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Isolation and Characterization of Major Seed Storage Proteins: I. Fabaceae family found in Sindh Pakistan

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Abstract: Seeds of nine medicinally important plants belonging to the family Fabaceae growing in Sindh, Pakistan (including ., *Trigonella foenum-graecum L.,Tamarindus indica L, Posralea corylifolia L,Sesbania aculeata L., Sesbania grndifolia L., Cassia occidentalis L, Cassia fistula L, Pithecellobium dulce Benth. and Acacia concinna L.)* were subjected used for the analysis of four major seed storage protein.Proteins were extracted from the seed flour by sequential steps of extractions including water (albumin), 5.0 M NaCl (globulin), 70% ethanol (prolamin), and150.2M sodium phosphate buffer,Ph8.0 (glutelin). Quantitative amount was carry out by the dye binding method of Bradford which revealed wide variations in terms of their concentrations and total yields. As expected, albumin fraction was found to be the highest in all plant seeds of family Fabaceae where, *P. dulci Benth* contains the highest and *T. indica L.* the lowest (80% and 23%) respectively. Globulin with second dominant protein fraction may also vary from 3% (*A. concinna*) to 43% (*T. foenum-graecam*). Likewise, prolamin was found to be significantly higher in *A. concinna* (65%), *C. fistula* (37%) and *P. corylifolia L* (33%) while, the lowest of around 10% in *T. foenum-graecam*. On the other hand, a consistent pattern of 2 to 18% of glutelin concentrations were observed in all plants (with exception of *T. indica L* having 31%). Comparative SEC FPLC profiles of the four major seed storage proteins extracted from *P.dulci Benth* nicely complement our work and provided a single step strategy for the purification of complex, multimeric and high molecular mass seed storage proteins from plant for wide biotechnological applications.

Keywords: Seed storage proteins, Fabaceae, Extraction, Protein estimation

1. INTRODUCTION

The family Fabaceae is a large and economically important family of flowering plants, the seeds of legume family biggest sources of protein and also provide high quality nutritional food source, the most important foods such as lentils, peas beans, soya and chickpeas are all legumes, a variety of Legumes are capable to change atmospheric nitrogen into nitrogenous compound of use to plants, legume seeds are also control anti-nutritional compounds (Pernas *et al.,* 2000). Plants are used in Hikmat, Ayurvedic and Allopathic and Homeopathic treatment commercially (Mehmood *et el.,* 2003) and provide all basic needs of life such as food, shelter, fodder, timber, medicines, fuel etc (Iqbal and Hamayon, 2006). Plant seeds are the major source of dietary proteins essential for supplementing protein where there was not enough meat and/or for vegetarians.

Seed storage proteins emphasize on their biological roles such as their evolutionary origin, modes of synthesis, modes of deposition and their defensive activity in human metabolism**.** The albumin and globulin are richly present in angiospermic plants both in monocot and dicot plant including cereals, fern spores, palms (Templeman *et al.,* 1987). Glutelin is highly resistant to pepsin so cannot be digested easily in human body.

In the present studies, seeds of nine medicinally important plants belonging to the family Fabaceae growing in Sindh, Pakistan including Trigonella foenum-graecum L., Psoralea corylifolia L., Tamarandus indica L., Sesbania aculeata, Sesbania grandifolia, Cassia occidentalis, Cassia fistula, Pithecellobium dulci Benth and Acacia concinna were subjected for the analysis of four major seed storage proteins.

2. MATERIAL AND METHODS

Material

Seeds of nine different plants from dicot Fabaceae family. All plant growing in Sindh Pakistan . Seeds are used for this study, plant were collected from different localities of Sindh (Nawbshah, Hydrabad, Jamshoro), and some were prvided by Hamdard University, Karachi.

Seed storage protein extraction

The seed were ground with mortar pistal.The flour was delipidated by solvent extraction by solvent extraction by stirring with pure hexane (1:10w/v), for 30 min repeated three times. After filtration flour was dried for overnight at room temperature. The seed flour was air dried under hood for 24 hrs and used for the micro-extraction method describe by Sammour et al. (1998) in water, high salt, alcohol and under alkaline buffering conditions.

In brief, seed flours (500µg) successively extracted (triplicate in eppendrof tubes) with (i) 500µl deionized water, (ii) 500µl 5.0M NaCl, (iii) 500µl 70% ethanol and (iv) 500µl 0.2M sodium phosphate buffer, pH8.0 for the extraction of four major seed storage proteins i.e. albumin, globulin, prolamin and glutelin, respectively. Each extraction was performed for 20min at

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350rpm/25°C using Thermomixer comfort (Eppendorf, Germany). All extracts were centrifuge at 14,000rpm for 15min at 4°C (Biofuge Primo R Heraerus, Japan) and after supernatant separated, extraction were repeated twice in order to remove all the remaining protein of each fractions. The supernatants of each fraction were pooled and store at -20°C until unless subjected to for protein profiling. Total protein concentration of all extracts was measured by modified dye binding assay of Bradford (1976) using bovine serum albumin as a standard. Measurments was performed in triplicate of in a microplate reader (Sunrise Tecan, Austria).

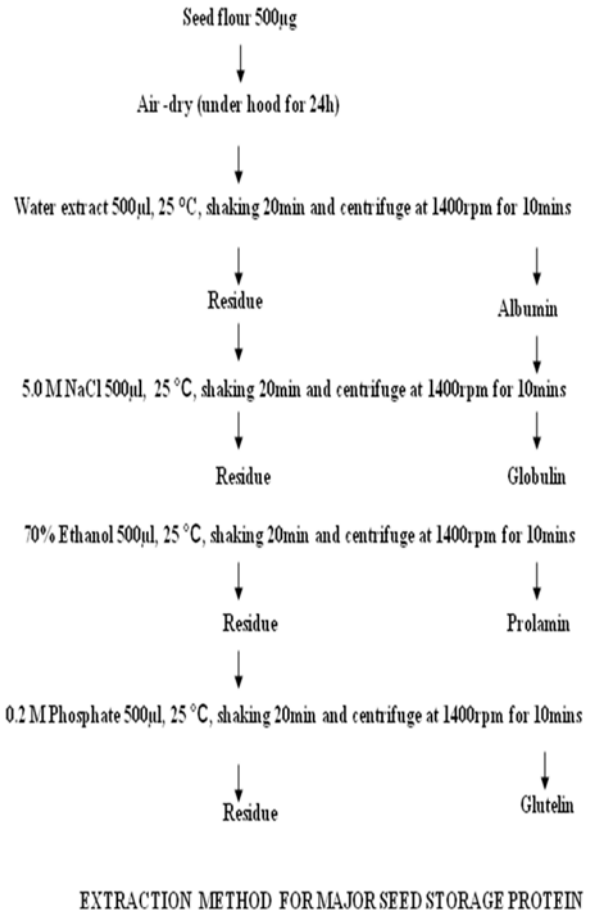


Fig. 1- Extraction Method For Major Seed Storage Protein

3. RESULT AND DISCUSSION

Storage proteins with indication to 50% of the total protein in seed and also important impact on their nutritional value in good deed of humans and animals and on their useful property in foodstuff processing. Osborne (1924) examine the proteins of seeds and notable more than a few kinds which were differentiate by their solubility (Blagoveshchenskii, I960, D. Boulter *et al* 1967).

The storage protein are generally categorized in four types due to their solubility: albumin water soluble, globulin salt soluble, prolamin alcohol soluble and glutelin alkaline soluble protein, nine seeds varieties belong to dicote Fabaceae family, results shown that major seed storage protein

They present in different concentration. **(**Table-1). As estimated, albumin fraction was found to be the highest in all plant seeds of family Fabaceae where, % yield in *P.dulce* (80.15%) and *S.aculeata L* (53.81%) *P.corylifolia.L, C.fistula, C.occidentalis*, (48.85 %, 45.54%, 40.65%) respectively) contains the medium while, the lowest concentration was observed in *T.indica, A.concinna*. *T.fonel, S.grandifolia,* (23.57%, 26.18%, 32.35%). Globulin with the second dominant protein fraction may also vary from in *T.fonel* (43.18%) to (2.63%) in *A.concinna*. Likewise, prolamin was found to be significantly higher in *A.concinna*. (65.46%), *C.fistula,* (37.64%), *P.corylifolia.L* (33.48%) *S.grandifolia,* (2618%) *T.indica,* (19.84%) *C.occidentalis*, (16.21%) *S.aculeata*(12.04%) while, the lowest of around (9.9%) in *T.fonel,* seeds. On the other hand, a consistent pattern of 2.64 to 18.41% of glutelin concentration was observed in all plants seeds (with exception of *T.indica, L* having (31.32%) highest concentration.The best known globulins, insoluble in water, Proteins are of big dietary importance and are directly concerned to necessary used for survival. (Casey, 1999).

4. CONCLUSION

The aim of the present study is search for high quality and cheaper source of protein **present in plant, which are nutritionally** important for community (Tangaduri et al 2001).Nine 9 seed varieties, Fabaceae family determine the (SSP) in different extraction method. A/C to new research plants and their seeds are major sources of foodstuff other, they also provided inexpensive protein source amongst community existing in developed country. work is provide information about these medicinal plants seeds used as a alternative sources for human diet after proper processing different species of legume seeds may contain different concentration and four type of SSP were observed in different solubility in different physiological conditions. Plant protein used as a food, so necessary to examine the association of protein value with giving out factor that influence the useful performance of protein foodstuffs.

Table 1. Comparison of the concentrations and total percent yield of the major seed storage proteins in the family Fabaceae found in Sindh, Pakistan.

S.NO.	Plant Name	Albumin *mg/g #%yield		Globulin *mg/g #%yield		Prolamin *mg/g #%yield		Glutelin *mg/g #%yield	
1.	*Acacia concinna L*	0.12	26.18	0.01	2.63	0.31	65.46	0.02	5.71
2.	*Cassia fistula L*	0.50	45.54	0.12	10.93	0.41	37.64	0.06	5.88
3.	*Cassia occidentalis L*	1.21	40.65	1.07	35.93	0.48	16.21	0.21	7.21
4.	*Psoralea corylifolia*	1.20	48.85	0.25	10.15	0.82	33.48	0.18	7.50
5.	*Pithecellobium dulce Benth.*	5.90	80.15	0.85	11.57	0.24	3.32	0.36	5.00
6.	*Sesbania aculeata L*	0.66	53.81	0.38	31.48	0.14	12.04	0.03	2.64
7.	*Sesbania grandifolia L*	0.48	36.01	0.25	19.38	0.35	26.18	0.24	18.41
8.	*Trigonella foenum-graecum L.*	0.49	32.35	0.66	43.18	0.15	9.9	0.22	14.47
9.	*Tamarindus indica L*	0.55	23.57	0.58	24.75	0.46	19.84	0.75	31.82

* Concentrations in mg/g of seed flour. Values are mean of three independent extractions.
# % yield of a particular protein in total protein contents of seed flour.

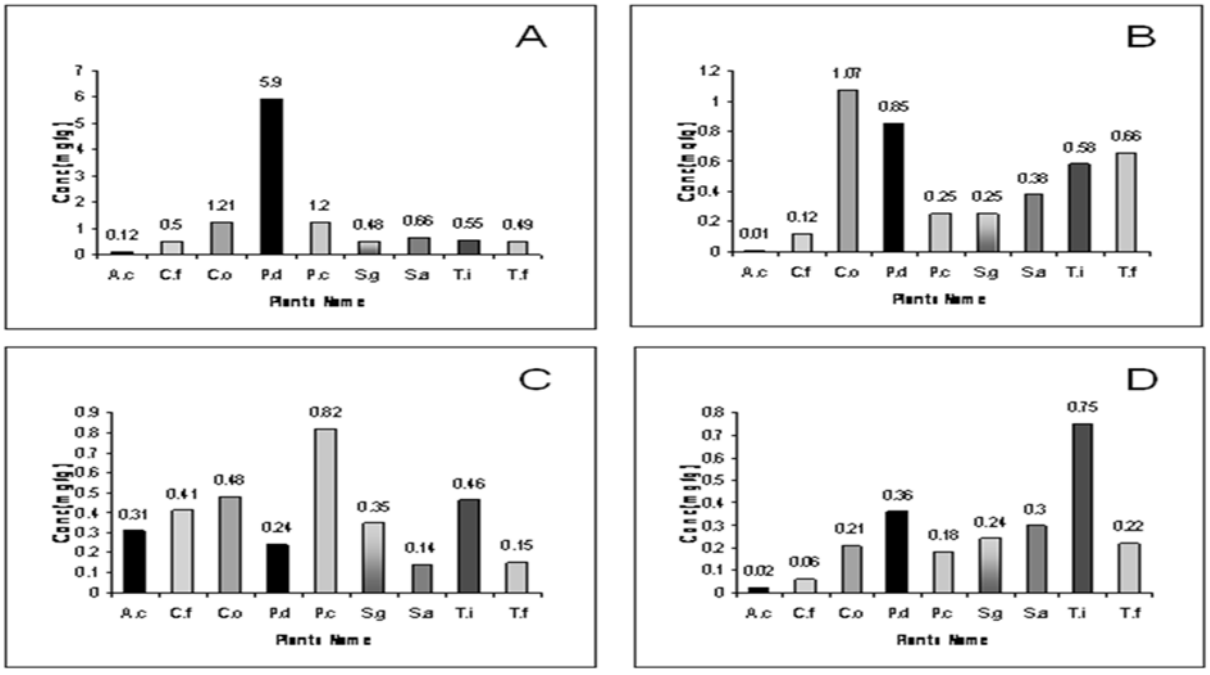


Fig. 2. Graphical representation of the four major seed storage protein concentrations (mg/g) measured from the seeds of family Fabaceae. (A) Albumin, (B) Globulin, (C) Prolamin and (D) Glutelin. Plants name are in abbreviation according to the list given in table 1. (Acacia concinna L, Cassia fistula L, Cassia occidentalis L, Psoralea corylifolia L., Pithecellobium dulce Benth. Sesbania grandifolia L, Sesbania aculeata L, Tamarindus indica L., Trigonella foenum-graecum L.),

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