Pak. J. Bot., 43(3): 1733-1734, 2011.

NOVEL PROTOCOL FOR ALBUMIN AND GLOBULIN DETECTION IN *PISUM SATIVUM* GENOTYPES USING SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

M. NISAR*[1], A. GHAFOOR[2], M.R. KHAN[3], SADDRUDINE SIDDIQUI[2], NASRULLAH KHAN[4] AND MUHAMMAD FAHEEM SIDDIQUI[4]

[1]Department of Botany, University of Malakand, Chakdara, Dir (Lower), Khyber Pakhtoonkhwa, Pakistan
[2]Institute of Agri-biotechnology and Genetic Resources, NARC, Islamabad, Pakistan
[3]Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan
[4]Department of Botany, Federal Urdu University, Karachi, Pakistan
**Correspondence author E-mail: mnshaalpk@yahoo.com / nisarhu@gmail.com*

Abstract

In the present study a novel protocol for the removal of Albumin and Globulin in 10 genotypes of *Pisum sativum* were developed by using Osborn solubility class protein extraction buffer (10mM Tris-Hcl buffer, pH 6.8, 0.5 M NaCl). One major allocation of protein band and one minor protein band corresponding to 10 kda and 22 kda, respectively, was detected in SDS-PAGE (untreated cases). There is a need to further improve this method so that the minor groups of albumin and globulin could also be investigated further to characterize through 2D gel electrophoresis.

Introduction

Denaturation of proteins may be defined as the disruption of secondary, tertiary and quaternary structure of the native proteins resulting in the alteration of the physical, chemical and biological characterization of the protein by a variety of agents. Denaturation of native protein take place by physical agents (heat, surface action, ultrasound of high pressure) and chemical agents like acid, alkali, salt urea and ethanol (Deb, 2002). Among the chemical agents sodium dodecyl sulphate (SDS) is an anionic detergent that denature protein by wrapping the hydrophobic tail around the polypeptide backbone. For almost all proteins, SDS binds to the protein, thus confirming a net negative charge to the polypeptide in proportion to its length (Shahid *et al.,* 2000).

The SDS also disrupts hydrogen bonds, block hydrophobic interaction, and substantially unfold the protein molecules, minimizing difference in molecular form by eliminating the tertiary and secondary structures. The SDS denatures and reduces polypeptides are flexible rods with uniform negative charge per unit area. Recently, biochemical and molecular genetic techniques have emerged as a complementary strategy in conjunction with traditional approaches in the management of plant genetic resources (Ayad *et al.,* 1997; Bretting *et al.,* 1995).

Study on population genetics, polyacrylamide gel electrophoresis (PAGE) is considered as a useful tool for most taxa (Ghafoor *et al.,* 2002; Nisar *et al.,* 2007; 2009). A lot of work has been reported for the identification and determination of seed storage protein using SDS-PAGE. But there is no single report, on the removal and detection of albumin and globulin in *Pisum sativum*, hence the protocol was developed to enhance the legume sciences.

Materials and Methods

Plant material: Seeds of 10 genotypes (DASAN, Climax, DMR-7, DMR-4, Shawnee, Fallon, 10607, 18349 and 3272) were obtained from the genebank of institute of Agri-Biotechnology and genetic resources, National Agricultural Research Center, Islamabad, Pakistan. The experiment was split into two groups i.e., Group-I and Group-II for albumin and globulin detection.

Extraction: For Group-I experiment, single seed from each genotype grounded to a fine powder with Mortar and Pestle and about 0.05g of it was taken in eppendorf tube and treated with 800 µl of 10mM Tris-Hcl buffer, pH 6.8, 0.5 M Na Cl_2 (Osborn solubility class protein extraction buffer, OSCPEB). The mixture was agitated by vortex mixer (1KA-VIBRAX-VXR) for one hour and centrifuged at 15000 rpm for 10 min., at 4°C. The supernatant containing albumin and globulin was discarded and pellet was treated with OSCPEB three times to remove maximal albumin and globulin. Thus the pellet obtained, was extracted with 400 µl of 0.05 M Tris-HCl buffer, pH 8.0, containing 0.2% SDS, 5 M Urea and 1% β-mercaptoethanol (SDS-PAGE protein extraction buffer). The content was mixed well with Automatic Lab Mixer and centrifuged again at 15000 rpm for 10 min. The supernatant was collected for SDS-PAGE analysis.

In another set of experiment (Group-II) in which extraction step with OSCPEB was omitted; 0.01g of fine powder was treated directly with SDS-PAGE protein extraction buffer. The content was agitated and mixed well for 1-2 min and centrifuged at 15000 rpm. The supernatant was saved and used for SDS-PAGE analysis. In all the samples of Group-I and Group-II, bromo-phenol blue was added as tracking dye.

SDS-polyacrylamide gel electrophoresis: Electrophoresis was carried out by the method of Laemmli (1970). Proteins were separated in 12.5% acrylamide mini-slab gel. After the run, the proteins in the gel were stained with Coomassi blue and the molecular weight estimates were made using standard size marker proteins (Nisar *et al.,* 2006).

Results and Discussion

Classification of plant storage protein, Osborn Methods is used (Landers & Hamaker, 1994; Steenson & Sathe, 1995; Iida *et al.,* 1997), which is based on the relative solubility of the target protein Osborn, (1924). Total seed protein profile has been investigated in *Pisum sativum* (Nisar *et al.,* 2006; Nisar *et al*., 2009). No report is available in albumin and globulin detection; hence a protocol was developed to identify this subunit. In the present study, Osborn solubility class successfully removed the albumin and globulin in *Pisum sativum* genotypes. In SDS-PAGE two proteins bands were appeared in untreated case of DASAN genotype, which correspond to molecular weight of 22 kda and 10 kda as shown Fig 1 lane 2. These two proteins bands were found

absent in lane 3. Similarly, same results were obtained from untreated and treated Climax, DMR-7, Shawnee, DMAR-4, Fallon, 10607-3, 18349 and 3272 (lane 4-19). The protein corresponding to 10 kda seems to be greater in amount in Fallon and 10607-3 (lane 12 and 14) as compared to proteins in DASAN, Climax, DMR-7 and 18349 (lane 2, 4, 6, 8 and 16). Very less amount of same protein is found in DMAR-4 and 3272 genotypes (lane 10 and 18), while the amount of 22 kda proteins seems to be smaller than 10 kda protein in all cases (untreated). Bands were hardly appeared in the gels. To investigate the minor group of the albumin and globulin the 2D gel electrophoresis is needed.

Table 1. Experimental design for both treated and untreated group of 10 pea's genotypes.

S. No	Genotypes	Group-1 treated with osborn solubility class extraction buffer solution	Group-2 not treated (untreated) with osborn solubility class extraction buffer solution
1.	DASAN	Both 22 kda and 10 kda protein band absent	Both 22 kda and 10 kda protein band present
2.	Climax	Bands absent	Bands present
3.	DMR-7	Bands absent	Bands present
4.	Shawnee	Bands absent	Bands present
5.	DMR-4	Bands absent	Bands present
6.	19658	Bands absent	Bands present
7.	Fallon	Bands absent	Bands present
8.	10607-3	Bands absent	Bands present
9.	18349	Bands absent	Bands present
10.	3272	Bands absent	Bands present

kda- kilo Dolton protein molecular weight

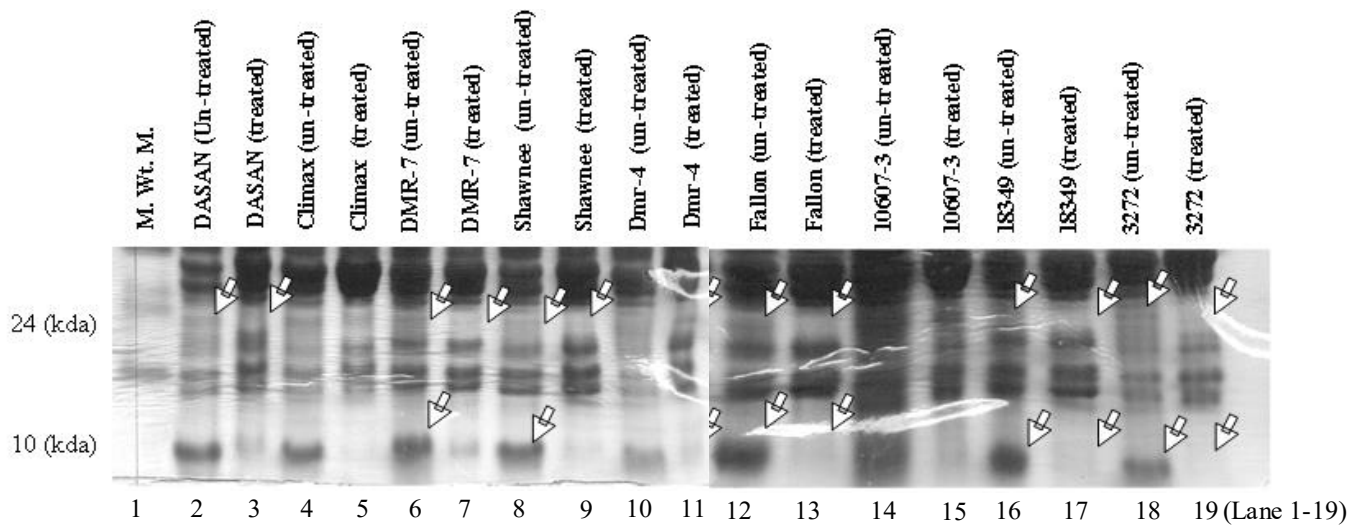

Fig. 1. Albumin and Globulin are absent in the treated samples and present in untreated samples. Arrow indicates the allocation of the band ~22 and 10 kda.

References

Ahmad., I. 2004. *Genetic diversity for Agro-Morphological and High Molecular Weight Glutenin Sub-units in Wheat (Triticum aestivum L.) Land Races*. University of Arid Agriculture Rawalpindi-Pakistan, Ph.D Thesis.

Ayad, W.G., T. Hodgkin., A. Jaradat and V.R. Rao. 1995. Molecular Genetic Techniques for Plant Genetic Resources. International Plant Genetic Resources Institute, Rome, 9-11 October, Rome, Italy

Bretting, P.K. and M.P. Widrlechner. 1995. Genetic markers and plant genetic resources. *Plant Breeding Reviews*, 13: 11-86.

Deb, A.C. 2002. *Fundamentals of Biochemistry*. 8th edn, pp. 84-85, New central book agency Ltd, India

Ghafoor, A., Z. Ahmad., A.S. Qureshi and M. Bashir. 2002. Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiate* (L.) R. wileczek based on morphological traits and SDS-PAGE, *Euphytica*, 123: 378.

Iida, S., M. Kusaba and T. Nishio. 1997. Mutants lacking glutelin subunits in rice: mapping and combination of mutated glutelin genes. *Theor. Appl Genet*., 94: 177-183

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage t4. *Nature*, 277: 680-685.

Landers, P. and B. Hamaker. 1994. Antigenic Properties of Albumin, Globulin, and Protein Concentrate Fractions from Rice Bran. *Cereal Chem,* 71(5): 409-411

Nisar, M., A. Ghafoor., M.R. Khan and A.S. Qureshi. 2006. Screening of *Pisum sativum* germplasm against *Erysiphe pisi* Syd. *Acta Biologica Cracoviensia Series Botanica,,* 48: 2:33-37.

Nisar, M., A. Ghafoor., M.R. Khan and Asmatullah .2009. First Proteomic Assay of Pakistani *Pisum sativum* L. Germplasm relation to geographic pattern. *Russian Journal of Genetics*, 45(7): 805-810.

Nisar, M., A. Ghafoor., M.R. Khan., H. Ahmad., A.S. Qureshi and Haidar Ali .2007. Genetic diversity and geographic relationship among local and exotic chickpea germplasm. *Pak. J. Bot*., 39(5): 1575-1581.

Osborne, T. B. 1924. *The Vegetable Protein*. pp154, Long Marks, Green and Co. London

Shahid, M., A. Ghafoor and A. Javid. 2000. *Laboratory Manual Characterization and Evaluation of Plant Genetic Resources.* NARC, PARC, Pakistan

Steenson, D. and S.K. Sathe. 1995. Characterization and digestibility of Basmati rice storage protein. *Cereal Chem*., 72(3): 275-280.

(Received for publication 7 January 2010)