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THE QUANTITATIVE ANALYSIS OF TRYPTOPHAN IN CHICKPEA

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Abstract:

legumes are a good and easily affordable source of proteins in human diet. It also play an important role in adding to soil fertility and biological soil conservation. The different type of legumes and fodder have proved an invaluable agricultural component particularly in developing and poor countries. As per their solubility patterns, plant proteins have been classified into albumins (water soluble), globulins (salt soluble), glutelins (alkali soluble) and prolamins (alcohol soluble). The albumins perform the metabolic function and other three fractions constitute the storage proteins. The tryptophan content of the globulins was the lowest and it varied from 0.52 g/100g protein in line '537' to 0.84 g/100g protein in line '5453'. On the other hand prolamins were richest in tryptophan concentration which was in the range of 1.42 g/100g protein in line '1422' to 2.88 g/100g protein in line '5455'. For improving the quality and quantity of seed proteins, purification, characterization and identification of nutritionally valuable protein fraction and polypeptides.

Key Words: Proteins, Seed, Tryptophan, Globulins, Albumins, Glutelins

Introduction

The two groups of plants like cereals and legumes have contributed a lot to the world agriculture and crop production. These feed as much as 70% of population in the world (Shewry et al., 1981). Out of two,legumes are a good and easily affordable source of proteins in human diet. It also play an important role in adding to soil fertility and biological soil conservation. The different type of legumes and fodder have proved an invaluable agricultural component particularly in developing and poor countries (Franco, 1978). Thus, keeping in view the increasing costs of synthetic fertilizers and constant depletion of natural nitrogen resources, our dependence on legumes as well as a renewable nitrogen resource in today's agriculture becomes very essential (Phillips, 1980). The legumes are found to be low in sulphur containing amino acids and tryptophan. Through the number of animal feeding

experiments have proved that low methionine in legumes protein act as a limiting factor in growth and protein utilization. For improving the protein quality of food grains a number of strategies like screening of germplasm with higher protein content and better amino acid composition, screening of lines with increased proportion of seed protein fractions containing higher amount of sulphur amino acids in legumes and identification of nutritionally superior polypeptides have been suggested from time to time. The work for improvement of food grain quality need an essential information from purification, characterization and developmental regulation studies of seed proteins. By using the new research techniques, genes for nutritionally better polypeptides have been cloned and suitably modified by site-directed mutagenesis for further used in genetically engineered crops. The legumes like *Pisumsativum, Viciafaba* and *Glycine max* have been extensively worked out for their seed proteins. As per their solubility patterns, plant proteins have been classified into albumins (water soluble), globulins (salt soluble), glutelins(alkali soluble) and prolamins (alcohol soluble). The albumins perform the metabolic function and other three fractions constitute the storage proteins (Boulter and Croy, 1997). The few albumin polypeptides have been degraded at the time of seed germination and thus have been assigned the storage function as in pea (Croy et al., 1984) and sunflower (Kortt et al., 1991). In legumes, the storage proteins are represented mainly by the salt soluble fractions called as globulin (Osborne and Campbell, 1898). These accounts for 60-70 % of seed proteins and are followed by albumins, glutelins and prolamins (Boulter and Derbyshire, 1978; Dhankher et al., 1990). In Pisumsativum, the globulins are further consisting of two sub-fractions called as legumin and vicilin (Osborne and Campbell, 1898). The legumin was non-coagulable at higher temperature whereas vicilin coagulate at higher temperature. The vicilin fraction of globulin have low sulphur content(0.18%) as compared to legumin (0.42%).

Materials and Methods

For tryptophan estimation in chickpea, about 70 lines as germplasm of Cicer arietinum (L) collected from ICRISAT(International Crop Research Institute for Semi AridTropis), Patencheru (Hyderabad), Pulse Research Laboratory, IARI, New Delhi, CCS Haryana Agriculture university, Hisar and Punjab Agriculture University, Ludhiana for the study of proportion of this particular amino acid.

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i) Preparation of defatted Seed Meal

The seed testa was removed and cotyledons were pulverized using grinder. The seed meal was stirred for 2h in cold hexane using 10 ml hexane for 1 gm of seed meal. The seed meal was centrifused for 10 min in bench centrifuge and defatting process repeated. The pellet containing seed meal was dried and hexane removed under vacuum.

ii) Separation of Protein Fractions

The 1 gm of defatted seed meal was extracted with 10 ml of 50 mM borate buffer of pH 8.0 by stirring on stirrer for 2h. The contents were centrifuged at 23.000 g in a Remi C-24 high speed refrigerated centrifuge at 4 C for 30 min. The process was repeated twice with borate buffer and the supernatant of each extraction pooled together. This was dialysed overnight in 33 mM acetate buffer of 4.8 pH. The precipitation so obtained were centrifuge at 23,000 g for 30 min. The supernatant have albumin fraction and the pellet have globulins. The residue was extracted with 10 ml of 0.1 N NaOH and centrifuged at 14,000 g. The extraction was repeated twic e to get the glutelins. For prolamins, 70% ethanol followed centrifugation at 9,000 g. The supernatant obtained through this have prolamins fraction. The four fractions are than used for estimation of tryptophan content in the seed (Chandna and Matta, 1990).

iii) Tryptophan Estimation

Tryptophan was estimated by the method of Spies and Chamber (1949) based on the reaction of tryptophan with p-dimethylaminobenzaldehyde followed by production of blue color on oxidation with sodium nitrite. The 5 ml p-dimethylaminobenzaldehyde and 0.5 ml of protein fractions were mixed and incubated at 25+ 2 C for 18 h in dark. This was followed by addition of 0.1 ml of 0.045 the per cent sodium nitrite solution and contents shaken thoroughly on vortex shaker. After allowing the color to developfro 30 min., absorbance of the sample was read at 590 nm. A standard curve was plotted between the tryptophan concentrations and their respective absorbance values and used for determining the amount of tryptophan in different protein fractions.

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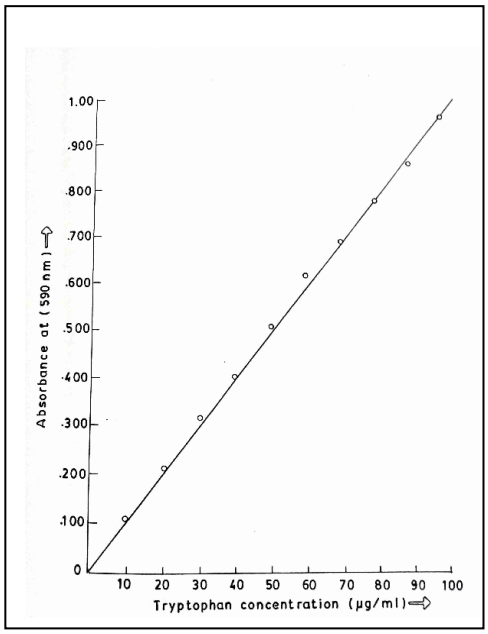
Result and Discussion

The tryptophan content of various protein fractions and relative contribution of tryptophan due to individual fractions is shown in table as follows

Sr. No.	Chickpea lines	Albumins	Globulins	Glutelins	Prolamins
1	5009	1.13	0.55	1.0	1.66
2	4918	1.20	0.81	1.37	2.31
3	5455	0.95	0.75	1.12	2.88
4	8397	1.04	0.68	0.95	2.0
5	537	1.12	0.52	1.08	2.01
6	5010	1.46	0.67	1.31	2.25
7	5015	1.09	0.56	1.10	2.10
8	5453	1.27	0.84	1.44	2.16
9	7679	0.98	0.60	0.99	1.90
10	1422	0.97	0.71	0.94	1.42
	S.E.	0.05	0.03	0.05	0.12

The standard curve for determining the amount of tryptophan as prepared by the method of Spies and Chamber (1949) is shown in Figure as below



The tryptophan content of the globulins was the lowest and it varied from 0.52 g/100g protein in line '537' to 0.84 g/100g protein in line '5453'. On the other hand prolamins were richest in tryptophan concentration which was in the range of 1.42 g/100g protein in line '1422' to 2.88 g/100g protein in line '5455'. The values of tryptophan content in albumin fraction varied between 0.95 g/100g protein in line '5455' to 1.46 g/100g protein in line '5010'. The glutelins were also similar to albumins in having their tryptophan concentration more or less in the same range i.e. varying from 0.94 g/100g protein (line '1422') to 1.44 g/100g protein (line '5453'). The contribution of given amino acid in the seed protein

due to a particular fraction was calculated using amino acid content of protein fraction and proportion of that protein fraction in the seed protein. The maximum contribution of tryptophan in the seed was due to globulins which varied between 43.2% to 54.9% in line '5015' and '8397' respectively. This was followed by albumins which contributed 20.2%(line'5455') to 26.1% (line'5009') of tryptophan of seed protein. The glutelins provided the tryptophan share in the range of 16.6% as found in line '8397' to 22.8% in line '5015'. Share of tryptophan in the seed protein due to prolamins was the lowest. This was found to be only 4.8% in line '5009' to 11.0% in line '5455' of chickpea.

A variety of reserve food materials are synthesized and deposited in the seed during its development to be utilized later at the time of seed germination by the growing seedlings. Seed proteins provide major source of nitrogen and sulphur to the young seedlings and have been rightly called as storage proteins. For improving the quality and quantity of seed proteins, purification, characterization and identification of nutritionally valuable protein fraction and polypeptides. Leguminous seeds are known to be poor in a few amino acids like tryptophan, methionine and cysteine. As the tryptophan content waasa found to be highest for prolamins and lowest for globulins. Though poorest in the content of this amino acid, globulins provide the maximum share of these in the seed protein because of their highest proportion of all the protein fractions in the seed. The proportion of any protein fraction should represent the out come of a number of events occurring during seed development.

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