PIGEON PEA STRAINS: DEFICIENT IN PHYTOHEMAGGLUTININ ACTIVITY

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PLANT proteins for human diet are derived mainly from legume grains. Legumes are rich not only in protein but also in lysine—the first limiting amino acid in cereal proteins[1]. Supplementation of cereals with legumes can, therefore, go a long way in alleviation of protein malnutrition. However, a major limitation in increased consumption of legumes is the presence of several toxic constituents, particularly protease inhibitors, phytohemagglutinins and flatulence causing oligosaccharides. Elimination or minimization of these toxic constituents is essential to improve the nutritional quality of legume proteins. The presence of several of the toxic constituents, including phytohemagglutinins, is determined genetically, and therefore, genetic manipulation is the most desirable way to achieve this goal[2]. This communication reports the presence of very low levels of phytohemagglutinin activity in several strains of pigeon pea (*Cajanus cajan*).

Semiquantitative determination of phytohemagglutinin activity was based on the method described by Liener[3]. One g of finely divided legume seed flour was shaken vigorously with 10 ml of 0.15 N sodium chloride solution for 2 hr, on a wrist action mechanical shaker. The contents were centrifuged at 10,000 g for 20 min. Clear supernatant (0.4 ml) was poured into depression (pit) 1, on a macrotitration plate. Normal saline solution (0.9% sodium chloride solution, 0.2 ml) was placed in each of the next 19 depressions. An aliquot (0.2 ml) of the contents was transferred from depression 1 to depression 2. Likewise, 0.2 ml of the contents were transferred from depression 2 to depression 3, after thoroughly mixing the contents in depression 2. This process of successive transfer was continued till the contents from depression 18 had been transferred to depression 19. An aliquot (0.2 ml) from depression 19 was finally discarded, so that the contents in each of the 20 depressions measured 0.2 ml and starting from depression 1, the extract was diluted 2-fold in each successive depression. The extract in depression 19 was diluted 2,62,144 fold. The depression 20 which lacked the extract, served as a blank. Trypsinized rabbit erythrocyte suspension (0.2 ml) was added to each of the 20 depressions. The plates were incubated for 1 hr at 37°C, in a hot air oven. At the end of the incubation period, contents in each depression were observed with naked eye, for agglutination in the form of gel-clot at the base of the depression, in comparison with the blank depression. The trypsinized erythrocytes suspension was prepared fresh every day, from normal healthy rabbit blood, according to the method described by Sage and Green[4].

Table 1 *Phytohemagglutinin activity in pigeonpea and lentil strains*

Pigeon pea		Lentil	
Strain	Dilution factor	Strain	Dilution factor
H–7244	2	L–9–12	4096
H–77208	4	LH–82–3	2048
H–77216	2	LH–82–4	2048
Prabhat	1	LH–82–6	2048
T–21	2	LH–82–7	2048
UPAS–120	2	LH–21	2048
		LH–311	2048

Six strains of pigeon pea viz H-7244, H-77208, H-77216 Prabhat, T-21 and UPAS-120 were analyzed for the presence of phytohemagglutinin activity. Very little activity could be observed in these strains (table 1). The activity did not increase even when the incubation period was increased to 24 hr. Literature on phytohemagglutinins in legumes appears to be silent as far as pigeon pea is concerned[2]. To ensure that the method employed was sensitive enough to quantify phytohemagglutinin activity at levels normally present in legume seeds, the activity was also determined in different strains of lentil (*Lens esculenta* L). Lentil seeds exhibited phytohemagglutinin activity even when the extract was diluted over 2000 fold. However, different strains of lentil except L–9–12, showed little variation in toxin activity. L–9–12 strain of lentil possessed higher phytohemagglutinin activity, compared with other lentil strains. It may, therefore, be concluded that the pigeon pea strains, included in the present investigation, have very little phytohemagglutinin activity and hence may serve as more useful sources of plant protein and lysine, from this point of view.

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1. Gopalan, C., Rama Sastri, B. V. and Balasubramanian, S. C., *Nutritive value of Indian foods*, National Institute of Nutrition, ICMR, Hyderabad, India, 1976.
2. Liener, I. E., *Toxic constituents of plant foodstuffs*, Academic Press, New York, 1980.
3. *Liener, I. E., Arch. Biochem. Biophys.*, 1955, **54,** 223.
4. Sage, H. J. and Geeen, R. W., In: *Methods in Enzymol.*, 1970, **28,** 332.

INTERACTION OF NITROGEN MOLECULES WITH PRE-ADSORBED H_2O_2 ON RUTILE TiO_2

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THE presence of hydrogen peroxide on the surface of TiO_2[1] and its interaction with molecular nitrogen has shown the formation of nitric oxide species[2,3]. This can occur only when the nitrogen molecule is fixed on the surface. Molecular nitrogen has been shown[4] to react with water vapour in the presence of TiO_2 under the stimulus of near UV radiation to produce ammonia and hydrazine. A direct relationship has been proposed to exist between the amount of NO formed and the initial pressure of nitrogen[5]. In this communication, a relationship that exists further between NO formation and the concentration of H_2O_2 on the surface of TiO_2 is considered.

Adsorption–desorption experiments were carried out in a conventional high vacuum system. Nitrogen gas ($P_{N_2} = 20\ Nm^{-2}$) was kept in contact with the sample before addition of H_2O_2. The change in pressure of nitrogen was monitored by using the mass spectrometer (V.G. Micromass 2A). After attaining a steady pressure of nitrogen in contact with TiO_2, vapour/liquid H_2O_2 was added to the sample. H_2O_2 was subjected to several freeze-pump-thaw cycles prior to admitting the nitrogen gas to the bulb containing the frozen H_2O_2. No significant change in the pressure of nitrogen was observed under this condition. Solid H_2O_2 was then melted so that H_2O_2 (vapour) came in contact with the surface of TiO_2. This position is represented by a in figure 1. The subsequent addition of H_2O_2 to the TiO_2 surface is represented by b in figure 1. In a blank experiment (in the absence of TiO_2), no significant change in the nitrogen pressure was observed during the melting procedure.

The decomposition of H_2O_2 into water and oxygen was found to be significant only when the liquid H_2O_2 was in contact with the surface TiO_2. Figure 1 demonstrates that vapour H_2O_2 when in contact with the surface (point a), produces very little evolution of oxygen. This may be due to the small probability of recombination of the reactive oxygen species (O*) to form oxygen molecules. In other words, more adsorbed NO is being preferentially formed by scavenging of the reactive oxygen species by molecular nitrogen, as shown:

$$H_2O_2 \rightarrow H_2O + O^*,$$
$$O^* + N_2 \rightarrow NO + N^*,$$
$$O^* + O^* \rightarrow O_2.$$

The direct addition of liquid H_2O_2 produced an enormous increase in the evolution of oxygen (point b, figure 1). This suggests that by the time nitrogen molecule diffuses through the liquid medium to

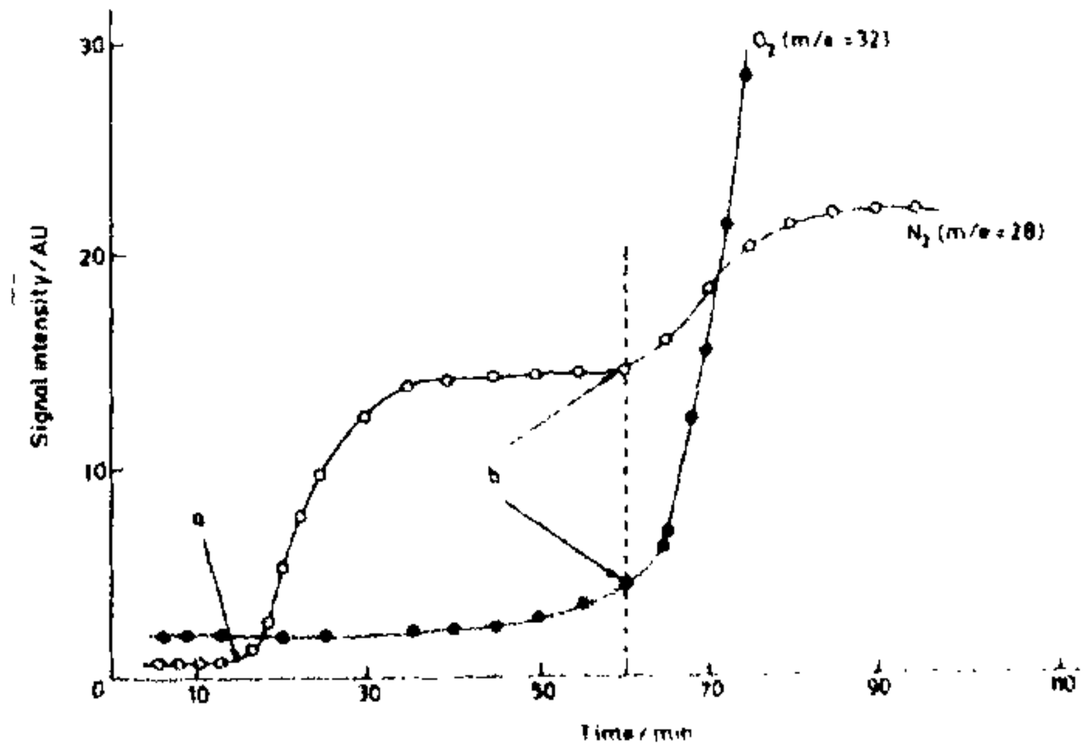

Figure 1. The simultaneous adsorption of nitrogen (m/e 28) and evolution of oxygen (m/e = 32) from the surface of TiO_2 moistured with H_2O_2. Point a–H_2O_2 (vapour) in contact with TiO_2 + N_2 (gas); point b–H_2O_2 (liquid) in contact with TiO_2 + N_2 (gas).