ronomic data on the actual production conditions for these varieties of *T. dicoccum*, their correspondence and segregation into rainfed and irrigated categories on the basis of the magnitude of area under moisture hysteresis curve and MRI could not be ascertained as was possible earlier[1,2]. Nevertheless, it is my conviction that the varieties listed in the upper half of the Table 1 should actually perform better than those listed in the lower half of the table, under rainfed field conditions.

Area under hysteresis loops bears significant positive correlations with MRI and percentage hydration of seeds and significant negative correlations with per cent initial moisture held by the saturated seeds, bringing out clearly that the seeds of varieties with larger area under hysteresis loops, hold less amount of moisture in them on initial saturation, a condition obviously undesirable for varieties meant for cultivation under arid and semi-arid conditions (Table 3). With final per cent moisture held by the seeds on resaturation after one cycle of dehydration and rehydration, the hysteresis area does not bear any consistent significant correlations within tetraploid *durum* and *dicoccum* wheats, but bear a significant positive correlation within varieties of hexaploid *aestivum* species (Table 3).

In conclusion, wide variations are observed in moisture absorption and desorption characteristics and in the area enclosed under the normalized moisture hysteresis curves for seeds of 30 *T. dicoccum* wheat varieties. The moisture desorption and absorption isotherms also reveal the variations in the rate and extent of permeability of moisture across the seed matrix and seed cuticular membranes from one variety to the other. It is believed that the data would help the wheat breeders in their selection of parents for improvement of *dicoccum* wheat for increased productivity/tolerance to drought under arid and semi-arid regions.

1. Moharir, A. V., Proceedings of DAE-BRNS Symposium on Nuclear Applications in Agriculture, Animal Husbandry and Food Preservation, March 16–18, 1994, Nuclear Research Laboratory, IARI, New Delhi, India.
2. Moharir, A. V. and Nam Prakash, *Curr. Sci.*, 1995, **68**, 316–326.
3. Cantrell, R. G., in *Plant Breeding Review* (ed. Janick, J.), Van Nostrand Reinhold Co., New York, 1987, vol. 5, pp. 11–40.
4. Martin, H. J., *J. Am. Soc. Agron.*, 1930, **22**, 993–1003.
5. Bayles, B. B., Taylor, S. W. and Bartel, T., *J. Am. Soc. Agron.*, 1937, **29**, 40–52.
6. Sandhu, A. S. and Lande, H. H., *J. Agron.*, 1958, **50**, 78–81.
7. Levitt, J., *The Hardiness of Plants*, Academic Press, New York, 1956, p. 278.
8. Dedio, W., *Can. J. Plant Sci.*, 1975, **55**, 369–378.
9. Kaul, R. and Crowle, W. L., *Z. Pflanzenzuchtg.*, 1974, **71**, 42–51.
10. Townley-Smith, T. F. and Hurd, E. A., in *Stress Physiology in Crop Plants* (eds Harry Mussell and Richard C. Staples), Wiley Inter Science and John Wiley, 1979, pp. 447–464.
11. Clarke, J. M. and Mc Caig, T. N., *Can. J. Plant Sci.*, 1982, **62**, 571–578.
12. Clarke, J. M. and Townley-Smith, T. F., *Crop Sci.*, 1986, **26**, 289–292.
13. Jones, H. G., *J. Agric. Sci.* (Cambridge), 1977, **88**, 267–282.
14. Clarke, J. M. and Townley-Smith, T. F., in *Crop Breeding: A Contemporary Basis* (eds Vose, P. B. and Blixt, S. G.), Pergamon Press, Oxford, pp. 137–162.
15. Clarke, J. M., Romagosa, I., Jana, S., Srivastava, J. P. and Mc Caig, T. N., *Can. J. Plant Sci.*, 1989, **69**, 1075–1081.
16. Mangelsdorf, P. C., *Readings from Scientific American with Introduction from Johan E. Hoff and Jules Janick*, 1953, pp. 94–103.
17. Harrington, J. F., in *Seed Ecology* (ed. Heydecker, W.), Butterworths, London, 1973, pp. 251–263.
18. Rao, M. V., in *Evolutionary Studies in World Crops* (ed. Joseph Hutchinson), Cambridge University Press, 1974, pp. 33–46.

ACKNOWLEDGEMENTS. I thank Dr M. G. Joshi, retired Principal Scientist for invaluable discussions and for critically going through the manuscript, to Dr A. B. Joshi for encouragement and to Mr Jagat Singh and Jagannath Mehto for all the help rendered.

Hydrolysing enzymes and respiration during ripening of tomato (*Lycopersicon esculentum*) fruits

Zeng Yanru, M. Pandey, N. K. Prasad and G. C. Srivastava

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110 012, India

Two varieties of tomato fruits, viz. Pusa *Ruby* and Pusa *Gaurav*, were studied with an attempt to get a better understanding of the relationship between respiration rate and hydrolysing enzymes during ripening. Pectin methyl esterase (PME) and polygalacturonase (PG) increased as ripening proceeded and reached a peak on 4th day after harvest followed by a decline. Respiration rate increased along with the activity of PME and PG with cyanide-resistant respiration being increasingly dominant compared to cyanide-sensitive respiration. There seems to be a close relation between the respiration (alternate) and hydrolysing enzymes during ripening of tomato fruit.

DURING ripening of climacteric fruits, a considerable increase in metabolic activities has been reported. Respiratory activity rises and shows a peak coinciding with a large volume of ethylene evolution in many fruits like banana[1] and avocado[2,3]. In tomato also hydrolysing enzymes like polygalacturonase (PG) and pectin methyl esterase (PME) have been reported to increase[3] during ripening.

In some fruits, the involvement of cyanide-resistant respiration has been reported to be responsible for raising temperature inside the fruits[4,5] required for increasing activity of enzymes. Whether there is any relationship between cyanide-resistant respiration and hydrolysing enzymes in tomato, which is also a climacteric fruit, is

not understood. In the present investigation the activities of PG and PME and cyanide-sensitive and cyanide-resistant respiration in ripening tomato are reported. Two varieties of tomato, Pusa *Ruby*, fast in ripening and Pusa *Gaurav*, comparatively slow in ripening, were used for the study. It was thought that the respiration rate and enzyme activities in two varieties would provide better understanding of their relationship during ripening.

Pusa *Ruby* and *Gaurav* were raised in small plots in the Division of Plant Physiology, IARI and normal cultural practices were followed. Fruits were harvested when they became mature but were still green and kept in an air conditioned lab maintained at 24 ± 2°C. Ripening-associated parameters such as PG, PME activity, total soluble sugar content, loss in weight and respiration rate in both varieties were measured every alternate day during the process of ripening.

PME activity was assayed following the method of Rouse and Atkins[6] and expressed as unit equivalent/g fr wt/min, while PG was estimated using the method described by Hobson[7] and expressed as mg glucose equivalent/g fr wt/h. Total sugar content was determined as suggested by Upmeyer and Koller[8] and expressed as mg sucrose equivalent to total carbohydrate/g fr wt. Respiration, including total as well as the cyanide-resistant and cyanide-sensitive, was measured on the basis of oxygen uptake by sliced tomato tissues (mesocarp) using a Clark-type oxygen electrode (Model DW2; Hansatech, UK). Salicylhydroxamic acid (SHAM, 5 mM) and KCN (5 mM) were used to check cyanide-resistant and cyanide-sensitive respiration respectively. A mixture of KCN and SHAM (5 mM each in concentration) was used to inhibit both types of respiration. Respiration rate was expressed as µmoles O_2 consumed/min/g d wt.

Data on PG, PME enzymes and sugar content in Pusa *Ruby* and Pusa *Gaurav* are given in Table 1. The activity of the two hydrolysing enzymes increased from the beginning and reached a peak on 4th day, thereafter followed by a decline. Similar observation was reported earlier by Tucker and Grierson[3]. Sugar content in the two varieties however showed a little different trend. In Pusa *Ruby* where ripening is rapid, the sugar content continuously increased reaching a peak on 4th day after harvest while in Pusa *Gaurav* where ripening is slow, the sugar content was high even from the beginning and started declining as the ripening proceeded. No specific explanation can be given at this stage for higher sugar content in Pusa *Gaurav* at the beginning of ripening except as a varietal character. This variety is not so sour as other commonly grown tomato varieties.

Data on cyanide-sensitive and cyanide-resistant respiration are reported in Table 2. It is interesting to note that respiration rate in Pusa *Ruby* (a fast-ripening variety) increased during ripening and reached maximum on 6th day and then showed a fall whereas in Pusa *Gaurav* the rate of increase was slow for a considerable number of days (8 days) and then reached a peak on 8th day. There was no cyanide-resistant respiration in unripe

Table 1. Polygalacturonase, pectinmethylesterase and sugar content in tomato fruits during ripening

	Pusa *Ruby*				Pusa *Gaurav*			
	Days after harvest				Days after harvest			
Parameter	1	4	6	8	1	4	6	8
PG activity	14.19	16.64	10.78	3.60	9.37	16.93	10.52	2.31
PME activity	0.086	0.155	0.149	0.182	0.079	0.224	0.082	0.12
Total sugar	40.48	49.94	33.85	37.70	48.44	47.38	32.39	37.95
Fr wt (g)	54.30	52.70	51.97	51.20	17.95	17.10	16.47	15.90

PG was expressed as mg glucose equivalent/g fr wt/h.
PME was expressed as unit equivalent/min/g fr wt.
Total sugar content was expressed as mg sucrose/g fr wt.

Table 2. Cyanide-sensitive and cyanide-resistant respiration in tomato fruits during ripening

	Pusa *Ruby*					Pusa *Gaurav*				
	Days after harvest					Days after harvest				
Treatment	1	4	6	8	10	1	4	6	8	10
Control	1.37	1.54	2.40	1.94	1.80	0.74	1.24	1.27	1.43	1.36
SHAM	1.44	1.00	0.90	0	0	0.82	1.18	0	0	0
KCN	0	0.56	1.44	1.92	1.65	0	1.52	1.20	1.35	0.70
KCN + SHAM	0	0	0	0	0	0	0.60	0	0	0

*Respiration rate: µmoles O_2/min/g d wt.

fruits of both the varieties and whatever respiration was present was all cyanide-sensitive respiration. These findings are supported by the results reported earlier[9]. Furthermore, in Pusa *Ruby* the increase in cyanide-resistant respiration was fast whereas in Pusa *Gaurav* it was slow. This point explains the ripening behaviour in these two varieties. The addition of inhibitors of cyanide-sensitive and cyanide-resistant system completely stopped the respiration and no oxygen uptake was noted.

It was reported that during ripening, respiration in climacteric fruit increases and the main component of this respiration is cyanide-resistant respiration, which is involved in thermogenesis in many ripening fruits[4,5] and in flowers[10]. These observations indicate that cyanide-resistant respiration is the major part of total respiration and it increases during ripening of tomato fruit along with the activity of hydrolysing enzymes and both the processes seem to be related to each other.

1. Tucker, G. A., *Biochemistry of Fruit Ripening* (eds Seymour, G., Talor, J. and Tucker, G.), Chapman and Hall, London, 1993, pp. 1–51.
2. Awad, M. and Young, R. E., *Plant Physiol.*, 1979, **64**, 306–308.
3. Tucker, G. A. and Grierson, D., *The Biochemistry of Plants*, Academic Press Inc., 1971, vol. 12, pp. 265–317.
4. Kumar, S., Patil, B. C. and Sinha, S. K., *Biochem. Biophys. Res. Commun.*, 1990, **168**, 818–822.
5. Kumar, S. and Sinha, S. K., *J. Expt. Bot.*, 1992, **43**, 1639–1642.
6. Rouse, A. H. and Atkins, C. D., *Florida Agri. Expt. Station Bull.*, 1955, **570**, 1–9.
7. Hobson, G. E., *Nature*, 1962, **195**, 804–805.
8. Upmeyer, D. J. and Koller, H. R., *Plant Physiol.*, 1973, **51**, 871–874.
9. Pandey, M., Zeng, Y. R., Prasad, N. K. and Srivastava, G. C., *Indian J. Plant Physiol.*, 1995, **38**, 182–183.
10. Chen, J. and Meeuse, B. J. D., *Plant Cell Physiol.*, 1972, **13**, 831.

Received 30 October 1995; accepted 3 April 1996

Mapping of assembled epitopes with microgram quantities of antigen: Identification of an epitope at the receptor binding region of human follicle stimulating hormone

G. S. Murthy and N. S. Srilatha

Primate Research Laboratory, Centre for Reproductive Biology and Molecular Endocrinology, Indian Institute of Science, Bangalore 560 012, India

Identification of epitopes by modification studies has been reported by us recently. The method requires milligram quantities of antigen and since several proteins are not available in large quantities they are not amenable for such an investigation. One such protein is human follicle stimulating hormone (hFSH) whose mapping of epitopes is of importance in reproductive biology. Here we report a method that uses microgram quantities of hFSH to map a β-specific epitope located at the receptor binding region. This identification has also been validated by the chemical modification method using heterologous antigen ovine follicle stimulating hormone (oFSH).

EPITOPE mapping is used to identify the region capable of binding to monoclonal antibodies (MAb) in an attempt to develop synthetic epitopes to be used in diagnostics, vaccines, etc.[1–4]. Linear peptide synthesis methodology, though popular, seldom provide, a sequence which has the same conformation as in the native molecule[5,6]. Recently, we reported a method for the identification of epitopic regions in their native conformation using chemical modification of antigen coupled to their immunoactivity as measured by solid phase radioimmunoassay (SPRIA)[7–9]. Even though this method is the first batch method reported for analysis of assembled epitopes, and compared to other existing methods is easier and adoptable in all laboratories, further improvement in the method can be envisaged in several directions. In the earlier method[7] need for antigen is rather high requiring milligram quantities and number of SPRIAs to be run are high. Any development which reduces both the quantity of antigen and number of SPRIAs required in epitope mapping would make the method extremely useful and economical. Several antigens are not available in mg quantities and one such antigen of importance in reproductive physiology is hFSH. Here we describe a method of epitope analysis which uses µg quantities of hSFH and eliminates the need for SPRIA in the analysis of assembled/sequential epitopes, and compare the results obtained with the conventional approach based on SPRIA of chemically modified antigen analogue, namely, oFSH.

MAb 68.K12.1D12 (referred to in this paper as 68.K12 – gift from Dr James A. Dias, Wadsworth Centre, WIH HD 18407) is a monoclonal antibody (MAb) against hFSH and binds to hFSH β-subunit. Displacement profile of [^{125}I]hFSH (obtained by iodination of hFSH by iodogen procedure) from immobilized MAb 68.K12 (Figure 1) shows that heterologous FSH, namely oFSH as well as its β-subunit binds to the antisera with 25% cross reactivity.

Chemical modification followed by gain or loss of activity with respect to a particular epitope has been used in our earlier studies[7,8] for mapping epitopes. Since hFSH or its subunits are not available in large amounts like human chorionic gonadotropin (hCG), we have modified iodinated hFSH and investigated the effect on