Electronic Journal of Plant Breeding, 6(3): 709-714 (Sep 2015)
ISSN 0975-928X

Research Article

Genetic analysis of divergence among advanced free threshable emmer wheat mutants derived through inter-specific hybridization followed by mutation for quantitative traits

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(Received:4th March 2015; Accepted 10th October 2015:)

Abstract
Ninety two mutant lines derived from inter-specific hybridization cum mutation involving emmer and durum wheat as source were evaluated for various quantitative traits. The mean values indicated existence of sufficient variability among the genotypes for all the traits studied representing pooling of positive alleles from both species. All the genotypes including parents were grouped into 11 clusters based on D^2 statistics. The inter-cluster distance and intra cluster distance indicated still there is scope to improve quality and yield traits through hybridization involving genotypes from different clusters. Yield, plant height and number of grains per spike contributed towards genetic divergence and these traits are major determinants of genetic variability in mutant populations. The present study indicated contribution of positive alleles for almost all the productivity traits and quality into durum background whereas, free threshability and test weight into dicoccum background and confer definite advantage of inter-specific hybridization cum mutation over simple inter-varietal hybridization.

Keywords : Emmer Wheat, threshability, genetic diversity

Introduction

In wheat there are three different cultivar groups *viz*., bread wheat, durum wheat and dicoccum wheat. In India about 90 per cent of the area is under bread wheat followed by durum wheat (9 %) and the remaining 1 per cent is under dicoccum wheat. Emmer wheat is tetraploid, self pollinated, non-free threshing, and is found in the few mountainous marginal areas of Italy (D'antuono *et al.* 1998) and in few states of India. Scientific studies on this wheat reveal that they are nutritionally superior as compared to commercially available wheat (Yenagi *et al.,* 1999). Hence, on account of being a special food, emmer wheat always fetches premium price in the markets as compared to bread and durum wheats. Dehulling in emmer wheat is a laborious process and involves additional expenses. The hulled character is the result of two differences in the structure of the spike: the semi-brittle joints between the rachis internodes, and the toughened glumes. Mackey (1954) reported that a polygenic system is scattered through all three genomes that counteracts rachis brittleness and tough glumes. Kerber and Rowland (1974) reported that the recessive allele tg as well as Q factor must be present for the expression of free threshing character in hexaploid wheat. Consequently, there is an exigency to develop amber grained, free threshing, high yielding and multiple disease resistant semi-dwarf varieties without affecting the grain quality. If desired genetic variability or a specific character is not available in a crop, then the mutation breeding is logic step. It is suggested that the application of

mutation treatment to hybrids may be one means of adding the variability inherent in the cross. Experiment by Gregory (1956) on peanut showed in terms of standard deviation, more variation in both irradiated parents and irradiated hybrids than in unirradiated hybrids and he hypothesized that the variation induced by irradiation might be cumulative with that of hybridization. Similar results were also observed in wheat (Ram *et al*., 1987). Hybridization followed by mutation can be considered as reliable tool for generating desirable segregants for economically important traits like free threshability and disease resistant a derived characters in dicocum wheat. Present syudy, we have utilized 92 advanced free threshing selected lines from the previous work (Pratibha, 2004) carried out at wheat improvement project, MARS, Dharwad More number of potential genotypes were recorded in F_2M_2 (g) population compared to their corresponding F_2's and F_2M_2 (EMS) and it was observed that effectiveness of physical mutagen in creating potential genotypes were more than the chemical mutagen (Pratibha, 2004). This material comprised of 2760 mutants, further the material was forwarded to M_4 and F_4M_4 during *rabi* 2009-10 from these 92 lines were selected based on rachis fragility and glume tenacity for diversity assessment.

Materials and methods

The mutant population used for present study were derived from inter-specific hybridization cum mutation involving 2 parents representing each of emmer (DDK 1001 and DDK 1025) and durum wheat (DWR 1006 and HD 4502). All combinations of crosses were done by taking dicoccum as female and durum as male. Each hybrid and the parental lines were treated with chemical mutagen, EMS at 0.2, 0.3 and 0.4 per cent concentration and similarly, they were exposed to 150 Gy, 200 Gy and 250 Gy {Gy=Grey (1Gray= 0.1 krad)} gamma rays treatments from Cobolt^{60} source at BARC Trombay, Mumbai. Further the material was forwarded by selfing. A total of 92 free threshable mutants of M_7F_7 generation were selected based on yield, threshability, tough rachis and soft glumes and they were sown in two seasons of *Kharif* 2012 and 2013 by following Randomized Block Design (RBD) design with 2 replications of 1m row length with spacing of 20 cm X 10 cm. The recommended packages of practices were followed to raise a good crop stand. The observations from 5 randomly selected plants were recorded for days to 50% flowering, days to maturity, plant height (cm), number of tillers per plant, number of grains per spike, awn length (cm), grain yield per plant (g), 1000-grain weight (g), spike length (cm), number of spikelets/spike, protein (%), seed color, threshability and raches. Threshability data of individual spikes was recorded after harvest. Spikes were threshed with hand, based on percentage of husked seeds obtained, classified as free threshable (0-30), medium free threshable (31-60) and hard to thresh non free threshable (61-100) and spikes were observed for presence of rachis (100%) and absence of rachis (0%). The genetic divergence of those genotypes was studied by employing Mahalanobis' (1936) D^2 technique. The varieties were grouped into a number of clusters with D^2 being treated as the square of generalized distance, according to the method described by Tocher (Rao, 1952).

Results and Discussion

The increasing demand of high yielding varieties with nutritional superiority, poses a formidable challenge for genetic improvement of tetraploid wheat and dicoccum wheat in particular. The analysis of variance is not a reliable basis for measuring the extent of genetic diversity. In order to overcome this problem and to quantify genetic divergence between any two genotypes or group of genotypes, the numerical measure of diversity was obtained with the help of D^2 statistics and constellations of genotypes into clusters was done.

Considerable amount of genetic diversity in the material representing different mutagenic treatments revealed no relation between mutagenic treatment and genetic diversity. On the basis of D^2 values, all the mutant genotypes were grouped into 11 clusters (Table.1). The maximum number of genotypes were in cluster II (25 genotypes), followed by clusters III (22) and I (21). The genotypes falling in the same cluster are more closely related and hence the clusters having the maximum number of genotypes, reflected narrow genetic diversity. However clusters VI, VII, VIII, IX and XI were distinct from the rest with each of them having single genotypes (SSD-6, SSD-56, SSD-70, SSD-6 and ER-36 respectively), indicating their uniqueness from breeding point of view. Many free threshable mutants grouped in cluster III with two checks of durum DWR 1006 and HD-4502 along with one dicoccum check DDK-1006, it may be due to durrumization of free threshable mutants and also may be due to pleiotropic affects of Q locus on morphological characters such as rachis fragility, glume shape and tenacity, spike length and plant height, and spike emergence time which is in line with the findings of Mackey *et al.* (1954), Jantasuriyarat *et al.* (2004), Muramatsu *et al.* (1963, 1986) and Kato *et al.* (1999, 2003).

It was noticed that, the fall of high yielding mutant line BULK-39 (455.5gm test weight with amber color grain, free threshable and fragile raches) with low protein mutant BULK-45 (8.2% protein content with amber colored grains) under same cluster (X), it may be conclussive evidence of high protein content were negatively correlated with yield. This negative correlation of high grain yield with high protein content was also recored previously by Subhashchandra *et al.* (2009) and Mevlut *et al.* (2009). Cluster I has got high protein content mutant line BULK-26 but it possess red grain color, low yield (169 gm) with free threshable and soft glumes. In general cluster II has got low yielding lines along with high protein content of amber color free threshable grain. In contraste, cluster X posses high yielding with low protein lines of red colored non free threshable, hardy glumes grains. Hence hybradization between these two cluster may helps to derive the segregents of high yielding, protein content along with soft glumes and free threshable spikes.

The cluster mean values for each character are presented in Table.2. The cluster VI and IX showed the lowest mean of days to flowering, maturity and plant height, while clusters XI, had maximum tiller number, grain number, protein content and grain color. The cluster VIII had the maximum mean values for spike and awn length. Cluster III exhibited maximum mean value for number of spikelets per spike, while cluster VI showed the minimum mean value for grain yield. The intra and inter cluster divergence among the genotypes was

varying in magnitude (Table.3). It is always desirable to look for genotypes having more than one desirable trait but belonging to different clusters based on cluster mean values. It revealed that intracluster distance was maximum for cluster IV followed by clusters V and III. The clusters VI, VII, VIII, IX and XI were having only one genotype and thus had zero value. The widest inter cluster distance was noted between clusters IV and X followed by clusters I and X with giving scope for hybridization programme to improve genotypes in those clusters. In general, the X cluster showed relatively good inters clusters distances in combination with other clusters attributing the importance of genotypes in that cluster. The distance between cluster IX and VIII was minimum indicating closer relationship between those clusters. These results are in line with the research findings of Shamsuddin, *et al.* (1985), Faris, *et al.* (2006), Arega, *et al.* (2007), Sharma, *et al.* (2008) and Ayed, *et al.* (2009).

The character contribution in cluster divergence (Table.4) revealed that the maximum contribution in cluster formation was attributed by grain yield (85.46) followed by number of number of tillers per plant (6.69), plant height (6.32) and grain weight (0.72), whereas, remaining traits played minor role in cluster divergence as attributed by present mutant wheat materials. Most of the lines selected were based on putative free threshability trait, hence the contribution of this traits for genetic divergence was nill, even though lines showed variation for this trait.

In summar, the 92 lines differ for traits like leaf angle, deposition of wax on leaf and on stem, plant stature shorter (50cm) to tall (106cm), less (12) to large (64) numbers of tiller, square head spike with green to black awns, spike compactness (normal to club shape), grain color (amber to red) protein content (8.22 to 15.65), test weight (33.30 to 57.65gm), raches fragility (non-fragile and brittleness of raches), glume shape and tenacity, hulled grain (non, medium and free threshable). Some of these lines exhibited threshability as that of durum types and quality of dicoccum types since contribution of positive alleles for almost all the productivity traits, quality and leaf rust resistance into durum background whereas, free threshability and test weight into dicoccum background. These results confer definite advantage of inter-specific hybridization cum mutation over simple inter-varietal hybridization. The study will also work as indicator for wheat breeders to evolve varieties with diverse genetic background to achieve sustainability in wheat production in the world.

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Table 1. Distribution of 92 mutant genotypes along with 4 checks into 11 different clusters

Sl No.	Cluster	Number of genotypes	Genotypes
1	I	21	ER-61, BULK-55, ER-54, ER-54, ER-24, BULK-26, BULK-39, BULK-7, BULK-7, ER-36, ER-36, SSD-58, SSD-4, ER-1, SSD-6, SSD-42, SSD-42, BULK-26, SSD-51, SSD-70, ER-24
2	II	25	SSD-55, BULK-91, SSD-7, SSD-9, SSD-6, BULK-53, BUKL-26, BULK53, BULK-7, ER-35, ER-33, SSD-52, BULK-50, ER-65, SSD-7, ER-36, ER-54, SSD-84, ER-63, ER-63, ER-65, ER-63, ER-24, ER-24, DDK-1001
3	III	22	BULK-7, SSD-10, SSD-7, BULK-61, ER-11, BULK-55, BULK-52, ER-36, BULK-92, SSD-2, SSD-4, SSD-3, BULK-26, BULK-52, BULK-26, ER-36, ER-61, ER-65, ER-14, DDK-1025, DWR-1006, HD-4502
4	IV	9	ER-54, ER-61, SSD-70, BULK-26, ER-63, ER-54, ER-63, BULK-54, ER-24
5	V	10	SSD-17, SSD-17, SSD-11, SSD-10, SSD-6, BULK-55, BULK-57, BULK-56, BULK-21, ER-67
6	VI	1	SSD-6
7	VII	1	SSD-56
8	VIII	1	SSD-70
9	IX	1	SSD-6
10	X	4	BULK-45, SSD-6 , BULK-39SSD-51
11	XI	1	ER-36

Table 2. Mean values of fourteen characters in eleven clusters of mutant lines.

Sl. No.	No. of clusters	DFF	DM	PH	NTP	SL	NSP	NGP	AL	GYP	TGW	P	COL	FT	RH
1	I	59.25	108.83	84.73	25.31	8.32	19.06	3.07	10.52	187.26	42.76	12.30	1.67	1.48	1.38
2	II	58.47	107.54	81.66	29.74	8.59	19.12	3.18	10.31	238.86	43.69	12.27	1.64	1.56	1.44
3	III	60.61	110.05	90.54	24.57	8.47	19.55	3.15	10.19	298.91	41.83	11.49	1.59	1.41	1.57
4	IV	59.28	109	78.23	30.72	8.66	18.25	2.83	10.08	126.50	43.04	12.02	1.56	1.39	1.56
5	V	66.2	114.65	89.63	29.05	9.03	18.78	3.23	11.09	365.40	40.58	11.36	1.70	1.45	1.50
6	VI	52.5	102.5	50.83	20	7.93	20	2.75	8.02	324.50	47.25	13.90	2.00	2.00	2.00
7	VII	54.5	104	63.08	62	5.83	17.25	2.25	7.17	255.50	46.00	11.85	2.00	1.50	1.00
8	VIII	53.5	103.5	62.33	46	9.58	15.75	3	11.42	197.00	49.15	13.70	1.00	1.50	2.00
9	IX	52.5	102.5	52.42	21	6.88	20	2	8.05	212.00	52.40	13.35	1.00	2.00	2.00
10	X	63.31	111.25	90.38	33.88	8.21	18.31	3.19	10.04	443.88	38.55	10.05	1.50	1.38	1.25
11	XI	61.5	110.5	103.58	56.5	5.6	15.75	3.25	7.77	257.00	50.00	13.90	2.00	1.00	1.50

DFF- Days to 50% flowring
DM- Days to maturity
PH - Plant height
NTP - No. of tillers/plant
SL - Spike length
NSS - No. of spikelets/spike
NGS - No. of grains/spike
AL - Awn length
GYP - Grain yield/plant
TGW - Thousand grain wt
P - Protein content
COL- Seed color
FT - Free threshability
RH - Rachis

Table 3. Average intra and inter cluster D^2 values of mutant genotypes. Diagonal value indicates intra cluster D^2 values

Clu	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	32.12	61.48	116.99	72.94	182.04	143.59	83.91	40.43	48.55	258.73	82.29
II		35.16	70.80	119.58	132.59	95.17	48.09	55.42	48.94	207.94	46.97
III			38.07	177.98	77.31	55.75	69.61	111.81	99.79	149.22	61.16
IV				48.92	243.20	203.10	137.80	81.45	96.56	320.07	139.58
V					41.83	66.68	122.33	174.80	162.07	86.30	116.85
VI						0.00	81.86	130.78	112.65	129.42	94.07
VII							0.00	61.08	61.22	194.11	42.29
VII I								0.00	31.60	250.25	74.54
IX									0.00	237.13	77.96
X										34.68	190.34
XI											0.00

Table 4. Per cent contribution of characters towards divergence in mutant population

Character	Contribution
Days to 50% flowering	0.61
Days to maturity	0.2
Plant height (cm)	6.32
No. of tillers/plant	6.69
Grain yield/plant (g)	85.46
1000-grain weight (g)	0.72
Spike length (cm), No. of spikelets/spike, No. of grains/spike, Awn length (cm), Protein (%), Seed color, Threshability and Raches	0