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Characterization of variation in seed protein fractions and total proteins in garden pea (*Pisum sativum* L.) genotypes

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ABSTRACT

Seed legume crops can be helpful in alleviating the protein crisis of the developing nations. Biochemical analysis of total seed proteins 8 pea genotypes ranged from 16.3-20.4% with highest protein content (20.4 %) exhibited by control Arkel. Different protein fractions obtained in 8 pea genotypes were for albumin, globulin, glutelin and prolamine. All the genotypes studied exhibit lesser percentage of albumin fraction than control (6.6%). The globulin content in the pea genotypes ranged from 6.3- 7.8 % whereas Glutelin content was much lesser in the genotypes compared to albumin and globulin content. It ranged from 2.9% to 3.9%. The total content of prolamine was found to be least in all the 8 pea genotypes with Arkel having the highest (1.9%) content.

Key words: *Pisum sativum*, seed proteins, protein fraction, nutrititive variability.

INTRODUCTION

Grained legume crops commonly known, as pulses constitute an indispensable agricultural asset to the economy of the world. They are rich in food proteins and appreciable amounts of vitamins and minerals (Deshpande 1992; Brahmaprakash *et al*. 2004). While cereals supply nearly 50% of the protein in the human diet, an unfavorable balance in amino acids (poor in lysine) requires complementary protein sources.

Legumes are good complements to cereals, as they are rich in lysine, but poor in sulfur containing amino acids (methionine and cysteine). Legumes are used as high-protein crops that play a secondary role to cereal or root crops which are higher yielding and supply much of the energy requirement in a diet. A large number of grain legumes and oil seed legume crops can be helpful in alleviating the protein crisis of the developing nations and have been labeled as the "Meat for the poor people" as protein from animal origin are less readily available and is beyond the reach of the poor man. The legumes have ecological importance as with the help of the bacteria in their root nodules, they fix atmospheric nitrogen and improve the soil fertility. Due to their nitrogen fixing ability and high nutritive value for fodder, legumes are commonly used as green manures. Legume seeds or pulses are second only to cereals as a source of human food and provide the much needed proteins to our predominantly vegetarian population (Chandra and Pental, 2003).

Pea possesses a considerable importance in agricultural economy of India. It's importance as a pulse and as a vegetative crop in human diet needs no emphasis. It contains high percentage of digestible proteins along with high proportion of carbohydrates, vitamins and mineral matter. Seeds of pea are nutritionally important due to its relative high content of essential amino acids which accumulated in cotyledon as storage proteins (Muntz 1982; Mandal and Mandal 2000; Sathe 2002). Pea vine is also used for making silage which contains an average of 7.1% digestible protein and 57.8% total digestible nutrients on dry matter basis.

The world pea production hovers around 12 million tons, Canada being the largest producer among all. In India, pea is grown in an area of 0.75 million hectares (ha) with production of 0.80 million tons and productivity of only 1067 kg/ha. Pea cultivation is about 80% in U.P followed by Bihar (6.8%). Unfortunately, the yield of pea is low in India as compared to the world average yield owing to the narrow genetic base and limited variability used in the development of local varieties (Kumar *et al.* 1997).

On the basis of their occurrence, proteins can be classified as leaf proteins, seed proteins and proteins of stem and root. All the storage seed proteins are referred to as metabolically inactive or reserve proteins. The storage proteins constitute the major proteins of the seeds. Osborne (1924) classified seed proteins on the basis of solubility in the different solvents into four types viz. albumins, globulins, prolamines and glutelins. In general seed proteins have relatively high nitrogen content as compared to those from animals (17-19%). They have also high content of arginine, decarboxylic acids and amide nitrogen (Danielson 1952), Prolamines and major seed globulins function primarily as nitrogen and carbon resources for germinating seeds.

Some investigators oppose that albumins are residual enzymes of other metabolic proteins but are not storage proteins. (Altshcul *et al*. 1966) while others such as Li *et al*. (1977) suggest that albumins contain true reserve proteins. Depending upon the variety and growing conditions, pea seeds contain on the basis of dry matter 18-37% protein. (Makasheva 1983). The hay of peas contain 16-20% protein, The green matter which remains after the harvesting of green pods is particularly valuable as it contains up to 22% proteins. After threshing the mature seeds, the empty pods contain 8-9% proteins.

MATERIAL AND METHODS

Biochemical studies included estimation of total seed proteins and protein fractions of the seeds of *Pisum sativum*. Seeds were collected at maturity from fifty plants selected randomly from eight genotypes of *P. sativum* viz Arkel, CHPMR-I, KTP-4, Mithi Phalli, NDVP-250, Pb-87, Pb-88 and VL-3 and then were used for subsequent analysis of the biochemical components. Genotype Arkel was used as control. Dry and clean seeds from the fifty plants of each genotype were ground to fine powder. The flour was sieved through fine muslin cloth to remove seed coats, husks etc. This fine flour was used for further analysis.

Protein Estimation

Total proteins were extracted from seed powder (50mg) with 5 ml of 0.1 N NaOH and then agitated over a cyclomixer. Homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. Proteins were estimated by Lowry *et al* (1951) method using Bovine serum albumin as standard.

Protein Fractionation

Seed meal for protein fractionation was prepared by grinding the grains (5g) of each genotype, followed by sieving. For defatting 2g of seed meal of each genotype was stirred with hexane/rectified spirit at room temperature. This was extracted twice with hexane/rectified spirit and the seed meal was then dried in oven at 60ºC for 72 hours. The procedure followed in the present investigation for protein fractionate was same adopted by Naik in 1968. For albumin fraction, water extract of the defatted flour was prepared by shaking the flour with distilled water for 1-5 hours in a shaker. Flour to solvent ratio of 1:5 (w/v) was used. The suspension was centrifuged and a clear supernatant was collected. The procedure was repeated thrice. Similarly for globulin fraction water extract of the defatted flour was prepared by shaking the flour with 1% sodium chloride solution followed by centrifugation at 10,000 rpm.

To prepare prolamine fraction an alcohol soluble extract of the flour was prepared by intermittently shaking the flour which was left behind after separation of albumin and globulin, with 70% ethanol. Supernatant were collected after centrifugation at 10,000 rpm. Then the flour i.e. the residue obtained after the above treatments was treated with 0.4% NaOH (thrice). It was shaken for 2 hour and centrifuged. The supernatant obtained represented the glutelin fraction. Then from above fractions, 5 ml of each fraction was used for further analysis.

RESULTS

The total seed protein content of 8 pea genotypes ranged from 16.3 - 20.4% (Table 1). From the table it is evident that the highest protein content (20.4 %) was exhibited by control Arkel and the lowest (16.3 %) was in genotype PB-88. The protein content did not vary significantly between CHPMR-I and KTP-4, and between NDVP-250 and VL-3 genotypes.

Different protein fractions obtained in 8 pea genotypes were for albumin, globulin, glutelin and prolamine (Table 2). All the genotypes studied exhibit lesser percentage of albumin fraction than control (6.6 %). The lowest albumin percentage was found in PB-88 (4.3%). The albumin fraction varies insignificantly between genotypes CHPMR-1, NDVP-250 and VL-3.

The globulin content in the pea genotypes ranged from 6.3 – 7.8 %. Except Mithi Phalli with highest (7.8 %) globluin content, all the remaining genotypes were found to have lesser globulin content than control Arkel (7.5%). Globulin content varied insignificantly between genotypes PB-87 and PB-88. The lowest globulin content (6.3%) was found in CHPMR-1 (Table 2).

Glutelin content was much lesser in the genotypes compared to albumin and globulin. It ranged from 2.9% to 3.9%. Only two genotypes viz. Mithi Phalli and PB-87 had higher glutelin content than the control (3.6%). The highest glutelin fraction was exhibited by Mithi Phalli (3.9%) and lowest by KTP-4 (2.9%). Glutelin content did not vary significantly between CHPMR-I, PB-88 and NDVP-250 genotypes (Table 2).

Of all the protein fractions, total content of prolamine was found to be least in all the 8 pea genotypes. Arkel had the highest (1.9%) whereas PB-88 had the lowest (0.52%) prolamine content (Table 2).

Table 1: Seed Protein content of eight pea genotypes

Genotype	Seed Protein content (%)
Arkel	20.4a
CHPMR-1	16.9b
KTP-4	16.9b
Mithi Phalli	19.7d
NDVP-250	17.7d
PB-87	18.1c
PB-88	16.3e
VL-3	17.8c

Mean = 18.0
S.E. = ± 0.47
Range = 16.3-20.4
Mean values not followed by the same alphabet, differ significantly from each other at 5 % level.

Table 2: Protein fractions of the pea seeds in eight genotypes

Genotype	Albumin (%)	Globulin (%)	Glutelin (%)	Prolamine (%)
Arkel	6.6	7.5	3.6	1.9
CHPMR-1	5.4	6.3	3.0	1.4
KTP-4	5.8	6.8	2.9	0.67
Mithi Phalli	6.3	7.8	3.9	0.99
NDVP-250	5.5	6.7	3.2	0.89
PB-87	6.1	7.3	3.8	0.94
PB-88	4.3	7.4	3.3	0.52
VL-3	5.6	6.5	3.6	0.64
Mean *	5.7	7.0	3.4	0.99
S.E.	± 0.23	± 0.18	± 0.12	± 0.15

*significant at 5% level of significance

DISCUSSION

The increasing need of protein rich products for human nutrition and animal feed has led to dependence upon pulses as a major and cheap source of protein. However, production of legumes and in particular peas for industrial use is at present limited by the variability in their biochemical composition (Gueguen 1991; Baniel 1998). As with other crops, variability in pea seed composition results from both genotypic and phenotypic factors (Casey *et al*. 1982; Gueguen and Barbot 1988). Protein content is a quantitative trait and hence component characters associated with this trait should be identified for indirect selection of genotypes for direct cultivation or for using as parent material in breeding programmes.

In the present investigation about 16.3 to 20.4% of protein content was observed in the 8 pea genotypes studied. Three genotypes Arkel, Mithi Phalli and PE-87 had more then 18% proteins and are the high protein genotypes and thus could be potential parents for breeding of high protein genotypes (Table 2). A study of European pea cultivars has indicated that morphological factors such as the position of pea within the pods or the location of the pod on the plant can determine seed protein content and consumption (Bertholdsson 1990; Cousin 1992). Similar studies need to be conducted on the present genotypes to study the effect of morphological factors on protein content. Pea proteins which are of major significance include the water soluble albumins and dilute salt soluble globulins. About 50- 65% of the seed protein is contributed by the globulin fraction that consists of two major components, legumin and vicilin. In the present study, highest amount of globulin and albumin is present in Mithi Phalli and Arkel respectively. Unlike cereal proteins, legume proteins are rich in lysine but are deficient in sulphur containing amino acids, methionine and cysteine. The albumin fraction has higher content of sulphur containing amino acids and hence Arkel, Mithi Phalli and PB-87 are potential genotypes for using as parents that can be crossed with high yielding genotypes to raise high yielding cultivars with higher methionine and cysteine content. However, genetic correlation among the various traits and influence of the environment need to be determined before formulating a breeding strategy (Vigeolas *et al* 2008).

CONCLUSION

With the growing demand or nutritional security, pulses are becoming ever more important as plant based source of protein in human nutrition. Besides the newly emerging, strong consciousness for health among the human population there is a genuine need for adopting a nutritionally complete crop predominantly vegetarian based diet. This trend conspicuous among the affluent countries is spreading to other human societies as well, owing to the realization that a predominantly plant based food system is healthy, eco-friendly and energetically less expensive, Consequently, the vegetarian based food profile with pulses as the major protein source (Domoney and Stittis 2006) likely to become more widely adopted leading to a greater demand for plant-based nutrients (Brahmaprakash *et al*. 2004). Several issues that mask the virtual productivity levels in pulses include energy content of pulses, solar energy utilization efficiency, inputs and marginalization of pulses. Pulses are the most neglected components of our food grains and are treated as “marginalized crops” and it is not unlikely that if they are given sufficient inputs, their yield performance could be as high as those of the cereals (Wang *et al* 2003).

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