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IMPROVING CARBOXYLATION EFFICIENCY - A NOVEL APPROACH TO INCREASE WATER USE EFFICIENCY IN SUNFLOWER

B.T. Krishna Prasad[1], M.S. Sheshshayee[1], R. Uma Sbaanker[1], T.G. Prasad[1], M. Udaya Kumar[1] and K. Virupakshappa[2]

1 Department of Crop Physiology, University of Agricultural Sciences GKVK, Bangalore 560 065 India
2 Project Co-ordinator, AICRP, Sunflower Unit

SUMMARY

Genotypic variation in yield under rainfed condition and at a given level of evapotranspiration mainly attributed to differences in dry matter produced per unit amount of water (Water Use Efficiency-WUE). WUE measured at single leaf level by gas exchange techniques correlate well with that measured by gravimetric method. WUE can be improved by either increasing the photosynthetic rate at a given stomatal conductance or by decreasing the stomatal conductance. Across species, photosynthetic rate is determined by the carboxylation efficiency at a given internal CO_2 concentration. Carboxylation efficiency in sunflower genotypes is mainly regulated by the efficiency of RuBisCO (A/RuBisCo). Selection of genotypes with efficient RuBisCO leads to increased photosynthetic rate and hence to higher WUE. We conclude that improvement of carboxylation efficiency could form an important strategy in increasing WUE and thereby the productivity both under rainfed and irrigated conditions.

Key words: Sunflower, carboxylation efficiency, water use efficiency, RuBisCO

INTRODUCTION

One of the most important strategies to improve productivity under rainfed conditions is to increase water harvesting and water utilization efficiency of a crop species. In most of the crop plants total productivity depends upon evapotranspiration (ET) x water use efficiency (WUE) x harvest index (HI). It has been shown that productivity among species or genotypes at a given level of ET can be mainly attributed to differences in WUE, that is, dry matter produced per unit amount of water used (Sashidhar, 1987).

In most parts of India, sunflower is being grown as a rainfed crop, where the crop experiences intermittant moisture stress. In the context of its importance as an edible oil crop, efforts are being increasingly laid to impove its productivity under rainfed conditions. In this regard much emphasis has been put to identify sunflower genotypes with high WUE.

Water use efficiency of a crop is generally measured by gravimetric methods. However, measurement of gas exchange parameters such as photosynthetic rates ('A') and

1 Address for correspondence: Dr. T.G. Prasad, associate professor, Department of Crop Physiology, University of Agricultural Sciences , G.K.V.K., Bangalore 560-065, India

stomatal conductances (g_s) are also often used to arrive at the intrinsic water use efficiency at a single leaf level. The ratio of 'A' to g_s sufficiently serves the purpose of estimating the intrinsic differences in WUE between the crop species and genotypes within a crop species.

Identifying genotypes for lower stomatal conductance is by far the most appropriate approach for increasing WUE. However, increasing the efficiency of photosynthesis at a given intercellular CO_2 concentration (Ci) would ensure a higher productivity under non stress conditions as well. Although photosynthesis is significantly limited by the substrate availability, increase in photosynthetic rates can be achieved by improving mesophyll ability to fix carbon. Therefore understanding the factors limiting or regulating photosynthesis is essential.

Once the substrate CO_2 is made available at the site of carboxylation, the rate of its fixation is governed by a number of mesophyll factors such as the photochemical reactions associated with the lamellar system (Terry and Taylor, 1984), factors associated with RuBisCo (Quick *et al.*, 1991), Calvin cycle and end product synthesis and export (Sharkey *et al.*, 1986; Stitt *et al.*, 1986). However, there are convincing evidences to prove that photochemical reactions do not limit photosynthesis under ambient conditions (Sharkey *et al.*, 1989; Shivashankara, 1991 and Sheshshayee, 1992). Similarly, the regeneration limitation associated with end product synthesis and export have also been shown not to limit photosynthesis under ambient CO_2 concentrations (Stitt, 1986; Campbell *et al.*, 1987; Sage *et al.*, 1988; Shivashankara, 1991).

Much of the limitations in 'A' seems to come from factors associated with RuBisCo such as its content, activation state and kinetics of catalysis.

With this in background, an experiment was conducted to evaluate the role of carboxylation and RuBisCo in determining assimilation rates in a few sunflower genotypes.

MATERIALS AND METHODS

Nineteen medium duration sunflower genotypes were selected from the germplasm collections maintained by the All India Coordinated Research Project on Sunflower, University of Agricultural Sciences, Bangalore. The genotypes were sown in field during the summer season of 1993. When the crop was 35 days old, gas exchange parameters, total soluble proteins and RuBisCo content were estimated on the top fully expanded leaf in each genotype.

Gas exchange parameters: gas exchange parameters such as assimilation rate ('A'), stomatal conductance (g_s) and internal CO_2 concentration (Ci) were measured using a portable photosynthesis system (ADC, LCA-2) on the top fully expanded leaf in genotypes and also in a few other crop species. The same leaf was detached for quantifying the total soluble protein and RuBisCo content.

Total soluble proteins: a simple dye binding method was adopted to estimate the total soluble protein. A known weight of the leaf was ground in phosphate buffer (pH 7.8) and an aliquot (50 μl) of the clear supernatent was mixed with 5ml of the coomassie brilliant blue dye (Bradford, 1976) and the blue colour intensity was read at 595 nm against a standard curve developed using Bovine Serum Albumin (BSA).

RuBisCO content: antibodies were raised against the holoenzyme of RuBisCo (Sigma) in New Zealand rabbits. Using the partially purified antiserum, an indirect ELISA technique was standardised for the quantification of RuBisCo content.

Efficiency of RuBisCo: as an indirect estimate of activation state of RuBisCo, the ratio of assimilation rate to unit RuBisCo content was computed. The A/RuBisCo ratio was termed *in vivo* efficiency of RuBisCo.

RESULTS AND DISCUSSION

Initially, WUE and a few parameters associated with it were measured in a few crop species. The measured WUE was correlated with the estimates of WUE based on gas exchange traits (Table 1). Correlation analysis between these parameters indicate that instantaneous gas exchange estimates reflect well the physiological traits of WUE estimated by gravimetric methods at a particular agroclimatic condition. Hence, measuring the gas exchange parameters is a much simpler and faster approach compared with cumbersome gravimetric methods.

Table 1: Relationship between gas exchange parameters and WUE associated growth characteristics in sunflower and amaranthus.

Gas exchange parameter	WUE associated growth characteristics	r value
Photosynthetic rate ('A')	DM/LAD ratio	0.644
Stomatal conductance (g_s)	Rate of water loss	0.964
Assimilation to conductance ratio (A/g_s)	Transpiration quotient (1/WUE)	-0.965

The ratio of A/g_s that is often used as an estimate of intrinsic WUE, can be enhanced either by increasing 'A' or by decreasing g_s. Although decreasing g_s and thereby transpiration rate is a more appropriate approach, attempts to increase 'A' may also lead to improving the A/g_s ratio and hence WUE.

It is therefore essential to quantify and identify various factors that may limit or regulate 'A'.

The initial slope of the CO_2 response curve, (dA/dCi), has often been considered as a reflection of the carboxylation efficiency of a leaf. CO_2 response curves were developed in a few species and the rate of increase in 'A' per unit increase in Ci at CO_2 compensation point (dA/dCi) was quantified (Table 2). The data indicated that the species that had higher dA/dCi also recorded higher 'A'. Regression analysis of 'A' and dA/dCi recorded a significant association between 'A' and carboxylation efficiency (Fig.1). This positive relationship between 'A' and carboxylation efficiency (dA/dCi) suggests that the mesophyll factors associated with carboxylation efficiency control assimilation rate to a significant extent. Thus increasing 'A' by improving carboxylation efficiency might help in achieving high WUE at a given stomatal conductance.

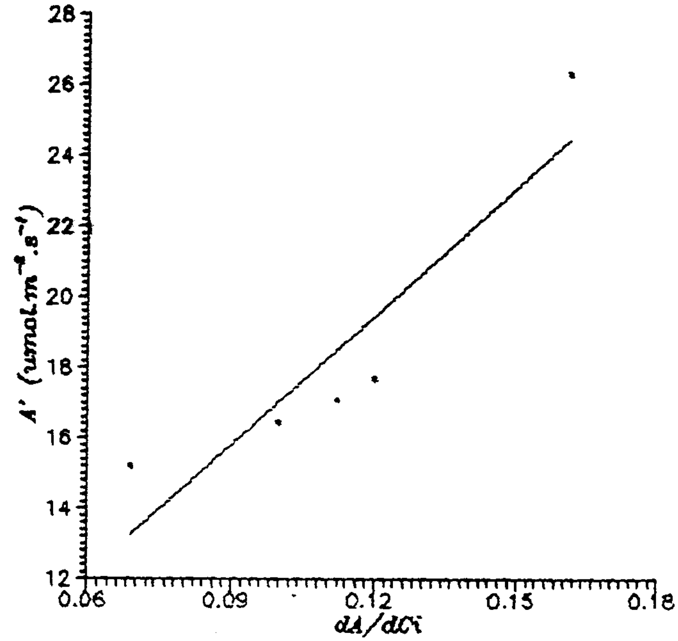

Figure 1. Relationship between 'A' and dA/dCi in a few crop species

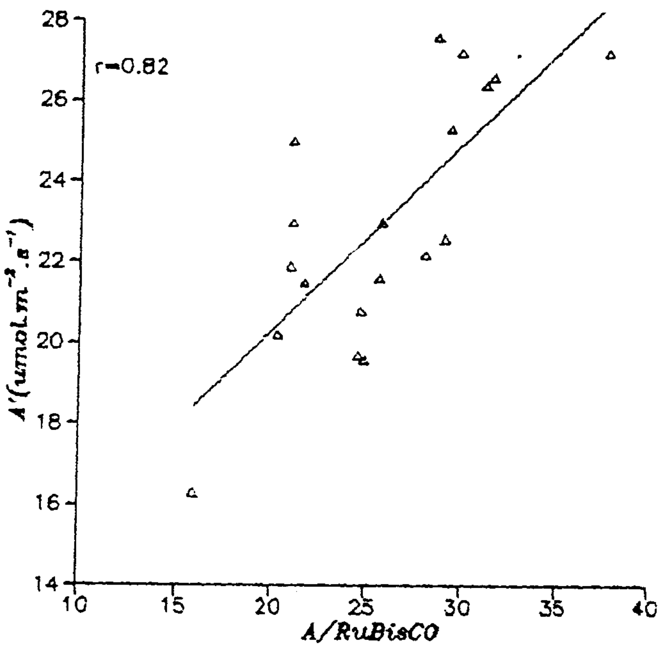

Figure 2. Relationship between A' and efficiency of RuBisCo

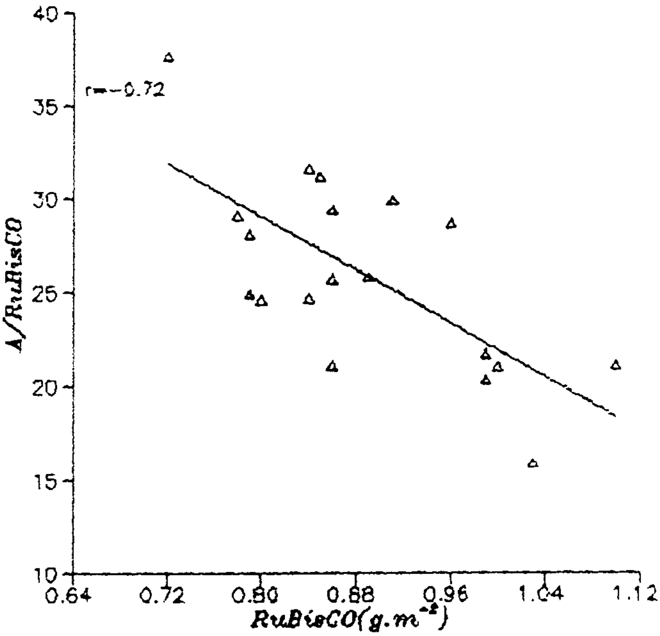

Figure 3. Relationship between RuBisCo content and its efficiency

Table 2: Carboxylation efficiency (dA/dCi) in a few species

Species	Assimilation rate ('A') (μmol.m^{-2}.s^{-1})	Carboxylation efficiency (dA/dCi)
Sunflower	26.32	0.161
Redgram	15.20	0.069
Soybean	17.10	0.112
Cowpea	16.45	0.100
Groundnut	17.70	0.120

Recently Farquhar *at al.* (1987) have shown an inverse relationship between carbon isotope discrimination and water use efficiency. More recently, the carbon isotope discrimination pattern has been used to select genotypes for higher water use efficiency (Acevedo, 1993). It has been shown by several workers that the discrimination values are positively correlated to the ratio of internal and ambient partial pressures of CO_2 (Pi/Pa ratio) (Condon *et al.*, 1990; Meinzer *et al.*, 1990; Gibson *et al.*, 1991). The Pi/Pa ratio is determined by the mesophyll ability to fix carbon as well as the stomatal diffusive characteristics. At a given g_s, the Pi/Pa ratios are largely determined by the carboxylation efficiency of a leaf. Carbon isotope discrimination has also been used to estimate the carboxylation efficiency of a leaf. These correlative studies indicate that genotypes having higher carboxylation efficiency are also better in their water use efficiency.

In vivo carboxylation efficiency of a leaf is governed by various mesophyll factors associated with photochemical reactions, RuBisCo, Calvin cycle enzymes and end product synthesis and utilization efficiencies. However, recent evidences indicate that carboxylation efficiency is not limited by photochemical reactions and end product synthesis aspects. Thus, the regulation of carboxylation efficiency is predominantly controlled by factors associated with RuBisCo (Mahabaleshwar *et al.*, 1993).

Table 3: Genotypic variations in assimilation rate, RuBisCo content in nineteen sunflower genotypes.

Genotype	('A') (μmol.m^{-2}.s^{-1})	RuBisCo content (g.m^{-2} leaf area)	A/RuBisCo ratio
1610	27.2	0.91	29.9
1599	27.2	0.72	37.7
BLC 180	26.6	0.84	31.6
1625	25.0	0.86	29.1
BLC 197	26.4	0.85	31.2
1630	25.3	0.86	29.4
BLC 177	27.6	0.96	28.7
1600	23.0	1.09	21.1
BLC 188	23.0	0.89	25.8
1648	22.6	0.78	29.0
1616	22.2	0.79	28.1
179	21.9	1.00	21.9
BLC 185	21.6	0.86	25.7
BLC 183	21.5	0.99	21.7
M 787-7-2	20.8	0.84	24.7
1630	20.2	0.99	20.3
832	19.7	0.80	24.6
62 B	19.6	0.79	24.9
339 B	16.3	1.03	15.9
CD (P = 0.05)	0.45	0.14	0.14

To assess the variability in factors associated with RuBisCo in sunflower genotypes, the enzyme content was quantified using an immunoassay technique. The data are presented in Table 3. A significant genotypic variation in 'A' was noticed in sunflower. However, though RuBisCo content per unit leaf area showed variation, the differences were not so marked among the genotypes. The ratio may also be considered as an indirect reflection of the *in vivo* activation state of the enzyme. A significant genotypic variation was noticed in the A/RuBisCo ratio suggesting that variation in the efficiency of RuBisCo might control the variability in sunflower genotypes. A strong positive correlation between 'A' and A/RuBisCo (Fig.2) further reiterated the fact that assimilation rate in sunflower genotypes was determined by the variation in the efficiency of RuBisCo. Hence selecting genotypes that have higher A/RuBisCo would be a plausible approach for identifying types that have higher carbon assimilation efficiency at a given substrate CO_2 concentration.

The data on A/RuBisCo was plotted against RuBisCo content of the leaf (Fig.3). A significant inverse relationship was evident from this plot suggesting that a genotype that has a higher RuBisCo content per unit leaf area down regulates the activation state of the enzyme, thereby determining a steady state assimilation rate. A genotype that has highly efficient RuBisCo, therefore might allocate lower amounts of total soluble proteins towards RuBisCo. Selecting the genotypes that have higher A/RuBisCO ratio with lower RuBisCo content will also improve the plant's protein use efficiency. These genotypes may have the relative advantage over the others in terms of having a high proportion of total soluble proteins that can be allocated to other rate limiting enzymes.

It is therefore possible to select genotypes that have higher carbon fixing ability by way of having more efficient RuBisCo with lower allocation of soluble proteins. These plant types should therefore have high 'A' at any given Ci. Since the carboxylation efficiency (dA/dCi) measured at low CO_2 concentrations is also high in these plants, the efficiency of carbon utilisation will also be significantly high. These events would certainly lead to relatively higher assimilation rates even when g_s is reduced. Therefore, we propose that selecting genotypes of sunflower for high A/RuBisCo ratio leads to improved productivity both under control and drought conditions. The genotypes that have high 'A' and higher A/RuBisCo ratios will certainly result in high water use efficiency.

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MEJORA DE LA EFICIENCIA DE LA CARBOXILACION, UN NUEVO METODO PARA INCREMENTAR LA EFICACIA DEL USO DE AGUA EN GIRASOL

RESUMEN:

La variación genotípica en rendimiento en condiciones de secano y a un nivel dado de evapotranspiración principalmente atribuidas a diferencias de materia seca producida por unidad de agua (Eficacia del uso de agua-EUA). La EUA medida a nivel de hoja por técnicas de intercambio gaseoso correlaciona bién con la medida por el método gravimétrico. La EUA puede ser mejorada bién incrementando la tasa de fotosíntesis a una conductancia estomática dada o mediante su disminución. A nivel de especies, la tasa de fotosíntesis es determinada por la eficacia de la carboxidación a una concentración interna de CO_2 dada. La eficiencia de la carboxidación en los genotipos de girasol es regulada principalmente por la eficiencia de RuBisCo (A/RuBisCo). La selección de genotipos con la eficiencia de RuBisCo conduce a incrementos de la tasa de fotosíntesis y por tanto a niveles de EUA mas altos. Se concluve que la mejora de la eficiencia de la carboxilación puede ser una estrategia importante en el incremento a la EUA, y por tanto de la productividad tanto en condiciones de secano como de riego.

AMÉLIORER L'EFFICACITÉ DE LA CARBOXYLATION - UNE NOUVELLE APPROCHE POUR AUGMENTER L'EFFICACITÉ DE L'UTILISATION DE L'EAU CHEZ LE TOURNESOL

RÉSUMÉ:

Les variation génotypiques observées pour le rendement sous différentes pluviométries et à un niveau d'évapotranspiration sont généralement attribuées à des différences de production de matière séche par unité de quantitté d'eau (water use efficiency - WUE). La WUE mésurée au niveau d'une feuille seule par la technique des echanges gazeux est bien corrélée aux résultats obtenus par gravitométrie. Elle peut tre améliorée soit par l'augmentation du rapport photosynthétique à une conductance stomatique donnée, soit la diminution de la conductance stomatique. Pour une espéce donnée le rapport photosynthétique est déterminé par l'efficacité de la carboxylation pour une concentration interne en CO_2. L'efficacité carboxylative des génotypes de tournesol étudiés est principalement régulée par le rendement de la RuBisCo (A/RuBisCo). La sélection de génotypes caractérisés par une RuBisCo performante conduit à augmenter le rapport photosynthétique et, de ce fait, à une WUE plus élevée. Nous en concluons que l'amélioration de l'efficacité carboxylative pourrait constituer une stratégie de choix par augmentation de la WUE et par voie de conséquence de la productivité tant en condition pluvieuse que sous irrigation.