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Breeding for free threshability in emmer wheat [*Triticum dicoccum* (Schrank.) Schubl.] through induced mutagenesis

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Abstract

Twelve hundred and sixty one advanced breeding lines derived through mutagenesis of the F_1s of 14 *Triticum dicoccum* x *T. dicoccum,* two *T. dicoccum* x *T. durum* and *T. durum* x *T. durum* crosses and six elite lines of *T. dicoccum* using Gamma rays treatment of seeds at equilibrium seed moisture content of eight per cent were studied for selecting superior genotypes with respect to various agronomic and quality traits. Based on rachis fragility and glume tenacity; 51 free-threshable dicoccum lines were selected comprising 48 from eight *T. dicoccum* x *T. dicoccum* crosses *viz.*, NP-200 x MACS 2912 (10), NP-200 x DDK 1009 (19), DDK 1001 x MACS 2928 (2), DDK 1013 x DDK 1001 (5), 266-10 x 248-4 (1), NP 200 x DDK-1015 (1), NP-200 x MACS 2336 (4) and DDK 1009 x MACS 2931 (6), one from the elite *T. dicoccum* line MACS 2931 and one each from the *T. durum* x *T. durum* crosses *viz.*, Bijaga Yellow x D 2571 and DWR 2006 x DWR 1005. Out of these 51 stable free threshable mutants, two lines derived from the cross NP-200 x MACS 2912 (line nos. 886 and 915) and one from DDK 1013 x DDK 1001 (line no. 206) were nutritionally superior with early flowering, increased seed weight and amber coloured grains. The line No. 206 recorded higher sedimentation value of 42ml, β-carotene of 3.90 ppm and protein content of 14.36 per cent. Hybridization followed by mutation created enormous variability in the study indicating its effectiveness as a breeding strategy for developing free threshability in emmer wheat.

Key words: Glume tenacity, free threshability, mutagenesis, rachis fragility

Introduction

Emmer wheat is tetraploid, self pollinated, non-free threshing, and is found in the few mountainous marginal areas of Italy [1] and in few states of India. Emmer wheat withstands higher temperature stress as compared to other cultivated wheat species and possesses high protein and dietary fiber content [2]. The products prepared from this wheat are more tasty and soft, have high satiety value with potential for baking, parboiling and popping [3] and also have low digestibility, low glycaemic index and therapeutic value in the management of diabetes [4-5].

The yield of semi-dwarf emmer wheat is comparable with *T. aestivum* and *T. durum* varieties and fetches 20-25 per cent more price. However, only 70 per cent grain recovery is possible on threshing because of fragile rachis and non-free threshability. Harvesting and threshing is difficult, time consuming and expensive. 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 [6] reported that a polygenic system is scattered through all three genomes that counteracts rachis brittleness and tough glumes. Kerber and Rowland [7] reported that the recessive allele *tg* as well as *Q* factor must be present for the expression of free threshing character in hexaploid wheat. Looking to its quality traits and resistance to fungal diseases, there is a great need to develop varieties with free threshability. The only source for introgressing this trait is *T. durum* as it crosses freely with emmer wheat. However, the extent of variation released by hybridization has been often reported to be inadequate to design varieties with free threshability trait. In order to create variability, several workers crossed diverse genotypes [1, 8, 9]. Appearance of high proportion of parental types in

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segregating populations and similar association in parents and F_1 populations suggests the strong conservative force resulting from linkage by inhibiting the frequency of genetic recombination. This resulted into bleak possibility for developing variability through hybridization. Hence, we have attempted hybridization between potential emmer and durum wheat lines and the crossed seeds were subjected to mutagenesis.

Material and methods

Twenty emmer elite lines and two durum lines were crossed in various combinations during 1997-98 winter to produce 14 *T. dicoccum* x *T. dicoccum* crosses, two crosses each of *T. dicoccum* x *T. durum* and *T. durum* x *T. durum.* The 18 F_1s produced were subjected to mutagen treatment along with six elite emmer wheat lines.

Mutagenic treatment

Gamma rays treatment

At Bhabha Atomic Research Center (BARC), Trombay, Mumbai, 300 seeds (without husk) of 18 F_1s and six elite emmer wheat lines were exposed to 150 Gy Gamma rays from Co^{60} source at equilibrium seed moisture content of eight per cent,

EMS treatment

About 500 grains (without husk) of 18 F_1s and six elite emmer wheat lines were presoaked in water for 14 hours. The presoaked seeds were treated with 0.5 per cent ethyl methane sulfonate (EMS) in 0.05 M phosphate buffer for six hours with constant stirring. After treatment, seeds were thoroughly washed with water for 4-5 times and sown immediately in the field.

Handling of mutagen treated material

During 1999-2000 winter, all the seeds of the mutagen treated F_1s (F_1M_1) were grown in 4m single row and 30-40 plants which survived were selfed. The selfed seeds were sown (F_2M_2) during 2000-2001 winter. The population ranged from 800-1200 in different crosses. From each plant four seeds were collected and bulked to constitute F_3M_3. Bulked populations were grown (2001-2002 winter) and again four seeds from each plant were collected and bulked. The selection for free threshability in all the three generations was ineffective probaby due to comparatively lower population size maintained and we decided to increase the population size. The F_4M_4 material was grown during 2002-2003 winter season (approximately 10,000 plants were maintained in each population). Selection for free threshability traits rachis fragility and glume tenacity was practiced and 1261 such plants with typical emmer wheat phenotype were isolated. During 2003-2004 winter, 1261 lines were planted head-to-row under Augmented design with three checks namely DWR 225 (*T. aestivum*), DWR 1006 (*T. durum*) and DDK-1001 (*T. dicoccum*) repeated every 20 progenies. The lines were sown in plots measuring 2.8m and 5.5m each consisting eight progenies of 1m length. The data were recorded on days to 75 per cent spike emergence, effective tillers per meter length, plant height (cm), flag leaf length (cm), flag leaf width (cm), peduncle length (cm), days to 80 per cent maturity, spike length (cm), number of spikelets per spike, number of seeds per spikelet, number of grains per spike, 1000 grain weight without husk (g), seed yield with husk (g/plant), rachis fragility and glume tenacity. The analysis of variance was performed and 51 stable free threshing lines were selected. During 2004-05 winter 51 selected lines were evaluated with three emmer wheat checks and one check each from bread and durum wheat under Randomized Complete Block Design with two replications. Each line was planted in three rows with spacing of 23cm between rows and 5cm between plants. In addition to above characters, the data were recorded on protein on dry matter basis (%), sedimentation value (ml) and β-carotene (ppm) [10]. The crude protein value was obtained by multiplying the nitrogen content by factor 5.7 and expressed in percentage. The sedimentation value indicates bread making quality of wheat and expressed in milliliter. Similarly, β-carotene imparts attractive yellow colour to the pasta products and is considered to be an important quality character. It is precursor of vitamin A, hence has immense nutritional importance. To obtain the values for β-carotene content (parts per million), the transmission reading of unknown sample was put in the equation i.e. β-carotene (ppm) = 0.0105 + 23.5366 x L Where, L = Apparent density (transmission value).

Results and discussion

The 1261 free threshable lines selected from F_4M_4 encompassed 976 lines derived from 14 crosses of *T. dicoccum* x *T. dicoccum,* 56 lines from two *T. dicoccum* x *T. durum,* 49 lines from two *T. durum* x *T. durum* crosses and as many as 180 putative free-threshable lines from six elite emmer lines (Table 1). Among the 1081 lines derived from 18 crosses, 662 were dicoccum types and 416 were durum types. From the mutant parent MACS 2931, 23 lines were dicoccum and two were durum

Table 1. Proportion of free threshable *T. dicoccum* and *T. durum* mutants* obtained through hybridization followed by mutagenesis

Total		*T. dicoccum*	*T. durum*
T. dicoccum* x *T. dicoccum			
DDK 1001 x MACS 2928	105	99(93)	6(7)
NP 200 x MACS 2912	79	36(46)	43(54)
NP 200 x MACS 2931	31	27(87)	4(13)
DDK 1013 x DDK 1001	82	78(95)	4(5)
NP 200 x DDK 1009 199(65)	305	106(35)	
266 10 x 248-4	73	67(92)	6(8)
NP 200 x DDK 1015	25	22(88)	3(12)
NP 200 x MACS 2336	28	7(25)	21(75)
264-17 x 264-10	28	26(93)	2(7)
64-1-2 x 248-4	25	21(84)	4(16)
264-1 x DDK 1013	26	26(100)	-
LK x DK-247	25	25(100)	-
246-10 x 241-3	28	10(36)	18(64)
DDK-1009 x MACS 2931	116	38(33)	78(67)
T. dicoccum* x *T. durum			
CBMIYC-34-3 x D-5230	32	32(100)	-
NP 200 x D-61 73	24	19(80)	5(20)
T. dicoccum* x *T. durum			
Bijaga Yellow x D-2571	25	6(24)	19(76)
DWR 2006 x DWR 1005	24	20(84)	4(16)

*Mutants classified based on morphological traits; Figures in parenthesis indicate percentage

Table 2. Putatitve free threshable[a] and quality[b] mutants[c] obtained through hybridization followed by mutagenesis

Crosses	Total	*T. dicoccum*	*T. durum*
T dicoccum* x *T. dicoccum			
NP 200 x MACS 2912	10	2	8
NP 200 x DDK 1009	19	5	14
DDK 100 x MACS 2928	2	2	-
DDK 1013 x DDK 1001	5	3	2
266-10 x 248-4	1	-	1
NP 200 x DDK 101 5	1	1	-
NP 200 x MACS 2336	4	1	3
DDK 1009 x MACS 2931	6	-	6
Elite dicoccum parent			
MACS 2931	1	-	1
T. durum* x *T. durum			
Bijaga yellow x D 257	1	-	1
DWR 2006 x DWR 1005	1	-	1

[a]-Classified based on rachis fragility and glume tenacity; [b]-Quality traits *viz.*, protein content, sedimentation value and β-carotene content; [c]-Classified based on morphological traits

types. Fifty one putative free-threshable dicoccum types were selected based on rachis fragility and glume tenacity. As many as 48 lines were identified from eight *T. dicoccum* x *T. dicoccum* crosses *viz.*, NP 200 x MACS 2912 (10), NP 200 x DDK 1009 (19), DDK 1001 x MACS 2928 (2), DDK 1013 x DDK 1001 (5), 266-10 x 248-4 (1), NP 200 x DDK-1015 (1), NP 200 x MACS 2336 (4) and DDK 1009 x MACS 2931 (6), one mutant from the elite *T. dicoccum* line MACS 2931 and one each from the *T. durum* x *T. durum* crosses *viz.*, Bijaga Yellow x D 2571(1) and DWR 2006 x DWR 1005 (1). Some of these lines impersonated the durum wheat for threshability. Hence these lines were further classified as dicoccum and durum types based on morphological traits (Table 2). Among free-threshable lines selected from *T. dicoccum* x *T. dicoccum* crosses, maximum percentages of durum types were obtained. Hybridization between emmer wheat lines should have resulted into dicoccum types but we obtained large number of durum type recombinants which may solely be due to mutation. They exhibited threshability as that of durum types and quality of dicoccum types. Thirty-four lines from nine *T. dicoccum* x *T. dicoccum* crosses were durum types. One mutant line from dicoccum parent (MACS 2931) exhibited amber grain colour, non brittle rachis, medium threshability, bolder seed (69.9 g) and yield of 282 g per plot, protein content of 18 per cent on dry matter basis with 43 ml of sedimentation value as that of bread wheat and 5.9 ppm of β-carotene as that of durum wheat. So hybridization followed by mutation in *T. dicoccum* x *T. dicoccum* crosses resulted in mutants which were free threshable as that of durum types and maintained quality of dicoccum intact. They also exceeded the parents (NP 200, DDK 1001) which have protein content of 16 per cent. We obtained two purely durum lines from two *T. durum* x *T. durum* crosses i.e. Bijaga yellow x D 2571 and DWR 2006 x DWR 1005. In these crosses, durum nature of parents was maintained intact. Though there was a compromise in β-carotene content (3.6 ppm) but good increase in protein content (14.36%) and sedimentation value (40 ml) was noticed. This indicated that there is greater possibility that high protein strains will also possess strong gluten (11). So the lines from *T. dicoccum* x *T. dicoccum* exhibited medium threshability as that of durum wheat retaining high protein content of emmer wheat and improvement in sedimentation value and β-carotene content. We also obtained dicoccum mutants in *T. dicoccum* x *T. dicoccum* crosses indicating the combination of hybridization and induced mutation resulting in improvement of locally adapted wheat varieties with regard to specific traits such as threshability and quality.

Frequency distribution of free-threshable lines for quality parameters

For 1000 grain weight, maximum number of plants from set II (NP 200 x DDK 1009) were fallen under the class interval 46-50 g followed by set I (NP 200 x MACS 2912) with a class interval 56-60 g (Fig. 1). This indicated that mutants obtained from *T. dicoccum* x *T. dlcoccum* crosses impersonated durum types. For protein content, large number of mutants were grouped in the class 11-13 per cent followed by the class with 14-16 per cent in the investigation (Fig. 2). For Sedimentation value, 11 mutants from the set II (NP 200 X DDK 1009) were categorized under the class 25-30 ml followed by class 31-35 ml of same set (Fig. 3) and for β-carotene content, maximum number of mutants (15) from the set II (NP 200 X DDK 1009) were grouped in the class interval of 4-5 ppm followed by set I (NP 200 X MACS 2912) of same class (Fig. 4). This indicated the usefulness of mutation in creating rare recombinants compared to simple hybridization for this trait.

Analysis of free-threshablc mutants for variability

Mean sum of squares clearly indicated significant variation among 51 putative free threshable dicoccum types for all the traits studied. Many workers [12-16] reported high variation for seed yield and other traits. The significant differences in mean sum of squares indicated large and novel genetic variability existing in the material. The two lines 886 and 915 belonged to cross NP 200 x MACS 2912 and line 206 from the cross DDK 1013 x DDK 1001 showed non-brittle rachis, free threshability and amber grain colour as that of bread wheat (Figs. 5, 7). The distinguished ancillary and quality features of these lines in comparison with checks are presented in Table 3. The plant height and days to maturity of the lines were on par with durum check (DWR 1006). There was improvement in 1000 grain weight (Fig. 6), protein content and days to flowering over three checks while, the sedimentation value and β-carotene content were comparable with three checks. Identification of these novel lines reflected on the reliability of hybridization followed by mutation as an effective tool for generating desirable mutants for economically important traits like free threshability, a very rare dominant mutation (q-Q) for the popularization of emmer wheat in all wheat growing areas.

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References

1. **D'antuono L. F., Galletti G. C. and Boahini P.** 1998. Fibre quality of emmer (*Triticum dicoccum* Schubler) and einkorn wheat (*T. monococcum* L.) land races as determined by analytical pyrolysis. J. Food Sci. Agril., **78**: 213-219.

Table 3. Characteristic features of three elite free-threshable amber grained mutants selected from 51 putative free-threshable lines obtained through hybridization followed by mutagenesis in comparison to checks

Sl.	Cross	Plant height (cm)	Days to flowering (days)	Days to maturity (days)	1000 grain weight	Protein content (%) (g)	Sedime-ntation value	β-carotene (ppm) (ml)
	NP 200 x MACS 2912							
1	Line no. 886	98.40	68	105	69.90	12.76	28.00	3.48
2	Line no. 915	92.00	68	110	58.80	15.96	32.00	3.90
	DDK 1013 x DDK 1001							
3	Line no. 206	100.40	70	110	69.60	14.36	42.00	3.53
	Checks							
	Bread wheat (DWR 225)	81.30	70	112	41.60	12.22	44.00	3.92
	Durum wheat (DWR 1006)	98.70	70	111	42.50	11.75	36.50	6.54
	Dicoccum wheat (DDK 1001)	73.00	75	110	33.21	13.83	31.53	3.45

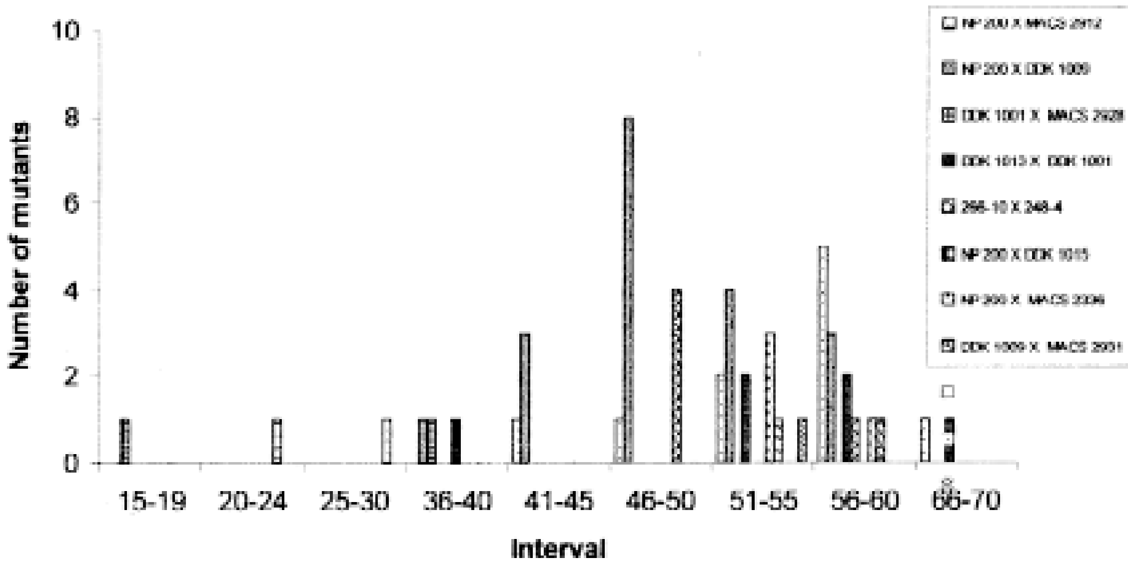

Fig. 1. Frequency distribution of 51 free-threshable mutants for 1000-grain weight

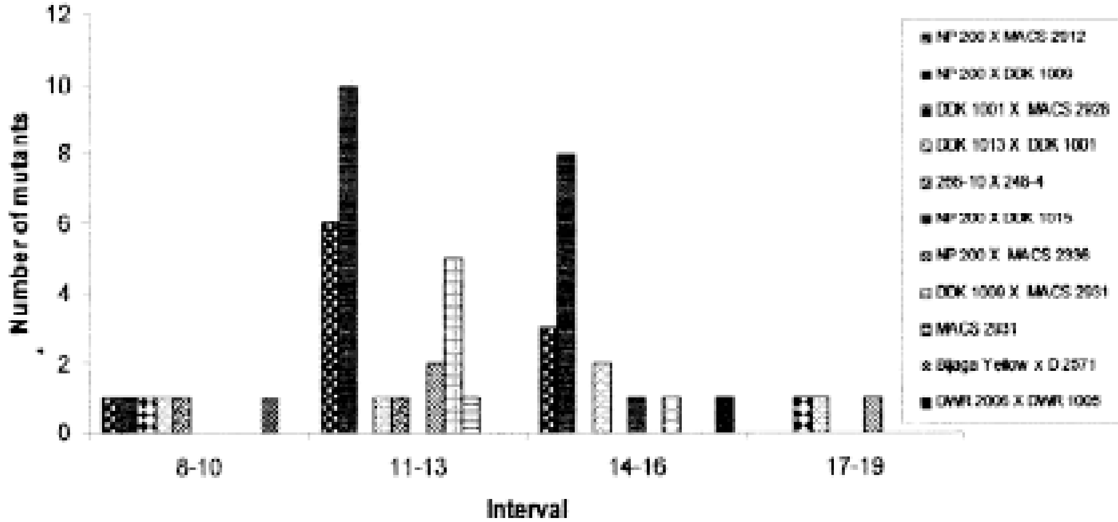

Fig. 2. Frequency distribution of 51 free-threshable mutants for protein content

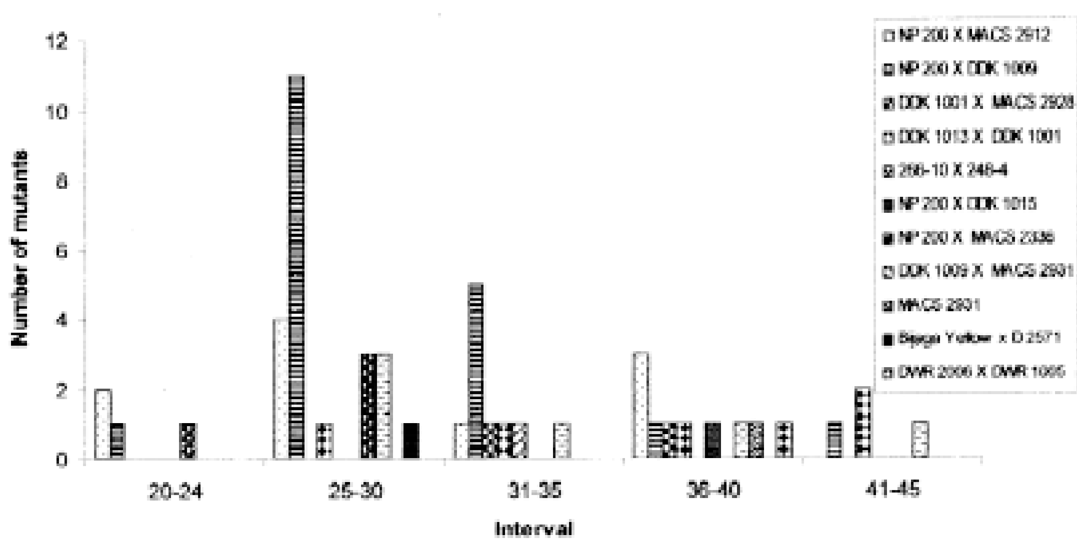

Fig. 3. Frequency distribution of 51 free-threshable mutants for sedimentation value

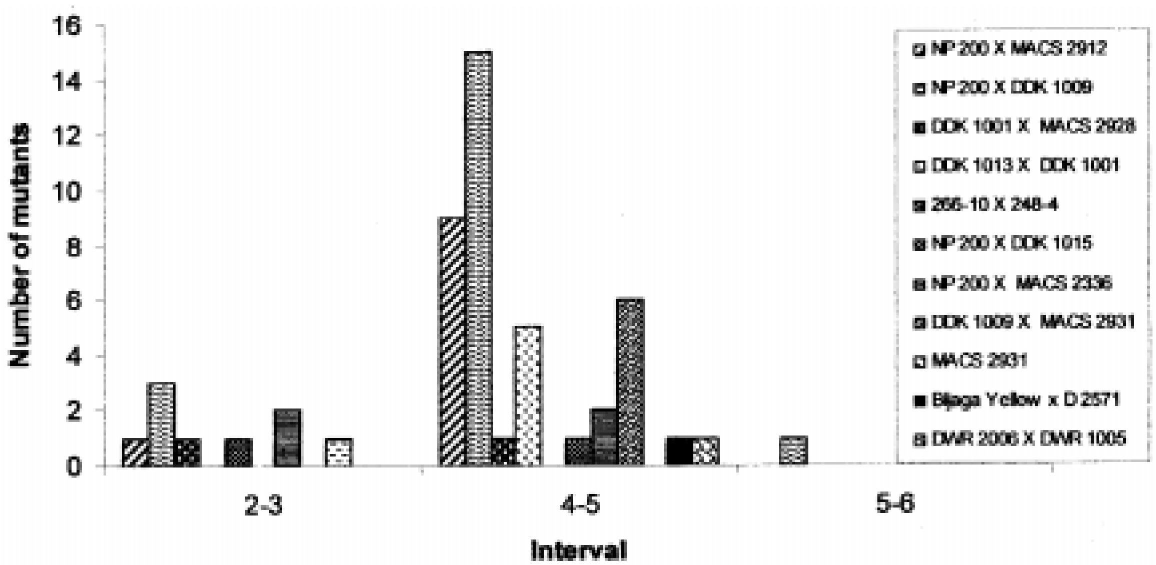

Fig. 4. Frequency distribution of 51 free-threshable mutants for β-carotene content

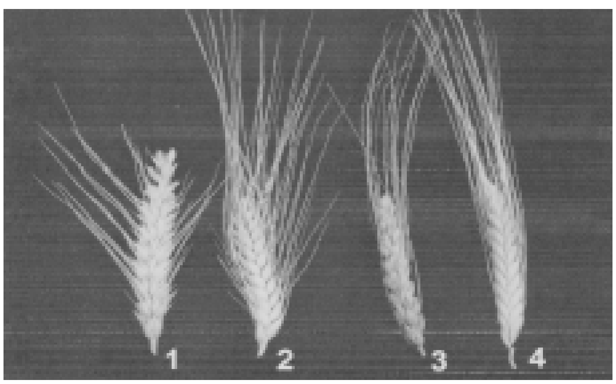

Fig. 5. Comparison of earheads of 1. *T. aestivum* var. DWR 225; 2. *T. durum* var. DWR 1006; 3. Free-threshable *T. dicoccum* mutant from NP 200 x MACS 2912 (886); 4. *T. dicoccum* var. DDK 1001

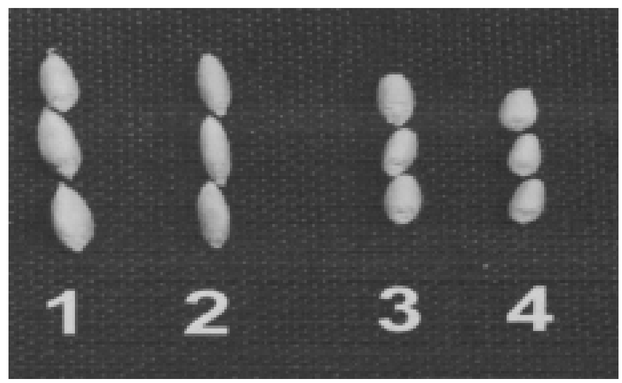

Fig. 6. Comparison of seeds of 1. Free-threshable dicoccum mutant - NP 200 x MACS 2912 (886); 2. DDK 1001 (*T. dicoccum*); 3. DWR 1006 (*T. durum*); 4. DWR 225 (*T. aestivum*)

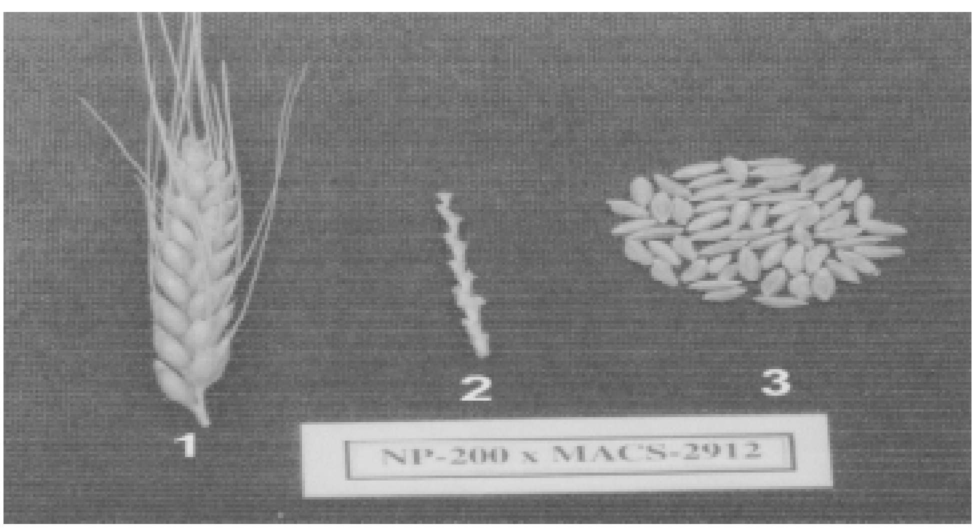

Fig. 7. A rare free-threshable *T. dicoccum* mutant-NP 200 x MACS 2912 (886) showing 1. spike; 2. Non-brittle rachis; 3. Seeds

2. **Bhuvaneshwari G., Yenagi N. B., Hanchinal R. R. and Naik R. K.** 1998. Nutritional and therapeutic qualities of *Triticum dicoccum* wheat varieties. Paper presented in the Fourth International Food Convention held at Mysore, Karnataka, India, 23-27 November 1998.
3. **Reddy N. M.** 1996. Suitability of wheat preparation of various food products. Unpubl. M.H.Sc. Thesis, Univ. Agril. Sci., Dharwad, Karnataka, India.
4. **Bhuvaneshwari G., Yenagi N. B., Hanchinal R. R. and Naik R. K.** 2003. Glycaemic responses to dicoccum products in the dietary management of diabetes. Indian J. Nutr. Dietet., **40**: 363-368.
5. **Yenagi N. B., Hanchinal R. R. and Suma C.** 1999. Nutritional quality of dicoccum wheat semolina and its use in planning therapeutic diets. Paper presented in XXXII Annual Meeting of Nutrition Society of India, Coimbatore, Tamil Nadu, India, 25-26 November, 1999.
6. **Mackey J.** 1966. Species relationships in triticum. *In*: Proc. 2nd Intern.Wheat Gen. Symp., 1963, Lund, Sweden. Hereditas (Suppl.), **2**: 237-276.
7. **Kerber E. R. and Rowland G. G.** 1974. Origin of the free threshing character in hexaploid wheat. Canadian J. Genet. Cyt., **16**: 145-154.
8. **Porfiri O., D'antuono L. F., Codianni P., Mazza L., Castanga R. and Jaradat A. A.** 1998. Evaluation of an Italian hulled wheat collection for agronomic and quality characters. Triticeae III. Proc. 3rd Intern. Triticeae Symp., 4-8 May 1998, Aleppo, Syria: 387-392.
9. **Tomar S. M. S., Kochumadhavan M. and Nambisan P. N. N.** 1989. Hybrid weakness in *Triticum dicoccum* Schubl. Wheat Information Ser., **69:** 21-23.
10. **Mishra B. K. and Gupta R. K.** 1995. Protocols for evaluation of wheat quality. Directorate of Wheat Research, Karnal, pp. 43-48.
11. **Lin Z. J., Zhon X. D., Jie S. H., Hu X. Y., Ding X. L. and Jin M. G.** 1989. Interrelationships among baking quality and other quality characteristics and yield traits in winter wheat. Acta-Agronomica Sinica, **15**: 151-159.
12. **Gupta S. K. and Verma S. R.** 2000. Variability, heritability and genetic advance under normal and rainfed condition in durum wheat (*Triticum durum* Desf.). Indian J. Agril. Res., **34**: 122-125.
13. **Shah M. A. and Deora V. S.** 2002. Genetic variability and association studies in wheat for grain yield and temperature tolerance parameters. Indian J. Agric. Res., **36**: 172-176.
14. **Singh S. P., Jha P. B. and Singh D. N.** 2001. Genetic variability for polygenic traits in late sown wheat genotypes. Ann, Agril. Res., **22**: 34-36.
15. **Nayeem K. A., Baig K. S. and Karad N. S.** 2002. Genetic variability and character association studies for export quality parameters in *T. durum.* J. Res. ANGRAU, **30**: 5-10.
16. **Dwivedi A. N. and Pawar I. S.** 2004. Evaluation of genetic diversity among bread wheat germplasm lines for yield and quality attributing traits. Haryana Agril. Univ. J. Res., **34**: 35-39.