Indian Journal of Biotechnology
Vol. 19, January 2020, pp 17-27

Seed storage protein phylogenetics of Indian wheat genotypes belong to *Triticum aestivum*, *T. dicoccum* and *T. durum*

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Received 21 August 2019; revised 11 November 2019; accepted 10 December 2019

Genetic diversity analysis of eighty six Indian wheat genotypes was performed using sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) electrophoresis based on their total seed storage protein. A dendrogram was constructed using the genetic similarity coefficient matrix based on SDS-PAGE, which categorized these genotypes according to their electrophoretic patterns into eight different clusters. These eighty six genotypes studied here belong to three different species of wheat. Out of these eighty six genotypes, two genotypes belong to *T. dicoccum*; eleven belong to *T. durum* and the remaining seventy three belongs to *T. aestivum*. In the dendrogram, all the eleven genotypes of the species *T. durum* (A-9-30-1, HD-4672, RAJ-1555, PDW-233, PBW-34, HI-8381, PDW-291, WH-896, PDW-215, HI-8498 and MACS-2846) were grouped in one cluster. The pedigree analysis of genotypes showed similar parentage with the electrophoretic profiling of the different wheat genotypes. From this study, it has been concluded that the total seed storage protein profile of wheat can be used as a marker for genetic diversity.

Keywords: Seed storage proteins, genetic diversity, *T. aestivum*, *T. durum*, *T. dicoccum*

Introduction

Wheat (*Triticum aestivum*) is a member of the family poaceae and supposed to be having its origin from Middle East Asia. Various valuable crops such as wheat, rice and maize are the member of this family and contribute to the economy of many countries and among these crops, wheat is the key staple food all over the world approximately in more than 50 countries[1]. In India, wheat is one of the three main cereals after rice and maize because of its consumption as a significant food source and its unique quality of bread production[2]. Wheat flour is used for making various products such as bread, noodles, biscuits, pasta, ethanol production and as animal feed[3]. As estimated there would be 70% rise in demand for wheat in the world in 2050 and to fulfill the nutritional requirement of the world population wheat production need to be doubled by the year 2050[4-5]. Wheat seed storage proteins play an essential role as being the primary source of nutrition and energy and are also very helpful in conferring bread-making quality[6].

Seed storage proteins could be used as a marker in plant breeding, in the studies of genetic diversity, as an important tool in crop improvement and for genotype classification[7]. These genetic markers have some limitations and a better method should be implemented in the form of protein marker such as use of seed storage proteins for genotype classification. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) seems to be most effective for protein based classification as it is fast, comparatively inexpensive, mostly unaffected by the growth atmosphere[8].

Seed storage proteins are more stable than other proteins as they do not denature during seed formation[9]. In a very short period, SDS-PAGE has become a convenient tool for genetic diversity analysis[10]. Seed storage protein analysis by SDS-PAGE is a trustworthy method because it does not get fluctuated by environmental conditions[11]. Seed storage proteins and nucleotide sequences are very useful and consistent approaches because these are generally independent of environmental variations[12]. In numerous investigations such as phylogenetic interactions, genetic diversity and genomic homologies, the seed storage proteins studies have been reported[13]. Characterization of proteins has also been used for interpreting genetic relationships among a group of cultivars. Based on seed storage protein profiling, genetic diversity analysis of wheat has not been

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studied earlier, especially for Indian wheat genotypes. Therefore, keeping in view very less data is reported on the phylogenetic relationship between various Indian wheat varieties based on seed storage protein profiling. The phylogenetic relationship among 86 Indian wheat genotypes belongs to three different species of *Triticum* like *T. aestivum*, *T. dicoccum*, *T. durum* was drawn on the basis of polypeptide pattern of total seed protein.

Materials and Methods

Plant Material

Indian wheat genotypes used in this study were kindly provided by the Central Soil Salinity Research Institute (CSSRI), Karnal, Haryana, India and Indian Institute of Wheat and Barley Research, (IIWBR) Karnal, Haryana, India.

Seed Protein Extraction

Seeds of each genotype were pulverized to a fine powder in a pestle and mortar. One hundred mg of seed meal of each genotype was defatted using 5% (w/v) diethyl ether at 4°C for 2 h followed by centrifugation at 6000 rpm for 12 min at 4°C in cooling conditions. The pellet obtained after centrifugation was vacuum dried. Total wheat seed protein was extracted using protein extraction buffer (Tris-HCl pH 6.8, 3% SDS, 2% glycerol, 0.7% 2-mercaptoethanol).

SDS-Polyacrylamide Gel Electrophoresis

The SDS-polyacrylamide gels (12%) were prepared according to the protocol specified by Laemmli[14]. Though, the reduced SDS-PAGE was conceded by using protocol given by Singh and Matta[15]. The protein marker ranged from 6.5 to 200 kDa (Sigma, USA) was used as standard protein molecular weights markers.

Phylogenetic Relationship

The presence (1) and absence (0) of the protein bands on the SDS-PAGE is used for the genetic diversity analysis of the polypeptide patterns of all the wheat genotypes. The binary data was used to calculate the pair-wise dissimilarity among these eighty six wheat genotypes. The phylogenetic relationship between all the wheat genotypes was carried out using SPSS (Version 16.0) software in the form of unweighted pair group method with arithmetic average (UPGMA) dendrogram as described by Singh and Matta[15].

Results

Electrophoretic Variation in Various Genotypes of Wheat

The polypeptide pattern of the total seed storage protein of the eighty six Indian wheat genotypes was performed to study the genetic diversity among these genotypes on the SDS-polyacrylamide gels under reducing conditions (Fig. 1; Table 1). The Indian wheat genotypes under this study with their parentage and other information regarding their development are listed in Table 2. The phylogenetic relationship was drawn between the wheat genotypes (Fig. 2)

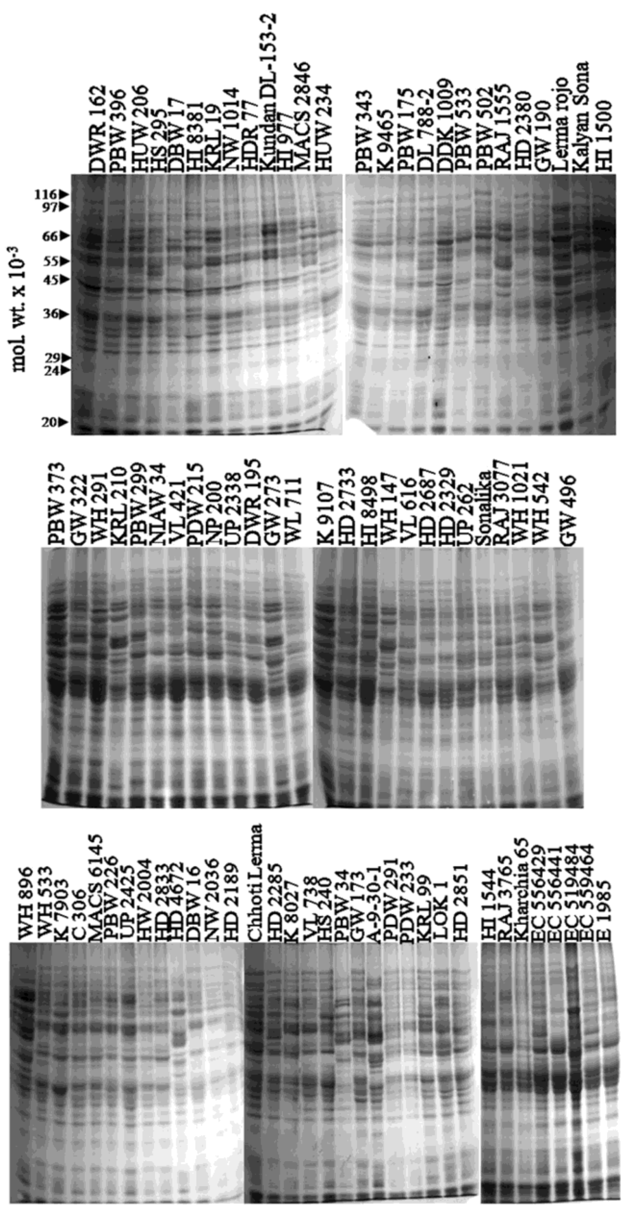

Fig. 1 — Polypeptide patterns of the total seed storage protein extracts of eighty-six Indian wheat genotypes on SDS-PAGE reducing conditions.

Table 1 — Electrophoretic variation of the polypeptides of the seed storage proteins of Indian wheat genotypes

Cluster no.	Wheat line	Molecular weight
I	DDK 1009	89.5, 78, 70, 66, 61, 59, 52.5, 45.5, 42, 41.5, 37.5, 35, 34, 32.5, 31.5, 30.5, 22.5, 21.5, 20
	NP 200	98.5, 89.5, 78, 66, 64, 61, 59, 52.5, 45.5, 42, 41.5, 37.5, 35, 34, 32.5, 31.5, 30.5, 22.5, 21.5, 20
II	HD 2687	98, 92.5, 88.5, 87.5, 75.5, 65, 56, 51, 46.5, 44.5, 42.5, 40.5, 39, 35.5, 34, 31.5, 29, 23.5, 22.5, 21.5, 21, 20.5
	HD 2329	98, 88.5, 75.5, 65, 56, 52.5, 51, 46.5, 42.5, 40.5, 39, 35.5, 34, 32.5, 31.5, 25, 23.5, 22.5, 21.5, 21, 20.5
	VL 421	97, 88.5, 80, 71.5, 64.5, 59.5, 56.5, 54, 43.5, 41, 40, 34.5, 33.5, 32, 26, 21.5, 20.5, 20
	UP 2338	97, 85, 80, 71.5, 66.5, 64.5, 54, 43.5, 41, 40, 34.5, 33.5, 32, 31, 26, 23.5, 21.5, 20
	UP 262	98, 82, 75.5, 66, 63.5, 61, 58.5, 49, 43.5, 41.5, 39, 36.5, 35, 34, 32.5, 31.5, 30.5, 28, 24.5, 22.5, 21.5, 20.5, 20
	GW 173	78, 72.5, 64.5, 61.5, 59.5, 55, 48.5, 42, 40.5, 38.5, 35.5, 34.5, 34, 32.5, 32, 29.5, 22, 21
	LOK 1	98, 96, 89, 78, 72.5, 64.5, 55, 51, 45, 42, 40.5, 38.5, 37, 35.5, 34.5, 32, 29.5, 22, 21
III	VL 616	96, 85, 66, 60, 57, 50.5, 43.5, 41.5, 39, 35, 34, 32.5, 26.5, 24.5, 21.5, 20
	Sonalika	98, 90, 81, 74, 69.5, 65, 59.5, 50, 47, 44.5, 41.5, 37.5, 36, 35, 32.5, 29.5, 24, 20.5, 20
	WH 1021	99.5, 91.5, 79, 71.5, 68.5, 63.5, 59, 54.5, 51.5, 48, 44, 41.5, 39, 37, 35, 34.8, 33.5, 32, 29.5, 22.5, 21, 20
	GW 322	97.5, 92, 84, 80.5, 73.5, 68, 60.5, 58, 55, 50, 42, 40.5, 35, 34, 33, 32, 27.5, 24.5, 21.5, 20.5, 20
	HDR 77	96.5, 90.5, 83, 79.5, 72.5, 64.5, 60, 54.5, 41, 40, 35, 33.5, 32.5, 30.5, 25, 21.5, 20.5, 20
	DWR 195	97, 84, 73, 64.5, 63, 60.5, 58, 47, 42.5, 40.5, 37, 35, 34, 33.5, 32.5, 31.5, 30.5, 22.5, 21.5, 20.5, 20
	GW 273	95, 88.5, 75.5, 66.5, 64.5, 62.5, 60.5, 47, 42.5, 41, 39, 37, 35.5, 34, 33, 31.5, 30.5, 25.5, 22.5, 22, 21.5, 21, 20.5, 20
	NIAW 34	97.5, 87, 75, 65.5, 63.5, 61, 56.5, 52.5, 41.5, 37.5, 35.5, 34.5, 34, 33, 32, 31.5, 30, 23.5, 22.5, 21.5, 21, 20.5, 20
	WH 533	98, 90, 81, 76, 70, 66, 63, 52, 43.5, 41.5, 39, 36, 34.5, 33, 31.5, 27, 24, 21.5, 20
IV	C 306	99, 95, 88, 82, 72, 61, 51, 49, 42.5, 41.5, 37, 35.5, 33.5, 32, 30.5, 25.5, 23.5, 21.5, 21
	HW 2004	99, 88, 72, 70.5, 66.5, 61, 57, 51, 49, 45, 42.5, 41.5, 37, 35.5, 33.5, 32, 30.5, 25.5, 21.5, 20
	MACS 6145	94, 85, 74, 70, 65, 60, 57, 56, 52, 50, 45, 43.5, 43, 39, 36.5, 36, 32.5, 32, 31, 30, 28, 23.5, 21, 20
	PBW 226	88.5, 82, 79.5, 75, 65, 61, 50.5, 42.5, 40, 38.5, 35.5, 35, 34, 33.5, 32, 31, 25, 23.5, 21, 20
	HD 2833	95.5, 88, 80, 75, 68, 65, 61, 50, 42.5, 40, 38.5, 35.5, 34, 33.5, 32, 31, 25, 23.5, 21.5, 21, 20
	GW 496	97.5, 91, 82.5, 78.5, 70, 65, 59.5, 57.5, 53.5, 45, 41.5, 40.5, 40, 35.5, 35, 34.5, 33.5, 32, 25, 22.5, 21.5, 20.5, 20
	HD 2285	98, 94.5, 89.5, 87.5, 80.5, 75.5, 65, 59, 53, 47.5, 43, 40, 38.5, 35.5, 35, 33, 31.5, 28, 24.5, 23, 21.5
	Chhoti Lerma	98, 90, 87.5, 76, 66, 63.5, 56, 46, 42.5, 41, 39, 36.5, 35.5, 34.5, 32, 31.5, 28.5, 23.5, 22.5, 21.5, 21
	PDW 291	98, 90, 87.5, 75, 66, 63.5, 58, 56, 45, 43.5, 39, 36.5, 35.5, 33.5, 32, 31.5, 29, 23.5, 22.5, 21.5, 21
	PDW 233	88, 75, 65, 63, 58.5, 48.5, 45.5, 43, 40, 38.5, 34.5, 33.5, 32.5, 31.5, 30, 22.5, 21, 20
	RAJ 1555	87, 70, 57, 55, 49.5, 43.5, 42, 39.5, 37, 34.5, 33, 32, 31, 30, 29, 27, 23.5, 21, 20.5
	PDW 215	88, 82, 75, 65, 58.5, 48.5, 45.5, 40, 39, 37, 35, 34.5, 33.5, 32.5, 30, 25.5, 23.5, 22.5, 20
	HI 8498	88, 75, 68.5, 65, 58.5, 57, 48.5, 45.5, 40, 37, 34.5, 33.5, 32.5, 32, 30, 22.5, 20
	A-9-30-1	94.5, 88, 82, 75, 69.5, 65, 58.5, 48.5, 45.5, 40, 39, 35.5, 34.5, 34, 32.5, 30, 25, 23.5, 22.5, 21.5, 20
	HD 4672	92, 88, 75, 73, 70.5, 65, 58.5, 55.5, 48.5, 43.5, 40, 39, 35.5, 34.5, 33.5, 32.5, 31.5, 22.5, 21.5, 20
	PBW 34	98.5, 88, 75, 65, 58.5, 54, 48.5, 45.5, 40, 34.5, 33.5, 32.5, 31.5, 30, 24, 22.5, 21.5, 20
	HI 8381	98, 92, 88, 75, 66.5, 65, 58.5, 48.5, 45.5, 43, 42, 40, 38.5, 37.5, 34.5, 32.5, 31.5, 30, 25, 22.5, 20
	MACS 2846	88, 75, 72, 65, 58.5, 48.5, 45.5, 40, 39.5, 36.5, 34.5, 32.5, 30, 25.5, 24, 22.5, 20
	WH 896	95, 88, 75, 71.5, 65, 58.5, 50, 48.5, 44.5, 42, 40, 38, 37, 35, 34.5, 33.5, 32.5, 30, 24, 22.5, 20
	KRL 19	96, 88.5, 78, 69.5, 63, 58.5, 54.5, 51, 45, 41.5, 40.5, 40, 34.5, 33, 32.5, 28, 25, 24, 21.5, 20.5, 20
	KRL 99	96, 78, 72, 69.5, 63, 60, 58.5, 51, 45, 41.5, 37, 34.5, 33, 32.5, 30.5, 28, 27.5, 25, 24, 21.5, 20.5, 20
	PBW 396	97.5, 95, 87, 77.5, 71, 62, 59, 53.5, 42.5, 40.5, 38.5, 34.5, 33, 32.5, 28.5, 25, 21.5, 20.5, 20
	HUW 206	95.5, 88, 81, 77.5, 71, 64.5, 62, 59, 54.5, 51, 46.5, 42.5, 40, 38.5, 34.5, 33, 32.5, 28, 25, 24, 21.5, 20.5, 20
	DBW 17	98.5, 89, 76, 66, 63.5, 58.5, 52, 43, 41.5, 39, 35, 34.5, 33.5, 32, 31.5, 30.5, 23.5, 22.5, 21.5, 20.5, 20
	HS 295	95.5, 82.5, 78, 71, 65, 62, 58.5, 54, 50, 45.5, 43.5, 40, 34.5, 33, 32.5, 28, 25, 21.5, 20.5, 20
	NW 1014	98, 89, 80.5, 75, 65.5, 63, 50.5, 42.5, 40, 38.5, 35.5, 34, 33.5, 32, 31, 25, 23.5, 21.5, 21, 20

(*Contd*.)

Table 1 — Electrophoretic variation of the polypeptides of the seed storage proteins of Indian wheat genotypes (*Contd*.)

Cluster no.	Wheat line	Molecular weight
V	HS 240	95, 75, 68.5, 63, 59, 56, 52, 44.5, 42, 36, 34.5, 33.5, 32.5, 30, 27, 25, 21.5, 20
	KRL 210	95, 78, 72.5, 71, 64, 60, 57, 43.5, 42, 39.5, 35, 33.5, 32.5, 30.5, 27, 26, 24.5, 21.5, 20
	PBW 299	96.5, 88, 80, 75, 69.5, 65, 61, 50, 42.5, 40.5, 35, 33.5, 32, 31, 25, 23.5, 21, 20
	WL 711	96, 82.5, 78, 71, 64, 59.5, 56, 52, 42.5, 40.5, 39, 34.5, 33.5, 32.5, 29, 25, 24, 21.5, 20.5, 20
	EC 556429	97.5, 88.5, 78, 71, 64, 61, 58.5, 56, 53.5, 50, 46.5, 42.5, 37, 35.5, 35, 34.5, 33, 32, 31, 29.5, 22, 21, 20
	EC 519484	91, 79.5, 70, 62, 59.5, 55.5, 50.5, 46.5, 43.5, 40, 37, 35.5, 35, 34, 33, 32, 29.5, 22, 21, 20
	K 7903	98, 89, 83, 80, 75.5, 66, 60, 50, 46, 42, 40, 35, 33.5, 32, 31.5, 26, 23.5, 21.5, 21, 20
	EC 556441	100, 88.5, 79.5, 75, 61.5, 58.5, 52, 42.5, 41, 36, 35, 33, 32, 31.5, 30, 22, 21, 20
VI	DBW 16	98, 90, 89, 87.5, 77, 68.5, 63.5, 59, 54.5, 52, 47, 44.5, 42.5, 41, 39.5, 38, 35.5, 34.5, 31.5, 29, 24, 22.5, 21
	HI 1544	100, 89.5, 79.5, 77.5, 67, 64, 59, 49, 44.5, 42, 38.5, 35.5, 34, 33, 30.5, 21.5, 20.5, 20
	RAJ 3765	95.5, 88.5, 80.5, 77.5, 63, 59, 53.5, 42.5, 40.5, 38, 34.5, 33, 32, 27, 25, 21, 20.5, 20
	UP 2425	95.5, 86, 79, 72, 67, 59, 50, 48.5, 46, 42, 40, 38.5, 35, 33, 32, 30.5, 25, 23.5, 21.5, 20.5, 20
	WH 291	98, 92.5, 88, 78.5, 64.5, 55, 50, 47, 43.5, 40, 38, 37.5, 36.5, 35.5, 33, 31.5, 30, 23.5, 21.5
	HUW 234	97.5, 91.5, 81.5, 76, 69.5, 63.5, 58.5, 55.5, 49.5, 45, 42, 37.5, 35, 33.5, 32.5, 23, 21.5, 20.5, 20
	DWR 162	98, 90.5, 88, 77, 65.5, 63.5, 59, 56.5, 52, 47, 45.5, 43.5, 41.5, 40, 38, 35.5, 32.5, 31.5, 29, 23.5, 22.5, 21.5
	VL 738	97, 85, 70, 68.5, 65, 59.5, 57, 52, 44.5, 42, 36.5, 34.5, 33.5, 32, 29.5, 24.5, 21.5, 20.5, 20
	HI 977	96, 84, 80, 75, 70, 65.5, 61, 50, 42, 40, 35.5, 34.5, 33.5, 32, 31, 25.5, 23.5, 21, 20
	Kundan DL-153-2	97, 88, 74, 64, 60, 57, 55, 45, 42.5, 40, 35.5, 34.5, 33.5, 33, 32, 30.5, 25.5, 22.5, 21.5, 20
	K 8027	96, 75, 73.5, 68.5, 62.5, 59, 55.5, 51, 48, 43, 42, 39.5, 35.5, 33.5, 32, 30.5, 25.5, 22, 21.5, 20
	Kharchia 65	97, 75, 73.5, 68.5, 63.5, 58.5, 55.5, 53.5, 43.5, 42, 40, 35.5, 34, 33.5, 32, 30, 23.5, 21.5, 20
VII	E 1985	96.5, 90, 78.5, 76.5, 71, 66, 59.5, 54, 49, 46, 39.5, 37.5, 35.5, 35, 34, 32.5, 30.5, 21.5, 20.5, 20
	K 9107	80.5, 74.5, 66, 64, 62.5, 60, 52.5, 46, 43, 40.5, 38.5, 36.5, 35, 34.5, 33.5, 33, 32, 31.5, 29, 26, 22, 21.5, 20.5, 20
	GW 190	87, 81.5, 70, 59.5, 55, 50, 46, 43.5, 42.5, 40, 39, 38, 37, 34.5, 33, 31.5, 30, 29, 27.5, 21, 20.5
	Lerma rojo	98, 88.5, 82, 74.5, 65.5, 63.5, 61, 57.5, 48.5, 43, 40.5, 38.5, 37, 35.5, 35, 33.5, 33, 32, 31, 29.5, 26, 23, 22, 21.5, 20.5, 20
	PBW 343	80, 78, 68, 62.5, 58.5, 52, 44, 42, 41.5, 38, 36.5, 32, 31, 30, 27.5, 23.5, 22, 20
	PBW 502	88, 79, 77.5, 63.5, 60, 57.5, 52, 44.5, 43, 42, 39, 37.5, 33.5, 32, 29, 25, 24, 20
	PBW 533	92, 82, 72.5, 64, 62, 60, 56, 45.5, 41, 39, 35, 34, 32.5, 31.5, 30.5, 23.5, 22.5, 21.5, 21, 20.5, 20
	HI 1500	80, 79, 69, 64, 62.5, 59, 55, 49, 44, 42.5, 39, 35.5, 32, 31.5, 30.5, 29.5, 24.5, 23.5, 21, 20.5
	EC 559464	98, 88, 79.5, 77, 70.5, 64, 61, 53, 47, 41, 38.5, 36.5, 35, 33.5, 32.5, 31, 30, 27, 23, 21, 20
	NW 2036	98, 91, 81.5, 77.5, 69, 64.5, 55.5, 51, 45.5, 43, 40, 37.5, 36, 35, 33, 32, 27.5, 24, 20
	HD 2851	90, 78, 71, 63, 60, 57, 52, 44.5, 41.5, 35.5, 33.5, 33, 31, 25, 22.5, 20
	PBW 373	90, 78, 68, 62.5, 58.5, 52, 47.5, 44, 43, 41.5, 38, 36.5, 32, 31, 30, 27.5, 24, 23.5, 22, 20.5
	WH 542	93, 84.5, 73, 67.5, 65.5, 63, 61, 58.5, 54, 44.5, 42.5, 40.5, 38.5, 36, 35, 34.5, 33, 32, 31, 23, 22, 21, 20.5, 20
	HD 2380	98, 89.5, 80.5, 77.5, 69.5, 59, 49, 44.5, 42, 40, 35, 34, 32.5, 30, 23, 21.5, 20.5, 20
VIII	HD 2189	80, 78, 64.5, 62.5, 57, 47, 44, 42.5, 39.5, 38, 35.5, 33, 32, 31, 30, 24, 23.5, 21, 20.5
	RAJ 3077	80, 78, 64.5, 62.5, 57, 47, 44, 42.5, 39.5, 38, 35.5, 33, 32, 31, 30, 24, 23.5, 21, 20.5
	HD 2733	98, 93, 88.5, 77, 68.5, 64.5, 61, 58, 54, 47, 44.5, 42, 40, 36.5, 36, 32, 30.5, 26, 23, 21.5, 21
	Kalyansona	85, 81, 70, 59, 54.5, 48, 42.5, 41.5, 38.5, 35.5, 34, 31.5, 31, 29, 27, 23.5, 20.5, 20
	PBW 175	88, 78, 67, 63, 60, 55.5, 50, 46.5, 44, 43, 40, 38.5, 37.5, 32.5, 31, 30.5, 27.5, 25.5, 21.5, 20
	DL 788-2	92, 82, 72, 64.5, 62, 59, 52.5, 48.5, 42, 40, 38, 36.5, 35, 34, 33, 32, 31.5, 30, 26.5, 23, 22.5, 21.5, 20
	K 9465	77.5, 70, 65, 63, 57.5, 54, 47.5, 44, 43, 40, 39, 35.5, 32, 31, 30, 28, 23.5, 20
	WH 147	90, 88, 82, 76.5, 70, 64, 60.5, 54.5, 51.5, 49.5, 46, 43, 41.5, 40.5, 39, 37.5, 36.5, 35, 32.5, 31.5, 29.5, 24.5, 22, 21

The total seed protein extracts of wheat genotype DDK 1009 and NP 200 shares similarity on the SDS-polyacrylamide gels under the reducing conditions. The differences were recorded at the position of the molecular weight of the polypeptides 98.5, 70 and 64 kDa. Similarly, wheat genotype HD 2687 and HD 2329 also shared a similarity in seventeen polypeptide bands and differences were observed at the molecular weight of 92.5, 87.5, 65, 45 and 39 kDa. The genotypes VL 421 and UP 2338 showed the

Table 2 — Pedigree and the classification of eighty-one Indian wheat genotypes

Sr. No.	Variety Name	Parentage	Year of release	Developed by	Species	Reference
1	DWR 162	KVZ/BUHO//KAL/BB	1993	Dharwad, Karnataka	*T. aestivum*	†
2	PBW 396	CNO67/MFD//MON S/3/SERI	1996	PAU, Ludhiana, Punjab	*T. aestivum*	‡
3	HUW 206	KVZ/BUHO//KAL/BB	1985	BHU, Varanasi, Uttar Pradesh	*T. aestivum*	‡
4	HS 295	CQT//AZ//IA555/ALDMLS/3/ALDMLS/NAFN/4/PJNS/PEL127669	1992	IARI, Shimla, Himachal Pradesh	*T. aestivum*	‡
5	DBW 17	CMH79A.95/3*CNO 79//RAJ3777	2006	DWR Karnal, Haryana	*T. aestivum*	@
6	HI 8381	JO69'S7/AA'S7 FGO'S	1994	Indore, Madhya Pradesh	*T. durum*	†
7	KRL 19	PBW 255/KRL 1-4	2000	CSSRI, Karnal, Haryana	*T. aestivum*	%
8	NW 1014	HAHN 'S'	1997	Faizabad, Uttar Pradesh	*T. aestivum*	†
9	HDR 77	PARTIZANKA/'HD 2204 // HD2204	1990	New Delhi	*T. aestivum*	†
10	Kundan DL-153-2	TANORI 71/ NP 890	1985	IARI, Delhi	*T. aestivum*	$
11	HI 977	GLL/AUST 61.157/CNO/NO 66/3/Y50E/3/KAL	1985	Indore, Madhya Pradesh	*T. aestivum*	†
12	MACS 2846	CPAN 6079 /MACS 2340.	1997	Pune, Maharashtra	*T. durum*	†
13	HUW 234	HUW12*2/CPAN1666// HUW12	1985	Varanashi, Uttar Pradesh	*T. aestivum*	†
14	PBW 343	ND/VG9144//KAL/BB/3/ YCO"S' /4A/EE#S "S"	1995	Ludhiana, Punjab	*T. aestivum*	†
15	K 9465	HD2160/K68	1997	Kanpur, Uttar Pradesh	*T. aestivum*	†
16	PBW 175	HD2160/WG1025	1989	Ludhiana, Punjab	*T. aestivum*	†
17	DL 788-2	K7537/HD2160/ HD2278//L24/ K4.14	1996	New Delhi	*T. aestivum*	†
18	DDK 1009	NP200*4//NP200/ALTRA84	1998	Dharwad, Karnataka	*T. dicoccum*	†
19	PBW 533	PBW 343/PBW 138//PBW 343	2005	PAU, Ludhiana, Punjab	*T. aestivum*	†
20	PBW 502	W 485 / PBW 343 // RAJ 1482	2003	Ludhiana, Punjab	*T. aestivum*	†
21	RAJ 1555	COCORIT'S' / RAJ 911	1982	Durgapura, Rajasthan	*T. durum*	†
22	HD 2380	HD2255 /HD 2257	1989	New Delhi	*T. aestivum*	†
23	GW 190	VEE/3/BB'S7SKA//ARJUN	1994	Vijapur, Gujarat	*T. aestivum*	†
24	Lerma Rojo	Y50/N 10B//L 52/3/2*LR	1965	New Delhi	*T. aestivum*	†
25	KalyanSona	PJ'S'/GB55	1967	CIMMYT, Mexico	*T. aestivum*	†
26	HI 1500	HW 2002*2//STREMPALLI/ PNC 5	2002	Indore, Madhya Pradesh	*T. aestivum*	†
27	PBW 373	ND/VG9144//KAL/BB/3/ YCO"S74/VEE#5 "S'	1996	Ludhiana, Punjab	*T. aestivum*	†
28	GW 322	GW173 /GW196	2002	Vijapur, Gujarat	*T. aestivum*	†
29	WH 291	HD1925/HD832//23584	1985	HAU, Hisar, Haryana	*T. aestivum*	†
30	KRL 210	PBW 65/2*PASTOR	2010	CSSRI, Karnal, Haryana	*T. aestivum*	%

(*Contd.*)

Table 2 — Pedigree and the classification of eighty-one Indian wheat genotypes (*Contd*.)

Sr. No.	Variety Name	Parentage	Year of release	Developed by	Species	Reference
31	PBW 299	BB/KAL//WL711/PBW65	1993	Ludhiana, Punjab	*T. aestivum*	†
32	NIAW 34	CNO79/PRL"S"	1995	Niphad, Maharashtra	*T. aestivum*	†
33	VL 421	SONORA64/YAQUI50[793]	1979	Almora, Uttarakhand	*T. aestivum*	@
34	PDW 215	RAJ 911//AA'S7D#2E/3/DWL 5002	1991	Ludhiana, Punjab	*T. durum*	†
35	NP 200	Selection local of rishi velly	1958	Wellington, Tamiladu	*T. dicoccum*	†
36	UP 2338	UP 368/VL 421 //UP 262	1994	Pantnagar, Uttarakhand.	*T. aestivum*	†
37	DWR 195	BONMARA -105-7	1994	Dharwad, Karnataka	*T. aestivum*	†
38	GW 273	CPAN 2084/VW 205	1997	Vijapur, Gujarat	*T. aestivum*	†
39	WL 711	S308/CHR//KAL	1977	Ludhiana	*T. aestivum*	†
40	K 9107	K 8101/K 68	1995	Kanpur, Uttar Pradesh	*T. aestivum*	†
41	HD 2733	ATTILA /3/ TUI /CARC // CHEN /CHTO/4/ATTILA	2001	New Delhi	*T. aestivum*	†
42	HI 8498	CR"S'-GS'S7A-9-30-1// RAJ911	1999	Indore, Madhya Pradesh	*T. durum*	†
43	WH 147	E4870/C286/C273/4/S339/PV18	1977	HAU, Hisar, Haryana	*T. aestivum*	‡
44	VL 616	Sonalika/CPAN 1507	1986	Almora, Uttarakhand	*T. aestivum*	†
45	HD 2687	CPAN2009 / HD 2329	1999	New Delhi	*T. aestivum*	†
46	HD 2329	HD1962/E 4870/3/K 65/5/ HD1553/4/UP262	1982	New Delhi	*T. aestivum*	†
47	UP 262	S 308 / BJ 66	1977	Pantnagar, Uttarakhand.	*T. aestivum*	†
48	Sonalika	1154-388 /AN/3/YT54/N10B/ LR64	1965	CIMMYT, Mexico	*T. aestivum*	†
49	RAJ 3077	HD2267/RAJ 1482/5/BB/ INIA66'S'/NAPO	1989	Durgapura, Rajasthan	*T. aestivum*	†
50	WH 1021	NYOT95/SONAK	2007	HAU, Hisar, Haryana	*T. aestivum*	†
51	WH 542	JUP/BJY"S"//URES	1992	HAU, Hisar, Haryana	*T. aestivum*	†
52	GW 496	HD 2285/4/CNO/NO//CC/ INIA66/3/KAL/BB	1990	Junagadh, Gujarat	*T. aestivum*	†
53	WH 896	STIL"S"/YAV'S7/PEN"S	1994	HAU, Hisar, Haryana	*T. durum*	†
54	WH 533	AGATHA/YACORA17	1993	HAU, Hisar, Haryana	*T. aestivum*	†
55	K 7903	HD1982/K816	1999	Kanpur, Uttar Pradesh	*T. aestivum*	†
56	C 306	RGN/CSK3 //2*C591/3/C217/ N14//C281	1965	HAU, Hisar, Haryana	*T. aestivum*	†
57	MACS 6145	C-306*9/CS-2A-2M-4-2[4280]; AJAIA-12/F3-LOCAL(SEL.ETHIO.135.85)//PLATA-13/3/SOMAT3/4/SOOTY-9/RASCON-37[4281];	2004	ARI, Pune, Maharashtra	*T. aestivum*	@
58	PBW 226	C591/RN//JN/3/CHR/HD1941	1989	Ludhiana, Punjab	*T. aestivum*	†
59	UP 2425	HD 2320/UP 2263	1999	Pantnagar, Uttarakhand	*T. aestivum*	†
60	HW 2004	C306*7//TR380-14#7/3 AG14	1995	Wellington, Tamiladu	*T. aestivum*	†
61	HD 2833	PBW226/HW1042//HD 2285	2005	New Delhi	*T. aestivum*	†
62	HD 4672	BIJAGA RED /PBW34 //ALTAR 84	1999	New Delhi	*T. durum*	†
63	DBW 16	RAJ3765/WR484//HUW468	1985	Dharwad, Karnataka	*T. aestivum*	†
64	NW 2036	BOW/CROW/BUC/PVN	2002	Faizabad, Uttar Pradesh	*T. aestivum*	†
65	HD 2189	HD 1963/HD 1931	1979	New Delhi	*T. aestivum*	†

(*Contd*.)

Table 2 — Pedigree and the classification of eighty-one Indian wheat genotypes (*Contd*.)

Sr. No.	Variety Name	Parentage	Year of release	Developed by	Species	Reference
66	Chhoti Lerma	L.R.64(SIB)/HUAR	1967	New Delhi	*T. aestivum*	†
67	HD 2285	249/HD2150//HD2186	1983	New Delhi	*T. aestivum*	†
68	K 8027	NP875/4/N10B/Y53//Y50/3/ KT54B/5/2*K852	1984	Kanpur, Uttar Pradesh	*T. aestivum*	†
69	VL 738	NS12.07 /LIRA "S" //VEE "S"	1996	Almora, Uttarakhand	*T. aestivum*	†
70	HS 240	AU /KAL-BB//WOP'S7 PAVON'S'	1989	Shimla, Himachal Pradesh	*T. aestivum*	†
71	PBW 34	AA'S'/FGO'S'	1985	Ludhiana, Punjab	*T. durum*	†
72	GW 173	TW275/7/6/1/LOK-1	1994	Vijapur, Gujarat	*T. aestivum*	†
73	A-9-30-1	A 206 / GAZA	1970	Ahmedabad, Gujarat	*T. durum*	†
74	PDW 291	BOOMER 21 x. MOJO 2	2005	PAU, Ludhiana, Punjab	*T. durum*	‡
75	PDW 233	YAV'S'/TEZ"S"	1995	Ludhiana, Punjab	*T. durum*	†
76	KRL 99	KRL 3-4/CIMK 2//KRL 1-4	2007	CSSRI, Karnal, Haryana	*T. aestivum*	%
77	LOK 1	S308 / S331	1981	Sano Sara, Gujarat	*T. aestivum*	†
78	HD 2851	CPAN 3004/WR 426//HW 2007	2003	New Delhi	*T. aestivum*	†
79	HI 1544	HD 2402 / HW 3007	2008	Indore, Madhya Pradesh	*T. aestivum*	†
80	RAJ 3765	HD 2402/VL639	1995	Durgapura, Rajasthan	*T. aestivum*	†
81	Kharchia 65	KHARCHIA LOCAL/ EG 953	1970	Durgapura, Rajasthan	*T. aestivum*	†

† Indian wheat database, Directorate of Wheat Research, Karnal, Haryana, India
http://www.indianwheatdb.com/SearchByVarName.aspx
% Central Soil Salinity Research Institute, Karnal, Haryana, India
http://www.cssri.org/index.php?option=com_content&view=article&id=135&Itemid=139
‡ Indian Agricultural Statistical Research Institute, New Delhi, India, http://www.iasri.res.in/wheat/variety/variety_det.asp
@ Genetic Resources Information System for Wheat and Triticale, http://wheatpedigree.net/sort/show/
$ Pathak, A., Raju, D., Rajendran, A., Kumar, M., Sarangapani, N. and Siddegowda, R.P., 2014. Transferability of rice SSR marker in wheat (Triticumaestivum). *Current Trends in Biotechnology and Pharmacy*, *8*(2), pp.204-212.
PAU- Punjab Agricultural University, *Ludhiana, Punjab*
BHU-Banaras Hindu University, Varanasi, Uttar Pradesh
IARI- Indian Agricultural Research Institute
CSSRI- Central Soil Salinity Research Institute, Karnal, Haryana
ARI-Agharkar Research Institute, Pune, Maharashtra
CIMMYT- The International Maize and Wheat Improvement Center
DWR- Directorate of Wheat Research, Karnal, Haryana
HAU- Haryana Agricultural University, Hisar, Haryana

differences at molecular weight 88.5, 85, 66.5, 59.5, 56.5, 31 and 23.5 kDa. The polypeptide patterns of GW 173 and LOK 1 shared similarity with further genotypes of the cluster II. Nine genotypes revealed the same polypeptide pattern of the total seed protein extracts and therefore placed in a single cluster, i.e., in cluster III. Based on electrophoretic patterns of fifteen wheat genotypes of *T. aestivum* and eleven genotypes of the *T. durum*, these genotypes are clustered in the cluster IV.

Phylogenetic Analysis of Indian Wheat Genotypes

A dendrogram was constructed using the genetic similarity coefficient matrix based on SDS-PAGE (Fig. 2). In dendrogram eighty six wheat genotypes were categorized according to their banding pattern on SDS-PAGE. These eighty six genotypes studied here belong to three species of wheat *T. aestivum*, *T. durum* and *T. dicoccum*. Out of these eighty six genotypes, two genotypes belong to *T. Dicoccum*, eleven genotypes belong to *T. durum* and remaining seventy three genotypes belongs to *T. aestivum*. On the basis of similarity matrix indices, a dendrogram was constructed from 86 wheat genotypes which were divided into eight different clusters. The cluster I include only two wheat genotypes, namely DDK-1009 and NP-200. The wheat genotypes HD-2687, HD-2329, VL-421, UP-2338, UP-262, GW-173 and

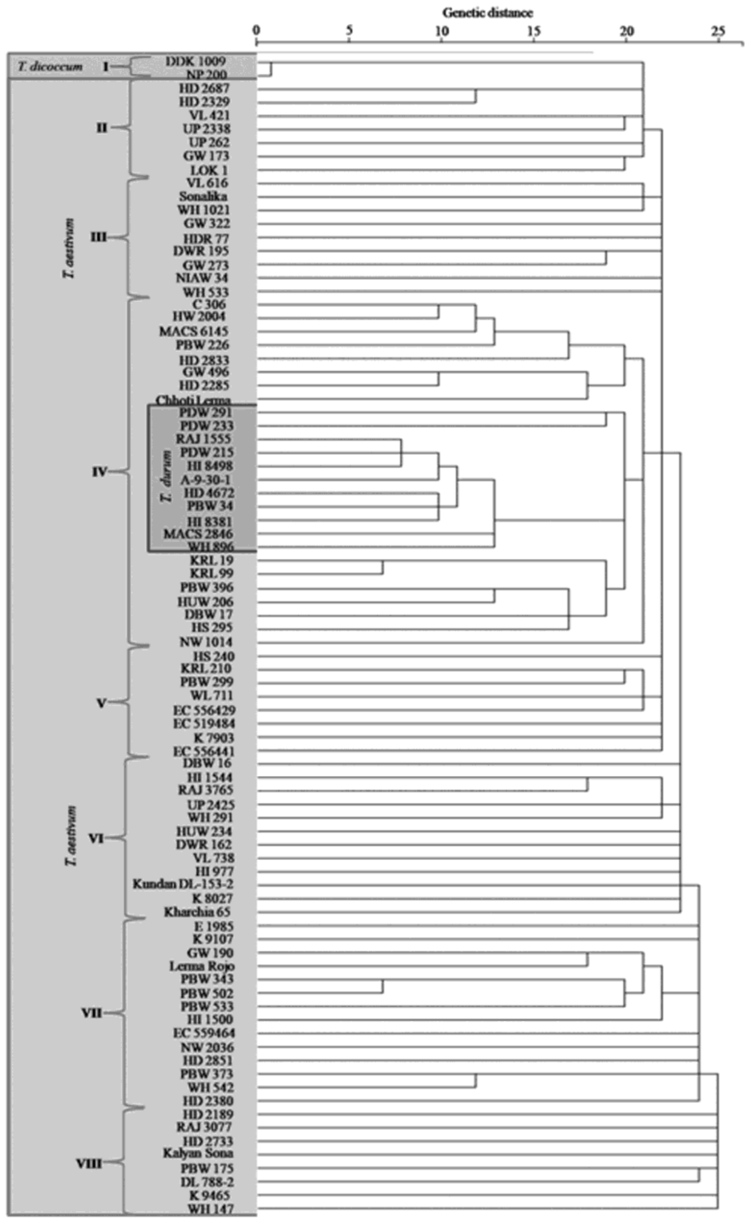

Fig. 2 — UPGMA dendrogram representing genetic distances among 86 Indian wheat genotypes belongs to three different species of the genus *Triticum*.

LOK-1 showed similar polypeptide patterns, hence placed in the cluster II.

Cluster III comprised of nine genotypes, namely VL-616, Sonalika, WH-1021, GW-322, HDR-77, DWR-195, GW-273, NIAW-34 and WH-533.

Cluster IV comprises twenty-six genotypes namely C-306, HW-2004, MACS-6145, PBW-226, HD-2833, GW-496, HD-2285, Chhoti Lerma, PDW-291, PDW-233, RAJ-1555, PDW-215, HI-8498, A-9-30-1, HD-4672, PBW-34, HI 8381, MACS-2846, WH-896, KRL-19, KRL-99, PBW-396, HUW-206, DBW-17, HS-295 and NW 1014.

Cluster V comprises eight genotypes namely HS-240, KRL-210, PBW-299, WL-711, EC-556429, EC-519484, K-7903 and EC-556441.

Cluster VI comprises twelve genotypes namely DBW-16, HI-1544, RAJ-3765, UP-2425, WH-291, HUW-234, DWR-162, VL-738, HI-977, Kundan DL-153-2, K-8027 and Kharchia-65.

Cluster VII comprises fourteen genotypes namely E-1985, K-9107, GW-190, Lerma Rojo, PBW-343, PBW-502, PBW-533, HI 1500, EC-559464, NW-2036, HD-2851, PBW-373, WH-542 and HD-2380.

Cluster VIII comprises eight genotypes namely HD-2189, Raj-3077, HD-2733, Kalyansona, PBW-175, DL-788-2, K-9465 and WH-147.

The pedigree of the genotype DDK-1009 shows that it is a descendant of NP-200 with which it also showed similarity in our study. There were various wheat genotypes that showed similarity in the dendrogram and also shared the same pedigree. The pedigree of these genotypes can be seen in Table 2. Wheat genotypes KRL-19 and KRL-99 have been developed from same parental genotype KRL-1-4 same as PBW-533 and PBW-502 both these genotypes also have the same parental genotype PBW-343 these also showed similarity in dendrogram indicating the close genetic relationship between these two genotypes. Wheat genotype VL-616 and WH-1021 have common parent Sonalika and present in the same cluster in the dendrogram. Three genotypes RAJ-1555, PDW-215 and HI-8498 have one parent in common, i.e., RAJ-911 they also clustered together; HI-8498 genotype developed from A-9-30-1 and as observed in the dendrogram these genotypes also showed similarity in their electrophoretic patterns. C-306 and PBW-226 both genotypes have one parent in common C-591 shares same pedigree; MACS-6145 and HW-2004 both genotypes have been developed from C-306; HD-2833 genotype have been developed from PBW-226 and HD-2285; HD-2833 and GW-496 shares the same pedigree and these all genotypes were also present in one cluster group of dendrogram. The HD-4672 genotype has been developed from PBW 34 and it also shared similarity with them. PBW-299 and KRL-210 have one parent in common PBW-65; the PBW-299 genotype has been developed from PBW-65 and WL-711, HW-2004, HD-2833 and PBW-226, also shared the same pedigree.

Discussion

SDS-PAGE has been considered as a powerful tool for genetic diversity identification for seed storage proteins because these proteins are free from environmental fluctuations[16]. It has also been used for analyzing seed storage proteins variability in various crops[17,18] and for differentiating different varieties and germplasm diversity[19]. The seed storage proteins have also been extensively studied by using gel electrophoresis all over the world. Based on seed storage proteins, genotype characterization has been studied in various crops such as wheat[20], mustard[21], black gram[22], *Solanum*[23], *Capsicum* and *Vigna*[24]. The electrophoretic study of seed storage proteins has been a tremendous tool because of the stability of protein profiles and also used to study the evolution and beginning of crops[25]. Protein profiling of seed storage proteins can also be implemented for the identification of varieties, to determine phylogenetic relationship between species, to characterize germplasm and for biosystematic analysis. Malik[26] found similar results from seed storage protein profiling of wheat and divided species *T. dicoccum, T. durum* and *T. triticale* in distinct groups. Pujar *et al*[27] studied pedigrees of 18 durum wheat genotypes and found a significant relationship between the groups formed by these genotypes in the dendrogram. Shuaib *et al*[28] performed a genetic diversity evaluation based on their protein profiling using SDS-polyacrylamide gel electrophoresis of thirteen wheat varieties. Quenum and Yan[29], it was concluded that cluster analysis performed on the basis of SDS-PAGE was remain consistent when cultivars under study belong to the same geographic location. This finding is further supported by Fazal *et al*, who reported similar results. SDS-PAGE techniques were effectively used for evaluating the genetic diversity in various researches[30].

Grewal *et al*[31] has reported a similar finding for 20 wheat genotypes with RAPD markers. Bean and Lookhart[32] stated that although numerous DNA markers are available for wheat for the assessment of genetic variation at intra population-level, yet seed storage protein remains highly valuable. Our dendrogram for wheat genotypes according to their protein profiling, clearly reflect their origins, parentage, and distribution. With some exemptions, this type of study was also reported for 28 durum wheat genotypes by Maccaferri *et al*[33] using SSR markers, and by Zhang *et al*[34] using DArT markers. Ishikawa *et al*[35] study showed that in breeding programs, pedigree-based association mapping is an advantageous method.

Choudhary *et al*[36] performed genetic diversity analysis of seven *Brassica napus* genotypes, based on their electrophoretic pattern of seed storage proteins; they concluded this technique is efficacious to differentiate the species. Khan and Ali[37] also recommended that wheat endosperm protein is valuable for the assessment of genetic variability and cultivars identification that helps in wheat breeding programs. Based on the seed storage-protein profiles on SDS-PAGE, Manivannan[38] have analyzed 21 pearl millet cultivars and suggested it as an efficient method to distinguish the varieties based on the electrophoretic patterns. By using electrophoretic patterns of the total seed storage protein, it is possible to get more distinct genotype among species which are also beneficial for the plant breeding programs as well as germplasm evaluation of various crops.

Acknowledgments

The financial assistance provided to NPS vide grant no. SB/YS/LS-334/2013 and SB/EMEQ-085/2014 by Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India, New Delhi and grant no. 41-515/2012 (SR) by the University Grants Commission, New Delhi is highly acknowledged. The financial assistance provided to MS vide grant no. CBT-3157-61 by the UGC-BSR fellowship is also highly acknowledged.

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