



OPEN Physiological, biochemical and enzymatic quality parameters of primed seed of rapeseed-mustard genotypes

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High-quality seed of improved genotypes is a critical key to well attract the consumers. Therefore, a two-year laboratory experiment was carried out at Department of Seed Science and Technology, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India in 2018 and 2019 in a triplicated factorial completely randomized design to determine the quality parameters of the newly produced seeds from plants of six rapeseed-mustard genotypes (Anushka and Sanchita (rapeseed), TBM-204 and TBM-143 (yellow seed coated mustard), Kranti and Pusa Bold (black seed coated mustard)) grown under five seed priming (KH_2PO_4 (0.15 mol), KNO_3 (0.1 mol), polyethylene glycol (PEG) 6000 (−0.3 MPa), distilled water, and dry seed). Pusa Bold was recognized to have greater germination percentage under application of KH_2PO_4 (86.32%). Further, KH_2PO_4 registered high root length (TBM-204) and seedlings fresh (TBM-204) and dry weights (Pusa Bold). Pusa Bold under application of KH_2PO_4 also expressed the highest soluble protein (23.05%), while Anushka had maximum oil content under seed treatment with KH_2PO_4 (46.30%) as well as lowest electrical conductivity ($0.91 \mu\text{S m}^{-1} \text{g}^{-1}$). Additionally, KH_2PO_4 had more impact on Pusa Bold regarding α -amylase activity, while TBM-143 treated with KH_2PO_4 recorded highest peroxidase activity. So, using KH_2PO_4 to prime Pusa Bold seeds can be an effective way to enhance germination, seedling vigour, and biochemical characteristics, which will increase oilseed crop productivity and quality. By encouraging sustainable agriculture and raising crop production, this strategy supports the UN sustainable development goal (SDG) 2 (zero hunger). Additionally, it meets SDG 12 (responsible consumption and production) through sustainable resource management and decreased environmental effect by optimizing input consumption and improving seed performance without genetic modification or excessive chemical use.

Keywords Anti-oxidants, Biochemicals and enzyme, Physiology and quality, Rapeseed-mustard genotypes, Seed germination and vigour, Seed priming with PEG-6000 and KH_2PO_4

Oilseeds, primarily used for food grains, have been neglected over the years, leading to a disparity between demand and supply, and high costs¹. To address this, farmers, researchers, extension workers, government, and private organizations must focus on cultivation and strengthening the demand-supply chain². Rapeseed and mustard are the second and third most important oilseeds in India and the world, correspondingly, and they are essential to the edible oil industry—promoting the agro-economy, generating value-added processing, and guaranteeing food security. This industry is important not just because it reduces a significant reliance on imports by meeting domestic cooking oil needs. Furthermore, rapeseed and mustard contribute value to India's agro-processing chains by supplying animal feed, biodiesel, nutraceuticals, lubricants, and condiments in addition to edible oil. Crop *Brassicaceae* include a wide variety of plants that are cultivated as vegetables, fodder, or as sources of sauces and oils. One of the most commercially significant agricultural products is the rapeseed-mustard. Eight distinct kinds of rapeseed-mustard—Indian mustard, toria, yellow sarson, brown sarson, gobhi sarson, Karan rai, black mustard, and Taramira—are grown in 53 countries worldwide. In 2018–19, the world's rapeseed-mustard area, production and productivity were expected to be 36.59 million hectares (mha), 72.37 million tonnes (mt), and 1980 kg/ha, respectively. India accounts for 19.8% and 9.8% of the world's total

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acreage and production, respectively³. Researchers find it difficult to increase the quality of oil and seed meal of rapeseed-mustard which is a necessary nutritional component in India. So, breeders in the quality improvement program will get particularly benefit from information on the nutritional and anti-nutritional composition of rapeseed-mustard oil and seed meal. The rapeseed-mustard's physiological and biochemical characteristics will help choose the ideal genotype for particular features⁴. According to Chauhan et al. (2020)⁵, there will be a 16.4–20.5 million tonnes demand for rapeseed-mustard in India by 2030, up from the current production of 9.26 million tonnes. This indicates the need for methods to boost rapeseed-mustard cultivation in a sustainable way. Under present climate change scenario, successful rapeseed-mustard cultivation faces challenges such as non-availability of improved seeds, traditional low-yielding varieties, poor germination, high seedling mortality, weaker seedlings, insect pest and disease problems, and loss of seed quality with aging⁶. In West Bengal, high temperatures or drought conditions hinder ideal germination and plant stand establishment of rapeseed-mustard. In such a situation, standardising the suitability of cultivars for a particular agro-climatic condition is crucial in producing good quality seeds of rapeseed-mustard.

In this context, seed priming is a promising agro-technological intervention for improving quality seed production. It involves pre-sowing controlled hydration, causing biochemical and physiological changes in seeds. This accelerates germination attributes, allowing radicle growth inside seeds and other metabolic activities without actual emergence of radicle⁷. This process is crucial under moisture stress conditions, as it allows radicles to protrude rapidly and uptake soil moisture and nutrients⁸. It offers numerous benefits including early emergence, uniform germination, vigorous root and shoot growth, early flowering, maturity, high water and nutrient use efficiency, reproductive organ development, drought, salinity resistance, and improved anti-oxidative activities⁹. Seed priming with external agents like KH_2PO_4 , KNO_3 , and poly ethylene glycol (PEG) is effective in various field crops, including oilseeds^{10,11}. Seed priming with KH_2PO_4 improves germination, emergence, and growth due to presence of phosphorus¹². KNO_3 elevates ambient oxygen levels, promoting seed germination and growth in studies¹³. PEG is an effective seed priming agent for field crops, improving membrane repair and germination through controlling the water absorption rate under saline condition¹⁴.

Healthy seed is a major determinant of ideal germination, vigorous plant stand, crop growth, development and yield⁶. Poor quality of seed leads to loss of viability, poor germination and seedling mortality. Seed quality deteriorates with prolonged storage of seeds, harsh agro-climatic conditions, insect pest and disease attacks, etc¹⁵. Under biotic and abiotic stress conditions, oxidative damage of seeds may also occur that further deteriorates the quality of the produced seeds. It is hypothesised that the crop grown from healthy seeds gets established in the field properly; grows well and produces the good quality seeds that can be stored or used for sowing next. Determination of various physiological, biochemical and enzymatic quality parameters of the newly produced seeds from the crop that were grown through various priming of seeds is a good indicator to assess the impact of seed priming on rapeseed-mustard genotypes. So far, research on impact of seed priming on rapeseed-mustard is limited, necessitating further evaluation of genotype performance under different seed priming options.

Understanding how various rapeseed-mustard genotypes react to seed priming processes in terms of seed quality and vigour—two crucial qualities that directly influence crop establishment and productivity—remains a critical gap despite considerable breeding and agronomic management efforts. Little is known about the effects of genotype and particular priming agents under controlled conditions on physiological and biochemical seed quality parameters, including germination rate, seedling vigour, protein and oil content, and enzymatic activities (like α -amylase and peroxidase). To improve oilseed performance, this gap must be filled to customize seed improvement techniques to genotypes. Considering all these, a two-year laboratory experiment was conducted in the Indo-Gangetic plains of West Bengal to assess the quality of freshly produced seeds from genotypes of rapeseed and mustard that were cultivated under the impact of seed priming. Finding the best seed priming treatment to improve germination, seedling growth, and biochemical traits, identifying genotype-specific reactions to these treatments, and recommending the best genotype-treatment combinations for increased seed quality and vigour in the Indo-Gangetic plains were the primary goals.

Materials and methods

Experiment details

The laboratory experiment was executed at Department of Seed Science and Technology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India in winter seasons of 2018 and 2019. The rapeseed-mustard seeds were produced and collected for this laboratory analysis from a split-plot designed field trial involving 6 rapeseed and mustard genotypes in main plots and 5 seed priming treatments in sub-plots conducted at District seed farm (A-B Block Farm) (23°50' N latitude and 89°E longitude), Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India in winter seasons of 2017-18 and 2018-19 (Table 1). The crop was sown on 16th and 12th November of 2017 and 2018, respectively. In Table S1 of the supplementary file, the weather observations for the two study years are shown. The laboratory analysis followed factorial completely randomised design.

Observations

In order to note the observations, matured seeds from 10 randomly selected and tagged plants excluding the border rows were procured and taken to the laboratory. The crop was matured when more than 80% of siliqua of the randomly selected plants were turned yellow colour. Different physiological quality parameters consisted of seed germination (%), root length (cm), shoot length (cm), seedlings' fresh weight (mg per 5 seedlings), seedlings' dry weight (mg per 5 seedlings), vigour index-I, and vigour index-II. Seed germination was considered after 5 days of seed placed of the test and seedling characters were studied after 7th day of germination. Among the biochemical and enzymatic quality parameters, oil content (%), total soluble protein content (%), electrical conductivity (EC) ($\mu\text{S m}^{-1} \text{g}^{-1}$), α -amylase activity (24 and 48 h) ($\mu\text{g min}^{-1} \text{g}^{-1}$), and peroxidase activity (24

Genotype and seed priming	Symbol
Main plot treatments: 6 genotypes	
Anushka (rapeseed)	V ₁
Sanchita (rapeseed)	V ₂
TBM-204 (yellow seed coated mustard)	V ₃
TBM-143 (yellow seed coated mustard)	V ₄
Kranti (black seed coated mustard)	V ₅
Pusa Bold (black seed coated mustard)	V ₆
Sub plot treatments: 5 seed priming options	
KH ₂ PO ₄ @0.15 mol	T ₁
KNO ₃ @0.1 mol	T ₂
Polyethylene glycol (PEG) 6000 @-0.3 MPa	T ₃
Distilled water or hydro priming	T ₄
Control or dry seed	T ₅

Table 1. Treatment details.

and 48 h) ($\Delta A \text{ min}^{-1} \text{ g}^{-1}$) were studied using various standard protocols. Individual year's observations on different physiological, biochemical and enzymatic quality parameters of seed and seedling of rapeseed-mustard genotypes under the influence of seed priming are shown in Tables S2-S15 of the supplementary file.

Analysis of physiological parameters

Germination percentage

100 seeds from each plot as per their treatments were taken inside the laboratory of Department of Seed Science and Technology, Bidhan Chandra Krishi Viswavidyalaya for estimation of germination percentage. There were four replicates of 100 seeds per plot to estimate germination %. Petri dish method was followed to estimate the germination percentage¹⁶ based on the following formula.

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds plated}} \times 100$$

Root and shoot lengths(cm)

Glass-plates were prepared treatment and replication wise for all the six genotypes. After 7th day of germination, glass-plates were taken out and ten randomly selected seedlings were plucked out gently with care from the filter paper of each plate using metal forceps and root and shoot lengths were measured using centimetre scale accurately. The average of root and shoot lengths was then chalked out.

Seedlings fresh and dry weights (mg per 5 seedlings)

After 7th day of germination, petri dishes were taken out and five randomly selected seedlings were plucked out gently with care from the filter paper of each dish using metal forceps and weights of freshly obtained five seedlings were recorded using digital weighing machine and expressed in mg. Further, those obtained seedlings were soaked using blotting paper and seedlings were kept in brown paper packets with proper labeling. Then, those packets containing seedlings were dried in a hot air oven at a temperature of 80 ± 2 °C till constant weights were achieved. Afterwards, dry weights of five seedlings as per their treatments were recorded using digital weighing machine and expressed in mg.

Vigour index-I and II

Vigour index-I and vigour index-II were estimated using the following formulas suggested by Abdul-Baki and Anderson (1973)¹⁷:

$$\text{Vigour index-I} = \text{Seed germination (\%)} \times \text{Average seedling dry weight (mg)}$$

$$\text{Vigour index-II} = \text{Seed germination (\%)} \times \text{Average seedling dry weight (mg)}$$

Analysis of biochemical and enzymatic parameters

Biochemical and enzymatic parameters such as oil, total soluble protein contents, electrical conductivity (EC) ($\mu\text{S m}^{-1} \text{ g}^{-1}$), α -amylase activity (24 and 48 h) ($\mu\text{g min}^{-1} \text{ g}^{-1}$), and peroxidase activity (24 and 48 h) ($\Delta A \text{ min}^{-1} \text{ g}^{-1}$) were estimated using the Soxhlet based solvent extraction method¹⁸, Lowry's method¹⁹, electrical conductivity method²⁰, colorimetric method²¹ and spectrophotometric method²², respectively. The detailed protocols of estimation of these biochemical and enzymatic parameters are mentioned in section S1 of Supplementary file.

Statistical analysis

Statistical analysis was performed on the laboratory data using the ‘analysis of variance’ method²³ (Table 2). Treatment means shown in tables were compared through least significant difference (LSD) at 5% level of significance. Treatment means shown in figures were compared using Duncan’s Multiple Range Test (DMRT) at the 5% significance level through Statistical Product and Service Solutions (SPSS) software (version 25.0). Germination percentages of seeds were subjected to angular transformation. Pearson correlation between all the physiological, biochemical and enzymatic quality parameters of seed and seedling comprising coefficients (r) was made using R software and represented as correlogram. Finally, a heat map-cluster analysis was made to observe the uniformity among the tested genotypes and seed priming treatments based on the physiological, biochemical and enzymatic quality parameters of seed and seedling using OriginPro (Learning edition) software.

Results

Analysis of variance (ANOVA) test

The ANOVA test was performed for all 14 physiological, biochemical, and enzymatic quality parameters to observe significant variations among genotypes, seed priming, and their interactions (Table 2). It was noted that rapeseed-mustard genotypes and seed priming separately showed highly significant variations (at 1% level of significance) for all 14 physiological, biochemical, and enzymatic quality parameters. Significant variations also existed between genotype and seed priming interactions for different physiological, biochemical, and enzymatic quality parameters, except seed germination, seedling dry weight and peroxidase activity (24 h). Specifically, significant differences were higher (at 1% level of significance) for the different characters except peroxidase activity (48 h), which showed significant variation at 5% level of significance.

Physiological quality parameters

Physiological quality parameters showed significant variations ($p \leq 0.05$) among the genotypes and seed priming interactions, except germination % and seedling dry weight. While considering the response of individual genotype towards the application of different seed priming treatments for germination (angular transformed value), Pusa Bold was recognized to have greater germination percentage under application of KH_2PO_4 @ 0.15 mol (V_6T_1), followed by Polyethylene glycol (PEG) 6000 @ -0.3 MPa (V_6T_3) and KNO_3 @ 0.1 mol (V_6T_2), which were respectively, ~5.9, 4.9 and 2.4% higher over no seed priming to that genotype. Sanchita (V_2) (~5.4% higher), Anushka (V_1) (~4.7% higher), TBM-204 (V_3) (~5.4% higher), Kranti (V_5) (~6.0% higher) and TBM-143 (V_4) (~5.7% higher) had higher germination percentage under application of KH_2PO_4 @ 0.15 mol (T_1) over their respective control (no seed priming) (T_5) (Table 3). Observations on root and shoot lengths depicted that KH_2PO_4 @ 0.15 mol produced maximum root length for TBM-204 (V_3T_1) (15.31 cm), which was ~14% higher than the control (T_5). It was also noticed that KH_2PO_4 @ 0.15 mol (T_1) also influenced best regarding root length in case of Anushka (V_1) (~26.4% higher), Pusa Bold (V_6) (~8% higher) and TBM-143 (V_4) (~1.2% higher) over control (T_5). On the other hand, Polyethylene glycol (PEG) 6000 @ -0.3 MPa (T_3) had more impact on Sanchita (V_2) (~30.5% higher over control) and Kranti (V_5) (~9.7% higher over control). Further, regarding shoot length, as compared to control, KH_2PO_4 @ 0.15 mol (T_1) had more impact on Anushka (V_1) (~45.7% higher), Sanchita (V_2) (~35.8% higher) and TBM-204 (V_3) (~27.4% higher), while KNO_3 @ 0.1 mol (T_2) had more impact on TBM-143 (V_4) (15.4% higher) (Table 3).

In case of seedlings’ fresh and dry weights, interaction between genotypes and seed priming options revealed that TBM-204 showed maximum seedlings’ fresh weight (~86.3 higher) under KH_2PO_4 @ 0.15 mol (V_3T_1), while in case of seedlings’ dry weight, seed priming with KH_2PO_4 @ 0.15 mol showed the best result in Pusa Bold (V_6T_1) (~64.7% higher), over control (Table 4). The lowest seedlings’ fresh weight (148.3 mg) and dry weight (12.00 mg) were observed from Anushka under no seed priming treatment (V_1T_5). Further, the combined influence of genotypes and seed priming options on vigour index-I showed that as compared to control (T_5), KH_2PO_4 @ 0.15 mol (T_1) had more impact on Anushka (V_1) (~32.7% higher) and TBM-204 (V_3) (~19.3% higher) (Table 5). TBM-204 (V_3) treated with KH_2PO_4 @ 0.15 mol (V_3T_1) recorded the highest vigour index-I. As compared to control, KNO_3 @ 0.1 mol (T_2) had more effect on TBM-143 (V_4) (~5.9% higher) and Pusa Bold (V_6) (~11.5% higher). Polyethylene glycol (PEG) 6000 @ -0.3 MPa (T_3) had better influence on Sanchita (V_2) (~31.9% higher) and Kranti (V_5) (~17.6% higher) over control. However, KH_2PO_4 @ 0.15 mol produced maximum vigour index-II of Pusa Bold (V_6T_1) (~67.6% higher over control), followed by Kranti (V_5T_1) (~65.9% higher over control), TBM-143 (V_4T_1) (~98.1% higher), Sanchita (V_2T_1) (~100.1% higher over control) and Anushka (V_1T_1) (~130.1% higher over control) (Table 5).

Biochemical quality parameters

Significant variations ($p \leq 0.05$) also existed among different genotype and seed priming interactions for the biological quality parameters. Regarding the seed oil content, the combination of all the genotypes with KH_2PO_4 @ 0.15 mol (T_1) produced the higher oil percentage (Fig. 1). As compared to control, Anushka was found to have maximum oil content under seed treatment with KH_2PO_4 @ 0.15 mol (V_1T_1) (~1.6% higher), followed by Polyethylene glycol (PEG) 6000 @ -0.3 MPa (V_1T_3) (~1.3% higher). Pusa Bold was noted to have lowest oil content under no seed priming (V_6T_5) (37.53%). In case of total soluble protein content, as compared to control (T_5), KH_2PO_4 @ 0.15 mol treatment on Pusa Bold (V_6T_1) showed highest total soluble protein content (~7.0% higher), closely followed by Kranti treated with KH_2PO_4 @ 0.15 mol (V_5T_1) (~10.2% higher) and Pusa Bold treated with Polyethylene glycol (PEG) 6000 @ -0.3 MPa (V_6T_3) (~5% higher) (Fig. 2). Lowest total soluble protein content was noticed from Anushka without seed priming (V_1T_5) (14.52%).

Further, interaction between genotype and seed priming option revealed that as compared to control (T_5), Anushka showed lowest electrical conductivity, when it was treated with KH_2PO_4 @ 0.15 mol (V_1T_1) (~16.5%

Parameters	Source of variation					
		Genotype (V)	Seed priming (T)	V × T (interaction)	Error	Total (Corrected)
Seed germination %	df	5	4	20	60	89
	SS	35.45	56.69	1.57	38.75	132.46
	MSS	7.09	14.17	0.08	0.65	
	F	10.98**	21.94**	0.12 ^{NS}		
Root length (cm)	df	5	4	20	60	89
	SS	61.07	18.76	51.52	21.57	152.92
	MSS	12.21	4.69	2.58	0.36	
	F	33.97**	13.05**	7.17**		
Shoot length (cm)	df	5	4	20	60	89
	SS	11.22	1.37	11.23	4.12	27.94
	MSS	2.24	0.34	0.56	0.07	
	F	32.66**	4.98**	8.17**		
Seedlings' fresh weight (mg per 5 seedlings)	df	5	4	20	60	89
	SS	1768693.06	121117.64	366092.36	162758.33	2418661.39
	MSS	353738.61	30279.41	18304.62	2712.64	
	F	130.40**	11.16**	6.75**		
Seedlings' dry weight (mg per 5 seedlings)	df	5	4	20	60	89
	SS	1678.32	2076.25	41.48	1269.00	5065.06
	MSS	335.66	519.06	2.07	21.15	
	F	15.87**	24.54**	0.10 ^{NS}		
Vigour index-I	df	5	4	20	60	89
	SS	1306411.91	423782.32	760427.37	268407.78	2759029.38
	MSS	261282.38	105945.58	38021.37	4473.46	
	F	58.41**	23.68**	8.50**		
Vigour index-II	df	5	4	20	60	89
	SS	667237.38	809297.19	26428.64	25599.61	1528562.82
	MSS	133447.48	202324.30	1321.43	426.66	
	F	312.77**	474.20**	3.10**		
Oil %	df	5	4	20	60	89
	SS	797.34	23.87	4.36	1.85	827.41
	MSS	159.47	5.97	0.22	0.03	
	F	5184.71**	194.01**	7.08**		
Total soluble protein %	df	5	4	20	60	89
	SS	615.11	23.01	3.09	2.43	643.64
	MSS	123.02	5.75	0.15	0.04	
	F	3037.89**	142.08**	3.81**		
Electrical conductivity ($\mu\text{S m}^{-1} \text{g}^{-1}$)	df	5	4	20	60	89
	SS	3.19	0.51	0.13	0.07	3.90
	MSS	0.64	0.13	0.01	0.00	
	F	550.65**	110.38**	5.60**		
α -amylase (24 h) ($\mu\text{g min}^{-1} \text{g}^{-1}$)	df	5	4	20	60	89
	SS	595.16	58.91	8.81	5.24	668.12
	MSS	119.03	14.73	0.44	0.09	
	F	1362.89**	168.64**	5.04**		
α -amylase (48 h) ($\mu\text{g min}^{-1} \text{g}^{-1}$)	df	5	4	20	60	89
	SS	847.55	70.02	6.90	4.05	928.52
	MSS	169.51	17.51	0.34	0.07	
	F	2511.26**	259.34**	5.11**		

Continued

Parameters		Source of variation				
		Genotype (V)	Seed priming (T)	V × T (interaction)	Error	Total (Corrected)
Peroxidase (24 h) ($\Delta A \text{ min}^{-1} \text{ g}^{-1}$)	df	5	4	20	60	89
	SS	0.05	0.00	0.00	0.00	0.05
	MSS	0.01	0.00	0.00	0.00	
	F	367.31**	42.85**	0.73 ^{NS}		
Peroxidase (48 h) ($\Delta A \text{ min}^{-1} \text{ g}^{-1}$)	df	5	4	20	60	89
	SS	0.04	0.00	0.00	0.00	0.05
	MSS	0.01	0.00	0.00	0.00	
	F	530.45**	58.39**	1.93*		

Table 2. Analysis of variance (ANOVA) of studied characters of rapeseed-mustard genotypes under the influence of seed priming. *df* degrees of freedom, *SS* sum of squares, *MSS* mean sum of squares, *F* ratio of between-group variance to within-group variance, *NS* non-significant. *significant at 5% level of significance, **highly significant at 1% level of significance.

Genotype × seed priming		Seed germination (%)	Root length (cm)	Shoot length (cm)
Anushka (V_1)	KH_2PO_4 @ 0.15 mol (T_1)	97.99 (81.97)	13.69	3.41
	KNO_3 @ 0.1 mol (T_2)	96.89 (79.90)	11.58	2.36
	PEG 6000 @ -0.3 MPa (T_3)	97.48 (80.93)	12.49	3.01
	Distilled water (T_4)	96.24 (78.86)	11.21	2.28
	Control (dry seed) (T_5)	95.86 (78.30)	10.83	2.34
Sanchita (V_2)	KH_2PO_4 @ 0.15 mol (T_1)	98.47 (83.11)	11.57	4.06
	KNO_3 @ 0.1 mol (T_2)	97.04 (80.16)	14.23	3.38
	PEG 6000 @ -0.3 MPa (T_3)	98.33 (82.70)	14.65	3.72
	Distilled water (T_4)	96.61 (79.42)	13.28	3.79
	Control (dry seed) (T_5)	96.23 (78.83)	11.23	2.99
TBM-204 (V_3)	KH_2PO_4 @ 0.15 mol (T_1)	99.19 (85.26)	15.31	4.23
	KNO_3 @ 0.1 mol (T_2)	97.79 (81.42)	13.06	3.47
	PEG 6000 @ -0.3 MPa (T_3)	98.99 (84.33)	13.15	3.33
	Distilled water (T_4)	97.49 (80.89)	14.59	3.76
	Control (dry seed) (T_5)	97.05 (80.22)	13.43	3.32
TBM-143 (V_4)	KH_2PO_4 @ 0.15 mol (T_1)	98.89 (84.08)	13.55	3.34
	KNO_3 @ 0.1 mol (T_2)	97.29 (80.58)	13.76	3.89
	PEG 6000 @ -0.3 MPa (T_3)	98.43 (82.94)	12.71	3.87
	Distilled water (T_4)	96.99 (79.98)	12.79	3.43
	Control (dry seed) (T_5)	96.70 (79.55)	13.39	3.37
Kranti (V_5)	KH_2PO_4 @ 0.15 mol (T_1)	99.36 (85.39)	14.43	3.37
	KNO_3 @ 0.1 mol (T_2)	98.38 (82.85)	13.93	3.99
	PEG 6000 @ -0.3 MPa (T_3)	99.16 (85.15)	15.16	3.99
	Distilled water (T_4)	97.78 (81.48)	13.50	3.53
	Control (dry seed) (T_5)	97.29 (80.57)	13.15	3.45
Pusa Bold (V_6)	KH_2PO_4 @ 0.15 mol (T_1)	99.49 (86.32)	15.17	3.40
	KNO_3 @ 0.1 mol (T_2)	98.62 (83.47)	14.88	3.93
	PEG 6000 @ -0.3 MPa (T_3)	99.32 (85.46)	14.54	3.32
	Distilled water (T_4)	98.39 (82.72)	13.76	3.14
	Control (dry seed) (T_5)	97.76 (81.51)	14.04	4.50
LSD ($p=0.05$)		NS	0.98	0.43

Table 3. Response of rapeseed-mustard genotypes towards seed priming on germination percentage, root length and shoot lengths. Data in the parenthesis indicates the angular transformed value of original data.

Genotype × Seed priming		Seedlings' fresh weight (mg per 5 seedlings)	Seedlings' dry weight (mg per 5 seedlings)
Anushka (V ₁)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	266.7	27.00
	KNO ₃ @ 0.1 mol (T ₂)	260.0	19.83
	PEG 6000 @ -0.3 MPa (T ₃)	180.0	21.67
	Distilled water (T ₄)	148.3	16.33
	Control (dry seed) (T ₅)	158.3	12.00
Sanchita (V ₂)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	306.7	28.33
	KNO ₃ @ 0.1 mol (T ₂)	325.0	21.50
	PEG 6000 @ -0.3 MPa (T ₃)	263.3	23.00
	Distilled Water (T ₄)	228.3	18.67
	Control (dry seed) (T ₅)	315.0	14.50
TBM-204 (V ₃)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	680.0	31.00
	KNO ₃ @ 0.1 mol (T ₂)	621.7	25.67
	PEG 6000 @ -0.3 MPa (T ₃)	608.3	28.50
	Distilled water (T ₄)	385.0	24.17
	Control (dry seed) (T ₅)	365.0	21.33
TBM-143 (V ₄)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	334.2	30.33
	KNO ₃ @ 0.1 mol (T ₂)	263.3	23.33
	PEG 6000 @ -0.3 MPa (T ₃)	368.3	25.83
	Distilled Water (T ₄)	355.0	21.50
	Control (dry seed) (T ₅)	358.3	15.67
Krantī (V ₅)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	538.3	36.83
	KNO ₃ @ 0.1 mol (T ₂)	485.0	29.17
	PEG 6000 @ -0.3 MPa (T ₃)	600.0	31.00
	Distilled water (T ₄)	646.7	26.67
	Control (dry seed) (T ₅)	458.3	22.67
Pusa Bold (V ₆)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	563.3	41.17
	KNO ₃ @ 0.1 mol (T ₂)	600.0	30.67
	PEG 6000 @ -0.3 MPa (T ₃)	586.7	32.33
	Distilled water (T ₄)	612.5	27.33
	Control (dry seed) (T ₅)	415.0	25.00
LSD (<i>p</i> = 0.05)		85.3	NS

Table 4. Response of genotypes towards seed priming on seedlings' fresh weight and dry weight.

lower), followed by Polyethylene glycol (PEG) 6000 @ -0.3 MPa (V₁T₃) (~14% lower), while TBM-204 showed highest electrical conductivity under no seed priming (V₃T₅) (1.63 μS m⁻¹ g⁻¹) (Fig. 3).

Enzymatic activity

Interaction between genotype and seed priming option for enzymatic activities varied significantly (*p* ≤ 0.05) except peroxidase activity (24 h). The α-amylase activity (24 h) showed that as compared to control (T₅), KH₂PO₄ @ 0.15 mol had more impact on Pusa Bold (V₆T₁) (~1.9% higher), followed by Polyethylene glycol (PEG) 6000 @ -0.3 MPa (V₆T₃) (~1.6% higher) (Fig. 4). Similar trend was noticed for other genotypes. Lowest α-amylase activity (24 h) was reported from produced seeds of Anushka, which was grown without seed priming (V₁T₅) (68.94 μg min⁻¹ g⁻¹). Regarding α-amylase activity (48 h), as compared to control (T₅), KH₂PO₄ @ 0.15 mol (T₁) had more impact on Anushka (V₁) (~6% higher), Sanchita (V₂) (~4.2% higher), TBM-204 (V₃) (~4.8% higher) and TBM-143 (V₄) (~6.4% higher) and Polyethylene glycol (PEG) 6000 @ -0.3 MPa (T₃) impacted best on Krantī (V₅) (~5.3% higher) and Pusa Bold (V₆) (~4% higher).

Additionally, interaction between genotype and seed priming option showed that Anushka without seed priming (V₁T₅) recorded lowest peroxidase activity (24 h: 0.383 ΔA min⁻¹ g⁻¹, 48 h: 0.452 ΔA min⁻¹ g⁻¹), while TBM-143 treated with KH₂PO₄ @ 0.15 mol (V₄T₁) recorded highest peroxidase activity (24 h: ~2.9% higher, 48 h: ~2.1% higher over control) (Fig. 5).

Correlation analysis

Pearson's correlation matrix shown in correlogram (Fig. 6) expressed that in most cases various physiological, biochemical, and enzymatic quality parameters significantly and positively correlated to each other (*p* < 0.01). In the correlogram, blank or no circle indicated non-significant correlation among the parameters. Strong positive and negative correlations between parameters were depicted in deep blue and dark red coloration, respectively which gradually faded as the correlation decreased. The strong positive correlation mostly existed between these parameters, especially, between root length and vigour index-I (*r* = 0.97), germination % and vigour index-II (*r* = 0.95), germination % and seedlings dry weight (*r* = 0.94) and between α-amylase activities at 24 and 48 h

Genotype × Seed priming		Vigour index-I	Vigour index-II
Anushka (V ₁)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	1676.0	529.5
	KNO ₃ @ 0.1 mol (T ₂)	1350.7	384.4
	PEG 6000 @ -0.3 MPa (T ₃)	1510.9	422.7
	Distilled water (T ₄)	1298.5	314.6
	Control (dry seed) (T ₅)	1263.3	230.1
Sanchita (V ₂)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	1540.1	558.2
	KNO ₃ @ 0.1 mol (T ₂)	1708.9	417.4
	PEG 6000 @ -0.3 MPa (T ₃)	1806.1	452.3
	Distilled water (T ₄)	1648.8	360.7
	Control (dry seed) (T ₅)	1368.9	279.0
TBM-204 (V ₃)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	1938.2	614.8
	KNO ₃ @ 0.1 mol (T ₂)	1616.7	502.0
	PEG 6000 @ -0.3 MPa (T ₃)	1630.7	564.3
	Distilled water (T ₄)	1789.0	471.1
	Control (dry seed) (T ₅)	1625.3	414.3
TBM-143 (V ₄)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	1670.6	600.0
	KNO ₃ @ 0.1 mol (T ₂)	1716.8	454.0
	PEG 6000 @ -0.3 MPa (T ₃)	1632.2	508.6
	Distilled water (T ₄)	1573.2	417.1
	Control (dry seed) (T ₅)	1621.3	302.9
Kranti (V ₅)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	1768.3	732.0
	KNO ₃ @ 0.1 mol (T ₂)	1762.6	573.8
	PEG 6000 @ -0.3 MPa (T ₃)	1898.8	614.8
	Distilled water (T ₄)	1665.0	521.3
	Control (dry seed) (T ₅)	1614.0	441.2
Pusa Bold (V ₆)	KH ₂ PO ₄ @ 0.15 mol (T ₁)	1847.7	819.2
	KNO ₃ @ 0.1 mol (T ₂)	1854.5	605.1
	PEG 6000 @ -0.3 MPa (T ₃)	1773.6	642.5
	Distilled water (T ₄)	1662.8	537.9
	Control (dry seed) (T ₅)	1812.4	488.9
LSD (p=0.05)		109.5	33.8

Table 5. Response of genotypes towards seed priming on vigour index-I and vigour index-II.

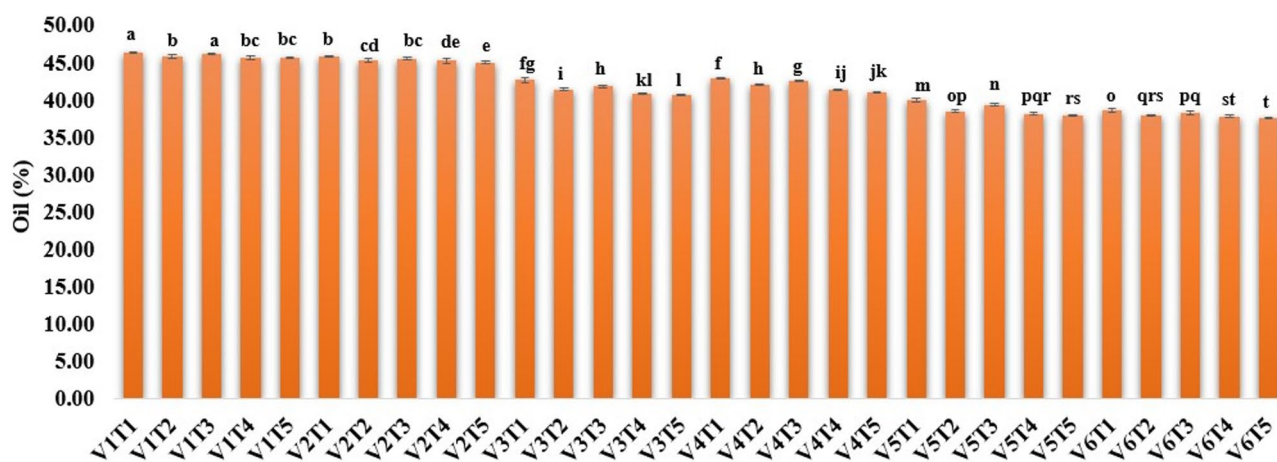


Fig. 1. Influence of seed priming on oil contents of produced seeds of rapeseed-mustard genotypes; Genotypes (V): V₁- Anushka, V₂- Sanchita, V₃- TBM-204, V₄- TBM-143, V₅- Kranti, V₆- Pusa Bold; Treatments (T): T₁-KH₂PO₄ @ 0.15 mol, T₂-KNO₃ @ 0.1 mol, T₃-PEG 6000 @ -0.3 MPa, T₄-Distilled Water, T₅-Control (Dry Seed); Means shown along with different letters are significantly different at p < 0.05 by DMRT.

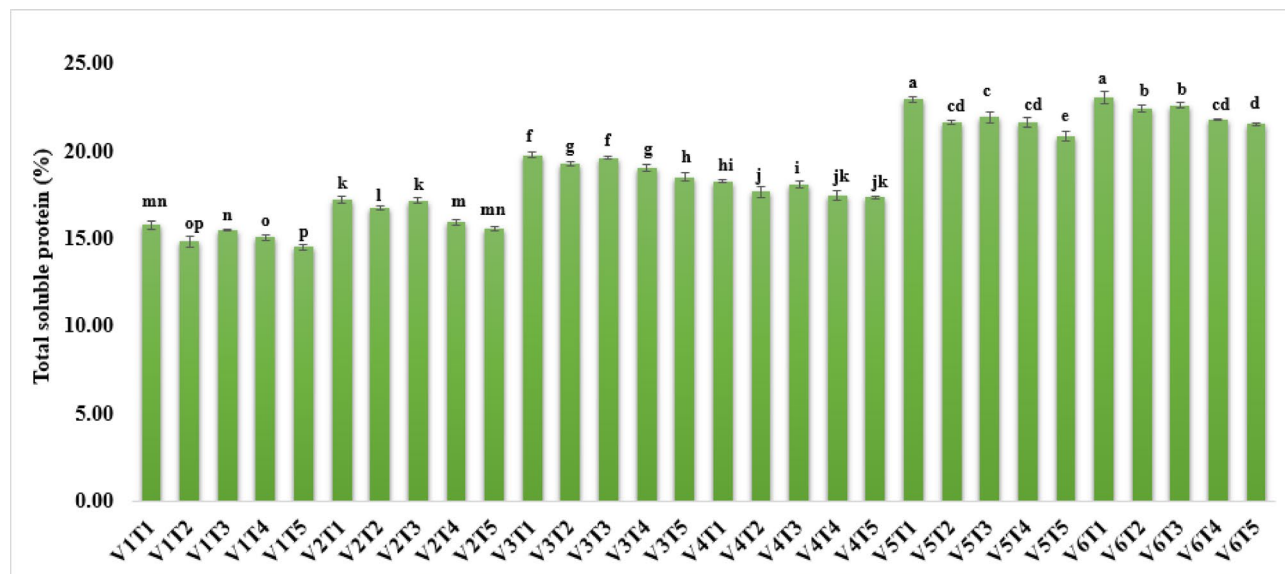


Fig. 2. Influence of seed priming on total soluble protein contents of produced seeds of rapeseed-mustard genotypes; Genotypes (V): V₁- Anushka, V₂- Sanchita, V₃- TBM-204, V₄- TBM-143, V₅- Kranti, V₆- Pusa Bold; Treatments (T): T₁-KH₂PO₄ @ 0.15 mol, T₂-KNO₃ @ 0.1 mol, T₃-PEG 6000 @ -0.3 MPa, T₄-Distilled Water, T₅-Control (Dry Seed); Means shown along with different letters are significantly different at $p < 0.05$ by DMRT.

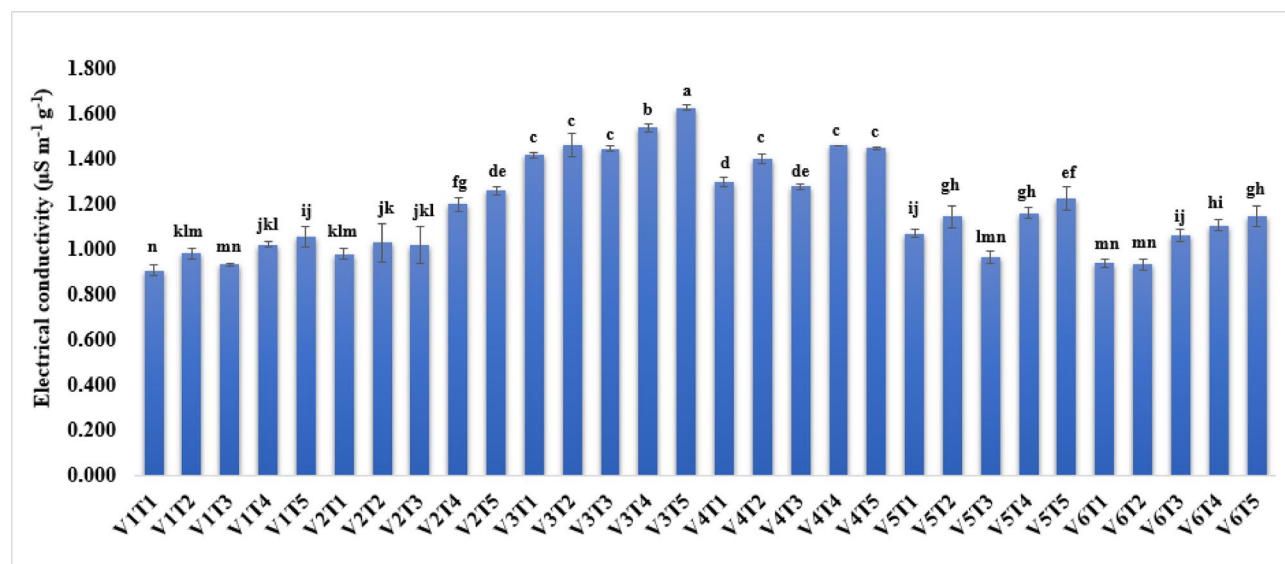


Fig. 3. Influence of seed priming on electrical conductivity of produced seeds of rapeseed-mustard genotypes; Genotypes (V): V₁- Anushka, V₂- Sanchita, V₃- TBM-204, V₄- TBM-143, V₅- Kranti, V₆- Pusa Bold; Treatments (T): T₁-KH₂PO₄ @ 0.15 mol, T₂-KNO₃ @ 0.1 mol, T₃-PEG 6000 @ -0.3 MPa, T₄-Distilled Water, T₅-Control (Dry Seed); Means shown along with different letters are significantly different at $p < 0.05$ by DMRT.

($r = 0.94$). The strong but negative correlation existed mostly between oil and total soluble protein ($r = -0.91$), seedlings fresh weight and oil ($r = -0.76$) and oil and α -amylase activity at 24 h ($r = -0.74$).

Cluster analysis

Clustering of interactions of rapeseed-mustard genotypes and seed priming options as shown in Fig. 7 expressed the similarities between various combinations, especially, V₆T₁ and V₅T₁; between V₁T₁ and V₂T₁ and others. Clustering different physiological, biochemical and enzymatic quality parameters also showed the similarities

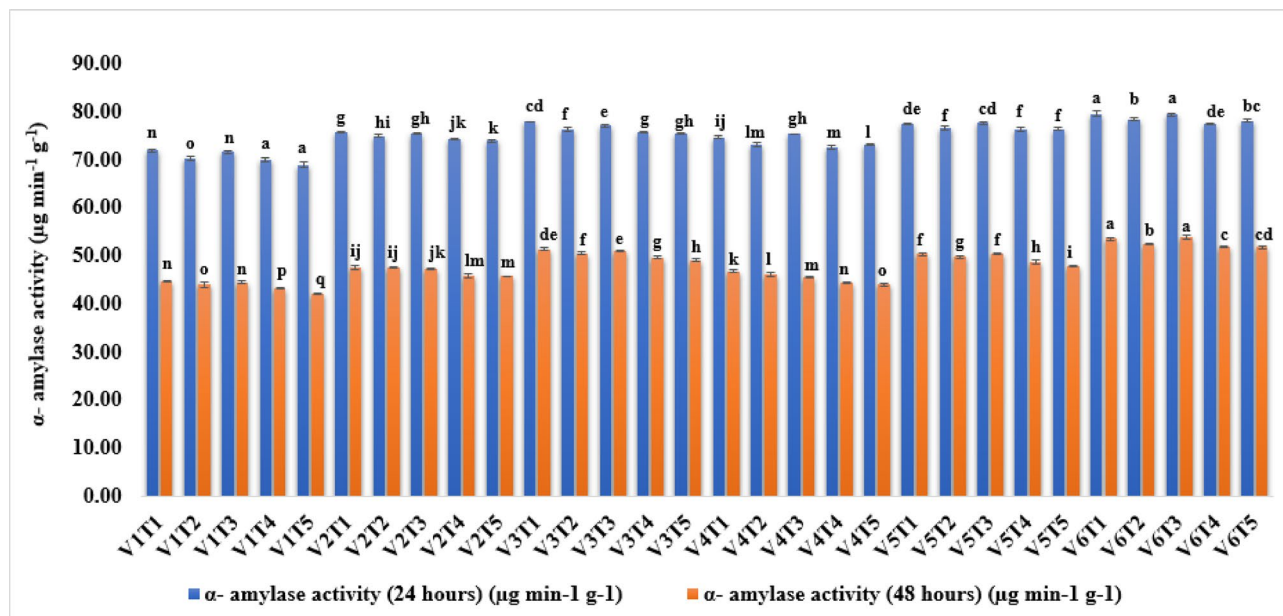


Fig. 4. Influence of seed priming on α -amylase activity (24 and 48 h) of produced seeds of rapeseed-mustard genotypes; Genotypes (V): V₁ - Anushka, V₂ - Sanchita, V₃ - TBM-204, V₄ - TBM-143, V₅ - Kranti, V₆ - Pusa Bold; Treatments (T): T₁ -KH₂PO₄ @ 0.15 mol, T₂ -KNO₃ @ 0.1 mol, T₃ -PEG 6000 @ -0.3 MPa, T₄ -Distilled Water, T₅ -Control (Dry Seed); Means shown along with different letters are significantly different at $p < 0.05$ by DMRT.

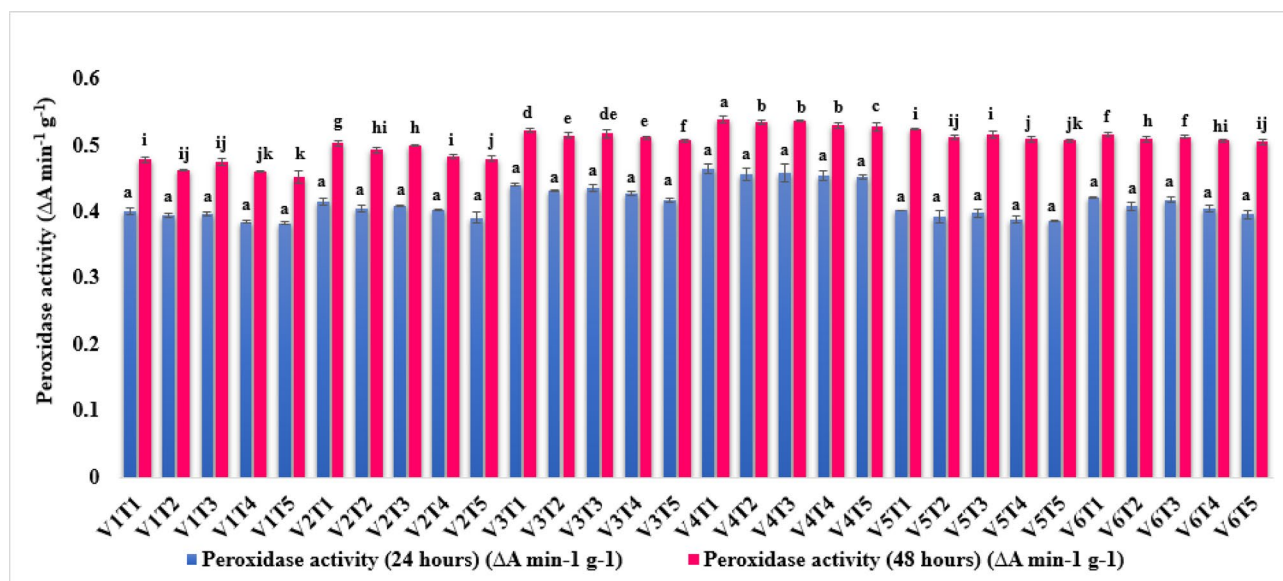


Fig. 5. Influence of seed priming on peroxidase activity (24 and 48 h) of produced seeds of rapeseed-mustard genotypes; Genotypes (V): V₁ - Anushka, V₂ - Sanchita, V₃ - TBM-204, V₄ - TBM-143, V₅ - Kranti, V₆ - Pusa Bold; Treatments (T): T₁ -KH₂PO₄ @ 0.15 mol, T₂ -KNO₃ @ 0.1 mol, T₃ -PEG 6000 @ -0.3 MPa, T₄ -Distilled Water, T₅ -Control (Dry Seed); Means shown along with different letters are significantly different at $p < 0.05$ by DMRT.

between α -amylase activities at 24 and 48 h, peroxidase activities at 24 and 48 h, germination % and seedlings dry weight, root length and vigour index-I etc.

Discussion

Inherent genetic variety and distinct interactions to the dominant agro-climatic situations during seed development are the two main causes of the difference in germination among genotypes²⁴. During seed filling,

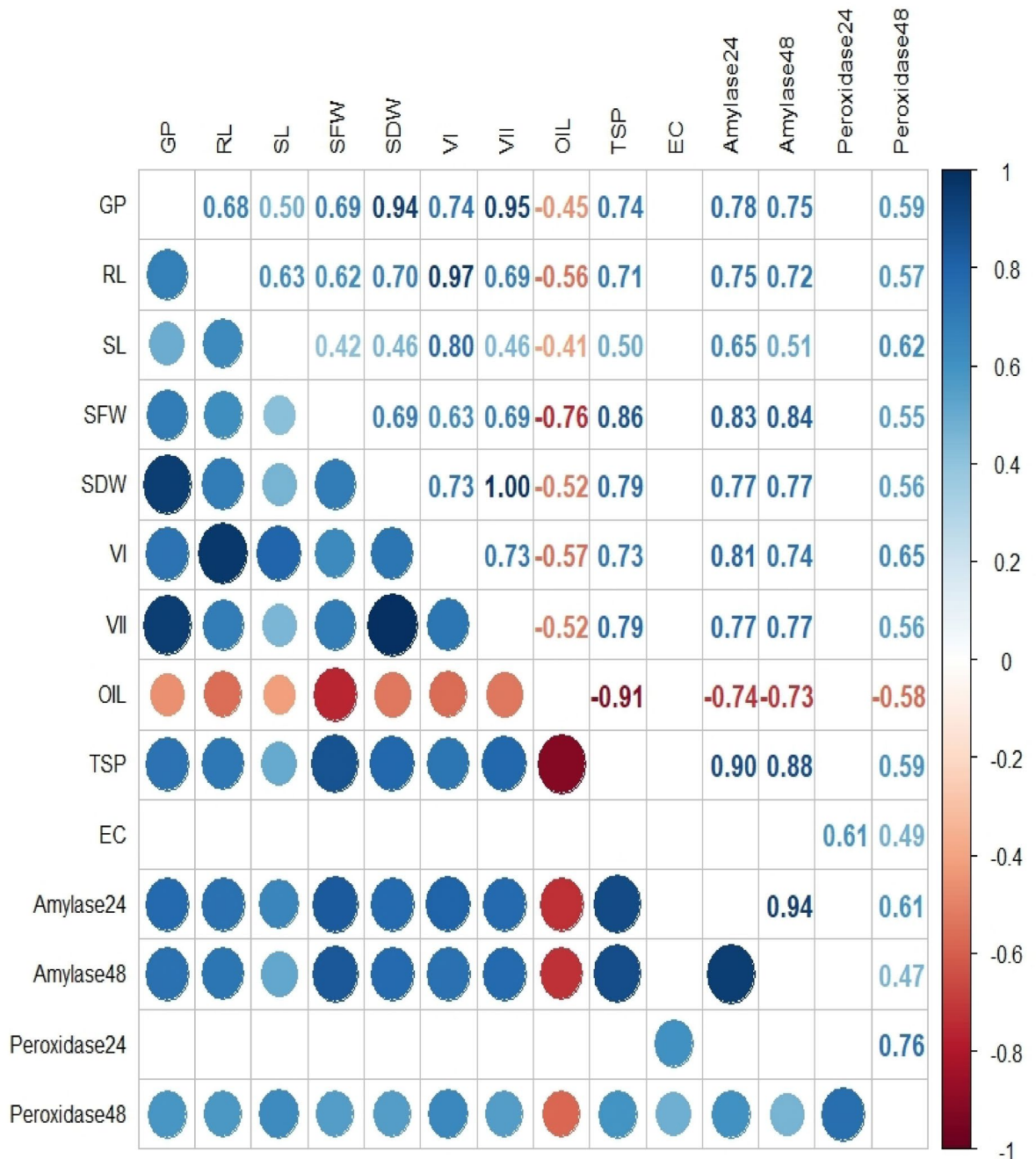


Fig. 6. Pearson's correlation matrix between physiological, biochemical and enzymatic quality parameters [coloured circles indicating highly significance ($p < 0.01$), blank indicating non-significance]; *GP* germination%, *RL* root length, *SL* shoot length, *SFW* Seedlings fresh weight, *SDW* seedlings dry weight, *OIL* oil content, *TSP* total soluble protein, *EC* electrical conductivity, Amylase24: α - amylase activity (24 h), Amylase48: α - amylase activity (48 h), Peroxidase24: Peroxidase activity (24 h), Peroxidase48: Peroxidase activity (48 h).

high ambient temperatures (> 30–35 °C) might hasten seed maturation, shortening the time needed for reserve accumulation and resulting in partial protein, lipid, and starch deposition. As a result, when seeds germinated, their metabolic potential was reduced. Furthermore, it was known that high temperatures inhibited the activity of α -amylase, which was essential for hydrolysing starch into soluble sugars required for the early growth of seedlings. High relative humidity (> 80%) during the late reproductive stages also raised the moisture content of the seeds, which weakened their vigour by encouraging either microbial infection or early sprouting. Cloudy weather or a shorter photoperiod reduced photosynthetic efficiency, which limited the amount of assimilates available to the developing seeds. This was especially true for oilseeds like rapeseed-mustard, where lipid synthesis was impacted. Besides, indeterminate flowering habit and exposure of developing seeds to existing

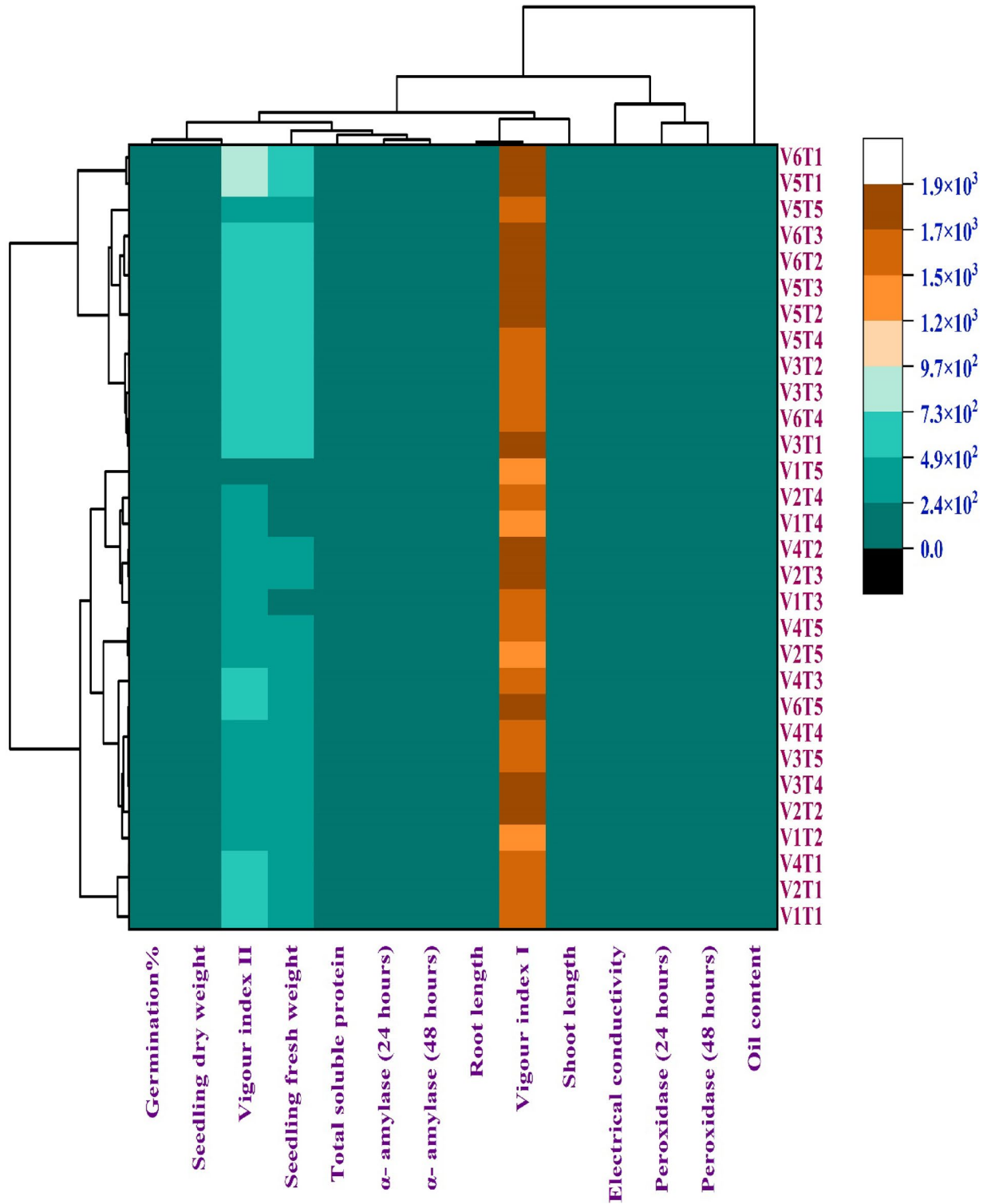


Fig. 7. Clustering of combinations of rapeseed-mustard genotypes and seed priming levels based on various physiological, biochemical, and enzymatic quality parameters and clustering of these parameters; Genotypes (V): V₁- Anushka, V₂- Sanchita, V₃- TBM-204, V₄- TBM-143, V₅- Kranti, V₆- Pusa Bold; Treatments (T): T₁-KH₂PO₄ @ 0.15 mol, T₂-KNO₃ @ 0.1 mol, T₃-PEG 6000 @ -0.3 MPa, T₄-Distilled Water, T₅-Control (Dry Seed).

weather conditions, probably exerted significant influence on biochemical activities and mobilization of food reserve on produced seeds and thereby, impacted on their germination²⁵. Seed priming with chemicals, specially, KH_2PO_4 influenced germination probably due to altered enzyme activity, resulting in accelerated metabolism. Besides, consistent supply of energy through phosphorus, when seeds were primed with KH_2PO_4 for various physiological, biochemical and enzymatic activities during germination phase probably resulted in improvement of seed germination percentage²⁶. Further, Bewley and Black (1982)²⁷ reported the activity of potassium in increasing ambient oxygen level by restricting oxygen availability for citric acid cycle. Sathish et al. (2011)²⁸ observed similar improvement in germination of hybrid maize seeds and stated that the result might be due to influence of seed priming on seed membrane integrity, increase in protein and nucleic acid, repair of seed quality deterioration caused by lipid peroxidation, electrolyte leakage etc. PEG 6000 also exerted positive influence on seed germination perhaps due to its beneficial influence on activity enzymes such as protease, amylase which accelerated the growth of seed embryo²⁹.

Apart from complex interaction of the inherent genetic trait and the agro-climatic condition for production of seeds, variable food reserves in seeds of the genotypes also perhaps exerted significant variation on root and shoot growth of seedlings³⁰. Seed priming with KH_2PO_4 probably resulted in increased food reserves which provided adequate supply of energy for various physiological, biochemical and enzymatic activities and resulted in ideal development of radicle and plumule lengths inside embryo. Presence of phosphorus in KH_2PO_4 might also play important role in development of roots. Seed priming with PEG 6000 also recorded high root length and it was perhaps due to improved metabolic activities as well as radicle development inside seed³¹. Seedling fresh and dry weights were the reflection of seedling growth (root and shoot). As emergence and initial seedling growth was totally dependent on food reserve of the seeds, variable food reserve possibly created variation in seedlings' fresh and dry weights of the genotypes. Seedlings' fresh and dry weights were improved under seed priming option probably due to greater accumulation of food reserve and thereby, developed high shoot and root growths (seedling growth) through translocation of dry matter to germinated seedlings³². Presence of phosphorus in KH_2PO_4 perhaps played important role in energy storage, energy transfer and oxidation reactions as phosphorus was the constituent of phospholipids, ADP and ATP³³. Thus, phosphorus utilized resources more efficiently in seeds and produced high seedlings' fresh weight. Further, seed priming with PEG 6000 also influenced seedling fresh and dry weights. Eesha et al. (2024)²⁹ earlier reported positive effect of PEG 6000 on seedling fresh and dry weights of lentil. By establishing a low water-potential environment that permitted seeds to progressively absorb water, osmopriming with PEG-6000 greatly improved seed germination and seedling vigour. Without causing early radicle emergence, this treatment had started vital metabolic processes such DNA repair, enzyme activation, and reserve mobilization³⁴. Research on a variety of crops, including, pea, carrot, caraway, and lentil, showed that PEG-6000 treatments generated more homogeneous and robust seedlings with longer roots and shoots, as well as a higher germination percentage and a shorter mean germination time²⁹. Furthermore, PEG-primed seeds accumulated compatible solutes (proline, sugars) and activated antioxidant enzymes (APX, CAT, POD, SOD) under osmotic or salinity stress, which decreased lipid peroxidation and stabilized membranes, improving stress tolerance during early growth³⁵. The preconditioned seeds from PEG-6000 osmopriming resulted in robust seedlings and stronger, more synchronized germination, which were essential for improved crop performance and field emergence.

Vigour index was the product of germination and seedling length or dry weight. In the present study, genetic trait and agro-climate's influence on produced seeds significantly impacted on variability of seed germination and seedling length or dry weight, which in turn, imposed variation in seedling vigour index-I & II³⁶. Further, seed priming, specially, with KH_2PO_4 improved germination, root and shoot lengths (seedling length) and dry weight and thereby, influenced vigour index-I & II. Specifically, it might repair the protein damage that occurred due to oxidative stress. Further, it probably maintained the normal function of metabolism pathways which, in turn, circulated energy required for biomass accumulation in essential structures and thus, produced vigorous seedling³³. Improvement of seed germination and seedling growth under chemical seed priming possibly due to improvement of ATPase activity³⁷ as well as anti-oxidant activities, specially, peroxidase³⁸ and their beneficial impacts on seed parts repair³⁹ and embryo development⁴⁰ reflected directly on seedling vigour index. Pandey et al. (2017)⁴¹ in cucumber and Dugesar et al. (2025)⁴² in black gram similarly reported improvement of seed germination and vigour index under seed priming with KH_2PO_4 and PEG 6000, respectively.

Regarding oil content, rapeseed genotypes were better performer than mustard genotypes. In the present study, besides the unique genetic trait in each of the genotype, nutrient and water availability, prevailing weather (temperature, relative humidity, rainfall, sunshine hours etc.), drought or heat stress during seed development also played important role in variable oil content among the genotypes⁴³. Increase of oil content through seed priming with KH_2PO_4 was observed due to the fact that phosphorus improved the oil content of seeds. Phosphorus was known to be the supplier of energy. In the present study, seed priming with KH_2PO_4 probably provided adequate energy for biosynthesis of oil⁴⁴. Furthermore, Pusa Bold performed best in registering protein content in produced seeds. It might be due to its greater adaptation to the ago-climatic condition, proper vegetative growth, specially, roots for proper uptake and utilization of nitrogen from soil⁴⁵. Seed priming with KH_2PO_4 recorded maximum total soluble protein content probably due to improved root system through which uptake and translocation of nutrients, specially, nitrogen from soil occurred to produced seeds. Accumulated nitrogen was the constituent of protein as observed in the present study. Besides KH_2PO_4 , the positive impact of PEG 6000 on protein content of canola was earlier reported by Elahi et al. (2023)⁴⁶.

Complex interaction of genetically unique genotypes with agro-climatic conditions created different impacts on seeds during development, specially, in their membrane integrity, moisture and lignin contents etc. which probably influenced the electrical conductivity of the seeds of genotypes differently. Higher electrical conductivity as observed in case of control indicated loss of membrane integrity which negatively impacted on seed quality⁴⁷. Reduction in electrical conductivity with seed priming, specially, using KH_2PO_4 indicated

that there might be restoration of seed membrane integrity, resulting in restriction of leaching of electrolytes⁴⁸. Further, low electrical conductivity under seed priming with PEG 6000 as observed in the present study was reported by Eesha et al. (2024)²⁹ in lentil.

In the present study, rapeseed genotypes showed low α -amylase activity as compared to mustard genotypes probably due to the influence of rainfall, relative humidity and temperature on the genotypes during seed development and maturity⁴⁹. Since mustard genotypes were of longer duration than rapeseed genotypes, increasing atmospheric temperature and rainfall towards seed ripening and maturity stages perhaps created marked influence in elevating the α -amylase activity of the produced mustard seeds, specially, in Pusa Bold. Seed priming enhanced α -amylase activity of seeds over control perhaps due to its beneficial activity during seed hydration, starch hydrolysis and rapid conversion of starch in to reducing sugar⁵⁰. Further, there might be activation of α -amylase activity under seed priming and for such activity, energy was supplied at sufficient level from the food reserve which was high in produced seeds due to beneficial impact of KH_2PO_4 or PEG 6000^{41,42}. α -amylase activity (48 h) was lower than α -amylase activity (24 h) which indicated depletion of food reserve due to energy generation for enzymatic activity.

In the present study, rapeseed genotypes exhibited lowest peroxidase activity than most of the mustard genotypes and it might be due to their short duration and less exposure to increasing temperature in spring or pre-summer times⁵¹. Improved terminal heat or drought stress as observed in case of mustard genotypes probably exerted direct influence on higher peroxidase activity. Peroxidase activity was known to vary for drought or heat stress and acted as a defensive mechanism against oxidative damage⁵². Higher activity of peroxidase indicated greater protection against stress⁵³.

Peroxidase activity increased under seed priming over control probably due to improvement of anti-oxidant properties as defense against oxidative stress. Adequate energy for such defense activity was supplied by food reserve of seeds which was accumulated due to beneficial impact of seed priming, specially, KH_2PO_4 . Seed priming with PEG 6000, on the other hand, might improve cell membrane stability and thereby, checked lipid peroxidation, which was possibly accompanied by increased levels of various anti-oxidant enzymatic activity, specially, peroxidase³¹. Increase of peroxidase activity (48 h) over that at 24 h under seed priming, thus, indicated seed quality maintenance⁵⁴. Valgimigli (2023)⁵⁵ reported that a decrease in peroxidase activity was perhaps positively correlated with lipid peroxidation. PEG 6000 induced the early production of α -amylase by carefully controlling water uptake into seeds through the creation of a mild osmotic stress environment. These enzymes converted stored starch into soluble sugars, such as fructose and glucose, which drove increased energy production and metabolic activity, resulting in more rapid and consistent germination³⁵. PEG priming concurrently increased peroxidase both in terms of gene expression and enzyme activity. Peroxidase expression and activity significantly increased following PEG treatment, according to several investigations with *Coronilla varia* seeds⁵⁶. Reactive oxygen species were detoxified, lipid peroxidation was decreased, membranes were stabilized, and even lignin biosynthesis was supported by converting coumaryl alcohol to hydroxy phenyl lignin, which increased the vigour of seedlings.

The diversity among genotype \times seed priming combinations based on important physiological, biochemical, and enzymatic seed quality parameters was depicted in hierarchical cluster analysis. The heat map showed patterns of similarity and divergence by visually grouping combinations into discrete clusters. With high values in germination percentage, seedling dry weight, vigour indices, and α -amylase and peroxidase activities, the combinations V_6T_1 (Pusa Bold \times KH_2PO_4) and V_5T_1 (Kranti \times KH_2PO_4) notably established a separate cluster, indicating excellent seed quality and vigour. On the other hand, unprimed pairings with persistently low trait values, as V_1T_5 (Anushka \times Control) and V_2T_5 (Sanchita \times Control), showed poor seed performance without priming. A common physiological underpinning for early seedling vigour was suggested by the clustering of characteristics, which also showed strong interrelationships, particularly among root length, vigour index I, and germination percentage. Likewise, there was a tight clustering of α -amylase and peroxidase activities at 24 and 48 h, suggesting coordinated enzyme activation under priming conditions. There was little linkage between the features linked to vigour and characteristics like electrical conductivity and oil content, which seemed more independent. Considering all these, the research demonstrated the genotype-specific character of seed priming responses and validated the efficacy of KH_2PO_4 priming, especially in Pusa Bold. These results are useful for refining seed improvement procedures to increase oilseed yields in a range of environmental conditions.

Conclusions

The current investigation confirmed that chemical seed priming had a beneficial effect on the physiological and biochemical quality parameters of rapeseed-mustard genotype seed and seedlings. Additionally, it showed that rapeseed-mustard genotypes adapted and performed differently under the agro-climatic conditions of the Indo-Gangetic plains of West Bengal via differences in their interactions with the environment. Hierarchical cluster analysis also confirmed the genotype-specific character of seed priming responses and validated the efficacy of KH_2PO_4 priming in achieving high quality seeds, especially in mustard genotype 'Pusa Bold'. Therefore, seed priming with KH_2PO_4 to mustard genotype 'Pusa Bold' can be recommended to oilseed growers of Indo-Gangetic plains of West Bengal for effective and high-quality oilseed production. This outcome helps achieve the UN sustainable development goal (SDG) 2 (zero hunger) by promoting sustainable agriculture and increasing agricultural production. Furthermore, by optimizing input consumption and enhancing seed performance without genetic modification or excessive chemical usage, it reduces environmental impact and satisfies SDG 12 (responsible consumption and production) through sustainable resource management.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Received: 20 March 2025; Accepted: 26 June 2025

Published online: 30 August 2025

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Author contributions

All authors contributed to the study conception and design. The research work was planned by Amitava Dutta. Material preparation, data collection and analysis were performed by Rupa Das. The first draft of the manuscript was written by Rupa Das. Saikat Biswas helped in data analysis and wrote the part of the manuscript. All authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-09325-z>.

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