Advances in Environmental Biology, 5(7): 1746-1750, 2011
ISSN 1995-0756

American-Eurasian Network for Scientific Information AENSI

This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLE

Rubisco and PEP-carboxylase Levels in Relation to Grain Development within a Spikelet of Wheat

Davood Eradatmand Asli and Alireza Houshmandfar

Department of Agronomy and Plant Breeding, Saveh Branch, Islamic Azad University, Saveh, Iran.

Davood Eradatmand Asli and Alireza Houshmandfar: Rubisco and PEP-carboxylase Levels in Relation to Grain Development within a Spikelet of Wheat

ABSTRACT

Relative levels of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and Phosphoenolpyruvate carboxylase (PEPC) were studied at different types of grain (bold and small) growing in the same spikelet of wheat (*Triticum aestivum* L. var. *PBW-343*). Ten labeled spikes were sampled five times, seven-day intervals started from seventh day after anthesis (DAA) up to 28th DAA, and at maturity. The behavior of Rubisco in two types of grains showed an increase in its levels upto 14th DAA in bold grains and 21st DAA for smaller grains followed by a gradual decrease with aging and it somehow was not detectable at maturity. The smaller grains possessed a lesser *per se* levels of Rubisco per unit basis with the highest gap at mid ripening stage. Analysis of data with regard to PEP-carboxylase activity revealed more or less the similar pattern as that of Rubisco activity. Its levels increased as the grains progressed upto mid ripening stage followed by a gradual decrease towards maturity. With regard to its distribution in bold and small grains, was apparent that the bolder grains possessed relatively higher levels of PEP-carboxylase at all stages of investigations. The higher quantum of PEP-carboxylase distribution in bold grains was maximum at 14th DAA.

Key words: Anabolic enzymes; PEPC; spikelet; *Triticum aestivum* L.

Introduction

An appraisal of parameters regulating cereals productivity divulges that their full potential to yield is still unrealized. One of the grey areas, which has remained untapped is the host of physiological and genetical barriers of developing kernels to grow to an optima and their manipulation by desirable traits and methodologies. The potential up gradation of components constituting the total yield in wheat (number of productive tillers m^{-2}, grains per spike and 1000-grain weight), would help to raise the production substantially. Though, significant milestones have been achieved in the first two parameters the last component, the individual grain weight has eluded scientific investigations and rather paradoxically has declined with the advent of high yielding varieties. A study into the physiology of grain yield shows the existence of variation among different varieties or genotypes or even the grains developing in the same ear [3,21,17,23,11]. It further discloses that the yield may be influenced by the availability of photosynthates to the developing sinks [24,19,20,7]. Various sugar responsive genes in plants potentially affect the partitioning [9] and have been stressed to be key determinant of plant productivity [10]. Dry matter partitioning also plays a paramount role in growth rate of sink organs [14]. Working on the grain growth in wheat and buckwheat variation among varieties was traceable to endogenous hormone production in variety vis-à-vis that in the ear [6,5]. A few biochemical components as advocated by Abrol *et al.* [2], Hakaka, [12] and Hasan and Kamal, [13], might be of significance in

Corresponding Author
Davood Eradatmand, Department of Agronomy and Plant Breeding, Saveh Branch, Islamic Azad University, Saveh, Iran.
E-mail: asli@iau-saveh.ac.ir

determining sink efficiency and/or the grain yield. In the present study, it is proposed to analyse the relative levels of two enzymes belonging to the anabolic enzymes namely, Rubisco and PEP-carboxylase in different grains growing in the same spikelet and to assess whether they are variable amongst the differentially participating grains or not.

Materials and methods

Crop management and sampling

The investigation was conducted with a common bread wheat (*Triticum aestivum* L. var. *PBW-343*), which was sown in circular earthenware pots (50x30x30 cm) containing 35 kg of soil mixed with farmyard manure (4:1). Eight seeds per pot were sown and after 15 days, seedlings were thinned to two. Hoagland's nutrient solution [15] was supplied to the pots. The plants were grown in a screen covered hall under otherwise natural conditions. Ten labeled spikes were sampled five times, seven-day intervals started from seventh day after anthesis (DAA) up to 28th DAA, and at maturity. Grains were usually taken from three different segments in the ear. The labeled samples of grains were brought to laboratory and separated to two types of grains (small and bold) and the following biochemical analysis was carried out in the above aged grains.

PEP-carboxylase (PEPC);

PEPC was studied according to the method of Vance and Stade [18] with some modifications as follows:

Extraction of the enzyme - Crude extracts were prepared by homogenizing 1 g fresh grain with acid-washed sand in a pre-chilled pestle and mortar in grinding medium (1 ml/1 g tissue) containing 50 mM tris-HCl (pH of 8.0), 50 mM $MgCl_2$, 5 mM, 2-mercaptoethanol and 1 mM EDTA. The homogenate was passed through four layers of cheese cloth, and filtrate centrifuged at 30,000 g for 30 minutes and the supernatant assayed for enzyme activities of PEPC. All the steps were carried out at 4°C.

Estimation of Enzyme - Phosphoenolpyruvate carboxylase was assayed spectrophotometrically at 30°C and 340 nm for at least 3 min in the assay media with a final volume of 1 ml. For the PEPC, the assay medium consisted of 10 mM $NaHCO_3$, 10 mM $MaCl_2$, 0.2 mM NADH and 4 mM PEP in 100 mM bicine-KOH buffer (PH of 8.5) optimized from Vance and Stade, [21].

Before placing samples in spectrophotometer 0.2 mM NADH was added as quickly as possible in to the test tube with a vigorous mixing well and the initial absorbance was recorded and thereafter every 30 sec for at least 3 minutes. Phosphoenolpyruvate carboxylase activity was expressed as mmole per min per gram fresh weight of sample. PEPC activity was calculated with decrease in absorbance for one minute and with the following formula:

mmoles per min 0.2 ml enzyme extract = Absorbance decrease/min × 0.1613 × 3 (volume of the reaction mixture in ml).

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco):

Rubisco was studied radiometrically according to the method of Bravdo and Pallas, [4] with some modifications, in terms of stopping the activity by glacial acetic acid. The enzyme is made to utilize labelled CO_2 as the substrate and the radioactivity in the products in counted as a measure of enzyme activity.

Extraction of the enzyme - Grains samples, weighing one gram were homogenized in chilled mortar and pestle. Enzyme was isolated in 8 ml medium of 100 mM tris-HCl buffer (pH of 8.0) containing 5 mM DDT, 20 mM $MgCl_2$, 0.5g PVP and 0.2 mM EDTA. The crude extract was filtered through four layers of muslin cloth and the filtrate was then centrifuged at 20,000 g for 30 minutes at 4°C. The supernatant was collected as the enzyme source.

Estimation of the enzyme - In a total volume of 250 ml, the assay mixture for Rubisco contained 98 mM tris HCl (pH of 7.8), 20 mM $MgCl_2$, 20 mM $NaH^{14}CO_3$ (specific activity 48.1 mci/m mole, activity 0.5 mci, obtained from BARC; Bombay). 20 ml of crude enzyme was incubated with all components except RuBP for 5 minutes at 30°C and the reaction was initiated by addition of RuBP. After 2 minutes the reaction was stopped by adding 250 ml of glacial acetic acid. Blank reaction mixture was prepared by adding all ingredients except RuBP. The samples were kept overnight in fume hood. Next day known volume was taken and added 10 ml of scintillation medium. Counting was done in liquid scintillation counter (Packard Tricarb, Liquid Scintillation Spectrometer).

Scintillation liquid was prepared by the method of Bray (1960). According to this 4 gm P.P.O., 0.2g PoPoP and 10 g nepthalene were dissolved in 1 litter of toluene. For dilution of $NaH^{14}CO_3$ solution, 1.0 ml of $NaH^{14}CO_3$ (0.5 mci) was diluted with cold $NaHCO_3$ solution so as to give a final concentration of 100 mM. Rubisco activity was estimated as:

$$(CO_2 \text{ fixed}) \text{dpmg}^{-1} \text{ f.w.h}^{-1} = \frac{\text{dPm} \times V_1 \times 60}{V_2 \times t \times w}$$

Where net dpm is the disintegrations per minutes minus back ground counts, V_1 is the volume of assay system, V_2 is the amount loaded for counting, t is the reaction time and w is the weight of the sample.

Result and discussion

In order to establish whether the bold and small grains were separate biological entities produced as a consequence of the specific biochemical process or were simple chance manifestations of development, the relative levels of a few enzymes belonging to the anabolic enzymes namely, Rubisco and PEP-carboxylase were studied in them at different stages of growth and development. The salient features of results are given below.

Ribulose 1,5-bisphoisphate carboxylase/oxygenase (Rubisco):

The scrutiny of the data with regard to Rubisco, the key enzyme regulating C fixation presented a few interesting correlations. A look into the Figure 1 depicts the behavior of Rubisco in two types of grains which showed an increase in levels of Rubisco upto 14 days in bold grains and 21 days for smaller grains post-anthesis stages followed by a gradual decrease with aging and it somehow was not detectable at maturity. The two types of grains namely bold and small significantly possessed its differential levels. Analysis of the data revealed that the smaller grains possessed a lesser *per se* levels of Rubisco per unit basis with the highest gap at mid ripening stage (45.3 percent lesser at 14 days after anthesis) with minimum gap recordable at 28 days post-anthesis stage (17.4 percent) (Figure 3).

Phosphoenolpyruvate carboxylase (PEPC):

Analysis of data with regard to PEP-carboxylase activity revealed more or less the similar pattern as that of Rubisco activity (Figure 2). Its levels increased as the grains progressed upto mid ripening stage (14 and 21 days after anthesis for bolder and smaller grains respectively) followed by a gradual decrease towards maturity. With regard to its distribution in bold and small grains, was apparent that the bolder grains possessed relatively higher levels of PEP-carboxylase at all stages of investigations. The analysis of the data revealed that higher quantum of distribution in bold grains was maximum at 14th DAA (43.5 percent lesser in smaller grains) and subsequent differences were to the tune of 24.3 and 16.4 percents short in smaller grains at 21 and 28 days post-anthesis stages respectively and it was somehow, not detectable at maturity (Figure 3).

The results of this present study bring forth, in no uncertain terms, the findings that the ear of wheat is a developing place for a definite number of grains which intern are separate biological entities endowed with their inherent potentials to grow and accumulate dry matter. This axiom was advocated by Abolina, [1] and is in line with the observations of innumerable workers [20,16,22,17,23].

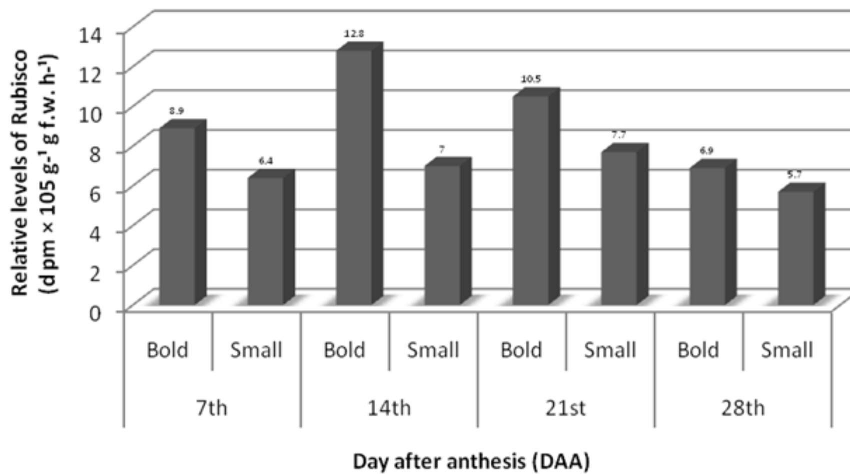

Fig. 1: Relative levels of Rubisco (d pm × 105 g^{-1} fresh weight h^{-1}) within developing grains (bold and small) of wheat (*Triticum aestivum* var. *PBW-343*)

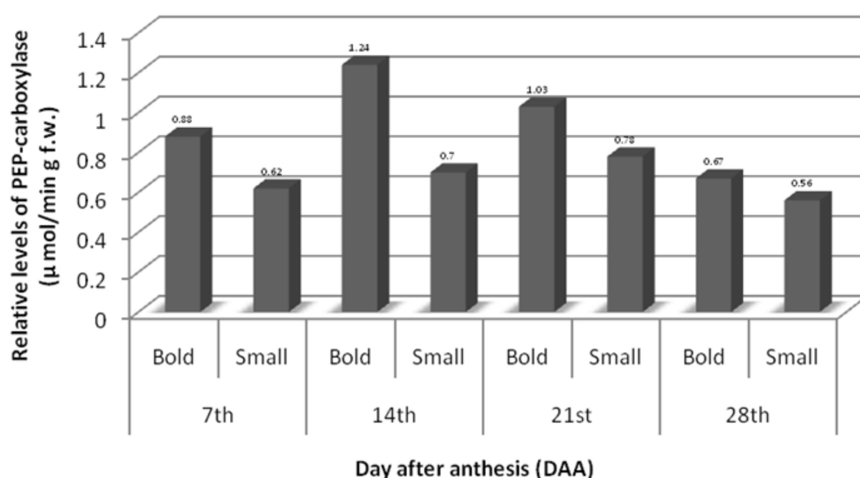

Fig. 2: Relative levels of PEP-carboxylase (µ mol/min g fresh weight) within developing grains (bold and small) of wheat (*Triticum aestivum* var. *PBW-343*)

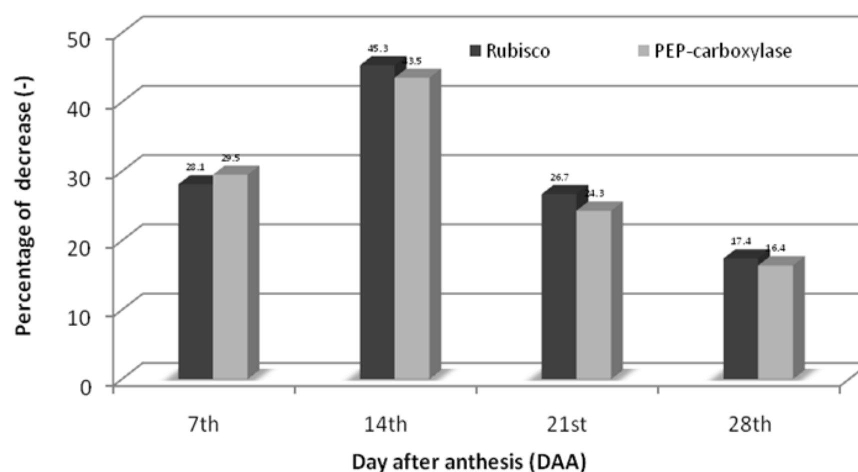

Fig. 3: Percentage decrease (-) in relative levels of Rubisco and PEP-carboxylase in small grains over their counterparts bold grains

References

1. Abolina, G.T., 1959. A study of the causes of variability in the development of wheat grain. Fizol. Rast., 6: 102-104.
2. Aborl, Y.P., P.A. Kumar, J.V.R. Nair, 1984. Nitrate uptake and assimilation and grain nitrogen accumulation. Adv. Cereal Sci. and Tech., 6: 1-48.
3. Asana, R.D., 1968. Some thoughts on possible mechanisms controlling yield. Indian J. Genetics Plant Breeding, 28: 21-30.
4. Bravdo, B., J.E. Pallas, 1982. Photosynthesis, photorespiration and RuBP carboxylase/oxygenase activity in selected plant genotypes. Photosynthetica, 16: 36-42.
5. Bray, G.A., 1960. A single efficient liquid scintillation for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem., 1: 279-285.
6. Dua, I.S., U. Devi, N. Garg, 1990. An appraisal of the hormonal basis of grain growth in buckwheat (*Fagopyrum esculentum* Moench). Fagopyrum., 10: 73-80.
7. Dua, I.S., O.P. Sehgal, 1981. Differential levels of growth promoters in the middle and peripheral grains of the same ear in wheat (*Triticum aestivum* Linn. emend. Thell). Proc. Indian Nat. Sci. Acad., B-47(4): 543-550.
8. Foulkes, M.J., G.A. Slafer, W.J. Davies, P.M. Berry, R. Sylvester-Bradley, P. Martre, D.F. Claderini, S. Griffiths, M.P. Reynolds, 2010. Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. Journal of Experimental Botany, 1-18.
9. Geiger, D.R., K.E. Koch, W.J. Shieh, 1996. Effect of environmental factors on whole plant assimilate partitioning and associated gene expression. J. Exp. Bot., 47: 1229-1238.

10. Gifford, R.M., J.H. Thorne, W.D. Hitz, R.T. Giaqunita, 1984. Carbon productivity and photo-assimilate partitioning. Science, 225: 801-808.
11. Gutam, S., V. Nath, G.C. Srivastava, 2008. Endogenous hormonal content during grain development in hexaplid and tetraploid wheat. Bangladesh J. Agril. Res., 33(3): 493-502.
12. Hakaka, K., 1998. Growth and yield potential of spring wheat in a simulated changed climate with increased CO_2 and higher temperature. European J. Physiol., 9(1): 41-52.
13. Hasan, M.A., A.M.A. Kamal, 1998. Effect of fertilizers on grain yield and grain protein content of wheat. J. Nat. Sci. Council of Sri Lanka., 26(1): 1-8.
14. Heuvelink, E., N. Bertin, 1994. Dry matter partitioning in a tomato crop: Comparison of two simulation models. J. Hort. Sci., 69: 885-903.
15. Hoagland, D.R., D.I. Arnon, 1939. The water method for growing plants without soil. Calif. Agric. Expt. Stn. Cir. 347.
16. Larsson, R.M., W.K. Hensen, 1992. Studies on seed quality of Triticale a-amylase and starch. Agro. Food Industry Hi-Tech., 3: 26-28.
17. Nautiyal, N., C. Chatterjee, C.P. Sharma, 1999. Copper stress affects grain filling in rice. Communications Soil Sciences and Plant Analysis, 30: 11-12.
18. Ravi, I., F.A. Khan, P. Sharma-Natu, M.C. Ghildiyal, 2001. Yield response of durum (*T. durum*) and bread wheat (*T. aestivum*) varieties to carbon dioxide enrichment. Indian J. Agric. Sci., 71: 444-449.
19. Sharma-Natu, P., M.C. Ghildiyal, 2005. Potential targets for improving photosynthesis and crop yield. Current Science, 88: 1918-1928.
20. Stoy, V., 1969. Inter-relationship among photosynthesis, respiration and movement of carbon. In: Eastin JD, Haskins FA, Sullivan CY, Vanbavel CHM (ed) Physiological aspects of Grain Yield. Amer. Soc. Agron. Crop Sci. Wisconsin, USA.
21. Vance, C.P., S. Stade, 1984. Alfalfa root nodule carbon dioxide fixation. II. Partial purification and characterization of root nodule phosphoenolpyruvate carboxylase. Plant Physiology, 75: 261-264.
22. Wang, Z., H.M. Yin, H. Cao, 1998. Source-sink manipulation effects of post-anthesis photosynthesis and grain setting on spike in winter wheat. Photosynthetica (Prague) 35: 453-459.
23. Yang, J., J. Zhang, Z. Wang, Q. Zhu, 2003. Hormones in the grains in relation to sink strength and post-anthesis development of spikelets in rice. Plant Growth Regulation, 41: 185-195.
24. Yin, Y., Z. Wang, M. He, J. Fu, S. Lu, 1998. Post anthesis allocation of source/sink change. Biologica Plantarum, 41(2): 203-209.