

## Protein at 69 kDa Expressed During the Initial Maturation Stage of Buckwheat (*Fagopyrum esculentum*) Seed Development<sup>1</sup>

Arun Nair<sup>2</sup> and Taiji Adachi<sup>2,3</sup>

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Common buckwheat belongs to the family Polygonaceae and is believed to have originated from China. Genus *Fagopyrum* has about 15 species distributed in different parts of the world (Tahir and Farook 1988). Common buckwheat is essentially self-incompatible and possesses two main types of flowers: pin and thrum. Due to allogamy, the individuals in a population are highly heterogenic and the seed storage proteins show high polymorphism. Buckwheat proteins are of high biological value as the grains are rich in proteins and carbohydrates. Compared to other cereals, buckwheat grains are rich in essential amino acids such as lysine, threonine, and tryptophan.

The main components of buckwheat seed proteins are globulins represented by 13S protein fraction with molecular mass of 280 kDa (Radovic et al 1996). Electrophoretic studies have been attempted by different authors to study protein polymorphism (Dolinšek 1980, Shevchuk 1986, Nishiyama et al 1991). To study the mechanism of seed storage protein accumulation in buckwheat seeds, we attempted SDS-PAGE of seed proteins at different developmental stages up to maturity.

### MATERIALS AND METHODS

Common buckwheat, *Fagopyrum esculentum* Moench. 'Miyazaki zairai' was grown in a glass house at Miyazaki University. On flowering, the pin types were selected as female parents and thrum as male parents. The flowers were hand-pollinated and seeds were collected at 5, 7, 10, 15, 20 and 25 days after pollination (DAP) for analysis. The testa was removed using a binocular microscope. The sample consisted of 50 whole seeds. The fresh weight of the young seeds varied from 1 mg (5 DAP) to 38 mg (25 DAP) at maturity. For single seed analysis, the seeds were dehulled and the three rinds of the achene were clipped and used as sample. The seeds thus sampled could be germinated and advanced for next generation.

Total seed proteins were extracted for 2 hr at room temperature under reducing conditions with 0.125M Tris HCl, 10% SDS, 1% 2-mercaptoethanol, 30% glycerol, 0.2% bromophenol blue. Protein extract (10 µL) was used per lane for electrophoresis.

Electrophoresis was performed on an SDS-PAGE discontinuous system as described by Laemmli (1970). The standard markers were obtained from Sigma Chemical Co. (St. Louis, MO). The molecular mass of sample proteins was estimated from a regression equation using a calibration kit (Sigma).

### RESULTS

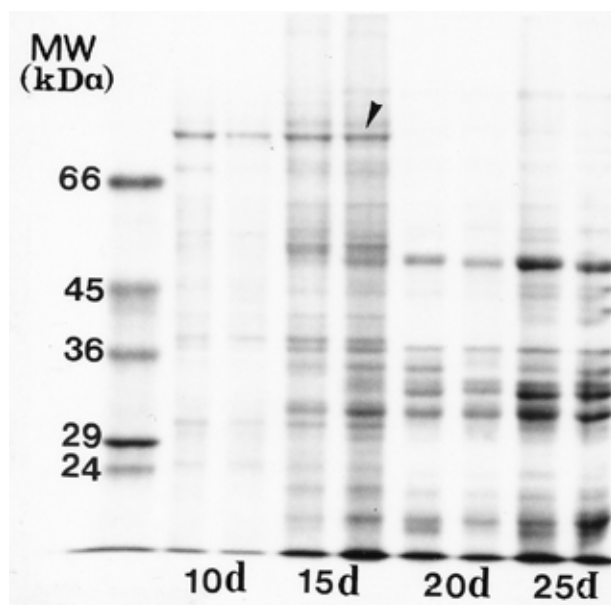
Seeds at 5 and 7 DAP did not reveal any bands (data not shown). Both faint and dark bands were visible from 10 DAP onward (Fig. 1).

The major protein band found among the developing seeds of 10 DAP was at 69 kDa and this band prevailed up to 15 DAP. At 15 DAP, darker bands were also visible at 57–58, 37–38, and 30–32 kDa. However, the 20 DAP protein profile showed darker bands at 57–58, 30–38, and 22 kDa, while the band at 69 kDa became faint or undetectable. This profile prevailed until maturity (25 DAP).

The band at 69 kDa was very faint or absent among the mature seeds tested. The single seed analysis ( $n = 600$ ) showed polymorphism for all the major bands except the 22-kDa protein band (Fig. 2).

### DISCUSSION

The protein accumulation in the seeds of common buckwheat began at 10 DAP. According to Maksimovic et al (1996), 57–58 kDa polypeptides were the main products at 9–11 days after flowering. However, we were able to detect a consistent major band at 69 kDa at 10–15 DAP. The 56–57 kDa band appeared as a major band after this period. Partial characterization of the major buckwheat storage protein by Rout and Chungoo (1996), revealed a high molecular weight globulin with a molecular mass of 280 kDa. Electrophoresis of this protein under denaturing conditions resolved the globulins into  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits with molecular mass ranges of 55–60, 32–44, and 16–29 kDa, respectively. Rogl and Javornik (1996), reported electrophoretic characterization of endosperm and cotyledonary proteins. The endosperm proteins separated mostly into one 54-kDa band. Hence, the 69-kDa protein band is unique and is expressed mainly during the initial maturation stage of buckwheat seed development. The single seed analysis of more than 600 individual seeds con-

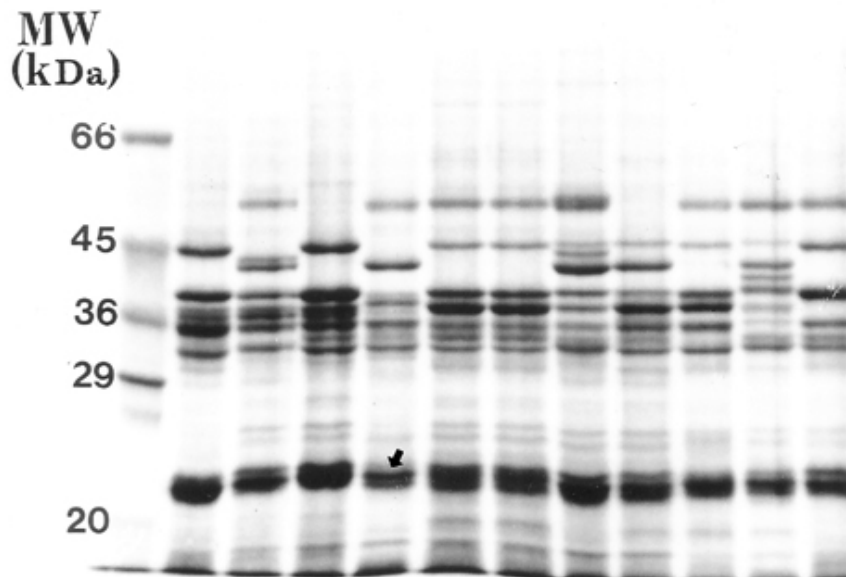


**Fig. 1.** SDS-PAGE electrophoretogram of seed proteins during developmental stages of *Fagopyrum esculentum* Moench. 'Miyazaki zairai'. Arrow indicates consistent band at 69 kDa up to 15 days after pollination. Protein bands then shifts to lower molecular weight regions.

<sup>1</sup> Contribution No. 115 from the Laboratory of Plant Breeding, Faculty of Agriculture, Miyazaki University, Gakuen Kibanadai Nishi 1-1, Miyazaki 889-2155, Japan.

<sup>2</sup> Applied Genetics and Biotechnology Division, Faculty of Agriculture, Miyazaki University.

<sup>3</sup> Corresponding author. Phone: +81-985-582811 ext 3115. Fax: +81-985-582884. E-mail: a0b207u@cc.miyazaki-u.ac.jp



**Fig. 2.** Single seed analysis of buckwheat seed proteins. All the major bands except 22 kDa (arrow) show polymorphism.

firmed that the 69-kDa protein band is not expressed in mature seeds of buckwheat and also that the absence is not due to polymorphism.

Although the function of the 69-kDa protein band is not very clear at this juncture, we postulate that either these proteins are produced initially and are used up during the developmental process, or they are proteolytically processed as a consequence of posttranslational modifications (Spencer et al 1980, Allen et al 1985) as the seeds mature. Hatano et al (1997), reported the presence of a binding protein (BiP) in pumpkin that is involved in cotranslational folding of nascent polypeptides and in the recognition and disposal of aberrant polypeptides. This BiP is a 70-kDa protein and is expressed only in the initial maturation stages. The N-terminus and internal fragments exhibited high identity to the sequence of soybean BiP (Kalinski et al 1995). The characteristics and molecular mass of 69 kDa for buckwheat is similar to those of soybean and pumpkin BiP and, hence, may be involved in the synthesis of seed storage protein during maturation. Further confirmation by isolating the total polyA mRNA during developmental stages and *in vitro* translation may reveal finer details.

### CONCLUSIONS

Electrophoretic analyses of buckwheat seed proteins during initial seed maturation stage (up to 15 DAP) revealed a high molecular mass (69 kDa) protein band. As the seeds matured, the banding pattern showed a shift toward low molecular weight regions. The characteristics of the 69-kDa protein in buckwheat seeds resemble the high molecular mass protein (70 kDa) from seeds of soybean and pumpkin that is involved in cotranslational folding of nascent polypeptides and in the recognition and disposal of aberrant polypeptides.

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