STUDIES ON SEED STORAGE PROTEIN METABOLISM IN COMMON BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH)

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ABSTRACT
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Seed storage proteins, intended as a source of nitrogen for germinating seedlings, constitute an important source of dietary proteins for human consumption. Although cereal grains and legume seeds are a major source of dietary proteins , the storage proteins in both are generally deficient in essential amino acids such as lysine , tryptophan and methionine . Over the years many attempts have been made to improve the amino acid composition in important crops through conventional breeding programmes. Molecular approaches towards improving the nutritional quality of seed proteins, however, provide alternative strategies to conventional breeding programmes . An important approach towards improving the amino acid composition of seed storage proteins could be to express the gene for a heterologous protein with a balanced amino acid composition . The generality of the approach by which foreign proteins rich in desired amino acids may be introduced is however constrained by scanty information in respect of suitable heterologous proteins . While a number of seed proteins rich in sulphur containing amino acids are available not many lysine rich seed storage proteins have been identified so far .

The North Eastern region of India is extremely rich in floristic wealth and is home to a large variety of traditional crops that could form an essential component of human diet in times to come. Common buckwheat (*Fagopyrum esculentum* Moench.) is one such plant that has been classified by IBPGR as an important but underutilized crop. The plant is a dicot psedocereal with a high protein content (18%) of its grains. Further unlike cereals, where the main storage proteins are the prolamins, the main storage protein in buckwheat seeds belongs to the globulin family . In order to use the available germpalsm for improvement programs , the characterization of seed storage proteins from the plant would be a prerequisite. The present investigation was undertaken to

1. determine the content and quality of storage protein in buckwheat seeds.

2. determine the extent of relationship between germination and mobilization of storage proteins in seeds of common buckwheat during early stages of germination.

3. Isolate and characterize the main seed storage protein of common buckwheat and to determine its homology with seed storage proteins of legumes and cereals.

In order to assess the protein quality and quantity of buckwheat seeds, the seeds of common buckwheat (*Fagopyrum esculentum* Moench), obtained from the Western Himalayas and the North Eastern Regional station of NBPGR, were analyzed for the content of total proteins. On dry weight basis, the seeds had a protein content of about 16 percent out of which more than 50 percent was present in the globulin fraction. Albumins, prolamins, glutelins and the residual proteins constituted 15.8, 7.83, 9.27 and 14.43 percent of the total protein content of the grains respectively.

The main storage protein in seeds of common buckwheat is localized within protein bodies present in the endosperm tissues of the seed. The protein bodies of common buckwheat are spherical in shape and range in diameter from 5-8 μm. The protein bodies are composed of an amorphous protein matrix with a single globoid inclusion. The protein matrix is enclosed by a single membrane. Electron microscopic examination of the isolated protein bodies of buckwheat seeds also revealed the presence of a membrane like boundary layer surrounding the globoid inclusion. The protein body of buckwheat seeds has been thus classified as of "complex" type.The protein bodies of buckwheat seeds had about 70 percent protein content; phytin and carbohydrates respectively constituted 25 and 2.7 percent of the protein body dry weight. An analysis of the pattern of accumulation of the main storage protein in buckwheat seeds revealed that the protein started to accumulate only after early mature stage of seed development.

When extracts from seeds at various stages of development were analyzed by double diffusion test with antibodies raised against the 280 kD protein from mature buckwheat seeds, no cross reactivity was observed with extracts from seeds harvested upto early mature stage of development. A precipitin line with extracts from seeds harvested at mid mature stage of development clearly indicated the presence of the protein at this stage.

Studies were carried out to determine the relationship between the levels of proteolytic activity and the mobilization of reserve proteins during early stages of buckwheat seed germination. Results of our investigations revealed a rapid uptake of water and mobilization of storage proteins in buckwheat seeds during early stages of germination. There was no marked change in the electrophoretic mobility of the main storage protein during the initial 24 hours of germination. Beyond 24 hours, the electrophoretic mobility of the protein increased with progressing seed germination. correspondingly there was no marked difference in the SDS-PAGE profile of the main storage protein extracted from ungerminated seeds and those allowed to imbibe water for 24 hours. After 24 hours a gradual decrease in the ammount of some high molecular weight subunits of the protein and increase in the levels of some low molecular weight proteins was observed with progressing germination. When tested by the Western blot assay, strong anti 280 kD activity could be detected in proteins extracted from seeds harvested at various stages of germination upto 96 hours. The immunoblot also confirmed the changes taking place in the electrophoretic mobility of the protein with progressing germination. These results indicate that during early stages of seed germination, the main seed storage protein in common buckwheat undergoes a partial cleavage leading to the release of some low molecular weight subunits. Results of the western blot assay obtained in the present investigation clearly establish that the 280 kD globulin of buckwheat seeds retained its antigenic identity even upto 96 hours of imbibition. Till this time the process of radical and plumule emergence is nearly complete.

A low level of proteolytic activity could be observed in the endosperm of buckwheat seeds upto 18 hours of germination. Increases in the activity of the enzyme occurred only after 18 hours of imbibition. In the embryo, however, the activity of the enzyme started to increase after after 6 hours of imbibition only. Corresponding with changes in the activity of proteases a marked increase in the tissue level of free amino acids was observed in the endosperm and embryo tissues with progressing germination after 14 hours. However, the content of soluble protein in these tissues showed a consistent increase throughout. Considered together with the changes taking place in the main storage protein and the levels of proteolytic activity in the seeds during germination, these results indicate that the 280 kD globulin may not be the source of amino acids that are utilized by the growing embryo during the initial stages of germination. Contributions towards this pool as a result of proteolytic cleavage of the main storage protein into small peptides and amino acids presumably start much later. The significant decrease in the level of free amino acids in the endosperm during the initial 2 hours of imbibition and the increase in the level of soluble protein in the embryo during the same period indicates that a pre-existing pool of free amino acids in the endosperm tissues could be the source of amino acids to be utilized for the synthesis of soluble proteins during the initial stages of germination.

The main seed storage protein of buckwheat was purified by $(NH_4)_2SO_4$ fractionation, dialysis and chromatography on Sepharose 6B column. The protein showed a molecular mass of 280 kD. Electrophoresis on a 7.5% polyacrylamide gel resolved the protein into a single band. Under denaturing conditions the protein resolved into three groups of subunits designated as α , ß and Γ with molecular weight ranging between (55-60 kD, α); (32- 44 kD, ß) and (16-29 kD , Γ). The 280 kD protein revealed an oligomeric structure typical to the 11-12S groups of legumin type globulins . When tested against antibodies raised against the 280 kD globulin from mature seeds of common buckwheat, the protein extract from ungerminated buckwheat seeds as well as the 280 kD globulin showed single precipetin lines indicating the antigenic homogeneity of the protein. The protein showed antigenic homology with 11-12S seed storage proteins from soya bean , mung bean and pea; the protein showed strongest homology with glycinin from soya bean seeds. No cross reactivity was observed for storage proteins extracted from broad bean , wheat , rice and grain amaranth. Based on the gross subunit composition and its antigenicity with seed storage proteins of other crops, as observed in the present investigation, it can be assumed that the 280 kD globulin of buckwheat seeds belongs to the legumin subfamily of proteins.

In order to determine which of the subunits of the 280 kD globulin had the highest lysine level, the protein was subjected to gel filtration on Sephadex G-120 and Sephadex G-50 after reduction and alkalization. Fractions of each peak were pooled together and lypholysed. Analysis of the partial amino acid composition of the separated proteins revealed that a protein which had separated under fractions 42-48 on Sephadex G-50 column had the highest (6.93 %) lysine content. Besides the protein was also rich in some sulphur amino acids.

On SDS-PAGE the purified subunit resolved into a single band corresponding to 26 kD molecular mass. The amino acid composition of the subunit revealed high levels of essential amino acids such as lysine, threonine, leucine and some sulphur containing amino acids. Compared to other seed storage proteins, the 26 kD subunit also showed a high content of glutamine and arginine . The role of high level of arginine is consistent with the role of crystalloid seed storage proteins as a nitrogen source during seed germination . The amino acid composition of the protein also closely matches the values of essential amino acids recommended by the World Health Organization for a nutritionally balanced protein.

The sequence of 17 N-terminal amino acid residues of the protein was established by automated sequencing. The sequence reads as:

GLY-ILE-ASP-GLU-ASN-VAL-CYS-THR-MET-LYS-LEU-ARG-GLU-ASN-ILE-LYS-SER

Alignment of the sequence with the corresponding regions of proteins from seeds of some economically important crops revealed considerable similarity in the sequences. The protein showed 73.3% sequence homology with the G_1, G_2, G_3 and G_4 precursors of soya bean glycinin. The second highest homology (66.7%) was shown with pea legumin. The least homology of 46.7% was observed with the ß subunit of 11S globulin from cucumber seeds. Out of the 17 residues compared, 6 were highly conserved and 5 residues matched closely in that they were amino acids with similar functional groups, thereby representing conservative replacements. Amongst the conserved bonds was the cysteine-threonine linkage at residue numbers 7, 8 and the asparagine-isoleucine linkage between residue numbers 14, 15. One of the significant aspects revealed by the alignment was the presence of lysine and serine at position numbers 16 and 17 respectively in the 26 kD globulin subunit of buckwheat seeds. None of the other sequence matched had these residues at position numbers 16 and 17.

The physical characterization of the 26 kD globulin subunit of buckwheat by circular dichrosim and fluorescence spectroscopy revealed that the protein had a predominantly α-helical structure. The protein had a 33% α-helix which under-phobicity .