambisol soil type, under different abiotic environmental conditions of PAR, temperature,  $\text{CO}_2$  and air humidity (Table 1). Hungarian energy grass produces 5 leaves in its vegetative period, stages can be defined as phenophases (2- to 5-leaved plant).

$\text{CO}_2$  gas exchange parameters and environmental data were in situ collected in 2 to 5 replicates on the upper fully expanded leaves of significant vegetative phenophases. Two-channel portable infra-red gas analysers were used for collecting data (LCA-2 and LCA-Pro+, ADC Ltd., UK) (Field et al. 1991, Long and Hällgren 1991, ADC 1994, 2007). Selected  $\text{CO}_2$  gas exchange parameters for projected leaf area as the measures of photosynthetic function (Larcher 2003) were calculated for the phenophases. Photosynthetic rate ( $A$ =assimilation) is a calculated parameter as the rate of carbon-dioxide exchange modulated by the environment in a differential open steady-state gas-exchange system (Field et al. 1991). Actual net assimilation rate ( $A_n$ ,  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ ), photosynthetic capacity ( $A_{max}$ ,  $\mu\text{mol m}^{-2} \text{sec}^{-1}$ ) and stomatal conductance for  $\text{CO}_2$  ( $g_s$ ,  $\text{mmol m}^{-2} \text{sec}^{-1}$ ) were used to characterize the environmental responses of the Hungarian energy grass. On one hand daily courses of the assimilation and the four main environmental factors were constructed for describing their relationship during the light-active period of the day. On the other hand remarkable values of PAR- $A_n$  response curves like light compensation point ( $I_c$ ) and assimilation capacity ( $A_{max}$ ) were calculated by the equation of  $y=Bx(x-A)/(K+x-A)$  in Origin 7.0, where  $y=A_n$ ,  $A=I_c$ ,  $B=A_{max}$ . These values are well-known and widely studied, that can be used for comparing plants with different photosynthetic pathway or activity. Stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{sec}^{-1}$ ) is a measure of the maximum rate of passage of carbon dioxide. Diffusion of  $\text{CO}_2$  into the leaves mainly driven by the stomatal aperture and linearly correlates with photosynthetic capacity (Wong et al. 1979). It plays an important role in the physiological processes of plant modulated by the environmental regime (Körner et al. 1979).

Table 1 – Soil characteristics and abiotic environmental conditions of experimental field (EF) and growing pots (GP). Soil data are after Dezső (2005)

	EF	GP
Altitude (m)	130	190
Soil type	cambisol	cambisol
Soil pH (H <sub>2</sub> O)	6,92 ± 0,63	6,92 ± 0,63
Humus content (m/m %)	1,76 ± 0,20	1,76 ± 0,20
CaCO <sub>3</sub> (m/m %)	0,84 ± 1,48	0,84 ± 1,48
Ca (mg/kg)	7156,97 ± 4297,72	7156,97 ± 4297,72
K (K <sub>2</sub> O) mg/kg	266,19 ± 76,69	266,19 ± 76,69
NO <sub>2</sub> +NO <sub>3</sub> (mgN/kg)	40,11 ± 9,47	40,11 ± 9,47
PAR (μmol m <sup>-2</sup> s <sup>-1</sup> ) (min-max)	62-1521	16-2999
Environmental CO <sub>2</sub> (ppm) (min-max)	105-441	315-456
Relative air humidity (RH %) (min-max)	13,8-64,9	2,8-15,6
Leaf temperature (T <sub>leaf</sub> C°) (min-max)	16,6-28,4	15,8-36,8

## Results and discussion

CO<sub>2</sub> gas exchange of a leaf influenced by number of environmental factors. Photochemical process of the photosynthesis is primarily dependent on the radiation, especially PAR, biochemical processes of it are affected by the availability of carbon-dioxide, temperature, water and nutrient supply. Daily changes of photosynthetic rate (A<sub>n</sub>) are as the leaf adapts to the changing environmental conditions. According to the daily course of net photosynthesis of the Hungarian energy grass, the four main environmental factors were variously effective for determining actual value of net assimilation (Fig. 1). The closest correlation were revealed between net photosynthetic rate and PAR so that was the most important environmental factor during the day. Carbon-dioxide supply was effective only for short periods of the day. Relative humidity of the air, controlling CO<sub>2</sub> availability through the stomata, was an effective external factor as well. Significance of leaf temperature was only indirect. Owing to the relative importance of external factors, light-photosynthesis data were selected for more detailed analyses.

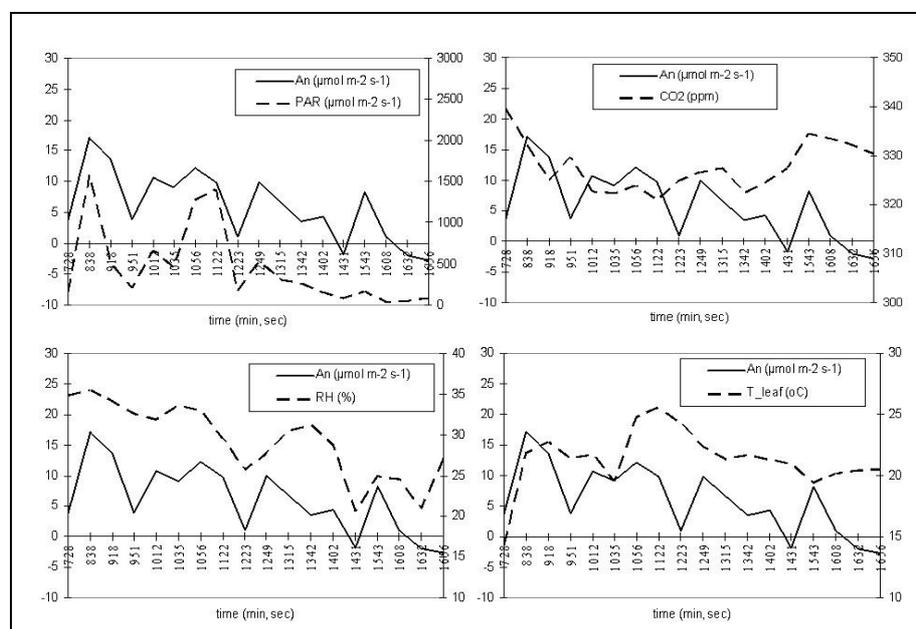


Figure 1 – Daily course of net assimilation (A<sub>n</sub>) and environmental parameters in the growing pots. PAR=photosynthetically active radiation, CO<sub>2</sub>=carbon-dioxide concentration, RH=air humidity, T<sub>leaf</sub>=temperature of the leaf.

The relationship between radiation (PAR) and net photosynthesis (A<sub>n</sub>) followed a saturation curve in all the cases (Figs 2 and 3). When photosynthetic CO<sub>2</sub> uptake and respiratory CO<sub>2</sub> release are in equilibrium, there is no net gas exchange detectable, light compensation point (I<sub>c</sub>) can be determined. Plants that respire more intensely, require

more light for compensation. In accordance our fitted curves light compensation point is always above  $100 \mu\text{mol m}^{-2} \text{sec}^{-1}$  that gradually increased through the phenophases and decreased just before the flowering in experimental field.  $I_c$  values are substantially lower and permanently decreased through the phenophases in growing pots.

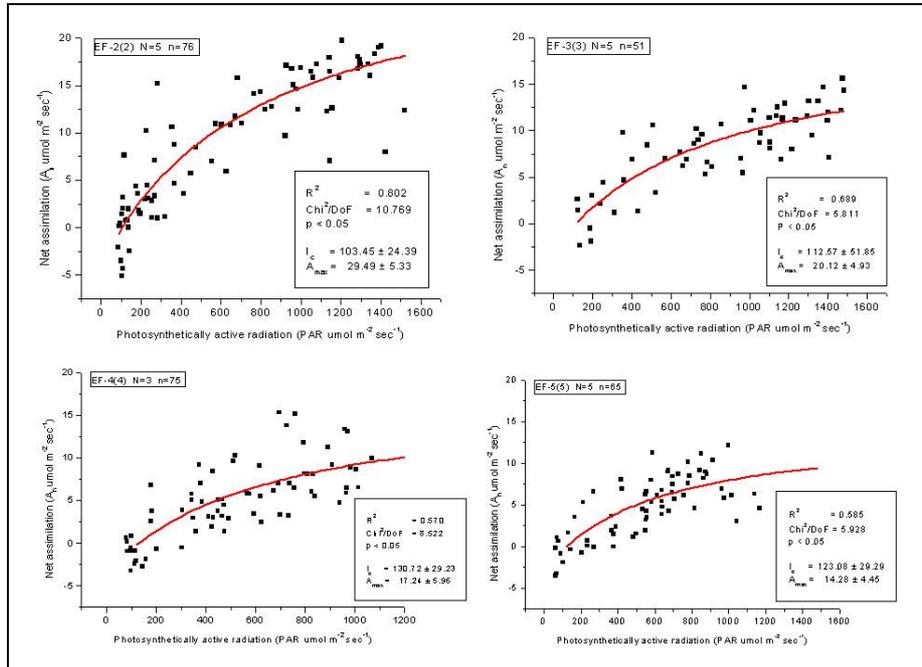


Figure 2 – Light-photosynthesis curves ( $A_n$ -PAR) in the experimental field (EF). Phenophase and leaf position e.g. EF 2(2), number of replicates (N), number of measuring points (n), coefficient of determination ( $R^2$ ), reduced chi-square value of fit, probability (p) and remarkable points of assimilation ( $I_c$ ,  $A_{\text{max}}$ ) are displayed.

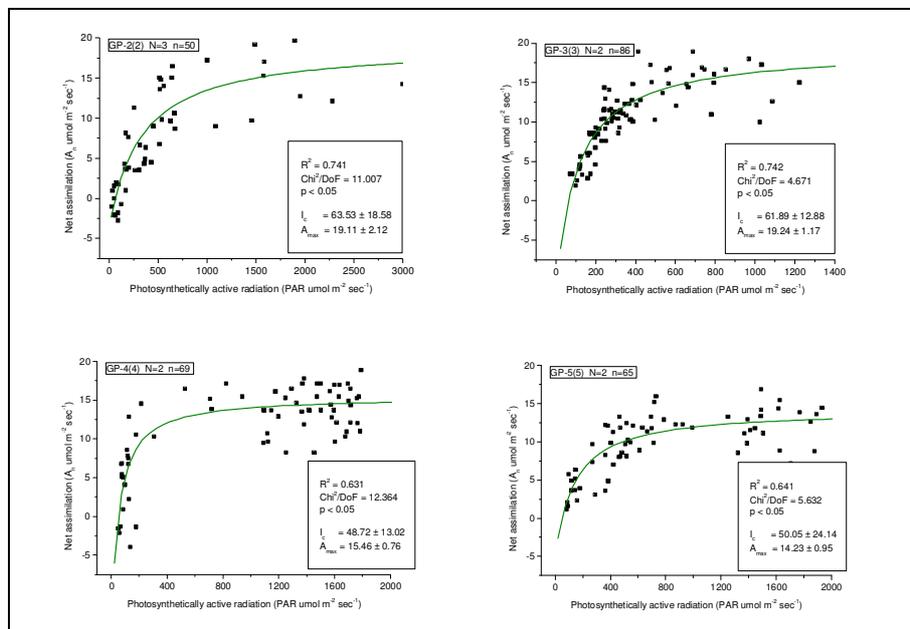


Figure 3 – Light-photosynthesis curves ( $A_n$ -PAR) in the growing pots (GP). Phenophase and leaf position e.g. GP 2(2), number of replicates (N), number of measuring points (n), coefficient of determination ( $R^2$ ), reduced chi-square value of fit, probability (p) and remarkable points of assimilation ( $I_c$ ,  $A_{\text{max}}$ ) are displayed.

Light saturated net photosynthetic rate as the photosynthetic capacity ( $A_{\text{max}}$ ) varied between 29,49 and 14,28  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  in the experimental field, between 19,11 and 14,23

$\mu\text{mol m}^{-2} \text{sec}^{-1}$  in the growing pots. Values of  $A_{\text{max}}$  permanently decreased through the phenophases at both of the experimental sites. By the curvature of light-photosynthesis relation Hungarian energy grass behaved like a C4 species with high  $I_c$  and  $A_{\text{max}}$  in the experimental field and like a C3 species with low  $I_c$  and  $A_{\text{max}}$  in the growing pots. Stomatal conductance for  $\text{CO}_2$  could be the measure of carbon-dioxide availability and changes in nearly the same proportion as the rate of assimilation of  $\text{CO}_2$ . Instantaneous stomatal conductance for carbon-dioxide of Hungarian energy grass varied between 0,05 and 0,35  $\text{mmol m}^{-2} \text{sec}^{-1}$  in the experimental field, and between 0,05 and 0,8  $\text{mmol m}^{-2} \text{sec}^{-1}$  in the growing pots. Values of  $g_s$  were not nearly or not linearly correlated in experimental field as in the literature cited, only in the growing pots by polynomial curves (Fig 4). Measured values of stomatal conductance are increasingly lower and their ranges are tapering. Correlation between stomatal conductance and net assimilation indicated by the coefficient of determination is getting better through the phenophases. It is established that stomatal regulation becomes more significant in the vegetative growing period.

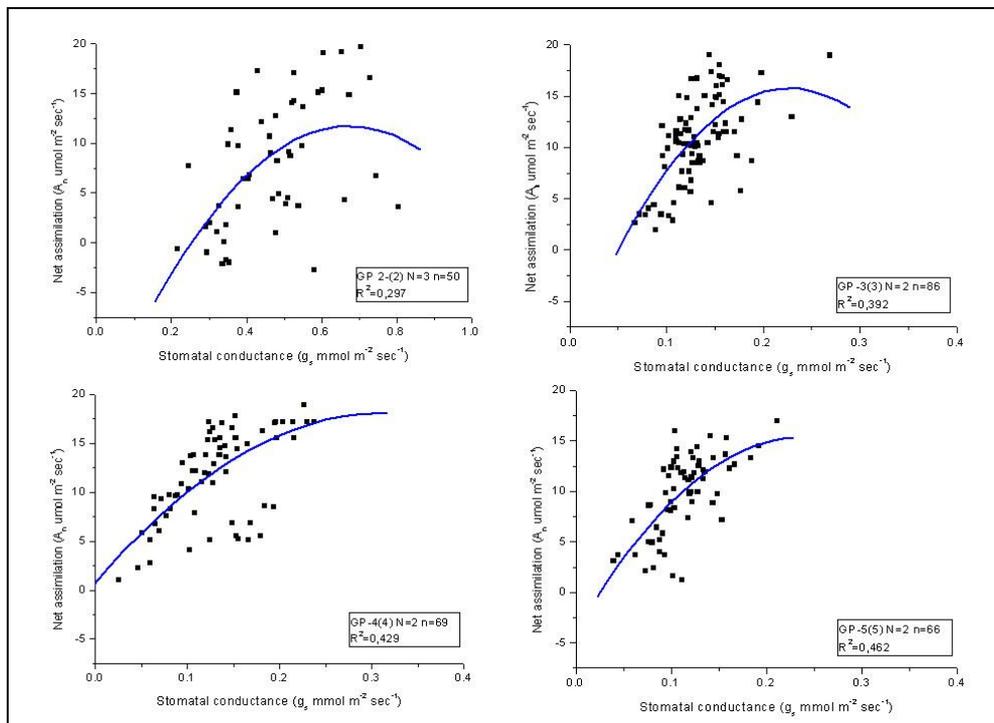


Figure 4 – Stomatal conductance for  $\text{CO}_2$  ( $g_s$ ) and net assimilation ( $A_n$ ) in the growing pots (GP). Phenophase and leaf position e.g. GP 2(2), number of replicates (N), number of measuring points (n) and coefficient of determination ( $R^2$ ) are displayed.

Assimilation capacity ( $A_{\text{max}}$ ) of the new Hungarian energy grass and some other C3 and C4 grasses, crops and trees were compared to discuss and estimate relative carbon-dioxide consumption (Table 2). It could be observed that Hungarian energy grass has a moderate photosynthetic rate among C3 crops: lower than traditional cereals (e.g. *Triticum aestivum*) but substantially greater and relatively high than other well-known energy grass (e.g. *Panicum virgatum*) or related native species (e.g. *Agropyron repens*). In comparison with C3 trees for energy purposes (e.g. *Salix* or *Populus* species) *Elymus elongatus* cv. has only a bit smaller assimilation capacity. Corresponding the data of some C4 crops (e.g. *Miscanthus x giganteus*) maximal assimilation capacity of Hungarian energy grass is near to their lower bound.

Table 2 – Assimilation capacity ( $A_{max}$ ) of several C3 and C4 crops and trees

	common name	species name	$A_{max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
C4 crops	Maize (Turkish wheat)	<i>Zea mays</i>	35-40
	Redroot amaranth	<i>Amaranthus retroflexus</i>	40-50
	Thornapple	<i>Datura stramonium</i>	50-60
	Giant Silver Grass	<i>Miscanthus × giganteus</i>	35-40
C3 crops	Couch-grass	<i>Agropyron repens</i>	5-10
	Hungarian energy grass	<i>Elymus elongatus</i> cv.	14-30
	Switchgrass	<i>Panicum virgatum</i>	8-10
	Wheat	<i>Triticum aestivum</i>	45
	Rice	<i>Oryza sativa</i>	40
C3 trees	Black locust	<i>Robinia pseudo-acacia</i>	30
	Poplar species	<i>Populus</i> spp.	20-25
	Willow species	<i>Salix</i> spp.	20-35

Many abiotic environmental factors have influences on the assimilation parameters of energy crops. Evaluating light-photosynthesis curves in two different localities we confirm that assimilation rate of Hungarian energy grass must be greater in natural field conditions under high light or air humidity and normal  $\text{CO}_2$  availability conditions. It points out to plasticity in light acclimation of leaf photosynthetic function according to changing environment. Regulatory function of stomatal conductance in carbon fixation become more significant as a reduction factor under low air humidity through the phenophases. Selected remarkable values of leaf assimilation ( $I_c$ ,  $g_s$ ,  $A_{max}$ ) are appropriate tools for calculation carbon-dioxide consumption and estimating plants to be a carbon-dioxide sink.

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