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Morphological characterization and genetic analysis in newly developed cytoplasmic lines of bread wheat

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Abstract

Wheat (*Triticum aestivum* L.) is the major cereal crop in India on which the food security rests. Determination of the best selection criteria is the most important aim in wheat breeding programs. To achieve high yield potential in wheat, hybrid breeding is a major innovative technique. A total of 17 newly developed CMS lines and seven restorer lines, were used as a experimental material with the objective to identify diverse cross combinations and classify morphological and morpho-physiological traits. Genotypes were evaluated for twenty one traits viz., growth habit, foliage colour, time of ear emergence, ear waxiness, flag leaf length, ear shape, ear density, presence of awns, awns length, awn colour, ear colour, grain colour, grain shape, days to heading, days to maturity, plant height (cm), peduncle length (cm), number of tillers/plant, relative water content (%), canopy temperature (%) and chlorophyll content (%). Sufficient variability was exhibited by most of the phenological traits.

The genotypes were also evaluated for variability parameters for eight metric traits. Significant genotypic differences were observed for all the traits studied indicating considerable amount of variation among genotypes for each character. The estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were moderate for peduncle length. High estimates of heritability were exhibited by most of the traits except chlorophyll content, canopy temperature and number of tillers/plant.

Based on Euclidean cluster tree analysis, genotypes were grouped in five clusters in such a way that genotypes within each cluster had higher similarity than between clusters i.e. the genotypes within the cluster had much smaller distance than the genotypes belonging to different clusters. Maximum divergence was present between clusters IV and V, followed by II and IV, clusters III and IV and cluster III and V. Cluster I was highly desirable as it consisted of genotypes which were good in respect to one or more traits and at least comparable in respect to other traits.

Keywords: genetic divergence, wheat, CMS lines and morpho-physiological traits

Introduction

Wheat (*Triticum aestivum* L.) is the world's second most important staple food crop for more than 35 percent of world's population next to the rice. It produces about 20% food resources of the world with high productivity and occupying a prominent position. India is the second largest wheat producer in the world with a production level of 93.50 million tonnes (FAO, 2016) ^[3]. Madhya Pradesh is the second largest producer state of wheat with area, production and productivity of 5.94 million hectare, 17.78 million tonnes and 2.9 tonnes, respectively (Anonymous, 2017) ^[1]. Madhya Pradesh wheat is known for its quality and sale at high premium rate in the market due to its quality. Looking to the target for wheat production at national level it is very difficult to meet out the demand of 109 mt by 2020 AD. The innovative approach is one of the alternatives to fulfill the demand. In this direction an effort was made using development of hybrid wheat genotypes using cytoplasmic male sterile lines.

A major problem in hybrid seed production of wheat is its low cross-pollination ability. Apart from above there are several problems in hybrid wheat development and among them, lack of genetic diversity among parents for harnessing heterosis, out-crossing/natural crossing potential, complete fertility restoration and efficient and cost effective hybrid seed production technology are notable. To achieve hybrid vigour, it is necessary to identify, suitable hybrids following cytoplasmic male sterile line approach.

In India, hybrid wheat development programme was initiated under the All India Coordinated Wheat & Barley Improvement Project (AICW&BIP) of the ICAR in 1995 in network mode.

Besides three-line breeding approach using CMS system, twoline approach using chemical hybridizing agent (CHA) was also explored. Experimental hybrids through CMS as well as CHA systems were developed, but their commercial exploitation had many bottlenecks.

Present study was conducted to decipher the extent of genetic variation and characterization of recently developed CMS lines of wheat based on morpho-physiological attributes which could be further utilized in hybrid breeding programs.

Material and Methods

Plant material for the study comprised of newly developed 17 CMS lines and 7 restorer lines, received from IIWBR, Karnal and Division of Genetics, IARI under CRP on hybrid wheat programme. The experiment was planted at Seed Breeding Farm, Department of Plant Breeding and Genetics, College of Agriculture, J.N.K.V.V., Jabalpur (M.P.) during Rabi 2017-18. Soil of the experiment is medium black with uniform topography and free from water logged conditions.

For the assessment of morphological variability, observations were made on 24 plants or parts of 24 plants, which was equally divided among two replications. For the assessment of colour characteristics, the latest Royal Horticultural Society (RHS) colour chart was used. All the genotypes were evaluated for twenty one morpho-physiological traits, viz., growth habit, foliage colour, time of ear emergence, ear waxiness, flag leaf length, ear shape, ear density, presence of awns, awns length, awn colour, ear colour, grain colour, grain shape, days to heading, days to maturity, plant height (cm), peduncle length (cm), number of tillers/plant, relative water content (%), canopy temperature (%) and chlorophyll content (%). Observations were recorded on randomly selected five competitive plants from each line.

Analysis of variance was done for partitioning the total variation into variation due to treatments and replications according to procedure given by (Panse and Sukhatme, 1967)^[11]. The estimates of phenotypic (σ^2 ph) and genotypic (σ^2 g) variances followed by genotypic and phenotypic coefficients of variation were worked out according to the method suggested by Johnson *et al.* (1955)^[4]. Broad-sense heritability (h²) was calculated as the ratio of the genotypic variance to the phenotypic variance according to Singh and Ceccarelli (1996)^[16]. Genetic advance as percentage of the mean assuming selection of the superior 5% of the genotypes was also estimated following the procedure elaborated by Singh and Chaudhary (2004)^[17]. The genetic divergence was estimated using Mahalanobis D² statistics (1928)^[6].

Result and Discussion

Twenty-four newly developed cytoplasmic and restorer lines of wheat were grouped according to different morphological traits as per DUS Guidelines. The frequency distribution of each trait was presented in figure 1.

A wide range of variation was observed for trait plant growth habit. Among the twenty four studied genotypes, four were found to have erect type, seventeen were semi-erect type and three were intermediate type. All studied genotypes were appeared as erect, semi erect or intermediate but none of them were near to prostrate habit. This is a favorable trait for the selection of high production and low grain and straw loss. Ruiz and martin (2000) ^[14] studied growth habit among Spanish landraces of durum wheat and show that 93 % of studied genotypes appeared as erect or intermediate.

For foliage colour, six genotypes were found to be pale green whereas twelve were green and six were dark green. When all the genotypes were grouped according to flag leaf length, fifteen were having medium (20.1-30 cm) and nine were having long (>30cm) flag leaf length. Khaliq *et al.*, 2008 ^[5] reported that flag leaf area and its active duration during grain filling had been considered as an essential trait in determining the grain yield. Flag leaf is of utmost importance in cereals like wheat, because it provides the maximum amount of photosynthesis assimilates to be stored in the grains.

Early time of emergence was found in all genotype. In case of ear shape, twenty-two genotypes were found to be typical 'tapering' style and one genotype were found to be as parallel sided and one was observed as club shaped. Lux type ear density was observed in eleven genotype, medium type in eleven genotypes and dense type ear density in two genotypes. Traits ear shape and ear density, plays a very crucial role in determination of grain yield in wheat. The large variation between the studied genotypes in terms of spike density reflects the genetic variation.

All the studied genotypes show the presence of awn. Sourdille, 2002 ^[18] revealed that the presence of awns can double the photosynthesis rates especially under drier conditions then enhance drought resistance. A very distinct variations among the genotypes were observed for traits awn length and awn colour. The wide variation of awns colour among studied genotypes reflects the variation in genetic structure among these genotypes.

Grain character was grouped into two categories, namely grain colour and grain shape. Eighteen genotypes showed amber, four showed white and two showed red colour grain. In grain shape, sixteen genotypes were oblong type, seven were elliptical and one was ovate type. Grain shape is one of the most important parameters used in classification, identification and study of variation in wheat varieties (Mebatsion *et al.* 2012)^[7].

Highly significant mean squares due to genotypes for all the traits in twenty four wheat genotypes revealed the presence of enough genetic variability in the material under study. This result implied that this population of wheat genotypes would respond positively to selection. The mean, the coefficient of variations (CVs) and the ranges for the quantitative plant characteristics are presented in table 1. Moderate CVs were obtained for the peduncle length and low CVs for number of tillers/plant, chlorophyll content (%), canopy temperature, relative water content, plant height, days to heading and days to maturity. Similar results have been given by Phougat *et al.* (2017) ^[12] for plant height and days to maturity; Yaqoob (2016) ^[20] for plant height, days to heading and days to maturity.

The range obtained for heritability in broad sense was 27.5 to 95.3. Maximum heritability was recorded for plant height (95.3), followed by days to heading (92.1), relative water content (88.9), days to maturity (85.2) and peduncle length (73.6). Other traits revealed moderate to low estimates of broad sense heritability. Present results are in agreement with the findings of Monpara (2011)^[9], Singh *et al.* (2012)^[7] and Nukasani *et al.* (2013)^[10]. The high heritability estimates would be helpful for breeding superior genotypes on the basis of phenotypic performance of quantitative characters.

To estimate D^2 values, correlated means of characters were transformed to standard uncorrelated means using Tocher's method. The statistical distance (Mahalanobis D^2) between pair of germplasms were obtained as the sum of squares of the difference between the pairs of corresponding uncorrelated value of any two genotypes considered at a time. All 24 genotypes used for the study were grouped into five clusters based on divergence analysis. Out of five clusters, Cluster I was the largest among all clusters comprising 10 genotype, followed by cluster II and III (5 genotype) and cluster IV (3 genotype). Cluster V was mono-genotypic (1 genotype) (Table 2). The discordance among diversity patterns and geographical distribution of genotypes found in this investigation implies that the parental lines for hybridization should be selected based on genetic diversity rather than the geographical distribution. These finding are in agreement with the result obtained by Dwivedi and Pawar (2005) ^[2] obtained highest number of genotype in cluster I.

The inter and intra cluster D^2 mean values are presented in table 3. The highest intra cluster distance was recorded in Cluster IV (37.33), followed by Cluster III (31.11), Cluster I (20.07) and Cluster II (16.06). The inter cluster distance was highest between the clusters IV and V (216.55), followed by cluster II and IV (184.79), III and IV (181.84) and cluster III

and V (153.84). Maximum percentage contribution towards genetic divergence was reported by relative water content (34.42%), followed by plant height (32.61%), days to maturity (9.42%), days to heading (9.06%) and canopy temperature (7.61%) (Fig 2).

The cluster mean values of different traits are presented in table 4. The highest cluster mean values were recorded for traits days to heading (70.78) in cluster III; plant height (116.38), canopy temperature (22.30) and number of tillers/plant (9.89) in cluster IV; days to maturity (121.67), peduncle length (21.78), chlorophyll content (52.07) and relative water content (88.28) in cluster V. Meena *et al.* (2014)^[8], Uddin *et al.* (2015)^[19] resulted highest mean value for chlorophyll content and peduncle length. The diverse cms lines from the present investigation will be used for the development of hybrid in wheat.



Fig 1: Variations for morphological traits in wheat ~ 2264 ~

Grain: Colour

Grain: Shape

Table 1: Kange, mean, genotypic and phenotypic coefficients of variation for different train	Table 1: Range, mean.	genotypic and pher	notypic coefficients	of variation for	or different traits
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Characters	Range		Maan	Coefficient of variation (CVs)		Heritability	Genetic
Characters	Min.	Max.	wream	Phenotypic	Genotypic	(h ² b)	advance
Days to heading	60.33	72.66	65.58	4.98	4.78	92.1	9.46
Days to maturity	109.00	121.66	116.69	3.02	2.78	85.2	5.31
Plant height	96.66	120.36	105.48	5.55	5.41	95.3	10.89
Peduncle length	16.54	23.95	20.38	10.48	8.99	73.6	15.89
Chlorophyll content (%)	40.93	54.69	48.38	7.40	5.76	60.5	9.23
Canopy temperature	20.13	24.63	21.06	6.76	3.54	27.5	3.83
Relative water content (%)	73.26	88.75	81.21	5.89	5.56	88.9	10.79
Number of tillers/plant	7.33	10.30	9.36	9.07	6.93	58.4	10.92

Table 2: Distribution of newly developed cytoplasmic wheat genotype in different clusters

Cluster No.	No. of genotype	G Genotype included in the cluster
1	10	CMS-83, CMS-85, CMS-86, CMS-87, CMS-88, CMS-92, CMS-95, CMS-96, CMS-2041, CMS-2046
2	5	CMS-82, CMS-89, CMS-91, CMS-94, CMS-97
3	5	R-37, R-38, R-936, R-939, CMS-90
4	3	R-7, R-9, R-10
5	1	CMS-84



Fig 2: Percentage contribution of different traits toward clustering

 Table 3: Inter and intra cluster D² values

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster1	20.07	51.87	90.30	78.14	63.36
Cluster2		16.06	56.45	184.79	43.69
Cluster3			31.11	181.84	153.84
Cluster4				37.33	216.55
Cluster5					0.00

Troita	Clusters					
Trans	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	
Days to 50% heading	66.47	62.07	65.00	70.78	61.67	
Days to maturity	118.77	117.20	112.67	114.00	121.67	
Plant height	107.34	100.92	100.39	116.38	102.66	
Peduncle length	21.13	19.17	20.20	19.81	21.78	
Chlorophyll content (%)	48.46	48.12	47.55	48.76	52.07	
Canopy temperature	20.69	20.83	21.45	22.30	20.37	
Relative water content (%)	82.17	82.65	75.24	83.23	88.28	
Number of tillers/plant	9.35	9.26	9.19	9.89	9.23	

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