JJBS

Volume 1, Number 2, Mar. 2008
ISSN 1995-6673
Pages 85 - 88

Jordan Journal of Biological Sciences

Effect of Chlorogenic and Caffeic Acids on Activities and Isoenzymes of G6PDH and 6PGDH of *Artemisia Herba Alba* Seeds Germinated for One and Three Days in Light and Dark

(Short Communication)

Farouk Al-Quadan *, Abeer Ibrahim, Fawzia M.R. Al-Charchafchi

Department of Biological Sciences., Faculty of Sciences, Hashemite University P. O. Box 330093. Zarqa 13133. Jordan.

Abstract

G6PDH and 6PGDH activities of *Artemisia herba alba* seeds during early stages of seed germination under conditions of light and dark were measured, in the presence of Chlorogenic or Caffeic acids; G6PDH (glucose 6 phosphate dehydrogenase) and 6PGDH (6 phospho gluconate dehydrogenase) activities were inactivated in the presence of both phenolic acids and were stimulated upon germination in the dark (Table 1 and Table 2). This novel approach provides evidence for the variable needs of pentose phosphate pathway activity, depending on concentrations of phenolic acids present and /or light conditions; moreover, a clear response of such germination condition was earlier expression of a second isoenzyme for G6PDH in the presence of 0.4 mM Chlorogenic or Caffeic acids with apparently variable inhibitory pattern.

الملخص

لقد اجري هذا البحث لدراسة تاثيراثنين من الأحماض الفينولية (الكافيك و الكلوروجنك) على نشاط إنزيمي جلوكوز ٦ فوسفات ديهيروجينيز و ٦-فوسفات جلوكونيت ديهيروجينيز المستخلصة من بذور نبات الشيح النامية في ظروف الضوء و الظلام لمدة ١ أو ٣ أيام في مستويات مختلفة من الحوامض الفينولية، وقد تبين أن نشاط الإنزيمين يزداد من اليوم الأول إلى الثالث في ظروف الضوء و الظلام، كما أن الإنزيمين يتم تثبيطهما في وجود حامضي كلوروجينيك و كافييك في ظروف الضوء و الظلام. وقد بينت النتائج الحاجة المتغيرة لنشاط تفاعلات سلسلة السكريات الخماسية المفسفره في وجود حامضي كلوروجينيك و كافييك. أما بالنسبة إلى أشباه الإنزيمين فقد بينت النتائج وجود شبيه واحد في اليوم الأول ثم يصبح شبيهين اثنين في اليوم الثالث من نمو البذور لكلا الإنزيمين في الظلام. كما بينت الدراسة أن وجود الأحماض الفينوليه عند الإنبات يؤدي إلى الظهور المبكر لشبيه جلوكوز ٦ فوسفات ديهيروجينيز كما يؤثر على احد الشبيهين أكثر من الأخر.

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Keywords: Chlorogenic acid, Caffeic acid, G6PDH, 6PGDH, Artemisia herba alba;

1. Introduction

Phenolic compounds were linked with seeds germination inhibition and dormancy in *Artemisia herba alba*. (Al-Charchafchi and Al-Quadan, 2006, Al-Charchafchi et al., 1987; Alam et al., 2001; Muscolo et al., 2001). Their concentrations in plants were subject to seasonal alterations (Solar et al., 2006). Phenolics accumulation played a protective role in strengthening the plant cell walls during growth by polymerization into lignins (Diaz, 1997).

Phenolic compounds are primarily synthesized through the pentose phosphate, and shikimate pathway. (Ali et al., 2006, Sgherri et *al*., 2004, Dixon, and Paiva 1995, Shetty et al., 2002; Randhir et al., 2005, Kova´cˇik et al., 2006).

Glucose-6-phosphate dehydrogenase (G6PDH E.C 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH E.C 1.1.1.44) are the key enzymes of pentose phosphate pathway, used in the biosynthesis of phenolic compounds (Andrei et al., 2002; Sükrü et al., 2003). In germinated seeds of *Artemisia herba alba*, activities of both enzymes were inhibited in presence of esculetin (Al-Charchafchi and Al-Quadan, 2006); whereas elevation of both activities were noticed in the dark (Robinson, 2000).

This study was undertaken to investigate G6PDH or 6PGDH activities in seeds of *Artemisia herba alba* germinated under the dual effect of inhibition of both enzyme activities (growth in presence of phenolic compounds) , and stimulation of both enzyme activities, that is the growth under dark condition; The timing of this study was designed at one and three days of germination, which represents an early stage of photosynthetic system development, and hence halt of $NADPH_2$ production from

* Corresponding author. e-mail: fquadan@hu.edu.jo

photosynthetic system; moreover, isoenzyme patterns of both dehydrogenases under such conditions were also investigated.

2. Materials and Methods

2.1. Plant Material

Seeds of *Artemisia herba alba* used in the study were harvested during December 2003 from the medicinal and aromatic plant garden of the Hashemite University/Zarka, Jordan. The seeds were kept in brown paper bags in refrigerator until used.

2.2. Germination and Growth

Germination was performed in 12 cm diameter petri dishes lined with one layer of filter paper (Whateman No.30) and moistened with 5 ml of distilled water or Chlorogenic or Caffeic acids at 0.2 and 0.4 mM. 100 seeds per dish (three replicates) were used for each treatment. All dishes were incubated at 20 °C in growth chamber under continuous illumination or darkness for 1, 3, and 5 days. Dark treatment was conducted by wrapping the dishes with aluminum foil immediately after seed imbibitions. Chlorogenic and Caffeic acids were prepared just prior to use and were wrapped in aluminum foil container.

2.3. G6PDH and 6PGDH Extraction Procedure

Extraction solution consisted of 100 mM Tris-HCl (pH 7.6), 2 mM EDTA Na2 [Scharlau, Spain] and 30 mM β-Mercaptoethanol [ARCOS, USA].The imbibed seeds and seedlings were suspended in 1 ml of extraction solution and were homogenized at 25000 rpm for 3 min using IKA homogenizer [Germany]. During homogenization, samples were immersed in ice bath. Homogenates were then centrifuged for 20 minutes at 10000 rpm at $4^{o}C$; supernatant were re-centrifuged two more times for 5 minute to remove lipid debris. Supernatants were used immediately for G6PDH and 6PGDH activity determination, or stored at -70 °C until time for analysis.

2.4. G6PDH and 6PGDH Assays

The assay procedure as described earlier (Al-Quadan and Al-Charchafchi 2006) was used. All assays were performed with GENSYS 5, USA spectrophotometer. Each assay consisted of 850 µ1 Tris-HCl buffer (10 mM, pH 7.6), 100 µ1 MgCl2 (67 mM), 10 µ1 glucose-6-phosphate or 6-Phosphogluconate (10 mM) [Sigma, Mo, USA] and 10 µ1 NADP+ (5mM) [BDH, England] in a total volume of 1 mL.

2.5. Protein Determination

Quantitative protein determination by the method of Bradford (1976), with bovine serum albumin (BSA) was used as standard.

2.6. Electrophoresis

Polyacrylamide gel electrophoresis and isoenzymes visualization was carried out as described earlier (Al-Quadan and Al-Charchafchi 2006) using 7.5% acryl amide and Bio-Red vertical cell [USA]. The gels were photographed using digital camera (JAI, Japan) interfaced to a computer [DELL, USA].

3. Statistical Analysis

ANOVA test was used to determine the level of significance within the *Artemisia herba alba* species regarding the effect of Chlorogenic acid or Caffeic acid on G6PDH and 6PGDH activities. Significance of differences was accepted when $p< 0.05$.

4. Results and Discussion

G6PDH and 6PGDH activities after one, and three days of seed germination under light or dark condition, in the presence or absence of different concentrations of Chlorogenic and Caffeic acids are presented in Tables 1 and 2.

G6PDH and 6PGDH activities were increased from day 1 to day 3 of growth under growth condition used ($P<0.05$). On the other hand, in the presence of 0.2 mM or 0.4 mM Chlorogenic or Caffeic acids both activities were significantly decreased ($P<0.05$) in comparison with absence of such phenolic compounds; the inhibition pattern was less pronounced on day three of seed germination; our explanation to this inhibition pattern may account either for the differential inhibition or sub cellular distribution of both isoenzymes. (Debnam and Emes (1999); Al- Quadan *et al.,* 1982; Hoover *et al.,* 1977). Chlorogenic and Caffeic acids inhibitory effect on the germination of *Artemisia herba alba* seeds (Al-Charchafchi and Al-Quadan, 2006) was strongly correlated to the inhibition of the very sensitive enzyme activities of oxidative pentose phosphate pathway.

The elevated activities of pentose phosphate pathway on day three in seeds germinated in the dark may be needed to compensate the short supply of NADPH resulted from the halt of photosynthetic pathway (Robinson, 2000). Number of isoenzymes of G6PDH and 6PGDH after one, and three days of seed germination under light or dark condition, in the presence or absence of different concentrations of Chlorogenic and Caffeic acids are presented in Table 3.

After one day of seed germination under light or dark condition, electrophoresis study showed only one G6PDH isoenzyme band (R_f 0.52); an additional slower isoenzyme band (R_f of 0.49) was present after three days of seeds growth in the presence of 0.4mM Chlorogenic or Caffeic acids. This slower band was also present on the third day of growth under light condition, but only if 0.4 mm Chlorogenic or Caffeic acids are present. The slow moving band had a lower relative intensity compared to the fast moving band.

Electrophoresis study showed one 6PGDH isoenzyme band (R_f 0.50) after day one of germination; an additional

faster isoenzyme band (R_f of 0.53) was present after three days of seed germination under all growth condition used. The fast moving band had lower relative intensity compared to the slow moving band

Table1: G6PDH specific activities (Units/mg protein) of *Artemisia herba alba* seeds after one and three days of germination under growth conditions indicated in presence of Chlorogenic acid or Caffeic acid. Values reported were mean of triplicate repeats ± SD

mM Chlorogenic or Caffeic acid	Chlorogenic acid				Caffeic acid			
	Light Treatment		Dark Treatment		Light Treatment		Dark Treatment	
	Day 1 G6PDH	Day 3 G6PDH	Day 1 G6PDH	Day 3 G6PDH	Day 1 G6PDH	Day 3 G6PDH	Day 1 G6PDH	Day 3 G6PDH
0	44 ± 11	49 ± 9	47 ± 12	70 ± 13	44 ± 11	49 ± 9	47 ± 12	70 ± 13
0.2	32 ± 8	36 ± 11	41 ±12	60 ± 9	26 ± 6	32 ± 7	42 ±13	64 ± 12
0.4	34 ± 8	39 ± 13	41 ± 12	63 ± 12	36 ± 9	42 ± 10	45 ± 12	65 ± 11

Table 2: 6PGDH specific activities (Units/mg protein) of *Artemisia herba alba* seeds after one and three days of germination under growth conditions indicated in presence of Chlorogenic acid or caffeic acid

mM Chlorogenic or Caffeic acid	Chlorogenic acid				Caffeic acid			
	Light Treatment		Dark Treatment		Light treatment		Dark Treatment	
	Day 1 6PGDH	Day 3 6PGDH	Day 1 6PGDH	Day 3 6PGDH	Day 1 6PGDH	Day 3 6PGDH	Day 1 6PGDH	Day 3 6PGDH
0	47±13	61±10	62±10	69±11	47±13	61±10	62±10	69±11
0.2	24±6	62±11	38±8	62±13	35±7	48±11	45±6	53±11
0.4	38±11	67±14	51±10	67±10	41±9	58±11	52±9	61±11

Table 3: Effect of Chlorogenic /Caffeic acid on number of G6PDH and 6PGDH isoenzymes from *Artemisia herba alba* seeds after 1 and 3 days of germination under light and dark condition.

Chlorogenic or Caffeic acid mM	G6PDH isoenzymes				6PGDH isoenzymes			
	Light Treatment		Dark Treatment		Light treatment		Dark Treatment	
	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
0	1	1	1	2	1	2	1	2
0.2	1	1	1	2	1	2	1	2
0.4	1	2	1	2	1	2	1	2

This study has indicated that activation and inhibition of pentose phosphate pathway enzyme activities in *Artemisia herba alba*, provides evidence for the variable needs of pathway activity depending on light conditions and /or concentrations of phenolic compounds presence; moreover, the early expression of second G6PDH isoenzyme as early as day three of germination in the presence of Chlorogenic or Caffeic acids, and with apparently different inhibitory patterns, elucidated the role played by those phenolic compounds in regulating pentose phosphate pathway activity.

Future study of Chlorogenic and Caffeic acids effect on purified isoenzymes should provide insight into this differential inhibition pattern.

Acknowledgment

Supported by a grant from the Deanship of Research and Graduate Studies, the Hashemite University.

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