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Research Article

QUANTITATIVE DETERMINATION OF TRYPSIN INHIBITOR ACTIVITY IN SOME SELECTED SPECIES OF THE GENUS *VIGNA SAVI*

***Deepak Devilal Pardhe**
Department of Botany, Sant Dnyaneshwar Mahavidyalaya,
Soegaon, Dist. Aurangabad - 431120, M.S.,India
**Author for Correspondence: deepgis2000@gmail.com*

ABSTRACT
Legumes have natural components such as lectins, amylase, saponins, phytic acid and trypsin inhibitors that may adversely affect their nutritional properties. Trypsin inhibitor, as the name indicates, interferes with digestion of proteins by inhibiting the action of the enzyme trypsin. A quantitative assay using natural substrate casein was developed to measure trypsin inhibitory activity in some selected species of the genus *Vigna savi*. The pertinent studies of selected species of the genus *Vigna savi* have indicated considerable variation regarding the trypsin inhibitor (TI) level. The trypsin inhibitor (TI) content values ranged from 51.40 to 455.80 TIU/ml/gm meal in selected *Vigna* species. The highest TI content (455.80 TIU/ml/gm meal) could be recorded in *Vigna aconitifolia* (Jacq.) Marechal , while the *Vigna mungo* (L.) Hepper - BDU 1 revealed the lowest TI content (51.40 TIU/ml/gm meal).

Keywords: *Casein, Enzyme, Legumes, Trypsin, Trypsin Inhibitor Activity*

INTRODUCTION

All food legumes are valuable sources of proteins, minerals, vitamins and occupy a very important place in human nutrition. The grain legumes occupy a unique position in world agriculture due to their high protein content. Legumes are very important for their value as food and fodder, role in biological nitrogen fixation and as industrial raw materials. The legumes are important as food plants (beans, gram, peas), as source of edible oil (soybean, ground nut) and also as tanbarks, timber, copal, gums, insecticides, cultivated ornamentals as well as the medicinal plants (Secmen *et al*., 1989; Tsevegsuren *et al*., 1998).

The genus *Vigna savi* contains several species that are of considerable economic importance in many developing countries. The annual worldwide production of the various *Vigna* species approaches 20 million hectares and virtually all of this production is in developing countries. It is a well recognized fact that the majority of food legume plants have the capacity to synthesize certain biochemically active substances commonly considered nutritional and anti-nutritional factors since they have been shown to affect animal and human nutrition. Proteinase inhibitors constitute a large and complex (in terms of composition) group of plant proteins. All these proteins share a common trait, the ability to form complexes with proteinases, within which the enzymes lose their activity (Laskowski and Kato, 1980; Bode and Huber, 1992; Valueva and Mosolov, 1999). The trypsin inhibitors were first reported by Read and Hass (1938). Later on, Bowman (1944, 1946) purified them and Kunitz (1945) isolated them in the crystalline form. The molecular weight of plant protease inhibitors ranges from 40-60 kDa. The plant protease inhibitor molecules are stable and often resistant to heat, pH extremes and proteolysis by proteases (Kassell and Williams, 1977; Ryan, 1981). The biochemical analysis plays an important role in determining the nutritional and antinutritional factors in legumes. The biochemical studies also facilitate to determine the scope for improvement in nutritional values and reduction in antinutritional factors by using various methods in legumes.

Among the trypsin, chymotrypsin, subtilisin and cysteine proteinase inhibitors, the trypsin inhibitors showed most polymorphism both within and between species of the genus *Vigna* (Konarev *et al*, 2002).

MATERIALS AND METHODS

Plate 1

The seed material used in the present research study obtained from Department of Pulses, Dr.Punjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.), India and Agricultural research station, Badnapur (M.S.), India respectively. The seed material comprises five varieties of *Vigna radiata*, two varieties of *Vigna mungo*, *Vigna umbellata*, *Vigna aconitifolia* and *Vigna unguiculata*, denoted by specific code names which are as per the following table 1.

Table 1: The list of selected species of the genus Vigna savi for TI activity

Sr. No.	Name of selected *Vigna* species	Code
1.	*Vigna mungo* (L.) Hepper -Tau 1	Sam 1
2.	*Vigna umbellata* (Thunb.) Ohwi and Ohashi	Sam 2
3.	*Vigna radiata* (L.) Wilczek AKM 8802	Sam 3
4.	*Vigna radiata* (L.) Wilczek- BPMR 145	Sam 4
5.	*Vigna mungo* (L.) Hepper - BDU 1	Sam 5
6.	*Vigna unguiculata* (L.) Walp.	Sam 6
7.	*Vigna radiata* (L.) Wilczek- NVL 1	Sam 7
8.	*Vigna radiata* (L.) Wilczek- BM 4	Sam 8
9.	*Vigna radiata* (L.) Wilczek- BM 2002-01	Sam 9
10.	*Vigna aconitifolia* (Jacq.) Marechal	Sam 10

Standardization of trypsin assay by using Casein

Trypsin stock solution was prepared by dissolving 10 mg of trypsin in 1 ml of 0.001 M HCl. This stock solution was diluted to prepare 1mg/ml working solution of trypsin in 0.001 M HCl. 2% Casein solution was prepared by dissolving 2 gm of Casein in 80 ml of phosphate buffer and completely dissolved by heating on a steam bath for 15 minutes. The solution was cooled, made to 100 ml with phosphate buffer. 10 to 100 µl trypsin was pipetted into a triplicate set of test tubes (one set for each level of trypsin) and the final volume of each tube adjusted to 2 ml. with the (0.1 M, pH 7.6) phosphate buffer. The tubes were set in a water bath at 37^0C. To one of the triplicate tubes was added 6 ml. 5% (w. /v.) trichloroacetic acid, this tube served as a blank. 2 ml. of the casein solution (previously brought to 37 ^{0} C) was added to each tube. The tubes were allowed to remain at 37 ^{0} C. for exactly 20 minutes, at which time the reaction was stopped by adding 6 ml. of 5% trichloroacetic acid to the experimental tubes. After standing for 1 hour at room temperature, the suspension was filtered and the absorbance of the filtrate was measured at 280 nm against the blank. A graph was plotted with absorbance versus concentration of trypsin and according to it, the optimum trypsin concentration to be used for the assay was determined.

The amount of proteinase inhibitor increased linearly with increasing amount of the sample extract. With casein as the substrate, trypsin inhibitor activity decreases in direct proportion to the level of inhibitor up to the point of about 80% inhibition. Hence, while using casein as a substrate in trypsin inhibitor activity, when expressed on a per ml/gm basis, it was maintained constant up to a level of about 80% inhibition.

Trypsin Inhibitor assay

Trypsin activity was measured by using the natural substrate Casein, as described after suitable modification by Kakade *et al*., (1969), originally described by Kunitz (1947). For trypsin inhibitor assay 100 µg of trypsin was found to be optimum from the earlier standardization. 0 to 1 ml. of aliquots of the sample extract containing trypsin inhibitor were pipetted into a triplicate set of test tubes (one set for each level of extract) and the volume brought to 1.0 ml with the phosphate buffer, 1 ml of the trypsin solution was added to each tube and the tubes were placed in the water bath at 37^0C. The remainder of the procedure was the same as that described in the standardization of trypsin assay. One trypsin unit (TU) is arbitrarily defined as an increase of 0.01 absorbance units at 280 nm in 20 minutes per 10 ml. of the reaction mixture under the conditions set. Trypsin inhibitor activity is defined as the number of trypsin units inhibited (TUI).

RESULTS AND DISCUSSION

As casein has been widely used as a natural substrate for measuring the trypsin inhibitor activity of natural trypsin inhibitors such as those which occur in *Vigna* species and other legumes, it was necessary to standardize the amount of protease by preparing trypsin standard curve. The pertinent studies of selected species of the genus *Vigna savi* have indicated considerable variation regarding the trypsin

inhibitor (TI) level.The trypsin inhibitor (TI) content values ranged from 51.40 to 455.80 TIU/ml/gm meal in selected *Vigna* species.

The highest TI content (455.80 TIU/ml/gm meal) could be recorded in *Vigna aconitifolia* (Jacq.) Marechal, while the *Vigna mungo* (L.) Hepper - BDU 1 revealed the lowest TI content (51.40 TIU/ml/gm meal). Among the five selected varieties of *Vigna radiata*, the TI content values ranged from 94.73 to 297.80 TIU/ml/gm meal. The highest TI content value (297.80 TIU/ml/gm meal) was observed in *Vigna radiata* (L.) Wilczek- BM 2002-01 whereas lowest TI content value (94.73 TIU/ml/gm meal) was shown by *Vigna radiata* (L.) Wilczek- NVL 1 among the *Vigna radiata* varieties.In case of two *Vigna mungo* varieties, the lower TI content value (51.40 TIU/ml/gm meal) could be seen in *Vigna mungo* (L.) Hepper - BDU 1, as compared to *Vigna mungo* (L.) Hepper -Tau 1 which has shown the higher TI content value (264.63 TIU/ml/gm meal).Other selected *Vigna* species also displayed a good amount of variability regarding TI content. The TI content value 272.70 TIU/ml/gm meal and 398.60 TIU/ml/gm meal was recorded in *Vigna umbellata* (Thunb.) Ohwi and Ohashi and *Vigna unguiculata* (L.) Walp. respectively.

Table 2: Trypsin inhibitor content in selected species of the genus Vigna savi

Sr. No.	Name of selected *Vigna* species	TIU/ml/gm of defatted seed powder ($\bar{x}$)	S.D.	± S.E.
1.	*Vigna mungo* (L.) Hepper -Tau 1	264.63	0.55	0.32
2.	*Vigna umbellata* (Thunb.) Ohwi and Ohashi	272.70	0.43	0.25
3.	*Vigna radiata* (L.) Wilczek AKM 8802	161.67	0.49	0.28
4.	*Vigna radiata* (L.) Wilczek- BPMR 145	217.0	0.20	0.11
5.	*Vigna mungo* (L.) Hepper - BDU 1	51.40	0.50	0.29
6.	*Vigna unguiculata* (L.) Walp.	398.60	0.40	0.23
7.	*Vigna radiata* (L.) Wilczek- NVL 1	94.73	0.38	0.22
8.	*Vigna radiata* (L.) Wilczek- BM 4	206.70	0.53	0.30
9.	*Vigna radiata* (L.) Wilczek- BM 2002-01	297.80	0.46	0.26
10.	*Vigna aconitifolia* (Jacq.) Marechal	455.80	0.65	0.38

S.D. = Standard deviation
S.E. = Standard error

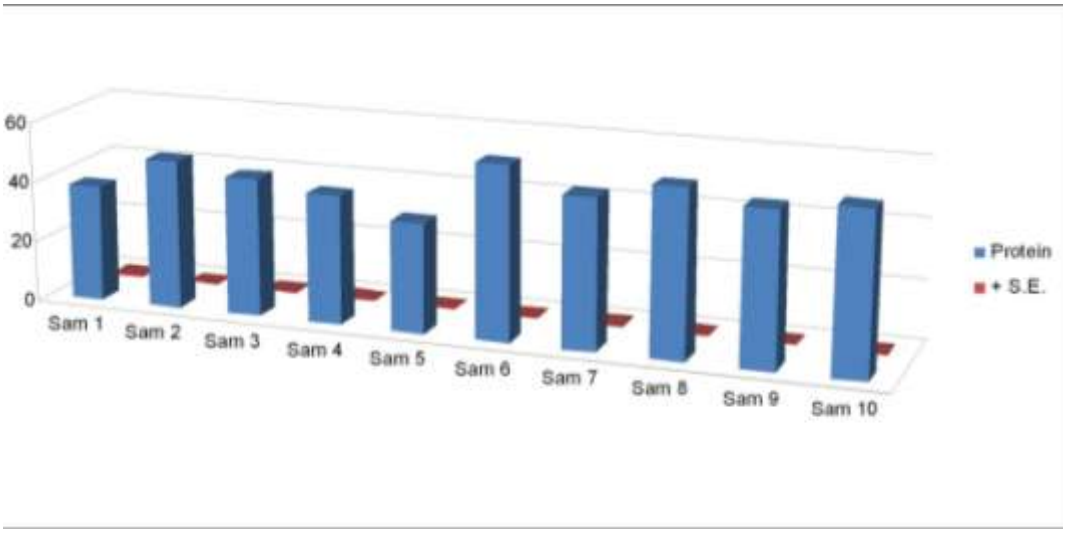

Plate A: Extractable seed protein content in selected *Vigna* species

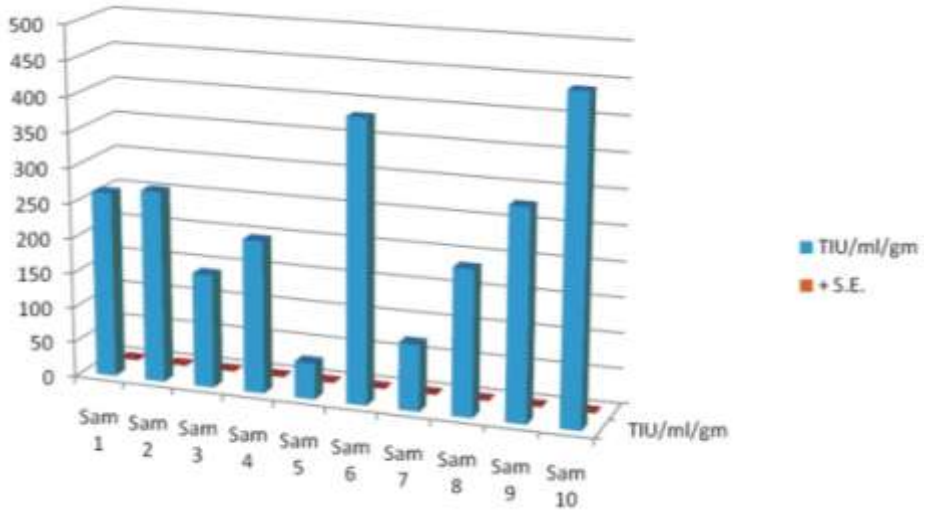

Plate A: Trypsin inhibitor content in selected Vigna species

CONCLUSION

It is evident from the results derived from the present research study that selected species of the genus *Vigna savi* have shown considerable variation regarding the trypsin inhibitor (TI) level. The trypsin inhibitor (TI) content values ranged from 51.40 to 455.80 TIU/ml/gm meal in selected *Vigna* species. The highest TI content (455.80 TIU/ml/gm meal) could be recorded in *Vigna aconitifolia* (Jacq.) Marechal , while the *Vigna mungo* (L.) Hepper - BDU 1 revealed the lowest TI content (51.40 TIU/ml/gm meal).

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