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Variation of physiological parameters and yield in rapeseed (*Brassica rapa* var. Toria) genotypes

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Abstract
A field experiment was carried out at the Instructional-cum-Research (ICR) Farm, Assam Agricultural University, Jorhat-13, Assam during *rabi* seasons of 2021-22 and 2022-23 with the aim to study the physiological characters, yield attributes and yield in a set of 22 diverse rapeseed genotypes. The experiment was laid out in randomized block design with three replications comprising of 22 different genotypes of rapeseed including TS-38 (Check), TS-46, TS-67, TS-36, TS-29, TS-75-1, TS-75-1TL, TS-75-2ME, TS-75-2-MM, TS-76-1, TS-76-2, JT-90-1, Panchali, Bhawani, CG Toria-4, TKM-20-1, TKM-20-2, JT-14-5, PT-2018-09, CG Toria-3, Tapeshwari and PT-303. The crops were grown following the recommended package of practice. All the physiological parameters and yield and yield attributes were taken following the standard methodologies. The results (pooled data of two years) of the study indicated a significant variation of physiological parameters *viz*. stomatal frequency, stomatal index, RLWC and chlorophyll content, yield attributes and yield among the genotypes. The genotypes TS-75-2ME and TS-38 exhibited more RLWC, total leaf chlorophyll, more yield and showed superiority in terms of seeds per silique, total number of seed per plant, seed yield per plant and harvest index.

Keywords: Genotypes, physiological parameters, rapeseed, stomatal index, yield attributes

1. Introduction
Rapeseed (*Brassica rapa* var. Toria) is one of the most popular and widely used oil seed crops among the people of Assam and North-East India. It contains 33-45% oil, 18-36% protein and other important fatty acids like linolenic acid, oleic acid, etc. It is also used as vegetable, edible oil, spices, preservatives, seed meal, fertilizer and feed. The total area under rapeseed in Assam is 2.89 lakh hectares with a total production of 1.86 lakh tones and the productivity is 6.44 quintal per hectare (Anonymous, 2022) [3]. Among the seven major annual edible oilseed crops cultivated in India, rapeseed-mustard contributes nearly 30 percent in the total production of oilseeds (Kumar *et al*., 2008) [11]. In the North-Eastern states, though Assam is the highest in terms of area of cultivation of rapeseed, increasing productivity has a major priority in the current scenario as it has the potential of increasing the productivity to great extent (Deka *et al*., 2018) [6]. To fulfil the increasing demand of edible oils, appropriate interventions must be paid for improvement of existing oilseed genotypes or introduction of new genotypes to the region. The suitable genotype for a particular region has to be identified based on physiological efficiency and yield potential. Important physiological characteristics such as Stomatal Index (SI), Relative Leaf water content (RLWC), chlorophyll content etc. can govern the path of increasing productivity of a variety (Malek *et al*., 2012) [12]. A systematic research works on physiological aspects of rapeseed genotypes in Assam is limited. Only a few research works on the existing rapeseed varieties with regard to physiological efficiency has been conducted. Indeed, there is a need to identify the most important physiological parameters which governs the productivity of rapeseed, more particularly in *rabi* season under rainfed condition of Assam.

2. Materials and Methods
The present experiment was carried out at the Instructional-cum-Research (ICR) Farm, Assam Agricultural University, Jorhat-13, Assam. The experimental farm is situated at 26°47 N latitude and 94° 12 E longitudes at an elevation of 86.6 m above mean sea level (MSL). The climate of experimental site of Assam Agricultural University, Jorhat is characterized by subtropical, humid climate with dry summer and cold winter. The soil of the experimental plot

was sandy-loam, acidic pH with medium levels of N, P and K. The seeds were collected from the Zonal Research Station, AAU, Shillongani, Nagaon, Assam. The experiment was laid out in Randomized Block Design with 3 replications and the crops were raised following the recommended package of practices. The data of both the years were pooled and the analysis of variance was done by the method of Panse and Sukhatme (1967) [15].

2.1 Physiological parameters

2.1.1 Stomatal frequency and stomatal index at 50 % flowering stage

Fresh, healthy, green leaf samples were collected from field and brought to the laboratory. Light coloured nail polish was smeared with little pressure on both the surfaces of the leaves and pressed with a cello tape. After pressing the tape for some time, it was removed from the leaf. The cello tape was then put on a slide and observed under low power objectives in a microscope. Stomatal density (No. mm^{-2} of leaf area) on abaxial and adaxial surfaces of the leaf was counted. Stomatal index was calculated according to the method of Meidner and Mansfield (1968) [13] using the following formula –

$$SI\ (\%) = \frac{SD}{ED+SD} \times 100$$

Where, SI=Stomatal Index
SD= Stomatal Density
ED= Epidermal pore Density

2.1.2 Total leaf chlorophyll

Leaf chlorophyll content was estimated after extracting by non-maceration method using Dimethyl Sulphoxide (DMSO) (Hiscox and Israelstam, 1979) [9]. The amount of chlorophyll content was calculated using absorption coefficients. The fresh leaf materials (0.1 g) in a test tube, containing 5ml of DMSO was kept in an oven at 65 ℃ for about 4 hours. Chlorophyll was extracted in a test tube and the volume was made upto 10 ml by using DMSO. The optical density of the extract was read at 663nm and 645nm using spectrophotometer. The chlorophyll content was determined by using the following formulae and expressed as mg/g leaf fresh weight.

$$Chl.a = [12.7(A_{663})-2.69(A_{645})]\times V/(1000\times W)$$

$$Chl.b = [22.9(A_{645})-468(A_{663})]\times V/(1000\times W)$$

$$Chl.(a/b) = Chl.a/Chl.b$$

$$Total\ chl. = [20.2(A_{645})+8.02(A_{663})]\times V/(1000\times W)$$

Where, A = Absorbance
V = Final volume (ml) of chlorophyll extract
W = Fresh weight (g) of the leaf tissue used for extraction

2.1.3 Chlorophyll stability index (CSI)

The leaf sample was heated in distilled water in bath at 56 °C for 30 minutes and extracted the chlorophyll by routine methods (as described in chlorophyll estimation). The chlorophyll content was compared with the normal samples kept at room temperature. The difference in two readings after heating at 56 ℃ was the chlorophyll stability index. The CSI was measured by using the following formula-

CSI (%) = Total chlorophyll in treated leaves/ Total chlorophyll in normal leaves × 100

2.2 Yield attributes and yield

All yield attributes and yield per plant were taken from five number of randomly selected tagged plants in each replication and the averages were computed.

2.2.1 Number of siliqua per plant

Five plants were selected at random, the number siliqua per plant was counted, and then the average value was calculated and expressed as number of siliqua per plant.

2.2.2 Number of seed per silique

Twenty number of siliqua were selected at random, the number of seeds per siliqua was counted, and then the average value was calculated and expressed as number of seeds per silique.

2.2.3 Number of seed per plant

The number of seed per plant was calculated by the following formula

Number of seed per plant = Number of siliqua per plant × Number of seed per siliquae

2.2.4 Seed test weight (g)

One thousand uniform sized, disease/pest free, well dried seeds were weighed and expressed as gram (g).

2.2.5 Stover yield (kg ha^{-1})

All the plants from $1m^2$ were harvested at physiological maturity from each plot and after proper drying seeds were separated and stover yield was recorded and converted into kg ha^{-1}.

2.2.6 Harvest Index (%)

Harvest index (HI) was calculated dividing the seed yield by total biological yield and multiplied by 100 as suggested by Nichiporovich (1967) [14]. It was expressed in percentage.

$$HI\ (\%) = \frac{\text{Seed yield}}{\text{Total Biological yield}} \times 100$$

2.2.7 Seed yield (g $plant^{-1}$)

The seeds from five plants were dried and weighed to record the seed yield per plant. Seed yield gram per plant was also recorded and converted into kg per ha.

3. Results and Discussion

3.1 Stomatal frequency and Stomatal index

The data for stomatal frequency and stomatal index are presented in the table 1. Significantly higher stomatal frequency was recorded in TS-75-1 (155.07) followed by Panchali (154.80) on the abaxia surface. On the adaxial surface, significantly higher stomatal frequency were found in the genotype Panchali (108.00) followed by TS-67 (104.90). On the other hand significantly lower abaxial stomatal frequency was found in the genotype TS-75-2ME (135.30**)** followed by TS-46 (136.74) on the abaxial surface and JT-90-1 (80.77) followed by TS-29 (81.94) on the adaxial surface. The range is similar to that of Akbar *et al.* (2020) [1], who reported density of stomata in abaxial surface in a range of 112.00–184.40 (No.mm^{-2}) and adaxial surface in the range of

69.28-105.20 (No.mm^{-2}) in all the studied species *viz.* Brassica *rapa* subsp. *Campestris, Brassica juncea* and *Brassica napus.* They also reported that stomatal index ranges from 16.55–25.00 in the adaxial surface and 19.96–21.05 in the abaxial surface which is similar with the trend of our findings.

Whereas, TKM-20-2 and PT-2018-09 showed significantly higher abaxial stomatal index with the value of 22.81 and 21.80, respectively, TS-38 (33.50) showed significantly higher adaxial stomatal index followed by Tapeswari (32.50). On the other hand, significantly lower abaxial stomatal index was recorded in TS-76-2 and TS-75-2ME with the value of 18.48 and 18.64, respectively. JT-90-1 (24.60) followed by Tapeswari (32.50) showed significantly lower adaxial stomatal index.

Significantly higher numbers of epidermal cell were recorded in the genotype TS-76-2 (608.05) followed by JT-14-1 (597.98) on the abaxial surface. On the other hand, significantly lower nubers of abaxial epidermal cell were found in the genotype TKM-20-2 (508.52). Genotype PT-303 (264.14) was recorded with significantly higher numbers of adaxial epidermal cell followed by Panchali (261.84). On the other hand, Tapeswari (197.36) showed the lowest numbers of adaxial epidermal cell.

Yarkhunova *et al.* (2016) [19] reported a significant difference in stomatal density among different genotypes of *Brassica rap* which is in corroboration with the current study. The findings of the current study are also supported by findings of Akbar *et al.* (2020) [1], who reported that the density of stomata is higher in abaxial surface (112.00–184.40) than the adaxial surface (69.28–105.20) in all the studied species *viz.* Brassica *rapa* subsp. *Campestris, Brassica juncea* and *Brassica napus.*. The difference in values may be due to genetic characteristics. The minute fluctuations due to year would be attributed for fluctuation in weather condition.

3.2 Chlorophyll a, Chlorophyll b, Total Chlorophyll and Chlorophyll stability index

There was significant difference among the genotypes for chlorophyll content as presented in the table 2. Significantly higher total chlorophyll content was seen in the genotype TS-75-2ME (1.62) and TS-38 (1.62). On the other hand, significantly lower total chlorophyll content was found in the genotype TKM-20-2 (0.81) followed by TKM-20-1 (0.85). If we consider chlorophyll a and chlorophyll b individually, significantly higher value of chlorophyll a was recorded in TS-38 (0.95) followed by TS-75-2ME (0.91) On the other hand highest chlorophyll b was recorded in TS-75-2ME (0.72) followed by TS-38 (0.67). The genotypes showing the lowest chlorophyll a and chlorophyll b were TKM-20-2 (0.47) and TKM-20-1 (0.33), respectively.

Significantly higher chlorophyll (a:b) ratio was found in TS-36 (1.97) followed by Panchali (1.96). On the other hand, CG-Toria-4 (0.97) followed by TS-76-2 (1.22) showed significantly lower ratio.

Highest chlorophyll stability index was recorded in TS-75-2ME followed by Tapeswari with the values of 33.56 and 33.01, respectively. On the other hand, TS-67 followed by CG-Toria-4 showed significantly lower index with the values of 27.10, and 27.52, respectively.

The variation in the values among the genotypes was might be due the genetic factors as reported by Dai *et al.* (2016). The result of the current study is also supported by the findings of Shekari *et al.* (2016) [17] who reported significant interactive effects of stress time and levels on chlorophyll '*a*' and '*b*' content, Chl. (a:b) ratio and total chlorophyll content in rapeseed (*Brassica napus* L.). Zhu *et al.* (2021) [20] also found variation in chlorophyll content between drought tolerant genotype and drought sensitive genotype in rapeseed (*Brassica napus* L.).

3.3 Relative Leaf Water Content (RLWC)

Significantly higher RLWC at 60 DAS was found in TS-75-2ME (67.97%) followed by PT-303 (67.19%). On the other hand significantly lower RLWC value was found in TS-29 (64.69%) followed by TS-76-2 (65.21%). The variation in values might be due to genetic factors of the genotypes as reported by Zhu *et al.* (2021) [22], who also reported a gradual decrease in RWC with increasing extent of drought stress in both drought-tolerant and drought-sensitive genotypes. Shekari *et al.* (2016) [17] also reported low amount of RWC and leaf water potential when applied only 30% water at siliqua formation period as compared to treated with field capacity in rapeseed (*Brassica napus* L.) which is in corroboration with the findings of the current studies.

3.4 Yield and yield attributes

The data on yield and yield attributing characters in rapeseed genotypes presented in table 3 indicated significant differences among the genotypes. Among the genotypes, significantly higher yield was found in the genotype TS-75-2ME followed by TS-38 with the values of 12.59 and 10.85 qha^{-1}, respectively. On the other hand, lowest yield was found in the genotype TKM-20-2 with the value of 4.23 qha^{-1}. There were significant variations in yield attributing characters among the genotypes. Significantly higher number of siliqua was found in the genotype TS-75-2ME (66.10) followed by TS-76-1 (58.66). On the other hand lowest number of siliqua was seen in the genotype TKM-20-2 (32.55) followed by TS-75-1 (35.96). Highest siliqua length was seen in the genotype TS-36 followed by JT-14-5 f with the values of 4.50 and 4.23cm, respectively. On the other hand, lowest was found in the genotype TS-46 (3.12) followed by TS-29 (3.36).

TS-36 (19.67) followed by TS-75-2ME (19.30) showed highest seeds per siliqua while, the genotype TS-46 (13.97) followed by JT-90-1 (14.17) showed lowest number of seeds per silique. Genotype TS-46 and TS-75-1 showed significantly higher seed test weight (1000) with the values of 4.83 and 4.77g, respectively. On the other hand, TS-75-1TL followed by PT-303 were found to be significantly lower with the values of 3.26 and 3.42 g, respectively. Total number of seed per plant was highest in TS-75-2ME (1246.28) followed by TS-76-1 (1034.13). On the other hand, TKM-20-2 (444.88) showed the lowest total number of seed per plant.

Finally, seed yield per plant was found to be highest in TS-75-2ME followed by TS-38 with the values of 5.02 and 4.24g, respectively. The genotypes TKM-20-2 (1.85) followed by JT-90-1 (2.16) was found to produce lowest seed yield per plant. Significantly higher Harvest Index (HI) was recorded in TS-75-2ME (25%) followed by TS-38 (24.01%). On the other hand significantly lower was observed in TS-75-1 (16.86%) followed by CG-Toria-4 (15.25%).

Awal *et al.* (2014) [4] reported significant variation in seed yield between the two species *Brassica campestris* and *Brassica napus* which is similar with the current study. Al-Juheishy *et al.* (2021) [2] revealed significant varietal differences in two rapeseed varieties in terms of number of branches per plant, dry weight of the plant, number of siliquaes per plant, the weight of 1000 seeds, seed yield and oil yield which in corroboration to the findings of the current experiment. Kumar (2015) [10] found that among the Toria varieties, TS-38 and TS-36 produced significantly higher values of yield attributes, *viz*., siliquae per plant, seeds per siliquae, length of the siliquae,1000 seed weight and seed yield. In the current study also it was observed. Our findings was also in corroboration with the findings of Samant *et al.* (2015) [16], Helal *et al.* (2016) [8], Gogoi *et al.* (2018) [7] and Tiwari *et al*. (2019) [18] where genotypic variation of yield and yield attributes observed.

Table 1: Stomatal frequency, epidermal cell and stomatal index of leaf surface at 50% flowering in rapeseed

Genotypes	Abaxial			Adaxial		
	Stomatal frequency (No.mm^{-2})	Epidermal cell (No. mm^{-2})	Stomatal index (%)	Stomatal frequency (No.mm^{-2})	Epidermal cell (No. mm^{-2})	Stomatal index (%)
PT-303	144.20	577.43	19.99	95.17	264.14	26.49
PT-2018-09	150.14	540.09	21.80	88.43	214.05	29.24
Panchali	154.80	577.82	21.13	108.00	261.84	29.22
Bhawani	138.77	567.20	19.67	89.07	243.88	26.76
TS-29	141.84	592.14	19.33	81.94	245.02	25.06
TS-36	143.14	582.97	19.72	92.77	238.72	28.02
TS-46	136.74	577.50	19.15	84.47	253.54	24.99
TS-67	147.90	545.29	21.35	104.90	253.76	29.28
TS-75-1	155.07	570.63	21.37	87.70	249.16	26.04
TS-75-1TL	140.70	580.25	19.52	93.64	260.57	26.44
TS-75-2ME	135.30	590.59	18.64	89.84	229.44	28.14
TS-75-2MM	138.57	587.30	19.09	91.90	229.51	28.70
TS-76-1	137.50	583.91	19.06	86.40	229.65	27.35
TS-76-2	137.84	608.05	18.48	91.90	246.56	27.25
JT-90-1	141.14	575.55	19.70	80.77	247.67	24.60
JT-14-5	141.53	597.98	19.14	82.23	235.53	25.92
TKM-20-1	140.14	580.11	19.46	84.53	212.98	28.48
TKM-20-2	147.40	508.52	22.81	86.40	238.91	26.60
Tapeswari	145.40	562.37	20.55	94.94	197.36	32.55
CG-Toria-3	141.60	581.60	19.58	89.57	250.02	26.38
CG-Toria-4	144.34	533.92	21.28	95.05	226.44	29.58
TS-38 (Check)	149.50	545.54	21.56	102.04	203.87	33.50
Mean	143.34	571.21	19.99	95.17	237.42	27.75
C D (0.05)	3.99	25.44	2.15	4.55	14.33	3.32

Table 2: Chlorophyll a, Chlorophyll b, Chlorophyll a/b, Total Leaf Chlorophyll, Chlorophyll stability index and Relative Leaf Water Content at 60 DAS in rapeseed

Genotypes	Chlorophyll a (mg.g^{-1} fr.wt.)	Chlorophyll b (mg.g^{-1} fr.wt.)	Chlorophyll (a/b)	Total chlorophyll (mg.g^{-1} fr.wt.)	Chlorophyll stability index (%)	RLWC (%)
PT-303	0.81	0.45	1.82	1.26	28.04	67.19
PT-2018-09	0.70	0.47	1.50	1.16	32.01	66.51
Panchali	0.65	0.33	1.96	0.98	31.29	66.46
Bhawani	0.66	0.65	1.02	1.30	29.81	66.10
TS-29	0.70	0.51	1.47	1.21	31.80	64.65
TS-36	0.69	0.38	1.97	1.07	31.43	66.26
TS-46	0.80	0.45	1.80	1.25	28.79	65.88
TS-67	0.54	0.39	1.39	0.92	27.10	66.78
TS-75-1	0.70	0.55	1.33	1.25	29.41	65.23
TS-75-1TL	0.49	0.36	1.42	0.84	31.32	67.05
TS-75-2ME	0.91	0.72	1.27	1.62	33.56	67.97
TS-75-2MM	0.70	0.39	1.79	1.09	31.88	65.26
TS-76-1	0.49	0.37	1.31	0.86	29.92	66.82
TS-76-2	0.65	0.54	1.22	1.19	28.77	65.21
JT-90-1	0.71	0.30	2.54	1.00	27.56	66.75
JT-14-5	0.80	0.45	1.80	1.25	29.64	65.68
TKM-20-1	0.53	0.33	1.62	0.85	30.06	65.99
TKM-20-2	0.47	0.34	1.40	0.81	31.53	66.78
Tapeswari	0.70	0.41	1.75	1.11	33.01	67.10
CG-Toria-3	0.89	0.55	1.61	1.44	31.77	65.51
CG-Toria-4	0.52	0.55	0.97	1.07	27.52	66.06
TS-38 (Check)	0.95	0.67	1.41	1.62	32.01	66.10
Mean	0.68	0.46	1.56	1.14	30.37	66.24
C D (0.05)	0.04	0.062	0.53	0.58	4.56	2.82

Table 3: Yield attributes and yield in rapeseed

Genotypes	Siliqua (No. Plant^{-1})	Length of silique (cm)	Seeds (No. Siliqua^{-1})	1000 seed weight (g)	Seeds (No.Plant^{-1})	Seed yield (g.plant^{-1})	Yield (q ha^{-1})	Harvest index (%)
PT-303	44.16	3.58	17.40	3.42	746.25	3.87	10.43	23.83
PT-2018-09	45.43	4.05	15.97	3.76	695.43	2.62	6.52	17.38
Panchali	43.22	4.09	16.47	4.58	681.52	3.12	8.34	18.53
Bhawani	47.50	3.64	16.77	4.34	770.37	3.33	9.21	20.60
TS-29	45.19	3.36	14.90	4.62	646.93	3.08	8.05	21.01
TS-36	36.65	4.50	19.67	4.35	690.10	3.64	9.99	20.20
TS-46	60.25	3.12	13.97	4.83	815.19	3.60	8.97	22.58
TS-67	54.43	3.56	17.33	4.23	909.16	2.97	8.16	21.06
TS-75-1	35.96	4.06	17.54	4.77	593.63	2.84	7.18	16.86
TS-75-1TL	45.47	3.36	15.47	3.26	671.64	2.19	4.99	18.32
TS-75-2ME	66.10	3.69	19.30	4.54	1246.28	5.02	12.59	25.00
TS-75-2MM	52.42	3.46	16.93	3.54	862.60	3.06	8.40	19.84
TS-76-1	58.66	3.58	18.13	3.48	1034.13	3.60	9.76	22.07
TS-76-2	44.52	3.49	15.47	3.84	663.65	2.55	6.30	19.56
JT-90-1	40.09	3.70	14.17	4.13	542.94	2.16	5.14	18.96
JT-14-5	52.72	4.23	15.14	4.67	769.12	3.59	9.56	23.83
TKM-20-1	37.30	4.23	14.70	4.46	519.94	2.32	5.79	19.53
TKM-20-2	32.55	3.73	14.44	4.13	444.88	1.85	4.23	17.24
Tapeswari	51.76	3.92	15.54	4.45	777.55	3.46	8.91	21.96
CG-Toria-3	40.10	3.57	17.53	4.43	672.55	3.20	8.10	21.06
CG-Toria-4	44.76	3.55	16.30	4.23	700.61	2.74	6.94	15.25
TS-38 (Check)	57.76	3.91	18.27	4.68	1020.51	4.24	10.85	24.01
Mean	47.14	3.74	16.50	4.21	748.86	3.14	8.10	20.39
C D (0.05)	6.67	1.43	4.86	0.26	182.04	1.01	1.614	2.15

4. Conclusion

The results of the study indicated a significant variation of physiological parameters viz. stomatal frequency, stomatal index, RLWC and chlorophyll content, yield attributes and yield among the genotypes. The genotypes TS-75-2ME and TS-38 exhibited more RLWC and total leaf chlorophyll content. The same genotypes showed more yield and showed superiority in terms of yield attributing characters viz., seeds per silique, total number of seed per plant, seed yield per plant and harvest index.

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