Different response of phosphoenolpyruvate carboxylase in two assimilating organs of *Hedysarum scoparium* on increasing soil water stress

Jia Jia Wang [1, a], Cong Xia Hu [1,b] and Chun Mei Gong [1,c *]

[1] College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China

[a] 846563931@qq.com, [b] 627463533@qq.com, [c] gcm228@163.com

* Chunmei Gong (gcm228@163.com)

Keywords: *Hedysarum scoparium*; Phosphoenolpyruvate carboxylase; Soil water stress.

Abstract. A typical desert shrub species, *Hedysarum scoparium* (C_3 plant) with green rachis, was analyzed on phosphoenolpyruvate carboxylase (PEPC) for considering increasing soil water stress as a probable driving factor to affect assimilation of plant in arid areas. The samples were planted in cultivation plots at depths of 1.4 m, 2.4 m and 3.4 m sandy soil respectively in this study. Water was periodically supplemented at the bottom of each cultivation plot to simulate soil water stress. The studies of activities of PEPC in two assimilating organs indicated that different photosynthetic organs possess different traits of CO_2 fixation. The results showed that PEPC presented both in leaflet and rachis of *H. scoparium*, and enhanced largely with the increasing soil depth. The result suggest that there is a adaptation strategy for desert species with green rachis expanding C_4 photosynthesis in *H. scoparium* under severe soil water deficit.

Introduction

The anatomical and photosynthetic physiological changes of photosynthesis can emerge from C_3 to C_4 as evolutionary adaptations to environment in land plants [1]. Soil drought is one of the most important factors promoting this variation [2]. There exist several photosynthetic key enzymes both in C_3 and C_4 plants. During the initial fixation of CO_2, phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) plays a key role in C_4 plants [3]. It increases in C_4 plants [4], but it increases, decreases or keeps unchangeable in C_3 plants [5].

Desert plants often experience a series of conditions that may cause stresses while water deficit is perhaps the fatal factor impacting adversely on plant growth and development [6]. Haloxylon ammodendron (Chenopodiaceae) and Hedysarum scoparium (Leguminosae) are two dominanted shrubs of the sandy deserts and arid regions across northwest China [7].

H. scoparium is a small-leaf desert species, and some photosynthetic attributes of H. scoparium leaflets is known well, but little is known about other assimilating organs. Previous research showed that C_4 photosynthesis can function within a single photosynthetic cell, without Kranz anatomy [2]. Desert C_3 species tend to develop their photosynthetic types with the increasing environmental stress, which results in emergence of C_4 pathway in assimilating organs.

In this study, *H. scoparium* was look upon as typical desert species to give clues for adaptation environment, especially the C_4 photosynthetic enzymatic adaptable characteristics of C_3 desert species. A specific hypothesis underlying the present studies was that desert C_3 species may develop their photosynthetic types and form some properties of C_4 mode in assimilating organs under water stress.

Materials and Methods

Experimental conditions and design. The study was carried out near the town of Minqin (38°38′N, 103°05′E), northwest China. The average annual precipitation is 115 mm, maily distributing from July to September. The aridity is 5.3, annual average evaporation is 2643.9 mm and annual average temperature is 7.8℃ with the highest temperature of 38.1℃ and the lowest temperature of -28.8℃.

The non-frost period reaches up to 165 days each year. The soil type is sandy soil and average depth of groundwater is 18 m.

The two years old seedlings of H. scoparium were grown outdoors in cultivated three chambers filled with sandy soil. Cultivated chambers were 1 m in length and width, and 1.6 m, 2.6 m or 3.6 m in depth. 3 depths of ground water (DGW) were established, 1.4 m, 2.4 m and 3.4 m [9]. The soil water content from every 20 cm in depth was monitored by measuring fresh and dry weight of soil.

Leaflet and rachis of *H. scoparium* were sampled and frozen in liquid nitrogen immediately after sampling.

Determination of water content. Three replicate soil samples were collected every 20 cm depth from holes that were randomly drilled in each treatment. Soil water content was determined gravimetrically after drying the samples in an oven at 105 ℃ for 24 hours.

The relative water content (RWC) of leaves were estimated by recording the fresh mass (FM) and the saturated mass (SM) of 0.5 g fresh leaf samples by keeping in water for 24 h, followed by drying in hot air oven till constant dry mass (DM) is achieved according to the method of Barrs et al. [10].

$$RWC(\%) = ((FM - DM)/(SM - DM)) \times 100\%$$

Enzyme extraction and assay. For extraction of photosynthetic key enzymes, aliquots of 100mg powdered plant tissue were carried out using a previously described method [9]. The activity of PEPC was monitored spectrophotometrically by the method described by Ting and Osmond [11]. The 1 ml assay medium contained 50 mmol L^{-1} Tris-HCl (pH 8.0), 5 mmol L^{-1} $MgCl_2$, 2 mmol L^{-1} DTT, 1 mmol L^{-1} $NaHCO_3$, 0.2 mmol L^{-1} NADH, 0.1 mmol L^{-1} EDTA, 3 units malate dehydrogenase and 20 μl extract. The reaction was initiated with 2 mmol L^{-1} PEP.

Statistical analysis. The data harvested and statistical significance of means of three replicates was judged by the least significant difference (LSD) test at 0.05 and 0.01 probability level.

Results and Discussion

Moisture state of desert plant. Soil moisture gradually increased with the increasing soil depth in three chambers. Accordingly, the RWC either in leaflets or in rachis of *H. scoparium* in different plot was varied, and the water potential / RWC in leaflets were all higher than that in rachis, and reached a peak at 15:00 as shown in Figure 1.

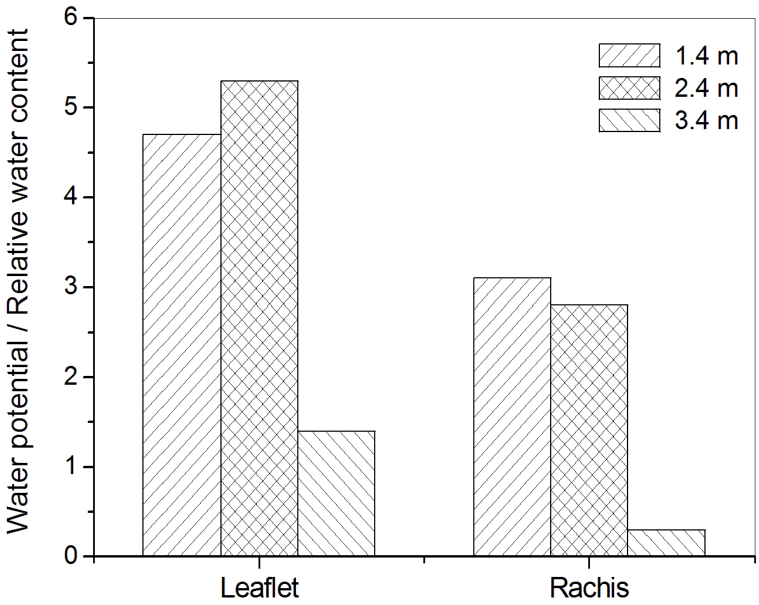

Figure. 1 Changes of water potential/RWC in leaflets and rachis of *Hedysarum scoparium* at three depths of ground water at 9:00.

Water potential / RWC can reflect the water availability. Water availability is an important factor affecting photosynthetic activity of plant species [12]. Our results showed that photosynthetic and growth latent capacity of leaflet and rachis was restricted with increase of soil depth, which indicated that rachis might develop its C_4 photosynthetic characteristics in severe water condition, also might explain that many species have formed assimilation organs like cylindrical green shoot which reduce transpiration and performed photosynthesis against overexploitation of ground water in Minqin areas. In addition, this result also corresponded to the fact that activities of SOD and POD in rachis were improved with increasing soil drought in *H. scoparium* during midday [13].

Activities of C_4 photosynthetic key enzymes. The activities of C_4 photosynthetic key enzymes in leaflets were all higher than those in rachis of *H. scoparium* at three depths of ground water. Activity of PEPCase in leaflets reached the top at 9:00 (ca. 4.4 μmol PEP min^{-1} mg^{-1} protein) in 3.4 m depths of ground water when it stayed a relatively lower level in rachis (ca. 3.3 μmol min^{-1} mg^{-1} protein). As the DGW is deeper and deeper, activity of PEPC in leaflets had a dropping and then had a little increase, and counterpart in rachis increased at the same time (Table 1), which is contrast to the changes of water content of plant. Meanwhile, we found changes of C_4 photosynthetic key enzyme consist with protein content changes of enzymes. This suggested the increase in activity was a result of protein synthesis rather than light-activation of pre-existing protein.

Table 1 PEPCase activities in leaflets and rachis of *Hedysarum scoparium* at 9:00 at three depths of ground water.

Organs	PEPC activity (μmol min^{-1} mg^{-1} protein)		
	1.4 m	2.4 m	3.4 m
Leaflet	4.28±0.75a	2.95±0.32b	4.36±1.21a
Rachis	2.06±0.52A	2.07±0.98A	3.31±0.94AB

Our results suggested that varying soil water stress plays a significant role in the distribution and ecophysiological features of different photosynthetic types and even develops the photosynthetic pathways. The intrinsic link between photosynthesis and biomass production suggests that photosynthetic response to drought probably play a major role in determining the ability of these species to persist in drought areas [14], also in consideration of global change. Furthermore, this activity was significantly high in each non-leaf organ [15]. As PEPC activity is positively and negatively regulated by metabolites [16], the ubiquity of these C_4 cycle enzymes in C_3 plants indicates that these 'C_3 isoforms' serve as the starting point for the evolution of the C_4 genes [17]. The multiple independent origin of C_4 photosynthesis suggested that the species evolution from C_3 to C_4 must have been relatively easy in genetic terms [18]. Our results suggest that there is the adaptable mechanism and evolutional direction of organs of C_3 desert species in deteriorated soil moisture circumstance, and under the conditions of high photon flux and high temperature in desert areas.

In conclusion, C_4 key photosynthetic enzymes in C_3 species *H. scoparium* presented an increasing thriving trend with the increasing degree of soil water stress. Therefore, we proposed a hypothesis that organs diversity of C_4 photosynthesis in C_3 desert species is an important indicator for plant growth, survival and evolutional transition in arid regions.

Acknowledgements

This work is financed by the National Natural Science Foundation of China (No.: 31070538) and the Fundamental Research Funds for the Central Universities (No.: QN2011026).

References

[1] C.M. Gong, P.B. Ning, G.X. Wang, Z.S. Liang, A review of adaptable variations and evolution of photosynthetic carbon assimilating pathway in C_3 and C_4 plants, J Plant ecol. 33 (2009) 206-221.

[2] R.F. Sage, The evolution of C_4 photosynthesis, New Phytol. 161 (2004) 341-370.

[3] U. Gowik, P. Westhoff, The path from C_3 to C_4 photosynthesis, Plant Physiol, 155 (2011) 56-63.

[4] C.H. Foyer, M.H. Valadier, A. Migge, T.W. Becker, Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves, Plant Physiol. 117 (1998) 283-292.

[5] A.E. Carmo-Silva, A.S. Soares, J. Marques da Silva, A. Bernardes da Silva, A.J. Keys, M.C. Arrabaça, Photosynthetic responses of three C_4 grasses of metabolic subtypes to water deficit, Funct Plant Biol. 34 (2007) 204-213.

[6] G. Han, Z. Zhao, Light response characteristics of photosynthesis of four xerophilous shrubs under different soil moistures, Acta Ecol. Sin. 30 (2010) 4019-4026.

[7] P.X. Su, T.T. Xie, Z.J. Zhou, C_4 plant species and geographical distribution in relation to climate in the desert vegetation of China, Sciences in Cold and Arid Regions. 3 (2011) 0381-0391.

[8] L.G. Zhang, F.J. Zeng, B. Liu, Z. Liu, G.X. An, H.F. Li, N. Yuan, Study of the photosynthesis characteristics and physical signs of four plants at the desert-oasis ecotone, Acta Ecol. Sin. 21 (2012) 103-111.

[9] C.M. Gong, X.W. Gao, D.L. Cheng, G.X. Wang, C_4 photosynthetic characteristics and antioxidative protection of C_3 desert shrub Hedysarum scoparium in Northwest China, Pak J Bot. 38 (2006) 647-661.

[10] H.D. Barrs, P.E. Weatherley, A re-examination of the relative turgidity technique for the estimating of water deficits in leaves, AJCS. 15 (1962) 413-428.

[11] I.P. Ting, C.B. Osmond, Photosynthetic phosphoenolpyruvate carboxylase characteristics of alloenzymes from leaves of C_3 and C_4 plants, Plant Physiol. 51 (1973) 439-447.

[12] L. Llorens, J. Peñuelas, I. Filella, Diurnal and seasonal variations in the photosynthetic performance and water relations of two co-occurring Mediterranean shrubs, Erica multiflora and Globularia alypum, Physiol Plantarum. 118 (2003) 84-95.

[13] C.M. Gong, J. Bai, G.X. Wang, C.L. Cao, The antioxidative protection strategies of different organs in *Hedysarum scoparium* under water stress at midday, J Nor For Uni. 24 (2009) 15-19.

[14] J. Gulías, J. Flexas, A. Abadía, H. Medrano, Photosynthetic response to water deficit in six Mediterranean sclerophyll species: possible factors explaining the declining distribution of Rhamnus ludovici-salvatoris, an endemic Balearic species, Tree physiol. 22 (2002) 687-697.

[15] X.L. Xu, Y.H. Zhang, Z.M. Wang, Effect of heat stress during grain filling on phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase/oxygenase activities of various green organs in winter wheat, Photosynthetica. 42 (2004) 317-320.

[16] Y. Kai, H. Matsumura, K. Izui, Phosphoenolpyruvate carboxylase: three dimensional structure and molecular mechanisms, Arch Biochem Biophys. 414 (2003) 170-179.

[17] R.K. Monson, The origins of C_4 genes and evolutionary pattern in the C_4 metabolic phenotype, In: Sage, R.F., Monson, R.K., eds. C_4 Plant Biology. San Diego: Academic Press. (1999) 377-410.

[18] J.M. Hibberd, J.E. Sheehy, J.A. Langdale, Using C_4 photosynthesis to increase the yield of rice-rationale and feasibility, Curr Opin Plant Biol. 11 (2008) 228-231.