NJ, according to the manufacturer's instructions updated in March 1979. The technique combines resin column chromatographic separation with colorimetric quantitation. Enzyme activities were assessed at 30 °C. Locally established ranges for a normal control population not participating in strenuous sports were 0–83 U/l total CK and less than 4% CK-MB. The paired Student's t-test was applied to the pre- and post-race data.

Results. Serum total CK, percent MB, and CK-MB activities before and immediately after the race are summarized in figure 1. The mean pre-race activities were close to the upper limits of normal. In three runners, the total CK, and in additional three, the CK-MB were greater than normal. These athletes trained in the two days before the race. The mean enzyme activities of the other runners fell into the middle of the normal range or were below detectibility.

Post-race total CK and percent MB activities doubled, while CK-MB activities more than tripled. The total CK increased in all runners. However, the percent MB declined in four athletes, in two of which the CK-MB also declined.

The correlation of the clocked running time of 17 or 15 athletes who completed the race and for whom times were recorded are given in figures 2 and 3. Increases were greater in the slower runners.

Discussion. Serum CK and CK-MB as well as other enzyme tests are important and sensitive tools for the diagnosis of acute myocardial infarcts and other myocardial injuries[1,2]. Increased CK and CK-MB are also found in healthy persons after long distance running[3–8], thus complicating the interpretation of enzyme changes in athletes with chest pain or other cardiac symptoms. In asymptomatic runners, the source of the circulating CK and CK-MB likely is not the myocardium, but rather injured skeletal muscle, according to numerous reports[3–5,7]. Most studies of exercise-related enzyme changes are performed in long distance runners[3–8], consistently documenting significant increases. Moderate exercise, however, is associated with inconsistent or smaller increases[8,9], presumably because of minor muscular injury. Symansky et al.[9] report absence of enzyme changes in competitive swimmers. Forces of 130–215 kg reportedly are exerted during down strokes during running, while forces of 2–9 kg are recorded during swimming[9], suggesting less trauma in swimmers. These findings argue for a correlation of the severity of trauma and the magnitude of enzyme increases.

Stansbie et al.[8] describe a direct correlation of serum total CK and the order in which marathon runners complete their races. The present study demonstrates a significant correlation of clocked running time and both CK and CK-MB increases. The greatest increases are found in the slowest runners, suggesting that longer running times are associated with greater trauma to skeletal muscle leading to enzyme leakage into the circulation. The slower runners are very likely also the less trained or less fit, older athletes.

Little is known about the time course of enzyme increases and subsequent normalization[5]. Therefore, alternatively, the greater increase in the slower runners may, because of different timing of sample collection in reference to the midpoint of the race, reflect the peak of enzyme leakage rather than greater trauma.

In view of the findings of ours, of Stansbie et al.[8], and others[5], increased serum CK and CK-MB activities in athletes suspected of having suffered recent myocrdial injury should be interpreted after exclusion of recent exercise related skeletal muscle trauma, especially in the slower, less fit, and older athletes.

---

* Acknowledgments. The authors wish to thank the members and president of the Columbia Track Club for their cooperation.

1 Roberts, R., Gowda, K.S., Ludbrook, P.A., and Sobel, B.E., Am. J. Cardiol. *36* (1975) 433.
2 Ali, M., Laraia, S., Angeli, R., Fayemi, A.O., Braun, E.V., Davis, E., and Palladino, P.H., Am. J. clin. Path. *77* (1982) 573.
3 Appel, F.S., and McGue, M.K., Am. J. clin. Path. *79* (1983) 716.
4 Reinhart, W.H., Staeubli, M., Koechli, H.P., and Straub, P.W., Clinica chim. Acta *125* (1982) 307.
5 Munjal, D.D., McFadden, J.A., Matix, P.A., Coffman, K.D., and Cattaneo, S.M., Clin. Biochem. *16* (1983) 195.
6 Priest, J.B., Oei, T.O., and Moorehead, W.R., Am. J. clin. Path. *77* (1983) 285.
7 Siegel, A.J., Silverman, L.M., and Holman, B.L., J.A.M.A. *246* (1981) 2049.
8 Stansbie, D., Aston, J.P., Dallimore, N.S., Williams, H.M.S., and Willis, N., Clinica chim. Acta *132* (1983) 127.
9 Symanski, J.D., McMurray, R.G., Silverman, L.M., Smith, B.W., and Siegel, A.J., Clinica chim. Acta *129* (1983) 181.

0014-4754/85/010039-02$1.50 + 0.20/0

Free amino acid pattern in stressed leaves of two contrasting resistant and susceptible cultivars of chick pea (*Cicer arietinum*)

G. Singh, P.S. Thakur and V.K. Rai

Department of Biosciences, Himachal Pradesh University, Simla-171005 (India), 9 September 1983

Summary. Drought resistant cv. C-214 of *Cicer arietinum* L. showed higher accumulation of α alanine, threonine, glutamine, β-alanine, arginine, γ amino butyric acid, valine, leucine, phenylalanine than the susceptible cv. G130 under water stress.

Key words. Cicer arietinum; free amino acids, drought resistance; water stress.

Higher proline accumulation has been proposed as an index of drought resistance in barley[1], but its usefulness could not be confirmed in chick pea, rice or barley[2–4]. Exogenous proline, leucine, asparagine, arginine, alanine, phenylalanine, γ amino butyric acid, glutamine and threonine protect membranes of *Vinca* against acid and alkali[5]. Here, the amino acid spectra in

Amino acids ± SE (mg/g dry wt) in cv. C214 and cv. G130 leaves at different water stress levels

Amino acid	Osmotic potential (MPa) 0 C214	 G130	 −0.1 C214	 G130	 −0.3 C214	 G130	 −0.6 C214	 G130	 −0.8 C214	 G130	 −1.0 C214	 G130
Cysteic acid	1.83 ± 0.17	1.44 ± 0.33	1.43 ± 0.23	0.99 ± 0.20	0.72 ± 0.14	0.43 ± 0.07	0.89 ± 0.08	0.22 ± 0.04	0.41 ± 0.07	0.21 ± 0.03	0.59 ± 0.17	0.16 ± 0.03
Aspartic acid	0.92 ± 0.09	0.39 ± 0.06	0.29 ± 0.08	0.75 ± 0.13	0.24 ± 0.09	0.64 ± 0.10	0.44 ± 0.05	0.33 ± 0.06	0.55 ± 0.09	0.32 ± 0.05	0.73 ± 0.13	0.46 ± 0.08
Glutamic acid	3.06 ± 0.51	0.86 ± 0.10	2.58 ± 0.32	2.48 ± 0.10	2.15 ± 0.65	1.93 ± 0.35	1.92 ± 0.08	2.44 ± 0.22	2.75 ± 0.35	3.30 ± 0.24	3.81 ± 0.37	3.32 ± 0.29
Serine + Glycine	3.67 ± 0.48	3.74 ± 0.07	1.72 ± 0.09	3.97 ± 0.15	7.78 ± 0.34	4.08 ± 0.39	3.40 ± 0.53	2.99 ± 0.30	3.30 ± 0.27	4.47 ± 0.35	3.29 ± 0.26	3.78 ± 0.57
Asparagine	6.72 ± 0.36	4.89 ± 0.12	7.16 ± 0.28	3.23 ± 0.43	4.06 ± 0.18	4.73 ± 0.25	2.66 ± 0.18	1.44 ± 0.14	3.16 ± 0.40	2.66 ± 0.54	3.22 ± 0.38	2.29 ± 0.37
α-alanine + Threonine	0.61 ± 0.11	0.86 ± 0.08	4.01 ± 0.43	0.75 ± 0.05	1.43 ± 0.07	1.29 ± 0.23	1.33 ± 0.07	1.11 ± 0.12	1.65 ± 0.32	2.34 ± 0.25	2.49 ± 0.29	2.06 ± 0.17
β-alanine	0.91 ± 0.18	4.60 ± 0.10	2.58 ± 0.19	5.47 ± 0.51	2.63 ± 0.47	6.01 ± 0.37	1.92 ± 0.12	2.66 ± 0.26	2.43 ± 0.25	3.41 ± 0.32	2.49 ± 0.43	3.67 ± 0.30
Glutamine	0.61 ± 0.01	0.86 ± 0.14	1.15 ± 0.25	0.75 ± 0.09	1.43 ± 0.19	0.86 ± 0.11	1.03 ± 0.14	0.66 ± 0.12	2.89 ± 0.29	1.07 ± 0.09	2.34 ± 0.19	2.75 ± 0.25
Histidine + Lysine	1.22 ± 0.20	0.58 ± 0.17	0.86 ± 0.12	–	1.67 ± 0.24	0.64 ± 0.08	0.89 ± 0.08	0.66 ± 0.08	1.65 ± 0.17	0.75 ± 0.03	3.22 ± 0.24	1.60 ± 0.06
Tyrosine	0.31 ± 0.08	0.58 ± 0.16	–	–	0.48 ± 0.16	1.29 ± 0.13	–	0.33 ± 0.05	0.41 ± 0.05	–	0.44 ± 0.08	0.46 ± 0.05
Proline	2.52 ± 0.32	2.30 ± 0.40	3.26 ± 0.14	2.92 ± 0.23	6.32 ± 0.29	7.16 ± 0.21	7.40 ± 0.50	8.16 ± 0.54	8.24 ± 0.13	17.00 ± 0.22	11.32 ± 0.32	17.90 ± 0.33
Arginine	2.41 ± 0.33	1.44 ± 0.31	2.58 ± 0.29	1.74 ± 0.22	7.88 ± 0.78	3.00 ± 0.41	5.17 ± 0.43	2.10 ± 0.15	7.28 ± 0.46	3.52 ± 0.35	6.00 ± 0.52	5.18 ± 0.52
Unidentified	0.61 ± 0.05	0.86 ± 0.16	–	0.50 ± 0.10	0.48 ± 0.06	0.86 ± 0.10	0.44 ± 0.06	0.55 ± 0.07	0.41 ± 0.01	0.21 ± 0.05	0.29 ± 0.05	0.34 ± 0.04
γ-amino butyric acid	1.53 ± 0.25	6.32 ± 0.62	2.87 ± 0.41	5.47 ± 0.31	9.07 ± 0.53	5.46 ± 0.44	6.05 ± 0.49	4.21 ± 0.37	8.38 ± 0.69	10.12 ± 0.22	7.91 ± 0.69	8.60 ± 0.68
Valine	0.61 ± 0.10	0.86 ± 0.06	0.86 ± 0.06	–	1.43 ± 0.31	0.86 ± 0.12	1.33 ± 0.17	0.44 ± 0.04	1.24 ± 0.20	1.07 ± 0.18	1.46 ± 0.15	1.60 ± 0.14
Leucine + Phenylalanine	0.92 ± 0.11	5.17 ± 0.70	0.86 ± 0.06	4.47 ± 0.23	5.73 ± 0.51	6.23 ± 0.53	4.58 ± 0.31	5.76 ± 0.63	5.08 ± 0.44	7.24 ± 0.31	4.25 ± 0.43	8.02 ± 0.62

Data presents mean values of 3 replicates. –, Present in traces.

stressed leaves of 2 contrasting cultivars of chick pea were compared to investigate the relationship of other amino acids with drought resistance.

Ten day old plants were stressed by flooding their roots with PEG (0–1.0 MPa) according to Zur (1966)[7]. Wilted leaves were homogenized in 80% boiling ethanol and the supernatant was evaporated to dryness. The amino acid mixture was recovered in 20% ethanol (1 ml g^{-1} fresh wt). Twenty amino acids were separated with 2-way ascending paper chromatography and identified with 0.1% ninhydrin spray[8]. Individual amino acid spots were eluted in 20% ethanol and the color read at 570 nm using a VSU II.

In general, free amino acids showed an increase, as was reported earlier[9–11]. The accumulation resulted from increased proteolysis[12], or reduction in protein synthesis[13], and increased synthesis of amino acids[12]. Different amino acids contribute differentially to the soluble pool (table). The following amino acids accumulated markedly under stress; proline (× 4 in C214 and × 8 in G130), α alanine plus threonine (× 6.5), γ amino butyric acid (× 6), leucine plus phenylalanine (× 6.2) and glycine (× 5) in C214 and glutamic acid (× 4), glutamine (× 3) and arginine (× 3) in G130. There was also some accumulation of histidine and lysine (× 2.7) in both the cultivars; β-alanine (× 2.8) and valine (× 2.4) in C214 and leucine and phenylalanine (× 1.6) in G130. On the other hand, β-alanine and valine in G130, aspartic and glutamic acids in C214, and cysteic acid, asparagine and an unidentified amino acid in both the cultivars decreased with stress. β-alanine and γ amino butyric acid declined beyond −0.3 and −0.6 MPa respectively in G130. Tyrosine and serine plus glycine showed no trend.

The resistant cv. C214 was marked by high accumulation of amino acids, as is mentioned in the summary, and proline ranks fifth among the amino acids which accumulate (fold increase). Thus, besides proline, other amino acids may also play a role in drought resistance.

Various roles have been assigned to the accumulating amino acids, such as nitrogen storage under stress conditions, osmoregulation to overcome stress effects[14–16], and the stabilizing of membranes[5].

1 Singh, T.N., Aspinall, D., Paleg, L.G., and Boggess, S.F., Aust. J. biol. Sci. *26* (1973) 57.
2 Singh, G., and Rai, V.K., Biol. Plant. *23* (1981) 86.
3 Mali, P.C., and Mehta, S.L., Phytochemistry *16* (1977) 1355.
4 Hanson, A.D., Nelson, C.E., and Everson, E.H., Crop Sci. *17* (1977) 720.
5 Rai, V.K., and Kumari, A., Experientia *39* (1983) 301.
6 Singh, G., and Rai, V.K., Ind. J. Ecol. *7* (1980) 246.
7 Zur, B., Soil Sci. *102* (1966) 394.
8 Ranjan, S., and Laloraya, M.M., Pl. Physiol. *35* (1960) 714.
9 Barnett, N.M., and Naylor, A.W., Pl. Physiol. *41* (1966) 1222.
10 Srivastava, A.K., and Kooner, N.K., Ind. J. exp. Biol. *12* (1974) 278.
11 Thakur, P.S., and Rai, V.K., Envir. exp. Bot. *22* (1982) 221.
12 Chen, D., Kessler, B., and Monselise, S.P., Pl. Physiol. *39* (1964) 379.
13 Nir, I.A., Mayber, P., and Klein, S., Pl. Physiol. *45* (1970) 173.
14 Palfi, G., Koves, F., Bito, M., and Sebeteyen, R., Phyton *32* (1976) 219.
15 Gornig, M., Drfier, W., and Heinke, F., Biol. Res. *15* (1977) 377.
16 Gornig, M., and Thien, B.H., Biochem, Physiol. Pfl. *172* (1978) 311.

0014-4754/85/010040-02$1.50 + 0.20/0