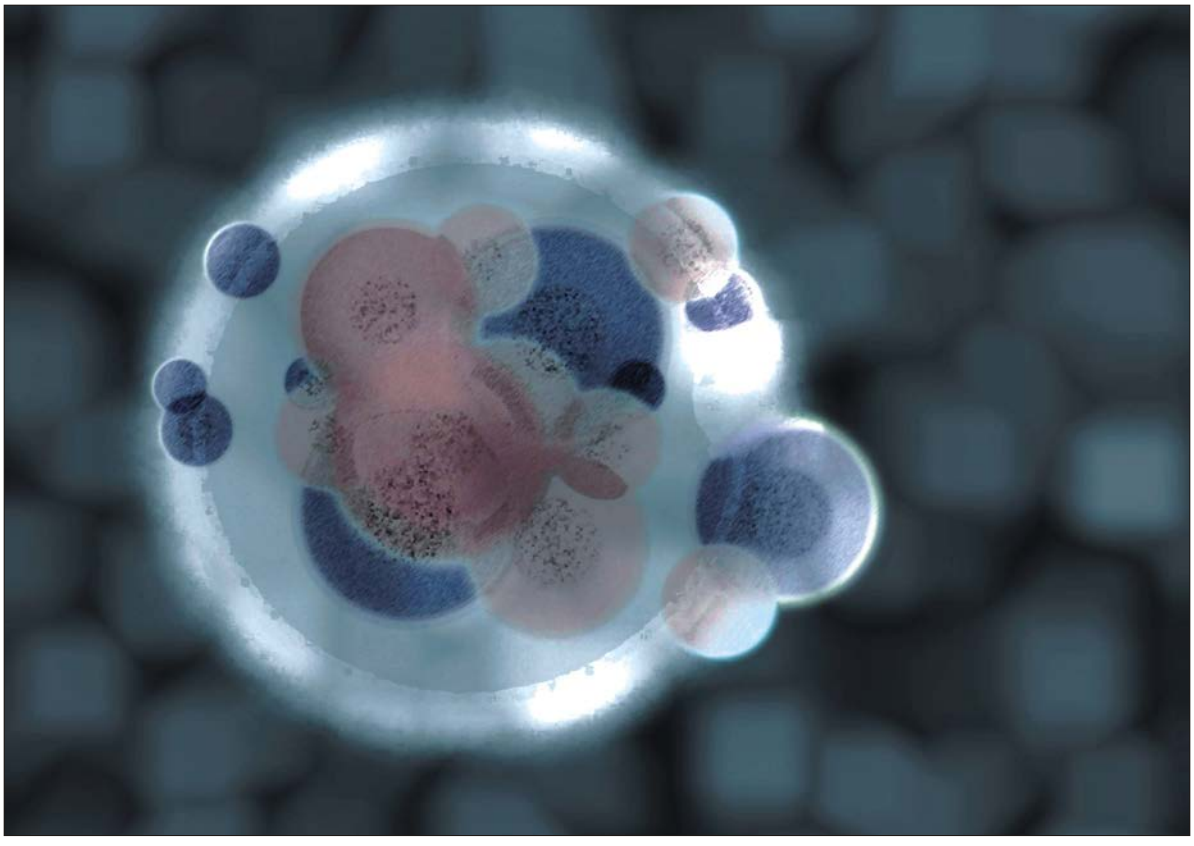

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Protein Profiling of *Brassica juncea* (L.) Czern var. Ensabi at Different Developmental Stages

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Abstract: *Brassica juncea* (L.) Czern var. Ensabi is a new local variety of mustard species, found only in Sabah and Sarawak, Malaysia. Electrophoretic characterization of plant proteins during development would be of great interest for comparison to other varieties of *Brassica* species. This study has been conducted to analyze protein profiles of *B. juncea* var. Ensabi at different growth stages. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of seed, shoot and root samples of seedling, before-flowering and after-flowering stages of the plant were performed on 10% polyacrylamide gel. Molecular weights of fractionated proteins were determined from a standard plot of log molecular weight versus relative mobility of marker proteins. Five polypeptides of ~66, 54, 39, 34 and 23 kDa, out of 11 protein bands noticed in seed proteins of Ensabi, matched closely with seed storage protein profiles of other varieties of *B. juncea*. The 29 kDa seed protein, commonly found in *B. juncea* (L.) Czern was also identified in Ensabi as S8. This protein expressed steadily in shoot samples at all growth stages. Interestingly, two seed proteins, S5 (~54 kDa) and S10 (~23 kDa) were remained expressed in both shoot and root samples throughout the plant development. Both common and unique proteins appeared at different growth stages of the plant. A comparison of the protein profiles at different growth stages suggested steady expression as well as up- and down-regulation of several genes encoding different proteins in *B. juncea* var. Ensabi.

Key words: Electrophoresis, Ensabi, growth phase, molecular weight, mustard, protein make-up

INTRODUCTION

Electrophoresis is generally employed for characterization and comparison of germplasm as well as evaluation of protein expression at different developmental stages of plants. Seed storage protein electrophoresis is commonly used as a tool to discover biosystematic relationship and genetic variation among crops for classification and varietal improvement purposes (Turi *et al*., 2010). Identification of promising genotype(s) for different traits using simple and inexpensive technique such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has been shown advantageous in breeding strategies (Kour and Singh, 2004; Sadia *et al*., 2009).

Brassicaceae or Cruciferae oilseeds such as mustard, canola and rapeseed are crops planted throughout the world mainly for vegetable oil production besides their use as protein-rich food i.e., animal feed and spices. In addition, the plants are also utilized as source of enzymes such as peroxidase to remove phenols from industrial waste (Noshin *et al*., 2003; Aluko and McIntosh, 2004; Coniglio *et al*., 2008; Das *et al*., 2010). *Brassicas* are best examples of amphidiploids which share molecular, morphological and physiological traits as a result of hybridization of the diploids. In general, mustard seed meal (*Brassica juncea* L.) contains good amount of protein e.g., 28-36% (Das *et al*., 2009). Electrophoretic analyses of seed storage proteins of many *Brassica* species (*B. juncea*, *B. campestris*, *B. rapa*, *B. carinata*, *B. olaracea*, *B. napus*) and their respective varieties have been well documented. These studies revealed genetic diversity i.e., presence of unique species-specific polypeptides as well as common proteins in polypeptide composition of each species. Consequently, this enabled researchers to understand homology and taxonomic relationship among them (Rabbani *et al*., 2001; Aluko and McIntosh, 2004; Kour and Singh, 2004; Sadia *et al*., 2009; Turi *et al*., 2010). For instance, *B. Carinata* contains polypeptides with molecular weights of 32.6 and 30.7 kDa which are also present in *B. rapa* and *B. napus* as species-specific polypeptides, respectively. However, these polypeptides are not present in *B. juncea* (Sadia *et al*., 2009). *Brassica juncea* (L.) Czern

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have been shown to contain a 29 kDa polypeptide, a common and major protein present in *Brassica juncea* seed meals (Aluko and McIntosh, 2004). This genetic information on *Brassica* germplasm is believed to be important to improve edible oil production and nutritional benefits of this crop (Turi *et al*., 2010).

Brassica juncea (L.) Czern var. Ensabi is a new local variety of mustard species found only in Sabah and Sarawak, Malaysia. A handful number of researches have been initiated to characterize allelopathic activity, physical and chemical properties of *B. juncea* var. Ensabi (Tossi and Bakar, 2007) in an attempt to explore the use of its extract as natural herbicide. Electrophoretic characterization of different polypeptides of *B. juncea* var. Ensabi would be of great interest for comparison to other varieties of *Brassica* species. Here, we present our data on protein profiling of this plant at different growth stages such as seeds, seedling, before-flowering and after-flowering stages, using SDS-PAGE and their comparison within each stage or different stages of development.

MATERIALS AND METHODS

All these experiments were conducted at RIMBA ILMU and Biochemistry Laboratory of the Institute of Biological Sciences, University of Malaya, Malaysia (July-December, 2009).

Chemicals: Bio-Rad (2000) prestained SDS-PAGE standards, broad range (Catalog No. 161-0318, Control 310004830) and reagents for SDS-PAGE were purchased from Sigma Chemical Co., USA. All other reagents used were of analytical grade purity. All the experiments were performed at 28°C unless otherwise stated.

Plant materials: Mature seeds of *Brassica juncea* (L.) Czern var. Ensabi were collected from Ensabi plants grown in the University of Malaya campus and stored with silica gel in darkness at 4°C before use. Fifty fresh seeds were placed in each petri dish, previously lined with 9 cm diameter Whatman No. 1 filter paper. The filter paper was moistened with 6 mL of water. The petri dishes were placed in growth chambers with a temperature regime of 25°C and exposed to fluorescent light with an intensity of 630 $Em^{-2}sec^{-1}$. All petri dishes were augmented with 6 mL of deionized water at 3 day intervals in order to maintain moisture conditions. Germinated seeds (complete seedlings) were removed from the petri dishes 14 days after sowing and used in subsequent experiments.

A part of seeds of Ensabi were sown in wooden boxes previously filled with garden soil of Malacca series in an insect-proof house. After germination, the plants were subjected to 12 h of natural sunlight outdoor (mean midday radiation of 1812 μmole photon $m^{-2}sec^{-1}$), 384 μmole photon $m^{-2}sec^{-1}$ inside the insect-proof house, mean ambient temperatures of 33±2°C (day) and 25±2°C (night) at Rimba Ilmu, University of Malaya, Kuala Lumpur, Malaysia. Plants were harvested at two different growth stages i.e., before- and after- (physiological maturity) flowering stages. These plants received a dose of fertilizer as 300 kg of NPK (15-15-15) ha^{-1} plus 150 kg of nitrogen ha^{-1} in split dose i.e., full NPK with half nitrogen at sowing and remaining half of nitrogen fertilizer 25 days after planting. Full dose of NPK was applied in the row below the seeds in the form of ammonium nitrate (34%), triple super phosphate and muriate of potash as the source of N (nitrogen), P (phosphorous) and K (potassium), respectively. These plants (before-and after-flowering stages, collected 52 and 86 days after germination, respectively) were immediately washed with double distilled water to remove soil or other adhered materials and used in other experiments.

Sample preparation: Roots and shoots of seedlings as well as each harvest (before-and after-flowering stages) were separated. Five grams of each material (seeds, shoots and roots obtained at seedling, before-and after-flowering stages, respectively) were soaked separately in 0.06 M sodium phosphate buffer, pH 6.8 overnight. These samples were grounded using mortar and pestle with 10 mL of the same buffer and filtered through four layers of cheese cloth to remove fiber debris. The filtrate of each sample was centrifuged at 5500 rpm for 30 min at 5°C. The supernatant was then vacuum filtered again with Whatman No. 42 filter paper. Finally, the last filtrate of each sample was stored at 4°C.

Protein estimation: Protein concentration in the plant samples was determined by Bradford (1976) method using BSA as the standard.

SDS-polyacrylamide gel electrophoresis: SDS-PAGE was performed to study the protein profiles of *B. juncea* var. Ensabi at different developmental stages according to the method of Laemmli (1970) on 10% (w/v) polyacrylamide gel in tris-glycine buffer, pH 8.3 containing 0.1% SDS. Sample incubation buffer also contained 5% (v/v) β-mercaptoethanol. About 10 μL of sample containing 5-20 μg of protein was loaded in each well and electrophoresis was carried out for about 2 h. The gel was stained with 0.2% (w/v) coomassie brilliant blue R-250 and destained in 5% methanol, 7% acetic acid solution. Different marker proteins along with their molecular

weights (adapted from technical bulletin of Bio-Rad (2000) prestained SDS-PAGE standards for Tris-HCl gel) given in parentheses: myosin (198, 510 Da), β-galactosidase (116, 254 Da), BSA (84, 796 Da), ovalbumin (53, 896 Da), carbonic anhydrase (37, 418 Da), soybean trypsin inhibitor (29, 051 Da), lysozyme (19, 809 Da) and aprotinin (6, 845 Da) were used for calibration. It is important to note that molecular weight values of some of these preparations are not matched with the reported values due to the formation of dye-protein complex as reported by the manufacturer (Bio-Rad, 2000).

Statistical analysis: Distances travelled by the protein band and bromophenol blue front were measured. Relative mobility (R_m) values of different protein bands were calculated using the following formula:

$$R_m = \frac{\text{Distance travelled by the protein band (cm)}}{\text{Distance travelled by the dye front (cm)}}$$

Data of log molecular weight (log MW) of different marker proteins were plotted against their R_m values. Regression analysis of these data yielded the following straight-line equation:

$$\log MW = -0.945\ R_m + 5.217$$

The above equation was used to determine the molecular weight of various fractionated proteins at different growth stages of *B. juncea* var. Ensabi.

RESULTS AND DISCUSSION

Electrophoretic patterns of protein samples obtained at different growth stages of *Brassica juncea* (L.) Czern var. Ensabi, namely, seed (S), shoot of the seedling (SH_S), root of the seedling (R_S), shoot of the plant before-flowering (SH_{BF}), root of the plant before-flowering (R_{BF}), shoot of the plant after-flowering (SH_{AF}) and root of the plant after-flowering (R_{AF}) showed presence of a number of fractionated proteins ranging from low to high molecular weights (Fig. 1a-c). The molecular weights of these proteins were determined using a standard curve between log molecular weight and relative mobility (R_m) of different marker proteins as described in 'MATERIALS AND METHODS' and listed in Table 1-4. A slight variation in the R_m values of certain marker proteins used in this study (Lane 1 of Fig. 1a-c) compared to those shown in the technical bulletin supplied by the manufacturer can be ascribed to the use of a low

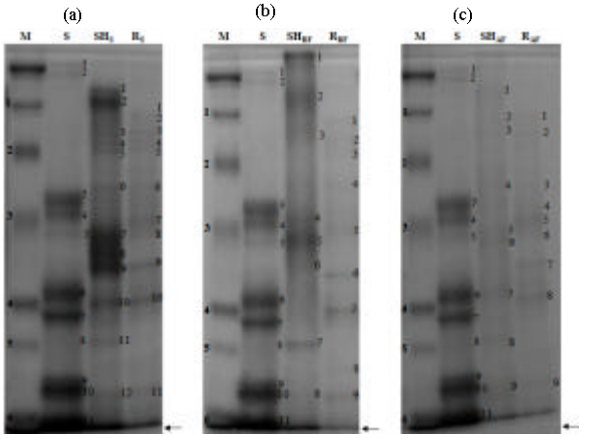

Fig. 1: SDS-PAGE pattern of Marker proteins (M), Seed (S), Shoot (SH) and Root (R) samples of *Brassica juncea* (L.) Czern var. Ensabi at (a) seedling (SH_S and R_S), (b) before-flowering (SH_{BF} and R_{BF}) and (c) after-flowering (SH_{AF} and R_{AF}) stages, performed according to the method of Laemmli (1970) on 10% polyacrylamide gel. The arrow shows the position of the tracking dye, bromophenol blue in each set. Marker proteins used were (Lane 1): 1. β-galactosidase; 2. BSA; 3. ovalbumin; 4. carbonic anhydrase; 5. soybean trypsin inhibitor and 6. lysozyme. Fractionated proteins in each preparation are numbered accordingly

percentage and non-gradient polyacrylamide gel against a gradient gel employed by the manufacturer. Consequently, both high molecular weight (myosin) and low molecular weight (aprotinin) protein bands were omitted in the construction of the standard curve. Use of the remaining six marker proteins in plotting the standard curve showed a strong correlation coefficient of 0.995. Furthermore, straight-line equation yielded similar molecular weights of marker proteins by using their R_m values.

A total of 11 major protein bands were noticed in seed (S) sample (Lane 2 of Fig. 1a-c) ranging in molecular weight from 140, 610 to 19, 327 Da (Table 1). Out of these fractionated proteins, seven proteins marked as S3, S4, S6, S7, S9, S10 and S11 were present in relatively higher amounts (Lane 2 of Fig. 1a-c). These proteins represent the storage, structural and biologically active proteins including enzymes associated with the hydrolysis of stored food (amylase, maltase, protease, carbohydrase and lipase), germination, aerobic/anaerobic respiration, lectins and enzyme inhibitors (Fukushima, 1991;

Table 1: Relative mobility (R_m) and molecular weight (MW) values of different proteins present in seed sample of *Brassica juncea* (L.) Czern var. Ensabi

Protein band	R_m	MW (Da)
1	0.073	140, 610
2	0.095	134, 037
3	0.424	65, 512
4	0.466	59, 790
5*	0.515	53, 743
6	0.668	38, 525
7	0.725	34, 031
8	0.786	29, 801
9	0.889	23, 817
10*	0.912	22, 655
11	0.985	19, 327

*Protein bands present in seed, shoot and root samples of *B. juncea* var. Ensabi at all developmental stages

Table 2: Relative mobility (R_m) and molecular weight (MW) values of different proteins present in shoot and root samples at seedling stage of *Brassica juncea* (L.) Czern var. Ensabi

Shoot sample (SH_S)			Root sample (R_S)		
Protein band	R_m	MW (Da)	Protein band	R_m	MW (Da)
1	0.121	126, 655	1	0.180	111, 404
2	0.153	118, 145	2	0.195	107, 826
3*	0.237	98, 409	3*	0.238	98, 195
4*	0.260	93, 605	4*	0.257	94, 218
5*	0.279	89, 814	5*	0.280	89, 619
6*	0.374	73, 042	6*	0.370	73, 680
7*'	0.500	55, 526	7	0.467	59, 660
8	0.550	49, 802	8*'	0.500	55, 526
9*	0.588	45, 850	9*	0.581	46, 554
10*	0.681	37, 450	10*	0.678	37, 696
11	0.779	30, 258	11*'	0.912	22, 655
12*'	0.912	22, 655			

*'Protein bands present in seed, shoot and root samples of *B. juncea* var. Ensabi at seedling stage. *Common proteins in both shoot and root samples of *B. juncea* var. Ensabi within this stage

Table 3: Relative mobility (R_m) and molecular weight (MW) values of different proteins present in shoot and root samples at before-flowering stage of *Brassica juncea* (L.) Czern var. Ensabi

Shoot sample (SH_{BF})			Root sample (R_{BF})		
Protein band	R_m	MW (Da)	Protein band	R_m	MW (Da)
1	0.027	155, 412	1	0.195	107, 826
2	0.137	122, 331	2	0.248	96, 082
3	0.225	101, 012	3	0.286	88, 457
4	0.458	60, 840	4	0.363	74, 811
5*'	0.518	53, 394	5*'	0.496	56, 012
6	0.573	47, 371	6	0.599	44, 766
7	0.786	29, 801	7	0.695	36, 327
8*'	0.916	22, 458	8	0.847	26, 097
			9*'	0.916	22, 458

*'Protein bands present in seed, shoot and root samples of *B. juncea* var. Ensabi at before-flowering stage. *Common proteins in both shoot and root samples of *B. juncea* var. Ensabi within this stage

Table 4: Relative mobility (R_m) and molecular weight (MW) values of different proteins present in shoot and root samples at after-flowering stage of *Brassica juncea* (L.) Czern var. Ensabi

Shoot sample (SH_{AF})			Root sample (R_{AF})		
Protein band	R_m	MW (Da)	Protein band	R_m	MW (Da)
1	0.110	129, 733	1*	0.189	109, 243
2*	0.187	109, 720	2*	0.236	98, 623
3*	0.230	99, 919	3*	0.379	72, 251
4*	0.374	73, 042	4	0.437	63, 685
5	0.494	56, 256	5	0.471	59, 143
6*'	0.521	53, 046	6*'	0.508	54, 568
7	0.661	39, 116	7	0.591	45, 552
8	0.790	29, 543	8	0.685	37, 126
9*'	0.918	22, 361	9*'	0.913	22, 606

*'Protein bands present in seed, shoot and root samples of *B. juncea* var. Ensabi at after-flowering stage. *Common proteins in both shoot and root samples of *B. juncea* var. Ensabi within this stage

Mandal and Mandal, 2000). Besides, proteins required for seed protection i.e., defense against micro-organisms and insects may also constitute the total protein content of the seed (Kelly *et al*., 1998). The number of protein bands appeared in the electrophoretogram (Lane 2 of Fig. 1a-c) shown in this study is relatively low compared to ~26 bands displayed in previous reports for seed protein profile of *B. juncea* (Sadia *et al*., 2009; Turi *et al*., 2010). This can be attributed to the relatively lower resolving power of non-gradient 10% polyacrylamide gel used in this study. The seed protein profile shown in this report was compared with the spectrum of seed proteins obtained from 13 *B. juncea* cultivars (Sadia *et al*., 2009). Five major peptides with molecular weight values of ~66, 54, 39, 34 and 23 kDa matched closely with S3, S5, S6, S7 and S10 proteins present in *B. juncea* (L.) Czern var. Ensabi (Fig. 1, Table 1). Appearance of S8, a protein of ~29 kDa in *B. juncea* var. Ensabi seed sample has been reported to be a commonly occurring polypeptide in *B. juncea* (L.) Czern (i.e., type: AC Vulcan and Commercial Brown) seed meals (Aluko and McIntosh, 2004). Dhawan and Nainawatee (1994) also showed presence of a 28 kDa water soluble polypeptide as the major protein in *B. juncea* seed meals besides 12 and 13 kDa polypeptides. Indeed, the protein (S8) remained expressed in shoot samples of *B. juncea* var Ensabi at all growth stages in the form of SH_S11, $SH_{BF}7$ and $SH_{AF}8$ (Fig. 1). Polypeptides with molecular weight of 66 and 34 kDa are two common proteins observed between *B. juncea* and *B. napis* as well as *B. campestris* and *B. juncea*, respectively (Dhawan and Nainawatee, 1994). These two polypeptides were also present in *B. juncea* var. Ensabi in the form of S3 and S7, respectively (Fig. 1, Table 1).

B. juncea seed meal is reported to contain calcium-soluble protein fraction (Aluko *et al*., 2004). The composition of this fraction from yellow mustard (*Sinapis alba*) seed meal has shown the presence of major polypeptides with different molecular weights i.e., 5, 15, 22, 28, 35, 50 and 55 kDa (Aluko *et al*., 2004). In view of it, four seed proteins, namely, S5 (~54 kDa), S7 (~34 kDa), S8 (~29 kDa) and S10 (~23 kDa) shown in this study (Table 1) seem to belong the same group of calcium-soluble protein fraction. Storage proteins in

Brassicaceae oilseeds (mustard) are classified into two major types, labelled as cruciferin or legumin type globulin (11S or 12S/300-360 kDa) and napin or napin-like albumins (1.7S or 2S/15-18 kDa) (Schwenke *et al*., 1981; Sjodahl *et al*., 1991; Lonnerdal and Janson, 1972; Crouch *et al*., 1983). Cruciferin is a hexameric protein where each subunit contains acidic/α (30 kDa) and basic/β (20 kDa) polypeptides linked by a disulfide bond (Dalgalarrondo *et al*., 1986; Delseny and Raynal, 1999). On the other hand, napin, an allergenic protein in Brassicaceae (i.e., Bra j 1 in *B. juncea*) (Monsalve *et al*., 2004) is composed of a large or heavy (10-12 kDa) and a small or light (4-6 kDa) polypeptides linked by four disulfide bonds (Gehrig and Biemann, 1996; Rask *et al*., 1998). Peptide sequences of 22, 28 and 35 kDa proteins of *S. alba* were shown to be composed of cruciferin peptides through MALDI-TOF analysis (Aluko *et al*., 2004). Due to the similarity of *B. juncea* var. Ensabi seed proteins S7, S8 and S10 (Table 1) with those of *S. alba* proteins, it can be suggested that these proteins may represent cruciferin peptides. Less resolving power of our non-gradient 10% polyacrylamide gel might be responsible for the lack of characterization of napin-like albumins in *B. juncea* var. Ensabi.

Interestingly, two seed proteins , namely, S5 and S10 of *B. juncea* var. Ensabi remained expressed in both shoot and root samples at all developmental stages in the form of SH_S7, $SH_{BF}5$, $SH_{AF}6$; R_S8, $R_{BF}5$, $R_{AF}6$ and SH_S12, $SH_{BF}8$, $SH_{AF}9$; R_S11, $R_{BF}9$, $R_{AF}9$, respectively (Fig. 1,Table 1-4). These seed proteins may possibly represent vital proteins or enzymes required for the growth, biochemical reactions and defense mechanism from the very early stage. Seed protein S11 of *B. juncea* var. Ensabi (Table 1) seems to represent trypsin inhibitor due to similarity in molecular weight with a trypsin inhibitor, BjTI (MW ~20 kDa) observed earlier in *B. juncea* (Indian mustard). This protein is supposed to be involved in the regulation of endogenous plant proteinases and protection of seed from diseases and pests besides its storage function (Mandal *et al*., 2002). Absence of this protein (S11) in shoot and root samples of different developmental stages further strengthens our prediction as BjTI was present only in seeds and not in other plant parts (Mandal *et al*., 2002). On the other hand, presence of seed protein S2 (MW ~134 kDa) in *B. juncea* var. Ensabi (Fig. 1, Table 1) contradicted an earlier report suggesting the absence of 135 kDa polypeptide in *Brassica* seeds (Aluko and McIntosh, 2004). In view of the very low R_m values of both S1 and S2 proteins and being outside the range of R_m values of different marker proteins used in this study, computed values of molecular weights of these proteins remain questionable. Further studies are needed to clarify the presence of high molecular weight proteins in *B. juncea* var. Ensabi.

When the seeds (embryos) germinated as seedlings, several new proteins, particularly in the molecular weight range ~74-127 kDa appeared both in shoots (SH_S) and roots (R_S) of seedlings (Lanes 3 and 4 of Fig. 1a) due to up-regulation of genes. In general, 12 and 11 protein bands appeared in shoot and root samples, respectively of seedlings of *B. juncea* var. Ensabi. Out of these bands, SH_S1, 2, 3, 4, 5, 6, 8, 9 and R_S1, 2, 3, 4, 5, 6, 9 represent newly expressed proteins in shoot and root of *B. juncea* var. Ensabi's seedlings, respectively (Lanes 3 and 4 of Fig. 1a, Table 2). The newly expressed proteins may account for cell division, enlargement and differentiation during growth and development of seedlings. Mustard plants in Brassicaceae family are known as hyper accumulators due to their ability to concentrate heavy metals in their different plant parts (Garg and Kataria, 2010). Therefore, the newly expressed polypeptides seem to have been evolved for heavy metal ion homeostasis and detoxification (Zhou and Goldsbrough, 1994; Cobbett, 2000). Three additional seed proteins, S4, S6 and S8 were remained expressed in seedlings as R_S7, SH_S10/R_S10 and SH_S11 (Fig. 1a, Table 1, 2), respectively besides S5 and S10 as described above and may serve similar functions. On the other hand, genes for remaining six seed proteins seem to be down-regulated once the seed transformed into seedling stage (Fig. 1a, Table 1, 2). This is possibly due to proteins/enzymes needed for catabolic reactions in seeds are no longer required as the dry mass of food storage declines at the end of seed dormancy for germination and seedling development (Mandal and Mandal, 2000).

A comparison of protein profiles of shoot and root samples of seedlings suggested several similarities as well as presence of unique proteins in each part. Proteins with molecular weight values of ~98, 94, 90, 73, 55, 46, 37 and 23 kDa were common in both shoot and root samples of seedlings in the form of SH_S3, 4, 5, 6, 7, 9, 10, 12 and R_S3, 4, 5, 6, 8, 9, 10, 11, respectively (Lanes 3 and 4 of Fig. 1a, Table 2), suggesting that these proteins share similar functional properties. Distribution of similar proteins (e.g., calcineurin or Ca^{2+}/calmodulin-dependent protein phosphatase, classical type of protein kinase c, stress related protein and phytochelatin synthase each of 55 kDa, glyoxalase I of ~58 kDa, an endogenous protein of 85 kDa) in different parts of seedlings has also been shown in different varieties of *B. juncea* (Sharma and Deswal, 2004; Deswal *et al*., 2004; Pareek *et al*., 1998; Deswal and Sopory, 1998; Gasic and Korban, 2005).

Proteins such as SH_S1, 2 and 8 as well as R_S1 and 2 were the distinctive polypeptides present in shoot and root of seedlings, respectively (Lanes 3 and 4 of Fig. 1a, Table 2). These proteins may represent the soluble enzymes or polypeptides available in leaves, particularly in chloroplast or stroma (SH_S1, 2 and 8) and in root (R_S1 and 2), responsible for photosynthesis and detoxification of heavy metal absorbed from soil, respectively.

Figure 1b (Lanes 3 and 4) shows SDS-PAGE pattern of *B. juncea* var. Ensabi at before-flowering stage, bearing a total of 8 and 9 protein bands in shoot (SH_{BF}) and root (R_{BF}) samples, respectively. Proteins, SH_S9 and R_S2, 4, 5, 6, 9, 10 in shoot and root samples of seedlings remained nearly akin in *B. juncea* var. Ensabi at before-flowering stage as SH_{BF}6 and R_{BF}1, 2, 3, 4, 6, 7, respectively (Table 2, 3). These are the proteins which possibly support continuous elongation of cells, growth and development of the plant during the primary growth stage of plant body, photosynthesis, transpiration, translocation and senescence. Proteins SH_{BF}1, 2, 3, 4 and R_{BF}8 are the new proteins appeared in shoot and root, respectively at before-flowering stage compared to seedling stage of *B. juncea* var. Ensabi (Lanes 3 and 4 of Fig. 1b, Table 3). The newly expressed proteins in shoot may include proteins responsible for initial growth of lateral buds and reproductive organs (flowers) of the plant. Apart from proteins SH_{BF}5, 8 and R_{BF}5, 9, all other proteins of shoot and root samples at before-flowering stage were totally distinct (Lanes 3 and 4 of Fig. 1b, Table 3). It seems probable that some of the expected common polypeptides of shoot and root samples were present in very low amount and hence were not visible on the electrophoretogram.

Protein profiles of shoot and root samples at after-flowering stage of *B. juncea* var. Ensabi are shown in lanes 3 and 4 of Fig. 1c. Poor visibility/low intensity of protein bands in both shoot and root samples may be attributed to either the low content of these proteins present in these tissues naturally or low recovery of proteins from these plant parts during extraction procedures. In spite of the above limitation, a total of 9 protein bands were visible in both shoot and root samples at this stage. Proteins which emerged at seedling stage of *B. juncea* var. Ensabi, particularly in root samples such as R_S2, 4, 6, 9 and 10 were remained throughout the plant growth. These proteins were represented as R_{BF}1, 2, 4, 6, 7 and R_{AF}1, 2, 3, 7, 8 at the before- and after- flowering stages, respectively (Table 2-4). Proteins S4/R_S7 and S6/SH_S10/R_S10 which were visible at both seed and seedling stages were seen in the form of R_{AF}5 and SH_{AF}7, respectively at the after-flowering stage (Fig. 1c, Table 1, 4). Shoot proteins SH_{AF}2, 3, 4, 6 and 9 were found to be similar to root proteins R_{AF}1, 2, 3, 6 and 9, respectively during this stage, whereas, proteins SH_{AF}1, 5 and R_{AF}4 were the unique proteins present in shoot and root, respectively at the after-flowering stage (Lanes 3 and 4 of Fig. 1c, Table 4).

In conclusion, both Fig. 1 and Table 1-4 have suggested the steady expression of several proteins as well as up-and down-regulation of several genes coding for different polypeptides in *B. juncea* var. Ensabi upon its gradual development from seed till physiological maturity stage. Indeed, present data on electrophoretic characterization of *B. juncea* var. Ensabi is the first report on the comparison of protein profiles of the plant at its different growth stages to the best of our knowledge. These findings can be further explored for precise identification of the major proteins present at different developmental stages of *B. juncea* var. Ensabi with 2D-gel electrophoresis and various molecular markers techniques.

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