

# Proteome Analysis of Seed Storage Proteins in Allogamous and Autogamous Buckwheat

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## Objectives

As an initial step, we aimed to separate proteins from total of allogamous buckwheat and autogamous buckwheat to perform *N*-terminal amino acid sequences and internal amino acid sequences of several proteins by gas-phase sequencing. This experiment shows a possibility to easily and rapidly identify a number of 2-DE separated proteins of buckwheat by gas-phase sequencing. Information from our study could provide a venue to buckwheat breeder to design their research strategies precisely.

## Materials and Methods

- 1) Materials: Allogamous ("Miyazakizairai") and Autogamous buckwheat.
- 2) Methods: seed embryo sample, two-dimensional electrophoresis, *N*-terminal amino acid sequence analysis, internal amino acid analysis (Cleveland peptide mapping), homology search of amino acid sequence were used for this analysis.

## Results and Discussion

Seed-proteins of allogamous buckwheat (*Fagopyrum esculentum* Moench.) (cv. Miyazakizairai) and autogamous buckwheat were separated by two-dimensional gel electrophoresis (2-DE) and characterized by gas-phase sequencing for proteome analysis. A total of 100 protein spots were resolved in 2-DE gels. Ten spots were identified as a characteristics of autogamous buckwheat. These proteins can be used as a marker for the identification of autogamous cultivar. Among them, 17 proteins were subjected to *N*-terminal sequencing. *N*-terminally blocked proteins (~80%) were subjected to Cleveland peptide mapping followed by microsequencing. The sequences obtained were compared with those of the SWISS-PROT and NCBI databases. A protein showed high sequence homology with 22 kDa allergen protein of buckwheat and seven with proteins such as allergen Ara H1, cysteine proteinase 2, ribosomal protein, superoxide dismutase from rice, *Arabidopsis*, peanut and maize. In autogamous buckwheat, the *N*-terminal sequence of three proteins were found homologous to ; glutelin type-A III precursor, glutelin type-B 2 precursor and putative 30S ribosomal protein S31 from rice.

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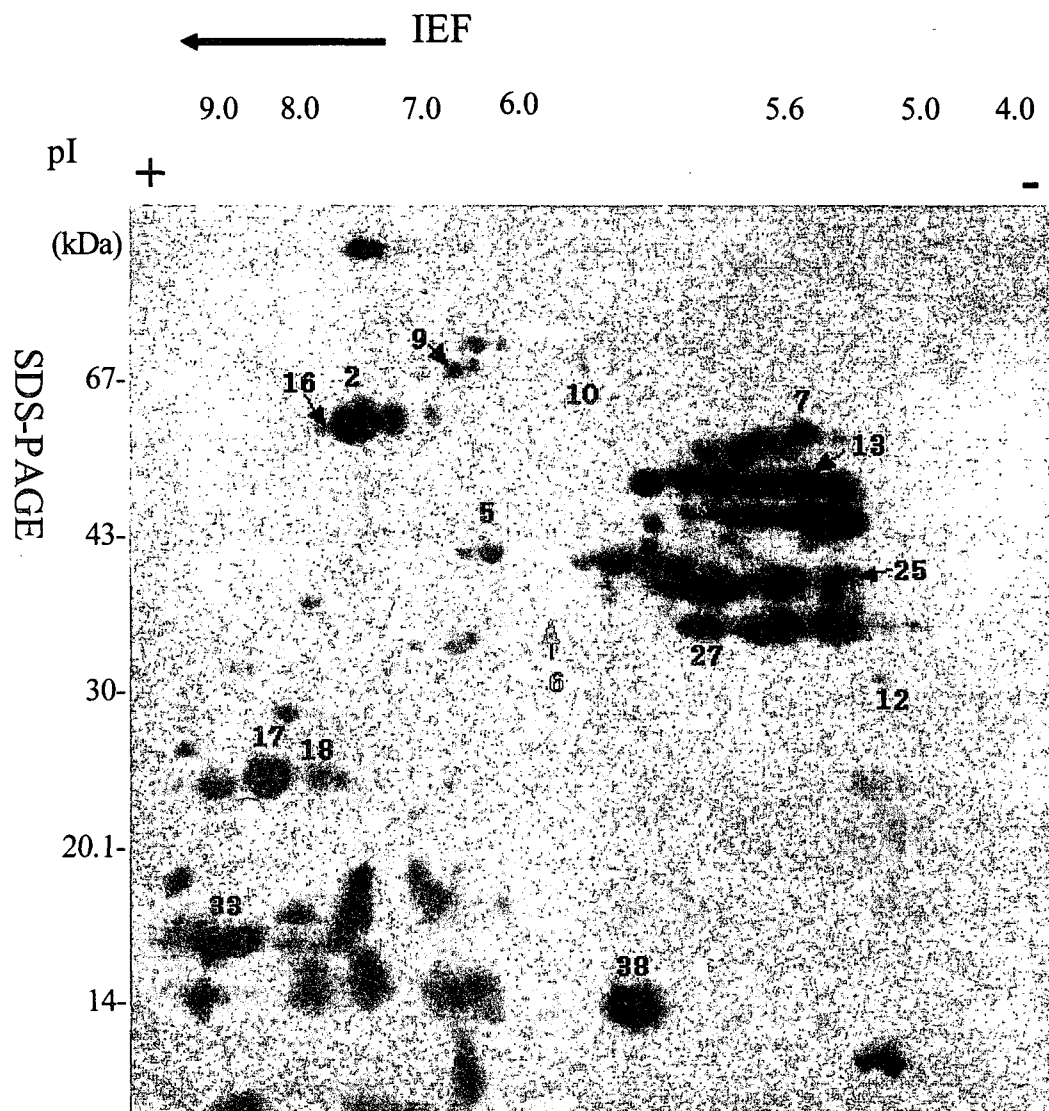


Fig.1. Two-dimensional gel patterns of the buckwheat embryo proteins. Allogamous buckwheat (cv. Miyazakizairai), first dimension (left to right), isoelectric focusing; second dimension (up to down), SDS-PAGE. The numbered spots were excised and analysed by Edman degradation.