

## Evaluation of newly developed white grain pearl millet lines against downy mildew incited by *Sclerospora graminicola*

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**ABSTRACT:** Pearl millet is an important staple food crop in some of the most marginal regions of Asia and sub-Saharan Africa. Pearl millet is endowed with various essential nutrients, minerals and vitamins and also has various nutraceutical properties. However, one of the most important constraints limiting wider commercial utilization of pearl millet defeating all bio-fortification and grain quality breeding activities is its dark grey colour of the grain. Thus consumer acceptance and industrial applicability need to be addressed; but physical interventions like post-harvest processing, packaging, etc is a not so pragmatic approach. Hence breeding programme aiming at developing pearly white grain colour of pearl millet was attempted at Indian Agricultural Research Institute, New Delhi and a number of breeding lines with white to cream grain colour of pearl millet were developed. While developing white grain coloured pearl millet attempts were also made to incorporate downy mildew resistance. The present study aims at grouping the 126 white grain pearl millet lines with downy mildew resistance along with other agronomic and quantitative characters through cluster analysis. The results revealed existence of six clusters at the euclidian genetic distance of 16.0. It was also found that, among the six clusters, cluster V was the largest and consisted of 85 genotypes, which was further subclustered into 5 groups-subcluster V.1(a), V.1(b), V.2(a), V.2(b) and V.3 with 16, 15, 14, 25 and 18 genotypes respectively and the second largest group was the clusters VI with 33 genotypes and cluster II consisted of two genotypes. Clusters I, III and IV formed unitary cluster with single genotype. This grouping assumes significant importance for use of the material in future breeding programmes of pearl millet.

**Key words:** Diversity analysis, Downy mildew, Pearl millet, White grain.

### INTRODUCTION

The food processing industry in India is a sunrise sector that has gained prominence in recent years. Among which cereals grain processing is the biggest component of food sector contributing 40% of its share. One of the major consequences of the industrialization of the food chain has been the reduction in plant and animal species that we eat. Currently over 80% of the population rely on just four staple crops-wheat, rice, maize and potato. India is the second largest producer of the wheat in the world. More than 65 percent of the wheat is converted into wheat products such as atta, together by organized and unorganized sector. Branded 'atta' (wheat flour) is an important item in this segment with an estimated market of US\$ 195 million. Most of it is used for direct consumption in the form of chapati, bread & bakery

products. Bread is slowly coming to be a staple product consumed by people of all economic classes in India. According to Ministry of Food Processing Industries, India (MOFPI), Total bread production in the country in 2014-15 was estimated at 15 million tons, growing at 5 per cent [1]. About 55 per cent of bread production comes from the organised sector. The per capita consumption of bakery products in the country is about 2 kg per annum, against 50-100 kg bread alone in western countries.

Due to continuous consumption of wheat and wheat products leads to various health concerns among people particularly occurrence of lifestyle diseases like diabetics and heart diseases. As many wheat-based foods are very disruptive to blood sugar (high glycaemic index), particularly when eaten in quantity leading to high glycaemic load results in 'pancreatic exhaustion' [2]. Also people with celiac

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disease can't tolerate gluten, a protein present in wheat. Gluten provides various rheological properties to the flour making it suitable for processing or extrusion. Even in small quantities of just 50 mg of gluten in the bloodstream triggers an immune response that damages the lining of the small intestine. This can interfere with the absorption of nutrients from food, cause a host of symptoms, and lead to other problems like osteoporosis, infertility, nerve damage, and seizures [3]. Hence, health concern peoples are now were focusing on food products such multigrain food products and gluten free diets.

Pearl millet is an important crop in some of the most marginal environments of Asia and sub-Saharan Africa. In India it is the fourth important food crop after rice, wheat and maize. It can be successfully grown where no other arable farming is possible due to extreme temperatures, poor and shallow soils and low water availability [4]. Pearl millet is also rich in vitamin, potassium, phosphorus, magnesium, iron, zinc, copper & manganese which were minimal in wheat. On the other hand, incidence of diabetes mellitus and gastrointestinal disorders are minimal among the population using pearl millet grain as staple food. Pearl millet is a gluten free grain and is the only grain that retains its alkaline properties after being cooked which is ideal for people with wheat allergies [5]. Pearl millet flour mixed with wheat flour is used for making baked products like breads, cakes, muffins, cookies and biscuits which adds a crunchy texture and brings variety in baked products. The utilization of millets for food is still mostly confined to the traditional consumers and population of lower socio-economic strata partly due to the non-availability of these grains in ready to use form [6]. Hence there exist a lot of scopes for improving the nutritional qualities of processed food by blending pearl millet flour with that of wheat.

However the presence of grey, brown or greenish pigmentation and off flavor in pearl millet (C-glycosyl flavones their metabolites) limits its use in bakery products [7, 8, 9]. Most of the hybrids and varieties in commercial market were of grey or brown grain colour, due to the presence of a thick pericarp and the presence of pigments in the aleurone layer. If there are no pigments present in the kernel and the pericarp is thin, the resulting colour of the kernel is pearly white. To make pearl millet a competitive food grain it becomes essential that besides the improvement in yield level, the consumer acceptance and industry acceptance are to be addressed. So far very limited attempts have been made to address this issue in pearl

millet limited to physical interventions like post-harvest processing, packaging, etc which is a not so pragmatic approach. Grey to dark grey grain colour is the single most constraint capable of defeating all bio-fortification and grain quality breeding activities, and hence is eligible for urgent attention from the plant biologists. The first report of pearly white lines showing xenia effect were reported by Patel et al., [10]. Later K.S Mangat [11] developed white grain inbred lines from the segregating germplasm lines through selections. But these lines reported to heavy incidence of downy mildew which further hampered the development of white grain hybrids. Think [12] reported that white grain lines were higher in reducing sugar level which may be reason for its susceptibility to downy mildew disease [13]. In this context research work was initiated to incorporate downy mildew resistance gene in white grain background through back cross breeding at Indian Agricultural Research Institute, New Delhi. Keeping this in view, the present investigation was undertaken to understand the genetic diversity existing in the newly developed DM resistant pearly white pearl millet lines using morphological traits.

## MATERIALS AND METHODS

### Experimental Layout

The experimental material consisted of genotypes selected from three different groups of white grain recombinant inbred lines (RILs) developed for mapping DM resistance genes derived from the cross between ICMR 09333 (R) and WGI 148 (S) to race Sg 384; WGI 148 (R) and WGI 58 (S) to race Sg 561 and WGI 58 (R) and WGI 148 (S) to race Sg 445 which is now onwards referred as white grain millet panel (WGMP). 121 lines were selected based on DM incidence % (<1) obtained during phenotyping carried out at International Pearl millet Downy Mildew Screening Nursery at ICRISAT, Patancheru during year 2012. The selected 121 genotypes, 3 parental lines and two checks (one susceptible: 7042 S and one resistant: 843-22B) were planted in augmented randomized complete block design [14] was laid in fifteen blocks with each block containing 2 checks with 8-9 test varieties, during rainy season of 2013 at IARI, New Delhi. A mixture of 7042 S and local susceptible material, DPR 7 were planted along with the test varieties to promote uniform spread of sporangial inoculum to test varieties. Highly susceptible line 7042 S and highly resistant line 843-22B as check planted along with test varieties helps to know the spread of

disease in the plot. Each line was sown in single row (3 m) with spacing of 50 cm × 10 cm. Field scoring of the disease was done at 60 days after planting (DAP) by counting the total number of plants and number of infected plants and percent disease incidence was calculated. The genotypes were evaluated for agronomic performance based on quantitative characters like plant height (cm), Spike length (cm), Spike girth (cm) and no. of productive tillers were considered for diversity analysis.

### Statistical Analysis

Descriptive analysis and ANOVA were performed using Indostat software v.9.2 developed by Indostat

services, Hyderabad, India. Cluster analysis, using Euclidean distances among genotypes was calculated and a dendrogram was generated using Euclidian's paired group method through PAST (2003) software.

### RESULTS AND DISCUSSION

The mean downy mildew incidence on 7042 S (indicator row) was more than 90% after 60 DAP, which indicated the uniform and good spread of the disease in the field. Similarly 843-22B which was used as resistant check carrying resistant genes from ICML 22 found to have very least infection of 0.03%.

**Table 1**  
**Summary of Augmented R.B.D. ANOVA**

Source of variation	d.f	PH (cm)*	SL (cm)*	SG (cm)*	NPT*	DM Incidence (%)*
Block	14	195.13\$\$	4.13\$\$	0.33**	0.74\$\$	146.40\$\$
Genotypes	125	517.91**	11.96**	0.36**	2.08**	854.14\$\$
Checks	1	15187.50**	313.63**	0.13\$\$	19.20**	46408.61**
Test Varieties	123	227.71**	6.10*&	0.30**	1.83**	241.24\$\$
Checks vs. Test Varieties	1	21542.01**	431.80**	8.88**	15.92**	30686.34**
Error	14	78.57\$\$	2.56\$\$	0.03\$\$	0.41\$\$	557.23\$\$

\* PH: Plant Height; SL: Spike length; SG: Spike Girth; NPT: No. of Productive tillers and DM Incidence: Downy mildew incidence (%).

Table 1 presents the mean sum of squares from analysis of variance for augmented randomized complete block design (ARBD) of five traits measured in 126 pearl millet genotypes. Block effects were found non-significant for the all traits except spike girth indicating that blocking was not important for the four traits that showed non-significant block effects. The two checks in each replicate as well as the test varieties differed significantly. Hence, the prime most criteria regarding grouping of pearl millet genotypes was well satisfied. Similar results were obtained while studying white grain genotypes [7, 8 & 11]

Phenotypic and genotypic coefficients of variation (PCV and GCV), broad sense heritability, and genetic

advance were calculated for all the characters (Table 2). The highest GCV and PCV were observed for number of productive tillers (35.62 and 41.08% resp.) and the lowest GCV and PCV were recorded for spike length (7.80 and 10.53% resp.). Results also showed narrow differences between PCV and GCV for most of the traits. All the characters exhibited high heritability above 60% except spike length. Among the traits, spike girth had relatively higher heritability of 89%. The genetic advance as percent of mean (GA%) ranged from 11.92% in spike length to 63.62% in no. of productive tillers. Among the traits, spike girth and no. of productive tillers exhibited higher percentages of genetic advance with high heritability.

**Table 2**  
**Estimation of genetic parameters of five characters among 126 pearl millet genotypes**

Characters	Mean	Range		GCV %	PCV %	h <sup>2</sup> <sub>BS</sub>	GA	GA as % of Mean
		Min.	Max.					
PH (cm)	136.50	70	195	8.81	11.13	0.63	18.74	14.37
SL (cm)	21.60	13	27	7.80	10.53	0.55	2.70	11.92
SG (cm)	2.75	1.21	4.23	16.94	17.97	0.89	0.94	32.90
NPT	3.12	1	6	35.62	41.08	0.75	2.00	63.62
DM Incidence (%)	12.68	0	99	8.73	11.00	0.79	7.63	21.55

GCV: genotypic coefficient of variability; PCV: phenotypic coefficient of variability; h<sup>2</sup><sub>BS</sub>: broad sense heritability ; GA: Genetic advance

**Table 3**  
**Cluster means of five quantitative characters with corresponding list of genotypes included in each clusters**

Clusters	Genotypes	PH	SL	SG	NPT	DM %
Cluster I	ICMR 09333	195	23.00	2.13	2.00	1.0
Cluster II	WGI 58, WGI 148	175	20.50	2.73	2.50	75.1
Cluster III	WGMP 9610	100	18.00	2.52	6.00	0.0
Cluster IV	843-22B	72	14.27	2.11	3.13	0.03
Cluster V 1(a)	WGMP 9502, WGMP 9516, WGMP 9594, WGMP 9619, WGMP 9635, WGMP 9867, WGMP 9898, WGMP 9949, WGMP 9962, WGMP 9990, WGMP 10024, WGMP 10033, WGMP 10065, WGMP 10080, WGMP 10103, WGMP 10115	135	23.56	2.94	3.75	1.72
Cluster V 1(b)	WGMP 9878, WGMP 9933, WGMP 9964, WGMP 9980, WGMP 9995, WGMP 10037, WGMP 10038, WGMP 10064, WGMP 10094, WGMP 10113, WGMP 10130, WGMP 10140, WGMP 9915, WGMP 10060, WGMP 9975	139	23.13	3.10	2.67	1.00
Cluster V 2(a)	WGMP 9505, WGMP 9524, WGMP 9543, WGMP 9675, WGMP 9862, WGMP 9983, WGMP 10005, WGMP 10006, WGMP 10007, WGMP 10095, WGMP 10099, WGMP 10102, WGMP 10110, WGMP 10126	125	23.29	2.92	2.71	1.13
Cluster V 2(b)	WGMP 9474, WGMP 9487, WGMP 9512, WGMP 9573, WGMP 9577, WGMP 9582, WGMP 9598, WGMP 9646, WGMP 9865, WGMP 9892, WGMP 9908, WGMP 9910, WGMP 9913, WGMP 9924, WGMP 9948, WGMP 10012, WGMP 10017, WGMP 10074, WGMP 10082, WGMP 10085, WGMP 10096, WGMP 10105, WGMP 10107, WGMP 10121, WGMP 10135	130	22.64	2.89	3.16	1.79
Cluster V 3	WGMP 9519, WGMP 9883, WGMP 9891, WGMP 9921, WGMP 9926, WGMP 9947, WGMP 9965, WGMP 9969, WGMP 9978, WGMP 9996, WGMP 10011, WGMP 10016, WGMP 10018, WGMP 10048, WGMP 10063, WGMP 10078, WGMP 10089, WGMP 10116	146.39	23.50	3.07	2.44	0.81
Cluster VI	WGMP 9480, WGMP 9485, WGMP 9489, WGMP 9493, WGMP 9551, WGMP 9563, WGMP 9580, WGMP 9583, WGMP 9584, WGMP 9588, WGMP 9599, WGMP 9600, WGMP 9606, WGMP 9647, WGMP 9655, WGMP 9658, WGMP 9659, WGMP 9661, WGMP 9663, WGMP 9664, WGMP 9667, WGMP 9863, WGMP 9896, WGMP 9928, WGMP 9932, WGMP 9942, WGMP 10000, WGMP 10001, WGMP 10034, WGMP 10051, WGMP 10052, WGMP 10119, 70425	117.61	22.07	2.68	3.59	4.35

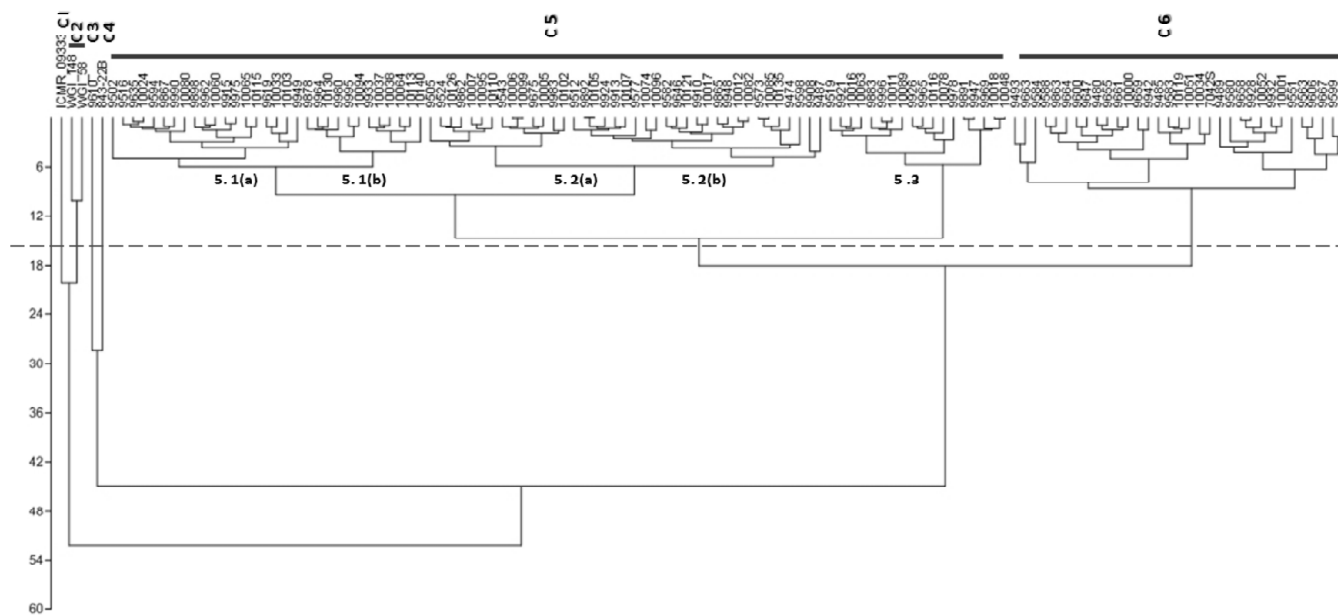


Figure 1: Dendrogram showing relationship among 126 pearl millet genotypes using five quantitative traits

Cluster analysis using all the five quantitative traits grouped the 126 genotypes into six major clusters at the euclidian genetic distance of 16.0 (Table 3, Figure 1). It was also found that, among the six clusters, cluster V was the largest and consisted of 85 genotypes, which was further subcluster into 5 groups-subcluster V.1(a), V.1(b), V.2(a), V.2(b) and V.3 with 16, 15, 14, 25 and 18 genotypes respectively and the second largest group was the clusters VI with 33 genotypes and cluster II consisted of two genotypes. Clusters I, III and IV formed unitary cluster with single genotype. Plant height contributed more towards divergence (62.67%) followed by DM incidence % (31.85%).

The cluster means of five different traits for six groups among 126 pearl millet genotypes are summarized in Table 3. Cluster I consists of a grey grained line ICMR 09333, which was found to have highest average plant height (195 cm). Also the genotype possesses resistance towards Sg 384 stain of downy mildew. Cluster II consists of two white grained inbred lines-WGI 58 and WGI 148 which are characterised to have high level of susceptibility to downy mildew incidence (75.0%). Cluster III included a white grain genotype from DM mapping panel-WGMP 9610 with highest number of productive tillers (6 no.s) and least DM incidence (0.0%). 843-22 B, a grey grained maintainer line carrying downy mildew resistant gene was got included in Cluster IV, characterised with short average plant height (72 cm) with shortest spike length of 14.27cm and spike girth

of 2.11cm. Cluster V got 85 genotypes all with thick panicle with average spike girth of 3.00 cm. Final cluster, Cluster V included genotypes with moderate DM resistance (~5%) but high tillering.

Cluster diagram provided information on the genetic distance existing among and between genotypes in clusters, which can be further utilised to develop parental lines that can throw highly heterotic hybrid combinations.

## CONCLUSION

The white grain genotypes with high level of DM resistance will serve as future material to develop white grain hybrids or varieties which can be used as composited flour with wheat in multigrain products or can be used as such as an alternative to wheat flour to develop gluten free diets.

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