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AGRONOMIC CHARACTERIZATION OF AN EMS-MUTAGENIZED POPULATION FOR SELECTING HIGH-YIELDING AND GLUTEN-ENRICHED INDUSTRIAL WHEAT

*Saiful ISLAM[1], Mohammad Mahmood HASAN[1,4], Tasnin Khan EUSUFZAI[1], Md. Ataur RAHMAN[1], Mohsina AFREEN[1], Fareyzul Haque ANSAREY[1], Tasnim Khandaker SAMIHA[5], Lutfur RAHMAN[2], M. HASANUZZAMAN[3], Abu Shamim Mohammad NAHIYAN[1]**

[1]Advanced Seed Research and Biotech Centre, ACI Limited, Dhaka, 1212, Bangladesh
[2]Bangladesh Agricultural University, Department of Genetics and Plant Breeding, Mymensingh, 2202 Bangladesh
[3]Hajee Mohammad Danesh Science and Technology University, Department of Genetics and Plant Breeding, Dinajpur, 5200, Bangladesh
[4]University of Florida, Department of Microbiology and Cell Science, FL, 32603, USA
[5]BRAC University, Department of Mathematics and Natural Sciences, Dhaka, 1212, Bangladesh
**Corresponding author: nahiyan80@hotmail.com, nahiyan@aci-bd.com*

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ABSTRACT

A mutant population was generated using a popular wheat cultivar of Bangladesh; BARI GOM-28, with ethyl methanesulfonate (EMS) to create diversity in terms of different agronomic characteristics and grain quality parameters. An EMS concentration ranging from 0.2% to 1.2% was used and the optimum was found to be 0.8%. This study was initiated with 16,000 seeds, where 1,581 lines survived under greenhouse conditions up to M_4 generation. Through 3 subsequent field trials, 3 promising lines, namely, 0037/17, 0020/17 and 0023/17 were selected with enhanced spike length, number of spikes per plant, number of spikelets per spike, number of grains per spike, grains weight per plant and 1000-grain weight. Such improved agronomic traits contributed towards a greater yield potential of 0037/17 (5.94-6.10 t ha^{-1}), 0020/17 (5.47-5.54 t ha^{-1}) and 0023/17 (4.97-5.20 t ha^{-1}) than BARI GOM-28 (3.63-3.69 t ha^{-1}) in the multi-location trial. Improvement in certain bread-making qualities like wet gluten content (>28%) and total protein content (~13%) was also observed and compared to BARI GOM-28 which had 22% wet gluten and 11% total protein. Therefore, these mutant lines could be used as a valuable resource for genetic studies to dissect the function of the genes controlling such desired parameters as well as superior breeding lines.

Keywords: EMS, gluten, mutation breeding, mutant population, *Triticum aestivum* L.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second-largest cereal crop in Bangladesh after rice (Hossain and Teixeira da Silva, 2013) and with improvements in lifestyle, people are consuming various bakery and confectionery items prepared with wheat flour (Karim et al., 2010), leads to increase the wheat demand. The total demand in Bangladesh is 8.5 million tonnes per year (Tonmoy, 2022), whereas, the total wheat production of 1.3 million tonnes, in the fiscal year, 2020-21 (FAOSTAT, 2020). With the current production rate (3.09 t ha^{-1}) of wheat in Bangladesh (FAOSTAT, 2020), it is impossible to meet the local demand and in 2020, Bangladesh imported more than 6.01 million tonnes of wheat to mitigate the demand. Therefore, Bangladesh placed as the 10th largest wheat importer in the world, which is equivalent to $1.28 billion (OEC, 2021). However, a major factor in the decreasing trend of wheat production is the failure of the grains from local cultivars to meet the industry quality threshold, leading to a decrease in the farmers' interest (Barma et al., 2019).

In addition, gluten, a storage protein of wheat, is responsible for forming the dough, the key ingredient in many industrial refined food products (noodles, pasta, wafers, biscuits, etc.). Gluten is present in wheat grains as a heterogeneous mixture of monomeric gliadin (texture & viscosity) and polymeric glutenin (strength & elasticity) (Kumar et al., 2019). Therefore, the gluten content in wheat is crucial for determining the quality of the flour and its milling properties. Other storage proteins of wheat grain, including prolamin, globulin and albumins also play vital roles in maintaining the viscoelasticity of the dough (Clare Mills et al., 2012). For instance, a high protein content (14%) is desirable for pasta, whereas, for cake and pastry, a lower protein content (7–11%) is required (Carson and Edwards, 2009).

Based on the above consideration, there is a significant need for high-yielding gluten-enriched wheat varieties to reduce the dependency on wheat imports in Bangladesh. However, improving wheat through breeding alone is very difficult because it is a crop with restricted genetic variability and a large genome size than most other crops (Venske et al., 2019). The transgenic approach could be used for genetic improvement, but, unfortunately, this is not always widely accepted by consumers (Slade et al., 2005). On the other hand, mutation breeding is a non-transgenic approach that has been used for decades to improve crops including rice, wheat, maize, soybean, barley, and many other crops (Jankowicz-Cieslak et al., 2017). Among the various mutation breeding techniques; Physical, chemical, or biological agents (Roy Chowdhury and Tah, 2013), chemical mutagenesis is comparatively a more feasible approach, as it is milder to crops and does not require any kind of special machines (Oladosu et al., 2016). Apart from this different chemical used to generate mutation in the plant, EMS (ethyl methanesulphonate) is the most convenient chemical to improve agronomic characteristics in plants since it induces a higher point mutation and produces no chromosomal aberrations (Kong et al., 2020).

In our study, we generated an EMS-mutagenized population of a Bangladeshi popular hexaploidy wheat variety, released by the Bangladesh Agricultural Research Institute (BARI) named BARI GOM-28. The specific objective of the study was to develop a gluten-enriched and high-yielding industrial-type wheat variety suitable for Bangladeshi conditions.

MATERIALS AND METHODS

Plant materials

A Bangladeshi mega variety, BARI GOM-28, was chosen as parent material and collected from the Bangladesh Agricultural Research Institute (BARI), Bangladesh.

Weather data

Weather data (temperature and precipitation) for Dinajpur Region were collected from Bangladesh Meteorological Department (http://www.bmddataportal.-com/#/) (Table 1). The hydrothermal Sielianinov (K) factor was calculated according to the formula given by (Ratajczak et al., 2020):

$$2K = (2P \times 10)\ 2\ /\ (2T \times L)\ 2$$

Here, K = hydrothermal Sielianinov factor, P = monthly precipitation, T = monthly temperature, and L = days of the month. The optimal value of K = 1; values < 1 indicate insufficient moisture, values < 0.5 indicate drought, values > 1 indicate sufficient moisture, and values > 1.5 indicate excess moisture for the plants.

Table 1. Weather conditions were recorded at a weather station in Dinajpur, Bangladesh from November 2017 to March 2018, November 2018 to March 2019, November 2019 to March 2020 and November 2020 to March 2021. Daily average temperature (°C) and total precipitation (mm) are shown in 10 days period

Season	Parameters	**Months** (10 days period)														
		Nov			Dec			Jan			Feb			Mar		
2017-18	Average dry bulb temp (°C)	23.73	23.12	19.58	20.29	18.93	18.52	12.76	12.41	15.08	17.95	20.01	21.89	23.85	24.30	25.42
	Total rainfall (mm)	0.00	3	0.00	20	21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10	0.00
	hydrothermal Sielianinov K factor		0.02			0.34			0			0			0.01	
2018-19	Dry bulb temp (°C)	23.53	21.26	20.49	18.56	17.72	15.85	15.98	16.73	18.31	18.17	19.82	21.13	20.65	24.61	24.75
	Total rainfall (mm)	7	0.00	0.00	0.00	5	0.00	0.00	0.00	0.00	0.00	0.00	60	10	21	0.00
	hydrothermal Sielianinov K factor		0.05			0.05			0			0.43			0.21	
2019-20	Dry bulb temp (°C)	24.73	23.24	21.72	19.10	16.91	13.65	16.33	15.81	15.24	16.23	18.78	20.13	21.87	22.89	24.95
	Total rainfall (mm)	45	130	0.00	0.00	0.00	7.3	47	0.00	0.00	0.00	0.00	6.7	8	0.00	0.00
	hydrothermal Sielianinov K factor		1.26			0.08			0.48			0.06			0.06	
2020-21	Dry bulb temp (°C)	28.97	25.75	24.68	24.68	25.75	22.53	23.51	24.52	21.05	21.19	25.43	26.27	28.50	31.00	31.02
	Total rainfall (mm)	4.08	0.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	hydrothermal Sielianinov K factor		0.01			0.00			0.00			0.00			0.00	

Generating EMS mutagenized wheat population

For optimizing EMS concentration to generate mutants of BARI GOM-28, different concentrations of EMS ranging from 0.2% (v/v) to 1.2% (v/v) with an interval of 0.1% (v/v) were used by following a previously developed protocol (Chen et al., 2012). A germination assay was done to set a standard EMS concentration that allowed around 40% of germination. Mutation frequency among the germinated seeds of BARI GOM-28 was also observed. After optimization, 16,000 seeds were used to generate the M_1 population (~6,400 seeds) in the greenhouse of Advanced Seed Research and Biotech Centre (ASRBC), Bangladesh. A single bold M_1 seed from each line was planted, and 2,957 different lines survived from these seeds to produce M_2 seeds. These lines were then re-sown until the M_4 generation, 1,581 seeds were eventually obtained.

Field trial of selected mutant lines

The 1581 seeds obtained from the M_4 generation were treated as individual lines and were subjected to field screening at the ASRBC Regional Research Station, Debiganj, Dinajpur Region, Bangladesh in 2016-17. The seeds were sown in a field plot with 1 m of row-to-row distance. Based on the number of effective tillers, length of spike, and number of spikelets per spike, 64 lines were selected. In 2017-18, these 64 lines were subjected to a performance yield trial (PYT). The unit plot size was 2.0 m × 0.4 m with 3 replications following Randomized Complete Block Design (RCBD) (Devesh et al., 2019). During this period, data were taken on the heading date, plant height, spike length, number of spikes per plant, number of spikelets per spike, number of grains per spike, weight of grains per plant, and 1000-grain weight and yield. Among them, 11 lines were selected with agronomic characteristics significantly better than the BARI GOM-28 and were then subjected to an observational yield trial (OYT) in the subsequent 2 seasons (2018-19 and 2019-20). During OYTs, RCBD design was followed with a 10 m^2 unit plot size and 3 replications and data were recorded on the above-mentioned parameters as PYT.

Grain quality analysis

The total protein content was measured following a previously established protocol (Mughal et al., 2020) of Bradford's method using bovine serum albumin (BSA). The wet gluten content was measured using a Double-head Gluten Tester-MJ-III (Zhejiang Top Instrument Co. Ltd). The procedure is available at the AACC Approved Methods of Analysis 11th Edition (AACC Method 44-01.01).

Ranking of the selected lines

To find out the best-performing lines in this study, in terms of both agronomic and flour quality aspects, a 1 to 3 scale-based ranking system was carried out (Nahiyan et al., 2014). The lines were given points according to their performance in different attributes like spike length, number of spikes per plant, number of spikelets per spike, number of grains per spike, 1000-grain weight, wet gluten content, and gluten index and yield.

Advanced Yield Trial (AYT)

Multi-location advanced yield trial (AYT) was conducted in the 3 districts of Dinajpur region; Dinajpur (25.6279° N, 88.6332° E), Thakurgaon (26.0274° N, 88.4646° E) and Panchagarh (26.3354° N, 88.5517° E) the major wheat-growing region of Bangladesh having loam and sandy-loam soil (Shirazy et al., 2017) in 2020-21 growing season. The soil pH of the three trial regions was in the range of 6.1 to 6.8. AYTs were done for yield performance evaluation with the top 3 lines and the experiment was carried out in RCBD with 3 replications with a unit plot size of 20 m^2.

Statistical analysis

Agronomic data, yield components and yield (Table 3 & 6) and quality parameters (Table 4), were examined by the individual and combined variance analysis and the means were compared by the Tukey's HSD test using the XLSTAT by Addinsoft, NY, USA.

Diversity analysis

DNA was extracted following the CTAB wheat genomic DNA extraction protocol (Doyle and Doyle, 1990). Seven SSR primers; Xpsp2999, Xpsp3000, Xgwm312, Xgwm33, Xgwm44, Xgwm533, and Xgwm674, of wheat were selected from the Grain Genes Database to investigate the experimental lines' diversity along with BARI GOM-28. Polymerase Chain Reactions (PCR) were obtained with the following thermal profile: annealing temperature according to SSR primers, e.g., Xpsp2999 (55° C), Xpsp3000 (55° C), Xgwm312 (55° C), Xgwm33 (56.9° C), Xgwm44 (56.1° C), Xgwm533 (53.3° C), and Xgwm674, (53.8° C). Dendrogram (UPGMA) was constructed based on Jaccard index coefficient similarity and distance matrix, using an online software DendroUPGMA (DendroUPGMA: Dendrogram construction using the UPGMA algorithm (urv.cat)). http://genomes.urv.cat/UPGMA/

RESULTS

Development of EMS-mutagenized wheat population

To determine a suitable EMS concentration required for generating enough mutant population, a germination test was performed and mutation frequency was observed. However, in this study EMS concentrations of 1.0%, 1.1% and 1.2%, corresponded to an inadequately low germination rate of 19%, 8% and 4% respectively (Fig. 1). Besides, seed treatments with less concentrated EMS solutions of 0.8% and 0.9%, a more preferable germination rate of 43% and 31% were observed respectively. The maximum mutation frequency (26%), was observed in seeds treated with 1.2% EMS concentration, while 18% mutation was noted when seeds were treated with 0.8% EMS solution. Afterward, a total of 16,000 seeds were treated with 0.8% EMS and germinated in pots. Around 6,400 seeds germinated, and the plants were allowed to grow to maturity. In this M_1 generation, 2,957 different lines survived and M_2 seeds were harvested. These lines were then advanced till M_4 generation, and 1,581 lines remained (Fig. 2).

Field evaluation of the mutant lines

The 1581 lines from M_4 generation, along with BARI GOM-28 as a parental, were subjected to field screening in 2016-17, and subsequently, 64 lines were selected for performance yield trial (PYT) in 2017-18 that outperformed the BARI GOM-28 (data not shown). From this trial, 11 lines were selected that showed significantly better performance in different agronomic parameters than BARI GOM-28. Two subsequent observational yield trial was performed with 11 selected lines during 2018-19 and 2019-20.

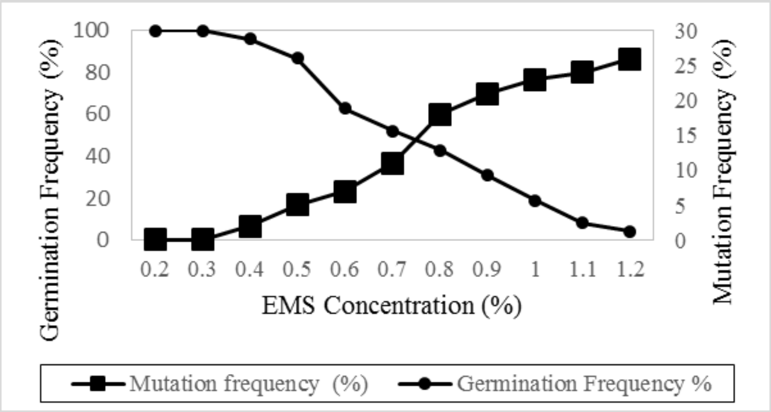

Figure 1. Optimization of EMS concentration along with mutation frequency of BARI GOM-28. EMS concentration was considered from 0.2% to 1.2% where 96% mortality and 26% mutation frequency was observed at 1.2% concentration.

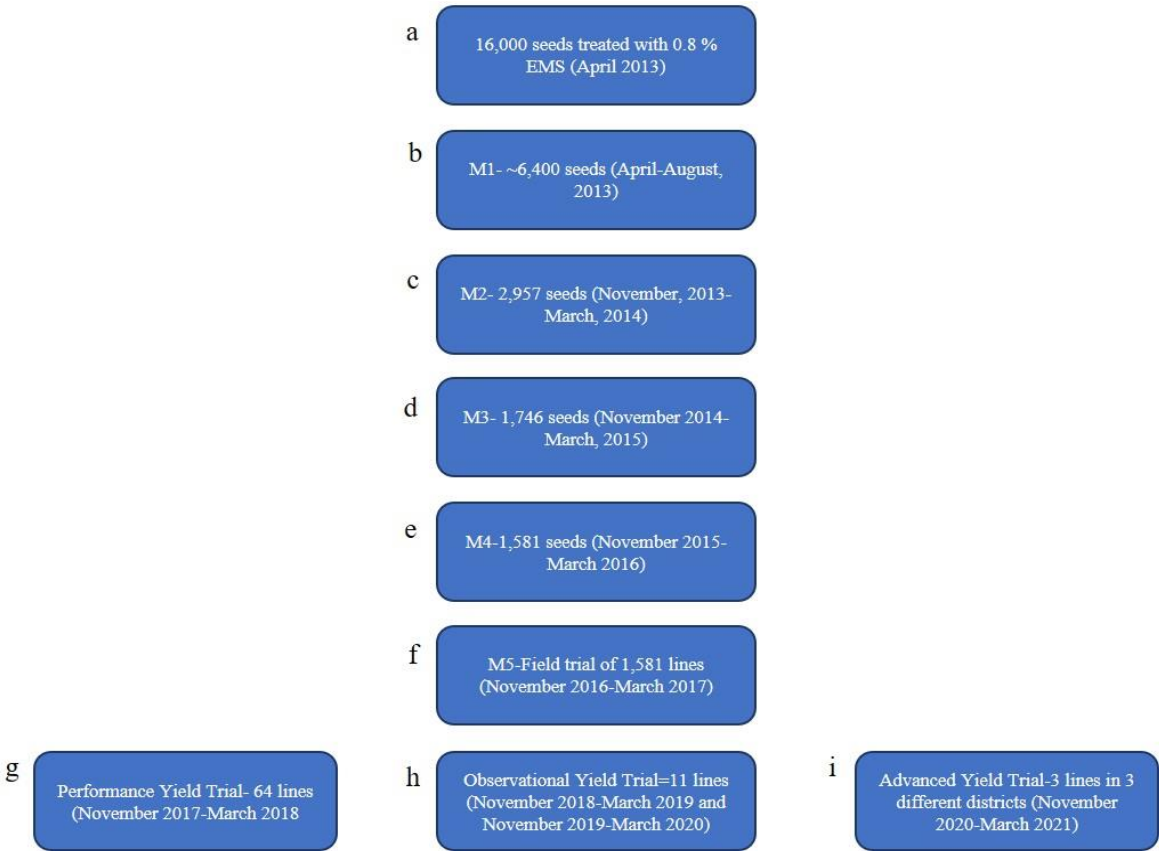

Figure 2. Schematic representation of EMS-mutagenized wheat population development followed by agronomic characterization. a) creation of mutant lines, b-e) propagation of mutant lines, f) evaluation of mutant lines in the field, g-i) agronomic performance trial of the selected mutant lines.

Heading and Plant height

In BARI GOM-28, the 1st heading was observed within 65-66 days after plantation (DAP), whereas 50% and 100% of the heading occurred by 67-68 DAP and 70-71 DAP, respectively (Table 2) which was similar to mutant lines 0023/17 and 0037/17. Whereas 0026/17, 0038/17 and 0065/17 showed the most delayed heading dates in 70-71 DAP for 1st heading and 75-77 days for 100% heading. The plants of 0020/17, 0037/17, 0043/17, 0036/17 and 0023/17 were significantly taller than BARI GOM-28 (Table 3). During 2019-20, 0020/17 gave rise to the highest plant height with an average of 98.97 cm, and the lowest height was recorded for 0030/17 with around 78.77 cm.

Spike morphology

A significant difference was observed among the 3 trials in the lines 0020/17 (6.37-6.63) and 0037/17 (6.60-6.80) compared to BARI GOM-28 (4.97-5.40), at *p* (0.05), in terms of the number of spikes per plant in 3 trial season (Table 3). Almost all the other lines had no significant differences in the number of spikes per plant with BARI GOM-28. The plants of 0020/17, 0037/17, 0019/17, 0043/17, 0036/17, 0026/17 and 0023/17 showed significantly longer spikes than BARI GOM-28 in all the 3 trials (Table 3). None of the selected mutant lines had shorter spikes than BARI GOM-28. The longest spikes were observed in line 0037/17, ranged 13.00 cm to 13.12 cm (Table 3) followed by line 0020/17 (12.67-12.90 cm), and 0023/17 (12.64-13.23 cm) in 3 consecutive seasons 2017-2020. Again, a significantly higher number of spikelets was observed in 0020/17, 0037/17, 0026/17 and 0023/17 than BARI GOM-28 in all the trials. On the other hand, 0050/17, 0030/17, 0036/17, 0038/17 and 0065/17 were found not significant in all the seasons at *p* (0.05) with regards to BARI GOM-28 (14.0). The maximum number of spikelets per spike was recorded for 0037/17 (19.96) in 2019-2020 and the lowest average number, 13.24, was recorded for 0065/17 (Table 3).

Table 2. Heading data of BARI GOM-28 and 11 selected mutant lines in days after plantation (DAP) during Observational Yield Trial (OYT)

Lines	1st heading			50% heading			100% heading		
	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
BARI GOM-28	66	65	65	68	68	67	71	71	70
0019/17	68	68	68	71	71	71	74	74	74
0020/17	70	70	70	72	73	72	75	76	75
0023/17	65	65	65	68	67	67	71	70	70
0026/17	71	70	70	72	72	72	75	75	75
0030/17	68	68	67	71	71	71	75	74	74
0036/17	68	67	67	71	70	70	74	73	73
0037/17	65	65	66	67	67	68	70	70	71
0038/17	70	70	70	72	72	73	75	75	76
0043/17	67	68	68	70	70	70	72	72	72
0050/17	66	67	67	70	70	70	73	72	73
0065/17	71	71	70	74	74	73	77	77	76

Grain parameters

Only 3 mutant lines, namely, 0020/17 (56.17-59.77), 0037/17 (59.33-59.90) and 0023/17 (53.37-54.62), consistently showed significantly higher numbers of grain per spike than the BARI GOM 28 during the 3 trial seasons (Table 3). The mutant line 0037/17 showed the best performance for this parameter, and 0050/17 (43.79-45.10) showed the least (Table 3). Moreover, the lowest weight of grains per plant in this study was found in 0038/17, 10.10 g, 8.96 g, and 9.13 g, which was significantly lower than the BARI GOM-28, 14.50 g, 14.33 g, and 14.57 g, respectively, in the subsequent 3 trial seasons. Whereas, only 3 mutant lines, 0020/17, 0037/17 and 0023/17, were found in this study that had a significantly higher weight of grains per plant compared to BARI GOM-28 for all the 3 seasons. However, the 0037/17 line had the maximum weight of grains per plant ranging from 20.37 to 21.70 g which prevailed as it always had the highest weight of grains per plant in all three seasons (Table 3). Furthermore, the total yield not only relies on the number of grains but also on the weight of the grains. Among all, 4 mutant lines, e.g., 0037/17 (65.27-65.51 g), 0020/17 (64.18-64.26 g), 0023/17 (60.81-61.20 g) and 0036/17 (60.09-60.16 g) showed significantly higher, at *p* (0.05), 1000-grain weight than BARI GOM-28 (54.24-54.37 g) in all the seasons.

Yield potential

The yield potential of all the selected mutant lines, except for 0038/17 (3.49 t ha^{-1}) and 0026/17 (3.42 t ha^{-1}), were significantly higher than BARI GOM-28 (3.40 t ha^{-1}) at 95% probability level in 2017-18. Contrastingly, in 2019-20, 0038/17 had a significantly higher yield potential but 0026/17 had a significantly reduced yield potential than BARI GOM-28. In 2018-19, lines 0037/17 and 0020/17 showed maximum yield potentials of 6.13 t $ha^{-1}$and 5.75 t ha^{-1}, respectively, whereas BARI GOM-28 correspondingly reached a maximum yield potential of 3.82 t ha^{-1} in the same season (Table 3). Similar to OYTs, in advanced yield trial 0037/17 showed the highest yield potential of 6.10 t ha^{-1} in Panchagarh and was significantly different from BARI GOM 28; 3.69 t ha^{-1} (Table 6). Among the 3 locations, 0037/17 always had the highest yield followed by 0020/17 (5.54 t ha^{-1}) and 0023/17 (5.20 t ha^{-1}).

Quality parameters

In terms of wet gluten content, all the mutant lines, except 0038/17 (20.53%) had higher wet gluten content than BARI GOM-28; 22.00%. Line 0037/17 showed superiority for its wet gluten content, 32.02%. The gluten index value was the highest for 0038/17 (95.19) and lowest for 0020/17 (64.14), in contrast to the BARI GOM-28 (92.88). Furthermore, BARI GOM-28 contained approximately 11.04% total protein, which was similar to 0038/17, 0043/17, 0030/17, 0036/17, and 0065/17, whereas total protein content was highest in 0037/17, 0019/17, and 0023/17, with values exceeding 13%.

Table 3. Diversity of important agronomic parameters in selected 11 mutant lines and BARI GOM-28 during Observational Yield Trial (OYT)

Material	Number of grains spike^{-1}			Weight of grains plant^{-1} (g)			1000-grain weight (g)			Yield potential (t ha^{-1})		
	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
BARI GOM-28	43.03±0.917a	39.26±1.568a	40.63±1.346ac	14.50±0.531ad	14.33±0.937ab	14.57±0.858ac	54.34±0.047a	54.24±0.032a	54.37±0.055a	3.40±0.030a	3.82±0.030a	3.61±0.027a
0019/17	48.67±1.040c	46.53±1.504ac	46.24±1.584ac	9.77±0.552f	11.87±0.686ab	12.00±0.649acd	50.79±0.149d	50.59±0.160d	50.60±0.161e	4.03±0.031d	4.08±0.029d	3.99±0.029d
0020/17	59.77±0.912b	56.17±1.122b	57.41±0.991b	19.90±0.959b	19.13±1.146b	20.27±1.081b	64.18±0.092b	64.24±0.110b	64.26±0.076b	5.68±0.027b	5.75±0.031b	5.69±0.028b
0023/17	53.37±0.751d	54.62±1.681d	53.53±1.475d	15.90±0.790c	15.03±0.660c	15.73±0.767d	60.81±0.085h	61.20±0.091h	61.2±0.087h	5.34±0.076g	5.29±0.114i	5.29±0.096g
0026/17	48.37±0.644c	48.17±2.004ac	49.81±1.345c	11.77±0.701ef	11.33±0.690ab	11.30±0.671ade	48.90±0.746f	49.00±0.610f	48.66±0.607g	3.42±0.056af	4.05±0.067g	3.25±0.074e
0030/17	44.17±0.791a	45.56±1.415ac	43.68±1.347ac	12.67±0.598ae	11.80±0.572ab	12.40±0.626ac	50.38±0.059e	50.28±0.061d	50.06±0.047a	4.39±0.037g	4.13d±0.037g	3.80±0.040d
0036/17	42.63±0.789a	41.59±1.158c	41.66±1.050ac	14.07±0.686acd	13.70±0.735c	13.83±0.576cd	60.16±0.237g	60.16±0.240h	60.09±0.220f	4.82±0.048h	4.95±0.038h	4.77±0.043f
0037/17	59.50±0.853b	59.33±1.238b	59.90±1.146b	20.37±0.792b	20.60±1.058b	21.70±1.074b	65.27±0.138c	65.51±0.170c	65.38±0.159c	6.12±0.029c	6.13±0.030c	6.02±0.027c
0038/17	44.47±0.800a	43.49±1.739a	44.21±1.345a	10.10±0.497f	8.96±0.483b	9.13±0.457e	48.29±0.292f	48.70±0.210g	48.44±0.220a	3.49±0.040af	3.39±0.031f	3.88±0.036d
0043/17	47.67±0.883c	46.74±2.830cd	46.20±1.440ac	13.70±0.869ade	12.10±0.946ab	12.27±0.889acd	50.52±0.067e	50.79±0.070d	50.66±0.082e	4.10±0.015eg	4.03±0.016g	3.89±0.017d
0050/17	45.10±0.814a	44.28±1.877a	43.79±1.860a	11.67±0.746ef	11.30±0.716ab	11.50±0.691acd	51.83±0.086e	51.68±0.094e	51.78±0.118d	4.01±0.020e	4.09±0.014dg	3.90±0.014d
0065/17	41.43±0.861a	39.81±1.811ac	40.90±1.764a	10.50±0.498f	10.03±0.517b	10.00±0.491de	48.35±0.108f	48.39±0.099f	48.43±0.092e	3.54±0.027f	3.23±0.027e	3.35±0.029e
Mean	48.18	47.13	47.37	13.74	13.35	13.73	54.49	54.57	54.49	4.36	4.41	4.29
P (Y)		ns			ns			ns			ns	
P (VxY)		ns			ns			ns			ns	

Material	Plant height (cm)			Spike length (cm)			Number of spikes plant^{-1}			Number of spikelets spike^{-1}		
	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20	2017-18	2018-19	2019-20
BARI GOM-28	85.83±1.427a	86.43±1.320a	88.37±0.651a	7.83±0.240a	8.13±0.214a	8.16±0.219a	5.23±0.257a	5.40±0.256a	4.97±0.251a	14.47±0.459ac	13.90±0.324ac	13.98±0.339ac
0019/17	81.27±1.446c	81.36±1.341f	80.90±1.419d	9.49±0.198ce	9.27±0.194c	9.25±0.173de	5.87±0.248abc	5.80±0.256abc	5.78±0.218cd	16.67±0.281e	15.20±0.343dfg	15.13±0.331c
0020/17	97.07±1.398b	98.17±1.318b	98.97±0.819b	12.67±0.429b	12.87±0.206b	12.90±0.222b	6.37±0.397b	6.50±0.436bc	6.63±0.419bef	19.80±0.227b	19.60±0.246b	19.83±0.209b
0023/17	97.13±1.059b	96.58±1.048b	96.30±1.010b	13.23±0.376b	12.65±0.246b	12.68±0.250b	6.00±0.296abc	6.13±0.295abc	6.06±0.283bdef	18.57±0.233f	19.25±0.308b	19.31±0.283b
0026/17	80.57±0.612c	81.47±0.612f	81.20±0.640d	9.66±0.114ef	9.67±0.173c	9.74±0.162e	5.87±0.278abc	5.70±0.276ab	5.10±0.277ac	15.73±0.355d	15.55±0.207fg	15.59±0.179de
0030/17	79.18±1.270c	78.80±1.270f	78.77±1.283d	8.43±0.272ad	8.62±0.175a	8.63±0.231ac	5.70±0.272abc	5.37±0.232a	4.93±0.230a	14.83±0.356ac	14.91±0.257dfg	14.97±0.258cde
0036/17	94.50±0.731bf	94.47±0.731be	93.93±0.606c	10.38±0.252f	10.37±0.164d	10.17±0.163f	5.60±0.290ab	5.56±0.290ab	5.47±0.261acd	14.50±0.295ac	14.68±0.203cdf	14.81±0.227cd
0037/17	95.33±1.106b	95.57±1.106b	94.33±0.736c	13.00±0.134b	13.12±0.152b	13.07±0.209b	6.60±0.344c	6.67±0.340c	6.80±0.337bef	19.93±0.253b	19.86±0.429b	19.96±0.423bde
0038/17	85.87±1.047a	85.98±1.047a	86.10±0.968a	8.87±0.345cde	8.57±0.177a	8.67±0.182acd	5.50±0.328ab	5.50±0.328a	5.13±0.295ac	14.67±0.277ac	14.64±0.285cdf	14.65±0.278cd
0043/17	91.87±1.036ef	91.54±1.036de	91.90±0.993c	9.41±0.277ce	9.29±0.201c	9.39±0.206e	5.60±0.351ab	5.63±0.347ab	5.23±0.238acd	15.43±0.298cd	15.81±0.386g	15.82±0.401e
0050/17	89.43±1.021de	89.23±1.021de	88.30±0.668a	8.79±0.250cd	8.64±0.209a	8.81±0.197cd	5.60±a0.261b	5.80±0.285abc	6.33±0.264bef	14.77±0.274ac	14.36±0.313cd	14.36±0.339c
0065/17	86.63±0.857ad	87.10±0.857ad	87.30±0.893a	8.31±0.235ad	8.36±0.190a	8.36±0.172ac	5.67±0.182abc	5.80±0.211abc	6.03±0.217bdef	13.97±0.297a	13.36±0.288a	13.24±0.282a
Mean	88.72	88.89	88.86	10.01	9.96	9.99	5.80	5.82	5.71	16.11	15.93	15.97
P(Y)		ns			ns			ns			ns	
P(VxY)		ns			ns			ns			ns	

Different letters in the same column indicate a significant difference according to the Tukey HSD test at P= 0.05; ns=non-significant, P(Y) and P(VxY) demonstrate the year-wise difference between the lines

Table 4. Wet gluten content, gluten index, and total protein content of 11 selected mutant lines and BARI GOM-28 during Observational Yield Trial (OYT)

Lines	Parameters		
	Wet gluten (%)	Gluten index	Total protein (%)
BARI GOM-28	22.00±0.333a	92.88±0.167a	11.04±0.033a
0019/17	29.44±0.030hi	89.58±0.033g	13.00±0.010b
0020/17	28.85±0.207gh	64.14±0.032c	12.96±0.023b
0023/17	30.22±0.007i	76.26±0.044d	13.11±0.013b
0026/17	23.56±0.073c	92.49±0.067a	10.05±0.029d
0030/17	27.89±0.033f	85.15±0.088e	11.05±0.013a
0036/17	26.65±0.058e	94.78±0.120h	11.03±0.033a
0037/17	32.02±0.033j	83.24±0.100b	13.20±0.020b
0038/17	20.53±0.067b	95.19±0.049h	11.10±0.027a
0043/17	28.62±0.067fg	86.04±0.029f	11.15±0.029a
0050/17	22.94±0.100c	92.27±0.044a	9.16±0.019c
0065/17	25.53±0.067d	86.53±0.058f	11.07±0.032a

Different letters in the same column indicate a significant difference according to the Tukey HSD test at P= 0.05

Correlation between total protein content and wet gluten content

There was a significant positive correlation observed between total protein and gluten content in the tested 11 mutant lines and BARI GOM-28 (Fig. 4; r^2=0.8514) in the 2019-2020 growing years (Table 4). The protein content was almost 85% determined by the gluten content and obtained regression equation $y = 0.3338x + 2.7943$

Dendrogram

UPGMA dendrogram indicates the segregation of 11 lines from BARI GOM-28 into two main clusters: cluster 1 & cluster 2 (Fig. 5). In cluster 2: line 0043/17, 0065/17 & 0030/17 are present with a minimum distance of 0.000 indicating similar characteristics and are diversified in character from BARI GOM-28.

Ranking of the selected lines

The ranking system was aimed to select lines with the best combination of different desired yield components and flour milling quality (Table 5). Based on the ranking value, we determined that 0037/17, 0020/17 and 0023/17 lines are overall the most elite ones considering all the attributes.

Table 5. Scoring of the 11 mutant wheat lines based on important characteristics and ranking during Observational Yield Trial (OYT)

Line	Plant height	Spike length	Number of spikes plant^{-1}	Number of spikelets spike^{-1}	Number of grains spike^{-1}	1000-grains weight	Wet gluten content	Gluten index	Total score	Rank
BARI GOM-28	2	1	1	1	1	2	1	3	12	7th
0019/17	2	1	2	2	1	2	3	3	16	4th
0020/17	1	3	3	3	3	3	3	2	**21**	**2nd**
0023/17	1	2	3	3	2	3	3	2	19	3rd
0026/17	2	1	2	2	1	1	1	3	13	6th
0030/17	3	1	1	1	1	2	2	3	14	5th
0036/17	1	2	2	1	1	2	2	3	14	5th
0037/17	1	3	3	3	3	3	3	3	**22**	**1st**
0038/17	2	1	1	1	1	1	1	3	11	8th
0043/17	1	1	2	2	1	1	3	3	14	5th
0050/17	2	1	2	1	1	2	1	3	13	6th
0065/17	2	1	2	1	1	1	2	3	13	6th

Plant Height: 75-85=3, 85-95=2, 95+=1; Spike length: 7-10 cm=1, 10-12 cm=2, 12+ cm=3; Number of spikes per plant: 4.5-5.5=1, 5.5-6.0=2, 6+=3; Spikelets per spike: 13-15=1, 15-18=2, 18+=3; Grains per spike: 35-45=1, 45-55=2, 55+=3; 1000-grains weight: 45-50g=1, 50-60g=2, 60g+=3; Wet gluten content: 20-24%=1, 24-28%=2, 28%+=3; Gluten index= <50=1, 50-80=2, >80=3

DISCUSSION

In this study, we aimed to develop industrial-type wheat lines that would meet both production and quality demands. Due to the lack of genetic variability in local cultivars, we adopted mutation breeding technology to generate a mutant population of wheat and characterize them. The seeds of BARI GOM-28 were treated with different concentrations of EMS to find out the optimal dose to generate about 40% germination, which was obtained with 0.8% of EMS (Fig. 1). This aligned with a previous study where a 40% germination rate was obtained in hexaploidy wheat with a similar dose of EMS (Chen et al., 2012). However, in this study, we found some lines that had similar headings to the BARI GOM-28, such as 0037/17, 0023/17, 0050/17, 0043/17, 0036/17, and 0030/17. The heading date in mutant lines is reported to be greatly affected by the genotype

(Yamagata et al., 1989). Furthermore, we obtained lines with a late heading date and also a similar heading date to the BARI GOM-28. This result indicates that mutation might have an impact on the heading date of some lines of the mutant population, while others remain ineffective. Moreover, the increased number of spikelets per plant and grains per plant is positively correlated with spike length and yield potentiality (Sakin et al., 2005). On the other hand, we observed that the lines taller than BARI GOM-28, e.g., 0037/17, 0020/17, 0043/17, and 0023/17, also had considerably longer spikes. Though some lines were shorter than BARI GOM-28, they had longer spikes (0019/17, 0030/17, 0026/17) as well. The lines that had spike length above 12 cm, i.e. 0020/17, 0037/17, and 0023/17, had about 19 spikelets and 45-60 grains per spike, which were significantly higher than the plants with shorter spikes and fewer plant height (Table 3). Improvement in yield performance in wheat by increasing the number of grains per spike has been reported (Flohr et al., 2018). As a result, we regarded increased spike length as a positive characteristic for increasing yield potential. Additionally, we found several mutant lines having a significantly higher number of spikes per plant, thus suggesting higher yield potential than the BARI GOM-28 (Table 3). Several tiller-inhibiting (Wang et al., 2018) and tiller-promoting (Naruoka et al., 2011) genes contributing to the enhancement of the number of spikes per plant have been identified. Therefore, we speculate that mutation might have disrupted the function of tiller inhibition genes or promoted the activity of promoting genes.

Table 6. Yield potential of best 3 mutant lines and BARI GOM-28 in 3 different districts of Dinajpur region

Location	Yield potential (t ha^{-1})		
	Dinajpur	Thakurgaon	Panchagarh
BARI GOM-28	3.63±0.115a	3.68±0.067a	3.69±0.120a
0020/17	5.54±0.088b	5.51±0.033b	5.47±0.088b
0023/17	5.11±0.040d	4.97±0.042d	5.20±0.030d
0037/17	5.94±0.033c	5.98±0.039c	6.10±0.027c
P (L)	ns	ns	ns
P(LxY)	ns	ns	ns

Different letters in the same column indicate a significant difference according to Tukey's test at P = 0.05, ns= non-significant, P(L) and P(LxY) demonstrate the location-wise yield difference in the same line

Finally, the grain weight was taken to find the best lines with multiple agronomic characteristics, which in turn will lead to higher yield potential. Many studies showed that improved grain weight has a direct positive correlation with yield (Aisawi et al., 2015). It is proven that selecting lines with a greater spike length and a higher number of spikelets per spike results in a higher number of grains along with a heavier 1000-grain weight (Gaju et al., 2009). We also found that the lines with longer spike lengths had more spikelets per spike, a higher number of grains, as well as bold grains (Fig. 3 and Table 3). Line 0037/17 had the longest spikes, the greatest 1000-grain weight (65.51 g), and also had the maximum weight of grains per plant, 21.70 g. Through selecting all these agronomic characteristics, we were able to conclude that all the 11 selected mutant lines have greater yield potential than the check.

Figure 3. Differences in a) plant height, b) root biomass c) spike length, d) spikelet shape and e) grain size among BARI GOM-28, and the best 2 mutant lines, 0020/17 and 0037/17. Here, 1= BARI GOM-28, 2=0037/17 and 3=0020/17.

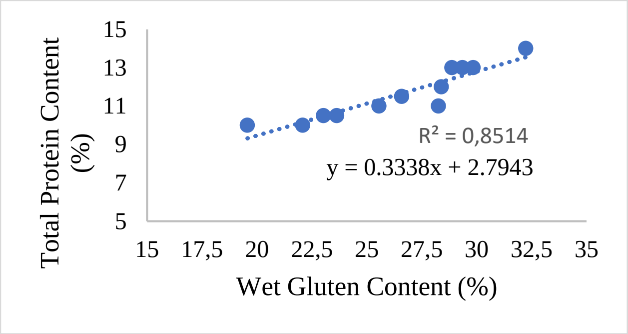

Figure 4. Correlation between total protein content and wet gluten content of BARI GOM-28 and 11 selected mutant lines

In order to achieve another major goal, to meet the wheat quality demand of the local industries, we performed various quality parameters on these 11 selected mutant lines (Table 5). Usually, a minimum of 25% wet gluten content is required for quality bread production (Bojnanska and Francakova, 2002). Eight mutant lines have more than 25% wet gluten content, whereas BARI GOM-28 had 22%. For instance, > 13% of protein content is required for pasta preparation, 10–12% is required for different kinds of bread, 8–9% is needed for cookies, and 10–13% is used for different kinds of noodles (Uthayakumaran and Wrigley, 2017). The total protein content varied among the selected 11 mutant lines in this study (Table 5). Three lines, 0037/17, 0019/17, and 0023/17, had ≥13.0% of total protein. Besides, the gluten index value of 80-100% indicates strong gluten, whereas 50-79% and less than 50% indicate medium to weak gluten. For wafers, a medium gluten index value is preferable (Oikonomou et al., 2015). In our study, although 0020/17 had more wet gluten content than the BARI GOM-28, it had a gluten index value of medium strength, whereas the gluten index of BARI GOM-28 is of high strength (Table 5). Afterward, 3 lines, namely, 0037/17, 0020/17, and 0023/17 were finally chosen as high-yielding gluten-enriched lines. These 3 lines and BARI Gom 28 were subjected to multi-location AYT within the Dinajpur region and we found that all the 3 chosen lines always outperformed the parent. The 0037/17 line showed the highest yield like PYT and OYTs followed by 0020/17 and 0023/17 (Table 6). Since the Dinajpur region is the wheat-growing region of Bangladesh, the high-yielding potentiality of the selected mutant lines may help to increase the total wheat production in the country (Shirazy et al., 2017). Moreover, according to UPGMA analysis represents that lines 0043/17, 0065/17, and 0030/17 are found in cluster 2 with a minimum distance of 0.000, showing comparable features and being different from BARI GOM-28 in characters. In contrast, the rest of the lines are in cluster 1 which is further branched into two sub-clusters. Only line 0019/17, from cluster 1, is in sub-cluster I. On the other hand, the rest of the lines are in sub-cluster II (Fig. 5)

Therefore, our developed mutant lines will play a vital role in meeting the demand for higher productivity and enhanced grain qualities that would also be suitable for industrial purposes. The creation of mutant lines of wheat using EMS has been carried out for a long time, and developing such mutant lines has not only proven to be effective for breeding purposes (Sakin et al., 2005) but also plays a vital role in forward and reverse genetic studies (Chen et al., 2012; Kong et al., 2020).

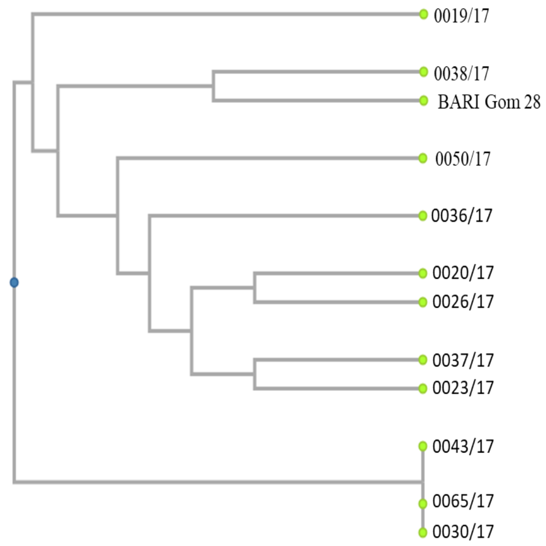

Figure 5. UPGMA dendrogram based on Jaccard index coefficient similarity and distance matrix, summarizing the data on the differentiation between 11 selected wheat lines and BARI GOM-28 according to gel electrophoresis bands obtained by using Xpsp 3000 SSR markers.

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