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ENZYMES OF GLUTAMINE METABOLISM IN TESTA-PERICARP AND ENDOSPERM OF DEVELOPING WHEAT GRAIN

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Key Word Index—*Triticum aestivum*; Gramineae; wheat; endosperm; testa-pericarp; development; glutamate dehydrogenase; glutamine synthetase; glutamate synthase; glutamate oxaloacetate transaminase, glutamate pyruvate transaminase.

Abstract—Glutamate dehydrogenase, glutamine synthetase, glutamate synthase, glutamate puruvate transaminase and glutamate oxaloacetate transaminase have been assayed in developing testa-pericarp and endosperm of two wheat varieties, namely Shera (11.6% protein) and C-306 (9.8% protein). On per organ basis, activities of all the enzymes studied, except glutamine synthetase, increased during development. Glutamine synthetase activity decreased during development in the testa-pericarp, whereas, no glutamine synthetase activity could be detected in endosperm of either variety at any stage of development. Compared to testa-pericarp, endosperm had higher activities of glutamate synthase and glutamate pyruvate transaminase. On the whole, enzyme activities in Shera were higher, as compared to C-306. Developmental patterns and relative levels of enzyme activities in the two varieties were more or less the same, when expressed on dry weight basis or as specific activities. The results suggest that ammonia assimilation in developing wheat grain takes place by the glutamate dehydrogenase pathway in the endosperm; and both by the glutamate dehydrogenase and glutamine synthetase–glutamate synthase pathways in the testa-pericarp.

INTRODUCTION

Glutamine is the major form of organic nitrogen in the phloem exudates of leaves [1] feeding the developing cereal grains. Amino acids derived from the leaves are transformed within the grain to provide the appropriate types of amino acids in the correct proportions for synthesis of grain proteins [2]. Enzymes of glutamine metabolism in developing grains of wheat varieties, namely Shera (11.6% protein) and C-306 (9.8% protein) have been reported earlier [3].

The assimilates from the phloem pass via the peduncle into the testa-pericarp en route to endosperm where protein synthesis is active. The testa-pericarp of immature grain remains green and active in photosynthesis [4] and thus provides the carbon skeletons required for the assimilation of ammonia and transformation of amino acids in the grain. Compartmentalization of the enzymes of glutamine metabolism between the chlorophyllous testa-pericarp and the nonchlorophyllous endosperm, may be of interest. The present paper reports activities of the enzymes of glutamine metabolism in the testa-pericarp and endosperm of developing grains of Shera and C-306 wheat varieties, with particular emphasis on compartmentalization of these activities between the endosperm and the testa-pericarp.

RESULTS

Since it was not feasible practically to separate testa-pericarp from endosperm at 10 days after anthesis (DAA) and 38 DAA; the values reported for these two stages are for the whole grain and have been published earlier [3]. Dry weight and total protein per organ increased during development both in endosperm and testa-pericarp (Fig. 1). Glutamate dehydrogenase (GDH), glutamate synthase (GOGAT), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities per organ also increased during this period (Figs 2 and 3). On the other hand, glutamine synthetase (GS) activity per organ decreased during grain development in testa-pericarp (Fig. 2b). However, no GS activity could be detected in endosperm of either variety at any stage of development.

Shera grain, as compared to C-306, had a much higher content of protein both in endosperm as well as in testa-pericarp (Fig. 1b). Testa-pericarp and endosperm in Shera had higher dry weights in the early and later stages of grain development, respectively, as compared to C-306 (Fig. 1a). Endosperm in Shera, as compared to C-306, had lower activities of GOGAT and GOT (Figs 2c and 3b) and higher activity of GPT (Fig. 3a). GDH activity was higher in Shera endosperm, as compared to C-306, only during later stages of grain development (Fig. 2a). GDH, GOGAT and GPT activities were higher in testa-pericarp of Shera, as compared to C-306 (Figs 2, c and 3a). GOT activity was also higher in testa-pericarp of Shera, as compared to C-306, for most of the period but became lower during later stages of development (Fig. 3b).

On per organ basis endosperm, as compared to testa-pericarp, had higher dry weight and protein content and activities of GOGAT and GPT (Figs 2c and 3a), whereas GS activity appeared to be localized exclusively in the testa-pericarp, GDH activity was more in endosperm as compared to testa-pericarp in C-306 while the reverse was found in Shera (Fig. 2a, b). GOT activity in Shera was lower in endosperm, as compared to testa-pericarp; however, the comparative pattern in C-306 was not uniform (Fig. 3b).

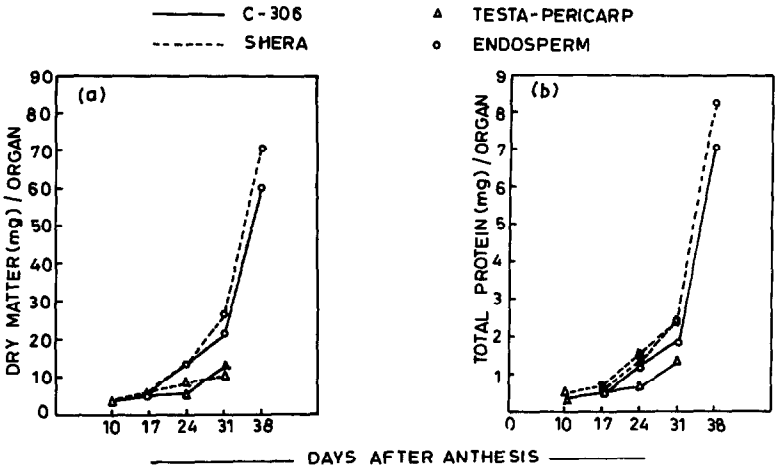

Fig. 1. Dry matter (a) and total protein (b) per organ in developing testa pericarp and endosperm of C-306 and Shera wheats.

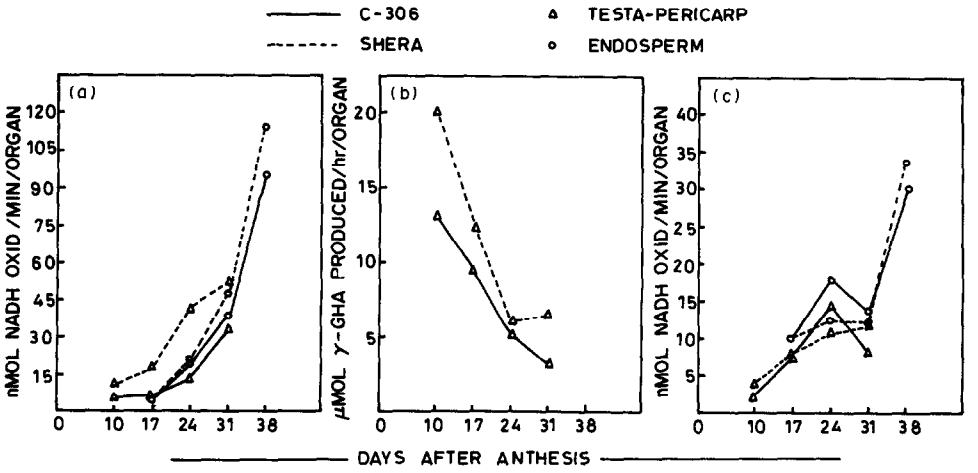

Fig. 2. Glutamate dehydrogenase (a), glutamine synthetase (b) and glutamate synthase (c) activities per organ in developing testa-pericarp and endosperm of C-306 and Shera wheats.

On a dry weight basis, enzyme activities followed more or less similar patterns as on a per organ basis (data not given). However, GDH, GOGAT and GPT activities did not increase during later stages of grain development. Specific activities of the endosperm enzymes did not increase at 38 days after anthesis. On the other hand, the specific activity of GOGAT decreased throughout development in endosperm.

DISCUSSION

Testa-pericarp plays an important role in the supply of carbon and nitrogen to the developing cereal grain particularly during early stages of grain development, when it is bright green. In developing barley grains, the pericarp changes from pale green to bright green early in development, and the green chlorophyll disappears during later stages of maturity [5]. Pericarp has a high rate of light dependent oxygen evolution and exhibits several enzyme activities associated with photosynthetic carbon reduction and nitrogen assimilation [5–7]. The present work has also clearly demonstrated the activities of GDH, GS, GOGAT, GOT and GPT in the testa-pericarp of wheat upto 31 DAA. The testa-pericarp is, therefore, active in assimilation of ammonia and transformation of amino acids. Testa-pericarp of Shera had higher activity of GDH than that of the endosperm, suggesting GDH to be active in the assimilation of ammonium ions translocated from the leaves to the developing grains, particularly in the testa-pericarp.

Glutamine synthetase activity was at its peak at 10 DAA in testa-pericarp (Fig. 2b). GS activity in the testa-pericarp may be associated with the synthesis of glutamine required for incorporation into soluble enzyme proteins that are synthesized in the testa-pericarp during the initial phase of grain development. The GS activity decreased to low levels at later stages (Fig. 2b) indicating that it may not play much of a role in the accumulation of storage proteins which had the highest rate of deposition during

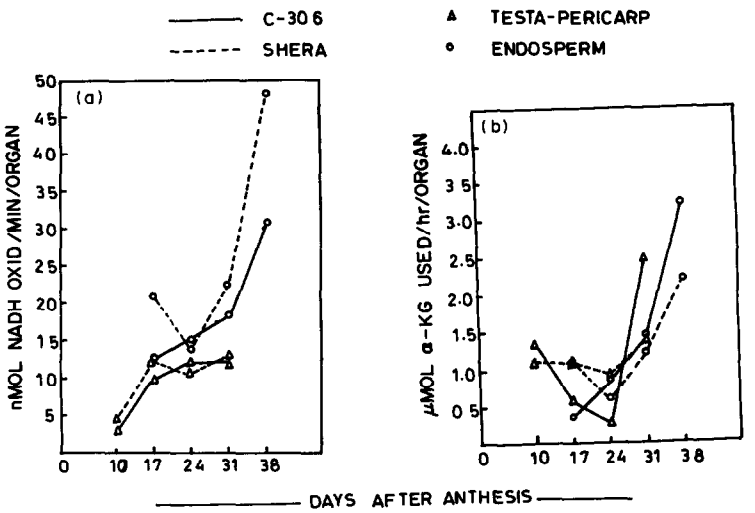

Fig. 3. Glutamate pyruvate transaminase [alanine aminotransferase] (a) and glutamate oxaloacetate transaminase [aspartate aminotransferase] (b) activities per organ in developing testa-pericarp and endosperm of C-306 and Shera wheats.

later phase of grain development (Fig. 1b). Further, endosperm had no detectable GS activity and it is the endosperm where storage proteins are deposited. Glutamine synthesized in testa-pericarp may, however, be translocated to endosperm where it may be acted upon by GOGAT or directly incorporated into storage proteins. Thus, GS in developing grain may have an indirect rather than a direct role in the deposition of storage proteins and therefore, GDH rather than GS appears to be more important in ammonia assimilation in developing grain.

GOT activity per organ increased with maturity (Fig. 3b). This enzyme is required for transformation of glutamate derived from ammonium ions assimilated either by the GDH pathway or the GS–GOGAT pathway [8]. Glutamate, once formed, is known to be immediately incorporated into proteins without extensive metabolism [9]. A part of glutamate in endosperm may, however, be utilized by GPT for the synthesis of alanine.

The results discussed above indicate that ammonia assimilation in developing wheat grain takes place by the GDH pathway in endosperm and by both the GDH and GS–GOGAT pathways in testa-pericarp. Though the two varieties under study were not isogenic, it may be suggested that the differences in protein accumulation between the two varieties might have been caused mainly by differential transport of glutamine from pericarp into the endosperm and lower utilization of glutamate by transaminases in endosperm of Shera as compared to C-306. It may, however, be confirmed by conducting similar studies on isogenic lines.

EXPERIMENTAL

Plant material. C-306 and Shera wheat plants were grown in pots in winter (rabi) season under identical environment and fertility conditions. Ears were harvested at 10, 17, 24, 31 and 38 days after anthesis (DAA), grains dehusked, testa-pericarp and endosperm separated and stored in liquid nitrogen. Separation of testa-pericarp from the endosperm of the grain was practically feasible only from 17 to 31 DAA. Hence, whole grains were used for analysis at 10 DAA and 38 DAA (mature grains). Glutamate dehydrogenase, glutamine synthetase, glutamate synthase, aspartate amino transferase (GOT) and alanine amino transferase (GPT) were assayed according to ref. [3]. Reaction mixture for these enzymes consisted of the following:

Glutamate dehydrogenase: 0.1 M Tris–HCl buffer (pH 7.6), 1.4 ml; 3 M NH_4Cl, 0.1 ml; 0.33 M α-ketoglutaric acid (neutral), 0.1 ml; 1 mM NADH, 0.2 ml and enzyme extract, 0.2 ml.

Glutamine synthetase: 0.2 M imidazole–HCl buffer (pH 7.5), 0.4 ml; 0.1 M $MgCl_2$, 0.4 ml; 2-mercaptoethanol (1:100 v/v), 0.4 ml; 0.5 M sodium glutamate, 0.2 ml; 0.5 M NH_2OH–HCl (freshly prepared by mixing equal vols of 1 M NH_2OH–HCl and 1 M NaOH), 0.2 ml; 0.1 M ATP, 0.2 ml and enzyme extract 0.3 ml.

Glutamate synthase: 0.1 M Tris–HCl buffer (pH 7.6), 0.5 ml; 0.2 M α-ketoglutaric acid (neutral), 0.2 ml; 0.2 M glutamine, 0.2 ml; 2-mercaptoethanol (1:100 v/v), 0.2 ml; 0.1 M KCl, 0.3 ml; 0.02 M EDTA, 0.2 ml; 1 mM NADH, 0.2 ml and enzyme extract, 0.2 ml.

Aspartate amino transferase (glutamate oxaloacetate transaminase): 0.2 M NaPi buffer (pH 7.5), 0.2 ml; 0.02 M α-ketoglutaric acid (neutral), 0.1 ml; 0.5 M aspartic acid (neutral), 0.3 ml; and enzyme extract, 0.2 ml.

Alanine amino transferase (glutamate pyruvate transaminase): 0.1 M Tris–HCl buffer (pH 7.6), 0.6 ml; 0.25 M α-ketoglutaric acid (neutral), 0.05 ml; 0.5 M alanine, 0.2 ml; lactate dehydrogenase, 2 units; 2 mM pyridoxal phosphate, 0.1 ml; 1 mM NADH, 0.2 ml; enzyme extract, 0.2 ml and water 0.8 ml.

Soluble protein was estimated according to ref. [10]. Total protein was determined by the microkjeldahl method [11]. Two independent extractions were carried out for each sample and each extract analysed in duplicate. Each value reported in this paper is the average of four coincident values.

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