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Experimental study on biochemical parameters and yield of M_4 Indian mustard mutants

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Abstract
To study the proximate biochemical composition and yield of twenty M_4 Indian mustard mutants, an experiment was conducted and work was subjected to the comparative evaluation of its chemical and biochemical parameters viz., total chlorophyll, nitrogen, phosphorus, potassium, protein, and oil. Among these twenty mutants, the highest chlorophyll, N, P, K at 25, 45, and 65 DAS, and protein and oil after harvesting was obtained from ACM_{18}, ACM_{12}, ACM_6, ACM_8 and ACM_4. In case of proximate analysis, the highest chlorophyll, N, P, K, protein and oil were recorded from ACM_{18}. The oil content of different mutants of mustard varied from (33.30 - 42.67%). ACM_{18} (28.13 %) contained the highest amount of seed protein. All the chemical and biochemical traits were showed highly significant and positive correlation with seed yield. Substantial genetic variability exists for chemical composition and nutritional traits which could be utilized to suggest the future strategy. Hence, these five mutants are recommended for breeding programme and testing.

Keywords: Mustard mutants, morpho-physiological parameters, yield

Introduction
Mustard specifically refers to *Brassica juncea* and *Erucasativa*. There are considerable differences in agronomic characteristics, yield and composition of seed oil between species and between varieties. Mustard is the third major source of edible vegetable oils in the world, after soybean and oily palm (Anonymous, 2011) [2]. The tender leaves of these cultivars serve as vegetable, while the seeds as a source of lubricating and cooking oil. The residue left after oil extraction (i.e., oil cake or meal) being rich in protein be used as livestock feed. It produces 9 k cal energy from 1g of oil per unit in comparison with other diets (carbohydrate and Protein). In a balanced diet for human health 20-25% of calories should come from fats and oils. The protein quality and quantity of *B. campestries* obtained oil cake is high. (Chowdhury *et al.*, 2014) [4]. Mustard is a high yielding oilseed with a reasonably high content of oil (Riley, 2004) [14]. Mustard seeds have high energy content, having 28-32% oil with relatively high protein content (28-36%).
The present research work has been designed to study biochemical analysis as well as their interrelation to the seed yield.

Materials and Methods
Dry healthy seeds of *Brassica juncea* (L.) Pusa bold and Bio-902 treated with gamma rays and EMS. The gamma rays treatment of 900, 1000, 1100, 1200, 1300Gy (Co^{60}) was done at BARC Trombay, Mumbai. Each of these treatments were treated with 0.5 per cent aqueous solution of EMS. The M_1 generation was raised during 2014-15 and individual plant in each treatment were harvested separately. The harvested seeds were used to raise M_2 generation.
During *rabi* 2015-16 mutants were identified from Pusa bold and Bio-902 (M_2 generation), these identified mutants along with 2 check (Pusa bold Bio-902.) were used in M_3 generation during *rabi* 2016-17.
The true breeding and stable mutants selected from M_3 generation were evaluated for biochemical and yield traits in M_4 generation in RBD with 3 replications during *rabi* 2017-18.The field experiment was laid out in Randomized Block Design (RBD) with three replications consisting of twenty two mutants. Observations on total chlorophyll, N, P, K were recorded at 25,45, and 65 DAS. Observations on seed oil and protein content were also recorded. Simple correlation of all chemical and biochemical parameters with yield was calculated as per following formula.

$$r_g = \frac{\text{gcov x y}}{(\delta_g x)\ (\delta_g y)}$$

r_g = genotypic correlation coefficient.
gcov x y = genotypic covariance between the character x and y
$\delta_g x$= genotypic standard deviation of x
$\delta_g y$= genotypic standard deviation of y

The chemical and biochemical parameters *viz*., leaf chlorophyll, nitrogen, phosphorus and seed protein content were estimated and recorded. Total chlorophyll content of dried leaves was estimated by colorimetric method as suggested by Bruinsma (1982) [3]. Nitrogen content in leaves was determined by micro-kjeldhal's method as given by Somichi *et al.* (1972) [16]. Phosphorus content in leaves was determined by vanadomolybdate yellow colour method as given by Jackson (1967). Potassium content in leaves was determined by flame photometer by di-acid extract method given by Jackson (1967). Nitrogen content in seed was determined by micro-kjeldhal's method as given by Somichi *et al.* (1972) [16] and same was converted into crude protein by multiplying 'N' per cent with factor 6.25. Oil content in seeds was determined by soxhlet's procedure as given by Sankaran, (1965). Seed yield hectare^{-1} was also recorded in quintal (q).The data were analyzed as per the method suggested by Panse and Sukhatme (1954) [11].

Results and discussion
Chemical and biochemical parameters
Chlorophyll content in leaves
At 25 DAS chlorophyll content in leaves ranged from 0.33 mg g^{-1} - 0.52 mg g^{-1}. Significantly maximum chlorophyll was found in mutants ACM_{18}, ACM_{12}, ACM_6, ACM_8, ACM_4, ACM_7, ACM_{10}, ACM_5, ACM_3, ACM_{20}, ACM_{19}, ACM_1 and ACM_2 (0.52, 0.52, 0.51, 0.50, 0.50, 0.49, 0.49, 0.47, 0.47, 0.46, 0.45, 0.44, 0.44 mg g^{-1} respectively). Similarly mutants, ACM_{11}, ACM_{14}, ACM_9 and ACM_{17} recorded moderate chlorophyll content in a descending manner when compared with check Bio-902 and Pusa bold. Mutants ACM_{13}, ACM_{15} and ACM_{16} recorded minimum chlorophyll content at this stage of observation and found at par with check Bio-902.
At 45 DAS chlorophyll content in leaves was increased as compared to 25 DAS stage and it ranged from 0.98 mg g^{-1} – 1.75 mg g^{-1}. Mutants ACM_{18}, ACM_{12} and ACM_6 produced significantly highest chlorophyll (1.75, 1.57, 1.56 mg g^{-1} respectively). Mutants ACM_8, ACM_4, ACM_7, ACM_{10}, ACM_5, ACM_3, ACM_{20}, ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14} and ACM_9 showed significantly moderate range of chlorophyll content when compared with check Bio-902 and Pusa bold. Mutants ACM_{13}, ACM_{15} and ACM_{16} recorded minimum chlorophyll at this stage of observation and found at par with check Bio-902.
At 65 DAS chlorophyll content in leaves was drastically reduced in all mutants and it ranged from 0.86–1.51 mg g^{-1}. Significantly more chlorophyll content was observed in mutants ACM_{18}, ACM_{12}, ACM_6, ACM_8, ACM_4 and ACM_7 (1.51, 1.48, 1.36, 1.34, 1.33, 1.31mg g^{-1} respectively). Mutants ACM_{10}, ACM_5, ACM_3, ACM_{20}, ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14} and ACM_9 recorded significantly moderate chlorophyll content in a descending manner when compared with check Bio-902 and Pusa bold, while mutants ACM_{17}, ACM_{13}, ACM_{15} and ACM_{16} recorded minimum chlorophyll content and these mutants were found at par with both the checks in chlorophyll content. The variation in chlorophyll content during flowering stages was significant among the mutants. High yielding genotypes showed superiority in leaf chlorophyll content and photosynthesis, Uddin *et al*. (2012) [17].
The chlorophyll is main pigment involved in photosynthetic process which is responsible for the production of dry matter and is represented by crops photosynthetic efficiency. The chlorophyll is main pigment involved in photosynthetic process which is responsible for the production of dry matter and is represented by crops photosynthetic efficiency.
Leaf chlorophyll content and seed yield were found strongly and positively correlated at all the stages of observations (r = 0.677**, 0.717**, 0.739* at 25, 45, 65 DAS respectively).
Gitte *et al.* (2006) [6] reported positive correlation of chlorophyll content and leaf area plant^{-1} with seed yield of safflower. The role of chlorophyll in the production of assimilates and in turn in governing the seed yield of safflower genotype has been observed as chlorophyll content was positively and significantly associated with harvest index and seed yield, while association of leaf area with harvest index and seed yield was insignificant which indicates its less contribution in sink development than that of chlorophyll content.

Nitrogen content in leaves
Nitrogen is the important constituent of protein and protoplasm and essential for plant growth. Nitrogen deficiency causes chlorosis and malfunctioning of the photosynthesis process. Plant cells require adequate supply of N for normal cell division and growth of the plant. Tender shoots, tips of shoots, buds, leaves contains higher nitrogen (Jain, 2010).
At 25 DAS maximum nitrogen content in leaves was estimated in mutants ACM_{18}, ACM_{12}, ACM_6, ACM_8, ACM4, ACM_7, ACM_{10}, ACM_5 and ACM_3 when compared with checks Bio-902 and Pusa bold. The rest of mutants ACM_{20}, ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14}, ACM_9 and ACM_{17} showed moderate nitrogen content in leaves and were found at par with checks Bio-902 and Pusa bold.
At 45 DAS significantly maximum leaf nitrogen was recorded in mutants ACM_{18}, ACM_{12}, ACM_6, ACM_8, ACM_4, ACM_7 ,ACM_{10} and ACM_5 over checks Bio-902 and Pusa bold. Rest of mutants ACM_3, ACM_{20}, ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14}, ACM_9 and ACM_{17} showed moderate nitrogen content in leaves in descending manner and were found at par with check Bio-902 and Pusa bold.
At 65 DAS significantly maximum leaf nitrogen was recorded in mutants ACM_{18}, ACM_{12}, ACM_6 and ACM_8. Mutants ACM_4, ACM_7, ACM_{10}, ACM_5, ACM_3 and ACM_{20} exhibited significantly moderate leaf nitrogen content but were found superior to checks Bio-902 and Pusa bold. Mutants ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14}, ACM_9 and ACM_{17} recorded minimum nitrogen content in a descending manner and were found at par with checks Bio-902 and Pusa bold.
From this data it is observed that leaf nitrogen content was decreased at 45 and 65 DAS when compared with 25 DAS stage. The decrease in nitrogen content might be due to fact that younger leaves and developing organs, such as seeds act as strong sink demand and may draw heavily nitrogen from older leaves. (Gardner *et al*., 1988) [5].
At (25, 45, 65 DAS) nitrogen content was found highly and positively correlated with seed yield (r = 0.713**, 0.707**, 0.687** at 25, 45, 65 DAS respectively).
Uke *et al*. (2011) [18] and Raut *et al*. (2012) [13] also reported positive and significant correlation of nitrogen content in leaves with seed yield in case of mustard.

Phosphorus content in leaves

At 25 DAS range of phosphorus recorded was 0.26 – 0.47 %. Maximum phosphorus content in leaves was estimated in mutants ACM_{18}, ACM_{12}, ACM_6, ACM_8, ACM_4, ACM_7 ACM_{10}, ACM_5, and ACM_3. Mutants ACM_{19}, ACM_1, ACM_2, ACM_{11} and ACM_{14} also showed significantly moderate phosphorus content in descending manner and these mutants were also found superior over checks Bio-902 and Pusa bold in phosphorus content. But mutants ACM_9, ACM_{17}, ACM_{15} and ACM_{16} recorded minimum phosphorus content and remain at par with the checks.

At 45 DAS range of phosphorus recorded was 0.16 - 0.38 %. Phosphorus content in leaves was significantly more in mutants ACM_{18}, ACM_{12}, ACM_6, ACM_8, ACM_4, ACM_7, ACM_{10} and ACM_5 when compared with checks Bio-902 and Pusa bold and remaining mutants also. Mutants ACM_3, ACM_{20}, ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14}, ACM_9, ACM_{17} and ACM_{13} were recorded significantly moderate phosphorus content and were found at par with checks Bio-902 and Pusa bold. Mutants ACM_{15} and ACM_{16} showed significantly least phosphorus content.

At 65 DAS range of phosphorus recorded was 0.26–0.12 %. Mutants ACM_{18}, ACM_{12}, ACM_6, ACM_8, ACM_4 and ACM_7 produced significantly highest phosphorus content followed by mutants ACM_{10}, ACM_5, ACM_3, ACM_{20}, ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14}, ACM_9 and ACM_{17} and check Bio-902. Similarly checks Bio-902 and Pusa bold also showed moderate phosphorus content. But mutants ACM_{13}, ACM_{15} and ACM_{16} showed least phosphorus content when compared with checks and rest of the mutants.

It is evident from data that phosphorus content in leaves increased gradually upto 45-65 DAS and decreased at 85 DAS. It might be because of translocation of leaf phosphorus and its utilization for development of food storage organ.

Phosphorus content in leaves had shown a very high degree of correlation at 25, 45, 65 DAS with yield (r = 0.809**, 0.816**, 0.801** at 25, 45, 65 DAS respectively).

Uke *et al.* (2011) [18] and Raut *et al.* (2012) [13] also reported significant and positive association of phosphorus content in leaves with seed yield in mustard.

Potassium content in leaves

At 25 DAS and 45 DAS potassium content in twenty mutants and two checks Bio-902 and Pusa bold ranged from 1.72–3.63% and 1.5–2.96% respectively.

Leaf potassium content at 25 DAS was significantly more in mutant ACM_{18} followed by mutants ACM_{12}, ACM_6, ACM_8, ACM_4, ACM_7, ACM_{10}, ACM_5, ACM_3, ACM_{20}, ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14}, ACM_9, ACM_{17} and check Pusa bold. Significantly less potassium content in leaves was noted in mutants ACM_{13}, ACM_{15} and ACM_{16} and these mutants were found at par with check Bio-902.

Leaf potassium content at 45 DAS was significantly more in mutants ACM_{18}, ACM_{12}, ACM_6, ACM_8, ACM_4, ACM_7, ACM_{10} and ACM_5. Mutants ACM_3, ACM_{20}, ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14}, ACM_9 and ACM_{17} also recorded significantly moderate potassium content in leaves in descending manner when compared with checks Bio-902 and Pusa bold. While less potassium content was recorded in mutants ACM_{13}, ACM_{15} and ACM_{16} and were found at par with checks Bio-902 and Pusa bold.

At 65 DAS potassium content in twenty mutants and checks ranged from 1.12 –1.87 %. Leaf potassium content was decreased significantly in all mutants as compared to 25 and 45 DAS stage. It is evident from data that potassium content in leaves increased gradually upto 25-45 DAS and decreased at 65 DAS. It might be because of translocation of leaf phosphorus and its utilization for development of food storage organ. At this stage mutants ACM_{18}, ACM_{12}, ACM_6 and ACM_8 recorded maximum potassium content followed by mutants ACM_4, ACM_7, ACM_{10}, ACM_5, ACM_3, ACM_{20}, ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14} and ACM_9. These all above mentioned mutants were found significantly superior over checks Bio-902 and Pusa bold in potassium content in leaves. But mutants ACM_{13}, ACM_{15} and ACM_{16} were found at par with checks Pusa bold and Bio-902 and recorded minimum potassium in leaves.

Potassium content in leaves had shown a high degree of correlation at 25, 45, 65 DAS with yield (r = 0.687**, 0717**, 0.642** at 25, 45, 65 DAS respectively).

Uke *et al*. (2011) [18] and Raut *et al*. (2012) [13] also reported significant and positive association of potassium content in leaves with seed yield in mustard.

Oil content in seed

Significantly maximum oil content was noticed in mutants ACM_{18}, ACM_{12}, ACM_6, ACM_8, ACM_4, ACM_7, ACM_{10}, ACM_5, ACM_3 and ACM_{20} over checks Bio-902 and Pusa bold. But mutants ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14}, ACM_9, ACM_{17}, ACM_{13}, ACM_{15} and ACM_{16} recorded minimum oil content and were found at par with check Bio-902 and Pusa bold. Oil content exhibited a positively significant correlation with seed yield (r = 0.627**).

Giri (2007) [7] also reported positive and significant correlation of oil content with seed yield in case of mustard, Kadam *et al*. (2009) [8] in case of groundnut, Moon *et al.* (2009) and Anandhan *et al*. (2010) [1] case of sunflower.

Protein content in seed

Significantly highest amount of protein was obtained from mutant ACM_{18} (28.13), followed mutants ACM_{12}, ACM_6, ACM_8, ACM_4, ACM_7, ACM_{10}, ACM_5, ACM_3, ACM_{20}, ACM_{19}, ACM_{20}, ACM_1, ACM_2, ACM_9, ACM_{11} and ACM_{14} in descending manner .These seventeen mutants were exhibited more protein contents over checks Bio-902 and Pusa bold. The Lowest amount of protein content was noticed by mutants ACM_{13}, ACM_{15} and ACM_{16} (22.29). But these three mutants were found at par with both the checks. Nitrogen is the constituent of the protein. Hence, increase in nitrogen content ultimately resulted in the increase in protein content in seeds of mustard in the present investigation.

The present values are more or less similar with the reported values of Chowdhury *et al*. (2014) [4] and Sarker *et al.* (2015) [15], However, these results are lower than those reported by many other authors. Prapakornwiriya and Diosady (2004) determined the protein 45.0% and 34.0% respectively and Sengupta *et al.* (2003) revealed that protein content of mustard were ranged from 44.2-44.7%.

Protein content exhibited a positive and significant correlation with seed yield (r = 0.599**).

Seed yield ha^{-1}(q)

Data recorded for seed yield plant^{-1}, plot^{-1}, ha^{-1} were showed significant variation. Mutants ACM_{18}, ACM_{12}, ACM_6, ACM_8, ACM_4, ACM_7 and ACM_{10} recorded significantly maximum seed yield over checks Bio-902 and Pusa bold and remaining mutants also. Similarly ACM_5, ACM_3, ACM_{20}, ACM_{19}, ACM_1, ACM_2, ACM_{11}, ACM_{14} and ACM_9 were showed significantly moderate seed yield when compared with checks Bio-902 and Pusa bold, while significantly minimum seed yield was recorded by mutants ACM_{17}, ACM_{13}, ACM_{15} and ACM_{16} when compared with two checks and remaining mutants under study.

Malek *et al*. (2017) [9] showed that the three mutants RM-01-07, RM- 10-07 and RM-04-07 produced significantly higher seed yield (1912, 1846 and 1862 kg ha^{-1}, respectively) which was 15.1, 12.1 and 11.1% higher than the mother variety Binasarisha-4 with seed yield of 1661 kg ha^{-1}. These three mutants had also the higher number of siliquae than the mother variety. This suggests that gamma rays irradiation can be fruitfully applied to develop mutants with higher seed yield and other improved agronomic traits in Oleiferous Brassica.

Table 1: Leaf chlorophyll, leaf N, P, K, protein and oil content in seeds of twenty mustard mutants

S. No.	Mutants	Leaf chlorophyll content (mg g^{-1})			Leaf nitrogen content (%)			Leaf phosphorus content (%)			Leaf potassium content (%)			Protein content in seeds (%)	Oil content in seeds (%)	Seed yield hectare^{-1} (q)
		25 DAS	45 DAS	65 DAS	25 DAS	45 DAS	65 DAS	25 DAS	45 DAS	65 DAS	25 DAS	45 DAS	65 DAS			
1	ACM_1	0.44	1.27	1.10	5.11	4.71	3.67	0.36	0.27	0.20	2.53	2.17	1.41	26.88	38	11.98
2	ACM_2	0.44	1.26	1.10	5.10	4.60	3.67	0.35	0.27	0.18	2.53	2.02	1.40	26.67	37.33	11.77
3	ACM_3	0.47	1.32	1.13	5.57	4.83	3.87	0.4	0.32	0.21	2.65	2.26	1.45	27.08	38.67	12.79
4	ACM_4	0.5	1.45	1.33	5.97	5.50	4.13	0.43	0.36	0.22	3.03	2.85	1.57	27.50	39.33	13.80
5	ACM_5	0.47	1.42	1.19	5.90	5.17	3.93	0.4	0.33	0.21	2.91	2.44	1.47	27.29	38.67	13.00
6	ACM_6	0.51	1.56	1.36	6.23	5.60	4.27	0.46	0.37	0.24	3.05	2.92	1.73	27.71	40.00	14.32
7	ACM_7	0.49	1.43	1.31	5.93	5.40	4.07	0.43	0.34	0.22	2.96	2.72	1.53	27.50	39.33	13.53
8	ACM_8	0.5	1.49	1.34	6.20	5.51	4.17	0.46	0.36	0.22	3.03	2.90	1.71	27.71	40.00	14.07
9	ACM_9	0.43	1.24	1.07	4.87	4.37	3.50	0.31	0.25	0.17	2.34	1.90	1.33	25.21	36.00	9.31
10	ACM_{10}	0.49	1.43	1.29	5.90	5.33	4.01	0.41	0.34	0.21	2.92	2.69	1.50	27.5	38.67	13.32
11	ACM_{11}	0.43	1.25	1.08	5.03	4.57	3.63	0.34	0.27	0.18	2.47	1.93	1.38	26.25	37.33	11.50
12	ACM_{12}	0.52	1.57	1.48	6.30	5.70	4.57	0.46	0.38	0.24	3.05	2.94	1.73	27.92	42.00	14.57
13	ACM_{13}	0.38	1.06	0.94	4.53	4.17	3.20	0.27	0.23	0.14	1.77	1.60	1.20	23.33	34.67	9.09
14	ACM_{14}	0.43	1.25	1.07	4.90	4.53	3.60	0.33	0.25	0.17	2.44	1.90	1.37	26.25	36.67	10.97
15	ACM_{15}	0.36	0.99	0.90	4.53	4.03	3.21	0.27	0.19	0.14	1.75	1.58	1.13	22.29	34.67	8.99
16	ACM_{16}	0.33	0.98	0.86	4.33	3.97	3.13	0.26	0.16	0.12	1.72	1.50	1.12	22.29	33.33	7.97
17	ACM_{17}	0.42	1.19	1.02	4.63	4.20	3.37	0.3	0.24	0.16	2.02	1.73	1.27	25.00	36.00	9.47
18	ACM_{18}	0.52	1.75	1.51	6.37	5.77	4.83	0.47	0.38	0.26	3.63	2.96	1.87	28.13	42.67	14.81
19	ACM_{19}	0.45	1.28	1.12	5.23	4.77	3.77	0.37	0.29	0.21	2.59	2.17	1.40	26.88	38.00	12.63
20	ACM_{20}	0.46	1.29	1.12	5.30	4.83	3.87	0.38	0.31	0.21	2.63	2.23	1.44	27.08	38.67	12.68
21	Bio 902	0.41	1.16	0.97	4.60	4.20	3.33	0.29	0.24	0.16	2.91	2.17	1.57	24.79	36.67	9.36
22	Pusa Bold	0.43	1.23	1.05	4.87	4.37	3.47	0.31	0.24	0.17	2.59	2.69	1.40	25.00	36.00	9.79
	SE (m) ±	0.028	0.080	0.072	0.33	0.30	0.23	0.023	0.018	0.012	0.14	0.14	0.09	1.11	1.67	0.54
	CD at 5%	0.080	0.23	0.20	0.95	0.86	0.68	0.066	0.050	0.034	0.42	0.41	0.25	3.16	4.79	1.55
	Correlation	0.677**	0.717**	0.739**	0.713**	0.707**	0.687**	0.809**	0.816**	0.801**	0.687**	0.717**	0.642**	0.599**	0.627**	-

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