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Technical Note

A Study of the Trypsin Inhibitor of Black Gram (*Vigna mungo* (L.) *Hepper*)

ABSTRACT

Trypsin inhibitor activity (TIA), water-soluble protein and total protein contents varied markedly among the twenty cultivars of Black Gram grains assayed. Although no relationship could be established between TIA and total protein content, significant positive correlation was seen between TIA and water-soluble protein. During the ontogeny of seed, the TIA and water-soluble protein increased gradually. However, during germination, TIA was found to decrease up to 48 h and this was followed by a sharp increase.

INTRODUCTION

Grain legumes are known to possess a number of antinutritional factors. Among these, the most studied is trypsin inhibitor (TI), since it is ubiquitously present in the plant kingdom (Ryan, 1981). One of the common legumes used in India is Black Gram, popularly known as Urd bean (*Vigna mungo*). Although the presence of TI in Black Gram and its partial characterization have been reported earlier (Kamalakannan *et al.*, 1981), detailed studies of TI in different cultivars, activity during seed development and germination and the localization of TI proteins in various parts of the seed have not yet been reported.

MATERIALS AND METHODS

The Black Gram grains were obtained from the Pulses Breeding Station of the School of Genetics, Tamil Nadu Agricultural University,

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Coimbatore. The grains were powdered to pass through a 100 mesh sieve. The TI was extracted from 5 g of the powdered sample with 25 ml of distilled water, by grinding at 0°C to 4°C with a pestle and mortar. The ground sample was kept in a refrigerator for 3 h, with frequent shaking, for the complete extraction of TI. Clear extract was obtained by centrifuging the sample at 15 000 *g* for 20 min at 0–4°C. One millilitre of the clear supernatant was made up to 10 ml with distilled water and used as a TI source. Trypsin inhibitor activity was assessed by the method of Kakade *et al.* (1974), using trypsin and benzoyl DL-arginine *para*-nitroanilide (Sigma Chemical Co., USA). The protein content was determined by the method of Lowry *et al.* (1951). One unit of TI activity corresponds to the amount of protein, in micrograms, which gives a 50% inhibition of enzyme activity under the conditions specified (Chrispeels & Baumgartner, 1977). The TIA was expressed as TIU/g of sample. Of the twenty cultivars analysed, cultivar CO5 was selected for further study since it is the most popular variety.

The husk and cotyledons were separated from CO5 grains, powdered separately and the TIA was determined in 5 g aliquots of both fractions.

The study of TI deposition during seed development was carried out on CO5 seeds in comparison with CO2 and CO3. The flowers were labelled on the day of blooming and the maturing seeds in the pods were collected at 5-day intervals in order to estimate TIA and water-soluble protein contents. The TI in germinated seeds was assayed as follows. Sound Black Gram seeds (CO5) were rinsed with 0·1% mercuric chloride for surface sterilization. The seeds were soaked in water for 5 min and allowed to germinate on dampened filter paper in clean Petri dishes at room temperature. About 5 g of the germinated seeds were used for assaying TIA after 12, 24, 36, 48, 60 and 72 h of germination.

RESULTS AND DISCUSSION

Varietal difference

The TIA, water-soluble and total protein contents varied significantly among the cultivars of Black Gram (Table 1). It is interesting to observe a significant positive correlation ($r = 0{\cdot}6474^{**}$) between the TIA and water-soluble protein. However, no significant correlation was observed between the total protein content and TIA. Since TI is a water-soluble

TABLE 1
Trypsin Inhibitor Activity, Water-Soluble Protein and Total Protein Contents of Certain Cultivars of Black Gram (*Vigna mungo*)

Sample No.	*Variety*	*TIU/g*	*Water-soluble protein (mg/g)*	*Total protein (%)*
1	CO3	829	82·9	17·5
2	CO4	772	80·7	18·3
3	CO5	922	93·4	22·2
4	ADT 3	681	69·5	21·2
5	COBG 1	858	76·6	19·8
6	COBG 2	814	69·0	22·2
7	COBG 8	775	81·3	19·4
8	COBG 9	662	65·8	17·5
9	COBG 10	895	87·9	19·0
10	COBG 271	650	74·6	21·5
11	JU.77–41	785	71·8	22·1
12	KM 2	727	71·1	20·2
13	Pant U 19	633	67·6	17·7
14	Pant U 26	609	82·8	23·3
15	Pant U 30	697	65·4	25·6
16	PDU 1	1 028	83·8	17·5
17	PDU 3	632	73·0	18·7
18	T 9	891	85·5	21·2
19	TMV 1	733	77·2	23·7
20	VG 191	716	69·4	21·8
	Mean	765	76·5	20·5
	SE	20·2	2·9	0·6
	CD	57·8	8·4	1·8

Values are the averages of three replications.
SE, Standard error.
CD, Coefficient of determination.

protein, the concomitant increase or decrease in TIA is exhibited in water-soluble protein content. Absence of significant correlation between TIA and total protein has also been reported in Soybean, Sunflower and Field Bean seeds (Roy & Bhat, 1974; Griffiths, 1979).

TIA in different parts of the seed

The whole grain contained 905 TIU/g. The cotyledon was found to contain 99·1% of the total TI activity whereas the husk contained only 0·9%. Earlier work on groundnut by Horii & Miyazaki (1973) showed

that the germ and endosperm of groundnut had very low TIA, but the seed coat had a high TI concentration. The study on TI of sesame seeds by Abo-Bakr & El Iraqui (1980) revealed the presence of TIA only in the cotyledons and their absence in the hulls. Probably, the distribution of TI in the different fractions of the grains varies between species.

Synthesis of TI proteins during seed development

TIA and water-soluble protein contents in the developing seed are presented in Table 2. Increases in the TIU/g and water-soluble protein content were noticed in the seeds of the three Black Gram varieties during seed maturation with a high positive correlation ($r = 10{\cdot}97^{**}$) between the two parameters. Irrespective of the varieties, the TIA increased as the seed matured but the increasing trend of TIA and the water-soluble proteins is not uniform among the varieties. Collins & Sanders (1976) measured the TIA of four varieties of Soybean during seed maturation and reported an increasing trend in all varieties but the rate of increase was not uniform. The presence of isoinhibitors has been reported in Soybeans and Garden Beans (Wilson & Laskowski, 1975;

TABLE 2
Profile of Trypsin Inhibitor Activity and Water-soluble Protein Contents During Seed Development

Stages	*Days after flowering*	*TIU/g*				*Water-soluble protein (mg/g)*			
		CO2	*CO3*	*CO5*	*Mean*	*CO2*	*CO3*	*CO5*	*Mean*
1	5	171	286	211	279	21·6	18·6	18·2	19·5
2	10	342	441	401	395	34·0	28·2	24·9	29·0
3	15	501	558	586	549	51·3	46·4	49·3	48·9
4	20	641	687	741	689	67·5	60·0	78·4	68·6
5	25	773	773	844	797	78·2	71·2	88·5	79·3
6	30	868	829	925	874	82·5	82·9	93·4	86·3
Mean	—	550	596	617	—	55·8	51·2	58·8	—
Stages (S)			CD				CD		
			7·1				2·6		
Variety (V)			5·0				1·8		
S × V			12·5				4·5		

Values are the average of three replications.
CD, Coefficient of determination.

TABLE 3
Effect of Germination of CO5 Black Gram on Trypsin Inhibitor Activity

Sample No.	*Duration of germination (h)*	*Trypsin inhibitor activity (TIU/g)*	*Water-soluble protein (mg/g)*
1	0	923	93·4
2	12	835	92·7
3	24	738	93·4
4	36	570	92·6
5	48	108	92·9
6	60	384	92·2
7	72	642	91·8

Values are the averages of three replications.

Odani & Ikenaka, 1978). The reason for the differential rate of activity of TI during seed development between cultivars may be due to the phased synthesis of isoinhibitors.

TIA during germination

Whilst a steady increase in the TIA was observed with the seed ontogeny, the reverse was true during early germination up to 48 h and this decreasing trend was followed by a sharp increase (Table 3). The water-soluble protein content remained constant. The decrease in TIA during the initial period of germination could be ascribed to the enhanced breakdown of the reserve protein for initiating the process of germination. Kakade & Evans (1966) also observed a similar decrease with Navy Beans. Kamalakannan *et al.* (1981) have also noticed the same trend in Black Gram in an unspecified variety. The increased activity after 48 h could not be explained with the present data. However, this trend suggests a possible rôle for TI in protein metabolism during the early developmental stages.

REFERENCES

Abo-Bakr, T. M. & El Iraqui, S. M. (1980). The presence of a trypsin inhibitor in sesame seeds. *Alex. J. Agric. Res.*, **28**, 151–4.

Chrispeels, J. M. & Baumgartner, B. (1977). Trypsin inhibitor in Mung bean cotyledons. *Pl. Physiol.*, **61**, 617–23.

Collins, J. L. & Sanders, G. G. (1976). Changes in trypsin inhibitory activity in some soybean varieties during maturation and germination. *J. Fd. Sci.*, **41**, 168–72.

Griffiths, D. W. (1979). The inhibitor of digestive enzymes by extracts of field bean (*Vicia faba*). *J. Sci. Fd. Agric.*, **30**, 458–62.

Horii, M. & Miyazaki, M. (1973). Changes in soybean trypsin inhibitor during processing. I. Trypsin inhibitor contents of soybeans, other beans and soybean products. *Reports of the National Food Research Institute* (Shokuryo Kankyusho Kenku Hokoku) No. 28, 52–8.

Kakade, M. L. & Evans, R. J. (1966). Changes in trypsin inhibitor activity of navy beans during germination. *J. Fd. Sci.*, **31**, 781–3.

Kakade, M. L., Rackis, J. J., McGhee, J. E. & Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Cereal Chem.*, **51**, 376–82.

Kamalakannan, V., Sathyamoorthy, A. V. & Motlag, D. B. (1981). Studies on blackgram (*Vigna mungo*) trypsin inhibitor. *J. Sci. Fd. Agric.*, **32**, 1172–6.

Lowry, O. H., Rosebrough, N., Farr, A. L. & Randall, R. J. (1951). Protein measurement with Folin-phenol reagent. *J. Biol. Chem.*, **193**, 265–70.

Odani, S. & Ikenaka, T. (1978). Evolutionary relationship among pulses. *J. Biochem.*, **83**, 747.

Roy, D. N. & Bhat, R. V. (1974). Trypsin inhibitor content in some varieties of soybean (*Glycine max*) and sunflower seeds (*Helianthus annus* L.), *J. Sci. Fd. Agric.*, **25**, 765–9.

Ryan, C. A. (1981). Proteinase inhibitors. In: *The biochemistry of plants.* (Stumpf, P. K. & Conn, E. E. (Eds)), Vol. 6. Academic Press, New York.

Wilson, K. A. & Laskowski, M. (1975). Proteinase inhibitors in garden beans. *J. Biol. Chem.*, **250**, 4261.

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