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ABSTRACT Wheat is the most important cereal crop of Pakistan which contributes 10% to the value added in agriculture and 2% to GDP. The objective of this study was to broaden the genetic bases through identification and evaluation of promising wheat mutants with higher yield and better quality. Genetic variability was induced in three wheat varieties Sarsabz, Kiran-95 and TD1 for the development of new ideotypes by gamma rays, EMS and combined treatment. From M₃ generation, 30 promising mutants were selected on the basis of phenotypic variations. These mutants were confirmed through SSR markers in M₄ generation and further characterized for quality traits through PINa-D1, PINb-D1 and waxy gene. As the physical trait grain texture affects the milling and marketing of bread wheat, the mutants were characterized based on their hardness. Among the 30 mutants along with three parents, the grain texture of 20 mutants was categorized as soft, 12 as hard and only one mutant was classified as ultra-hard. Our results showed five types of waxy phenotype (Type 1, 2, 3, 5 and 8) in which five mutants were non-waxy, three were waxy and 19 along with parents were partial waxy. Hardness index was ranged between 56 to 89 indexes. Seed diameter was varied from 2.51 to 2.86 and moisture contents were between 10.13 to 11.70. Highest gluten contents 45 were found in L23 and lowest were found in L8 and L11 (37). Maximum zeleny value of 87.3 was recorded in L12 and 23. Maximum falling number was observed in L5 (729 seconds) and minimum was 425 seconds in mutant L23. In this work new bread wheat ideotypes were developed for the identification of plant architecture and grain quality traits which could play a role in future wheat breeding. This work reports genetic characteristics of grain texture and starch content at gene level that could help in the improvement of modern cultivars for the purpose of milling and value addition to wheat product.

MATERIAL & METHODS

Induced genetic variability

- Gamma Rays**
 - C¹³⁷ source (Nigo 5, Belgaria)
 - 30.86 Gy minute⁻¹
 - 0, 50, 100, 150, 200, 250 and 300 Gy
- EMS**
 - Distilled water (1 hours)
 - EMS treated (over night)
 - Washing (2 hours)
- Combine Treatment**
 - Seeds were irradiated
 - treated with EMS

For new plant ideotypes

LABORATORY STEPS

DNA Isolation (MATAB)

DNA Quality and Quantification

0.8% Agrose gel

260/280nm

PCR reaction with primers

Gel Electrophoresis

Gel documentation

Data analysis

DNA Extraction and gene specific marker analysis: Isolation of pure DNA is pre-requisite for the molecular marker studies. Fresh young leaves were collected from field at seedling stage from thirty mutants and isolated the DNA by using MATAB method (Bibi et al. 2010) DNA stock of each mutant kept in refrigerator at -20°C. The extracted DNA was quantified as method described by Bibi et al. 2012.

Characterization of promising mutants

- Simple Sequence Repeats markers
- Characterization of promising mutants
 - Grain texture genes PINA, PINB
- Characterization
 - Waxy genes GBSS

According to the guidelines outlined in AACC (1990), each wheat mutant was evaluated for total protein percentage, moisture content, gluten content, falling number, and Zeleny (sedimentation value). Protein was also assessed using a grain analyzer. Wheat samples were ground using a laboratory mill 3100 Perton in accordance with the recommended procedure (AACC, 1990).

RESULTS

Figure 4-7 Field view of M₃ generation (Rabi 2012-13).

Figure 4-8 Wheat crop at maturity in M₃ generation (Rabi 2012-13) at NIA Farm.

Figure 4-9 Early mature mutant observed in M₃ generation (Rabi 2012-13).

Figure 4-10 Dark green and broad leaf mutant observed in M₃ generation (Rabi 2012-13).

Figure 4-11 Phenotypic variation in spike morphology.

Figure 4-12 Identified a stem etia mutant without phenotypic effect in M₃ generation (Rabi 2012-13) at NIA Farm.

Figure 4-13 (L1-xx, SE4/12-1, SE4/12-1, SE4/12-2, SE4/12-3, SE4/12-4, SE4/12-5, SE4/12-6, SE5/12-7, SE5/12-8, SE5/12-9, SE5/12-10, TC14/12-1, TC14/12-2, SE5/12-12, SE5/12-13, SE5/12-14, SE5/12-15, SE5/12-16, SE5/12-17, SE5/12-18, SE5/12-19, SE5/12-20, SE5/12-21, SE5/12-22, SE5/12-23, SE5/12-24, SE5/12-25, SE5/12-26, SE5/12-27, SE5/12-28, SE5/12-29, SE5/12-30, SE5/12-31, SE5/12-32, SE5/12-33, SE5/12-34, SE5/12-35, SE5/12-36, SE5/12-37, SE5/12-38, SE5/12-39, SE5/12-40, SE5/12-41, SE5/12-42, SE5/12-43, SE5/12-44, SE5/12-45, P= Parent)

Figure 4-14 Molecular weight profile of 30 wheat genotypes with wheat PPI1 (GBSS) marker.

Figure 4-15 Molecular weight profile of 30 wheat genotypes with wheat PPI2 (GBSS) marker.

Accession	Moisture	Water	Dissever	Hardness Index	Gluten	Zeleny	FN(Sec)
L1	88.4321.1	88.9	2.68	81.8	38.0 ^{AB}	85.3 ^{AB}	588.0 ^{AB}
L2	88.4321.2	88.8	2.77	88	39.0 ^{AB}	85.3 ^{AB}	575.0 ^{AB}
L3	88.4321.3	88.8	2.77	82	37.3 ^B	85.3 ^{AB}	554.0 ^{AB}
L4	88.4321.4	88.9	2.73	82	38.0 ^{AB}	86.3 ^{AB}	516.0 ^B
L5	88.4321.5	88.8	2.86	82	38.0 ^{AB}	85.3 ^{AB}	729.0 ^A
L6	88.4321.6	88.2	2.85	79	38.0 ^{AB}	84.0 ^{AB}	624.0 ^{AB}
L7	88.832.7	88.3	2.85	88	38.0 ^{AB}	86.3 ^{AB}	562.0 ^B
L8	88.832.8	88.4	2.72	84	41.0 ^{AB}	86.3 ^{AB}	528.0 ^B
L9	88.832.9	88.3	2.88	79	38.0 ^{AB}	85.3 ^{AB}	516.0 ^B
L10	88.832.10	88.2	2.69	84	40.5 ^{AB}	84.0 ^{AB}	561.0 ^B
L11	TC14/12.1	88.3	2.72	79	37.3 ^B	84.3 ^{AB}	498.0 ^B
L12	TC14/12.2	88.8	2.82	79	44.0 ^{AB}	87.0 ^A	569.0 ^{AB}
L13	88.832.12	88.4	2.76	77	39.5 ^{AB}	86.3 ^{AB}	568.0 ^B
L14	88.832.13	88.2	2.88	80	40.0 ^{AB}	86.0 ^{AB}	614.0 ^B
L15	88.832.14	88.2	2.67	82	38.0 ^{AB}	85.0 ^{AB}	575.0 ^{AB}
L16	88.832.15	88.9	2.87	80	39.0 ^{AB}	84.0 ^{AB}	541.0 ^{AB}
L17	88.832.16	88.2	2.65	89	40.0 ^{AB}	86.3 ^{AB}	671.0 ^B
L18	88.832.17	88.8	2.68	80	37.0 ^B	84.0 ^{AB}	572.0 ^{AB}
L19	88.832.18	88.3	2.64	84	40.5 ^{AB}	86.3 ^{AB}	473.0 ^B
L20	88.832.19	88.7	2.78	79	42.3 ^{AB}	86.0 ^{AB}	717.0 ^A
L21	88.832.20	88.7	2.69	81	41.0 ^{AB}	86.0 ^{AB}	581.0 ^{AB}
L22	88.832.21	88.2	2.87	88	42.0 ^{AB}	86.3 ^{AB}	597.0 ^B
L23	88.832.22	88.7	2.79	77	45.0 ^A	87.3 ^A	425.0 ^B
L24	88.832.23	88.9	2.72	79	42.0 ^{AB}	85.3 ^{AB}	532.0 ^{AB}
L25	88.432.24	88.3	2.76	85	39.0 ^{AB}	84.0 ^{AB}	627.0 ^B
L26	88.832.25	88.3	2.69	83	38.0 ^{AB}	84.0 ^{AB}	572.0 ^{AB}
L27	88.832.26	88.9	2.76	79	39.5 ^{AB}	84.0 ^{AB}	585.0 ^{AB}
L28	88.832.27	88.7	2.81	88	37.3 ^B	83.3 ^{AB}	545.0 ^{AB}
L29	Kiran-95	88.4	2.68	84	45.0 ^A	86.0 ^{AB}	584.0 ^{AB}
L30	TD1	88.8	2.69	82	38.5 ^{AB}	82.3 ^{AB}	542.0 ^{AB}
Parent	88.7	2.86	77	39.636	85.282	568.55	

CONCLUSION: Thirty new wheat ideotypes have been generated through mutation breeding. According to results, five types of waxy phenotype i.e. Type1, 2, 3, 5 and 8 were observed in which five mutants were non-waxy, three were waxy mutant and nineteen mutants along with parents were partial waxy mutants.

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