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**SHORT COMMUNICATION**

# CHARACTERIZATION OF ENDOSPERM PROTEIN FROM DIFFERENT VARIETIES OF WHEAT ISOLATED BY TRITON X-114

**VINOD KUMAR DHATWALIA[1]*, O.P. SATI[1], M.K. TRIPATHI[2] AND VERSHA RAI[1]**

[1]Department of Chemistry, H.N.B. Garhwal University, Srinagar, Uttarakhand
[2]Department of Biotechnology, ICFAI University, Dehradun, Uttarakhand

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**The wheat (*Triticum aestivum*) is one of the most abundant sources of energy and protein for world's population. A new basic protein has been Isolated from wheat endosperm by Triton X-l14 phase partitioning. This protein isolated from wheat endosperm has been named puroindoline. The puroindoline, endosperm-specific proteins involved in wheat seed hardness, are small proteins reported to have *in vitro* antimicrobial properties. Antimicrobial peptides play a role in the immune systems of animals and plants by limiting pathogen infection and growth. Puroindoline may also be a membranotoxin that might play a role in the defense mechanism of plants against microbial pathogens.This protein is isolated and characterized from eight different varieties of Indian wheat.It is concluded that endosperm protein profiles could be useful markers for studying diversity and classification of adapted cultivars.**

***Key words*: Endosperm, puroindoline, triton X-114, wheat.**

In India, there are three types of cultivated wheat: (i) *Triticum aestivum* (bread wheat), (ii) *Triticum durum* (durum wheat) and (iii) *Triticum dicoccum* (dicoccum wheat). Wheat belongs to the sub-tribe Triticinae of tribe Triticeae in the grass family Poaceae. Plants and animals produce antimicrobial peptides as part of their natural defense systems to control disease-causing microorganisms (Broekaert *et al.* 1992, Hancock and Scott 2000). These peptides may act against bacteria and/or fungi and may be expressed constitutively or induced following infection. Plants contain numerous antimicrobial peptides that may be involved in protecting the plant from pathogens (Krishnamurthy *et al.* 2001). During maturation and germination, seeds are very sensitive to viruses, fungi and bacteria (Blochet *et al.* 1993). Many proteins involved in the microbial defense mechanism of plants have been identified and some of them have an effective antimicrobial activity (Collinge and Slusarenko 1987 and Dixon and Harrison 1990). Thionins were the first antimicrobial proteins to be isolated from wheat endosperm half a century ago and they have been the subject of several studies. (Garcia-Olmedo *et al.* 1989). These low molecular weight basic and cystine-rich proteins are toxic to various microorganisms and animal cells and this cytotoxicity is most probably exerted through their interaction with membranes, especially with membrane lipids. Recently, by using the TX1 14 phase partitioning method generally used to isolate proteins that are tightly bound to membrane lipids (Bordier 1981). We report the isolation, estimation and characterization of Puroindoline protein from eight different varieties of Indian wheat.

The seeds of wheat varieties were obtained from Agriculture university Palampur, Himachal Pradesh. The eight varieties of wheat were: (1) BL-616, (2) HS-240,

*Corresponding author, E-mail: vinodguru07@yahoo.com
Present address: Upper Sodhowala (near Dolphin Institute), P.O. Manduwala, Dehradun

(3) Sonalika-308, (4) HPW-155, (5) HPW-42, (6) HPW-89, (7) HS-295 and 8). HPW -184. All were new improved hybrid varieties and resistant against the pathogens.

The endosperm proteins were isolated by following the procedure of Blochet *et al.* (1993) with some modification.10 gm sample was weighed, washed with distilled water and dried. The soluble proteins were extracted at 4°C by grinding the sample with 50ml of Tris KCl buffer pH 7.8. Homogenate was centrifuged at 5000 x g for 20 min. Residue was extracted at 4°C with 25ml of Tris KCl buffer containing 4% TX114 and centrifuged again at 5000×g for 20 min. Supernatant was heated to 30°C for 1hr and centrifuged for 15 min at 5000×g. Upper detergent poor phase was discarded and same volume of fresh Tris KCl buffer containing 0.06% TX114 was added. Solution was mixed for 1hr at 4°C and phase partitioning procedure was repeated. Upper phase was discarded and the lower detergent rich phase was removed and proteins were precipitated overnight with 50ml of diethyl ether and ethanol in 1:3 (v/v) at -20°C. This was centrifuged at 2000×g for 20 min. Protein pellet was re-extracted at -20°C three times with 50ml of diethyl ether-ethanol and finally with 50ml of diethyl ether (Centrifuged at 2000×g). Protein pellet was dried overnight. Dried protein pellet was dispersed in 3 ml of phosphate buffer (pH 7.8). Suitable aliquot (1ml) of extracted protein solution was taken. Protein was estimated by Lowry's (1951) method.

SDS-PAGE was performed by using the procedure of Laemmli (1970). Loaded 30µl (1µg/µl) protein sample in wells. Along with protein marker in one of the well.Placed the gel in a trough containing staining solution for 3-4 hours or it can be kept for staining overnight. Destained the gel with destaining solution till a clear background of the gel was obtained.

The SDS-PAGE profile of endosperm protein of all varieties was performed and protein mixture of molecular weight 10 kDa to 100 kDa was observed. The SDS-PAGE band pattern indicated similar band profile for all the wheat varieties except presence of some intense bands in some of the varieties (Fig. 1). The varieties HPW-184 and BL-616 show 80% similarity with one another. Endosperm protein level of all the eight varieties

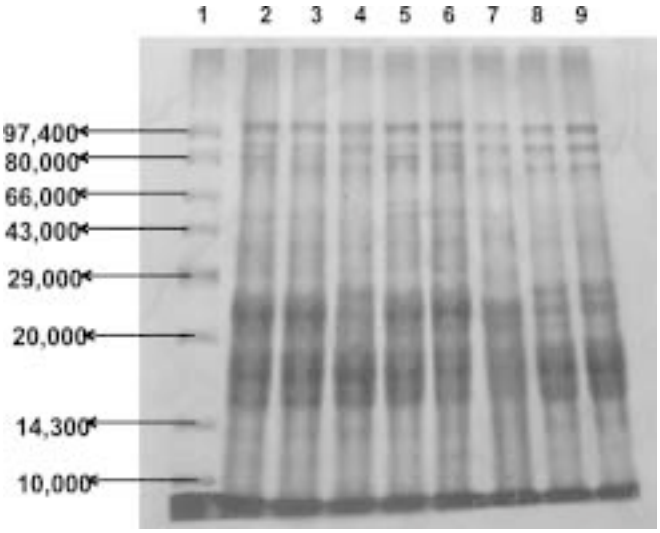


**Fig. 1. SDS-PAGE profile of endosperm protein of wheat varieties. Lane (1) Molecular weight marker, (2) HPW-184, (3) HS-295, (4) Sonalika-308, (5) HS-240, (6) HPW-155, (7) HPW-42, (8) BL-616, and (9) HPW-89**

varied from the concentration 0.236 mg/g to 0.141 mg/g (Fig. 2). BL-616 has high (0.236 mg/g) concentration as compared to other varieties. This is similar to the endosperm protein of endosperm flour as reported by (Blochet *et al.* 1993). Analysis of puroindoline protein from eight varieties suggested that endosperm protein is a determining factor of finding difference and similarities between these varities. Significant correlations between these varieties were detected due to similar band patterns and concentration of puroindoline protein. This work may help in improving the efficiency of wheat breeding programs in cultivar development.

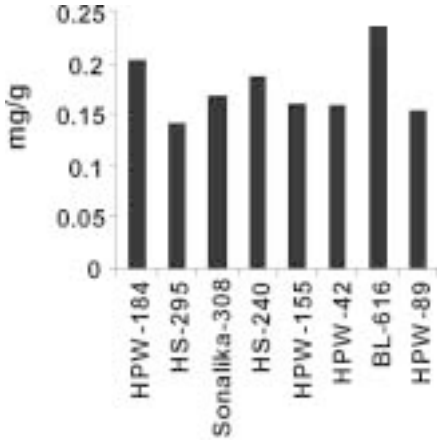


**Fig. 2. Endosperm protein content in wheat varieties**

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