

# Biological priming: An eco-friendly alternative for inducing salinity tolerance and augmenting plant growth in *Brassica juncea*

## ABSTRACT

High salinity injury is one of the critical factors that limit crop yields and quality internationally especially in hot and semi hot areas. *Brassica juncea* otherwise called as Indian mustard is a significant oilseed crop tender to saline stress. In this work, a possibility of the biological priming treatment application as the environmentally friendly method for increasing the plants' salt tolerance and growth rate of *Brassica juncea* plants is described. This study was carried out at Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh during rabi 2021-22. The experiment was carried out in factorial complete randomized design including 15 genotypes of mustard treated with 16 treatments of NaCl (20, 40 and 60%) and PGPR (*Bacillus cereus*, *Aeromonas media* and *Rhizobium alamii*). Various quantitative characters and qualitative characters were observed and studied. In this research we only highlighted the seed yield, chlorophyll content, proline and protein content as these observations are vital for salinity tolerance. The traits which exhibit positive effects on seed yield would be considered for the breeding programmes. Hence, RLM-619, RH-761, and P21-KANTI genotypes with PGPR-1, PGPR-2, and PGPR-3 treatments are most effective for increasing seed yield per plant, using strategies like plant breeding, genetic engineering, and seed bioprimering for improving the tolerance against the salinity. Hence, this study clearly discusses the effectiveness of the biological priming strategy as a cost effective and eco-friendly method of combating the effect of salinity stress as well as enhancing plant growth and yield of *Brassica juncea* in salt affected zones of agriculture.

*Keywords: Bio-priming, Brassica juncea, PGPR, Salinity stress*

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## INTRODUCTION

*Brassica juncea*, or Indian mustard, is a vital oilseed crop grown in India. It is ranked second in Asia in terms of acreage and production, after rapeseed (Mahla et al., 2014). Its production and area are further increased by the fact that it is mostly grown in states like Gujarat, Rajasthan, Madhya Pradesh, Haryana, and Uttar Pradesh (Latwal et al., 2019). With its cultivation spanning over 6.2 million hectares and an annual production of 9.3 million tons of seeds, mustard plays a critical part in India's agricultural economy (Verma & Baigh, 2012). Approximately 85% of India's oilseed production comes from seven important states, including Madhya Pradesh, which accounts for 40% of the country's production and GDP (Shukla & Gupta, 2020). Indian mustard is a valuable source of edible oil for cooking and other uses because of its oil content, which can range from 38 to 50% (Shakeel et al., 2019). The crop's adaptability is demonstrated by the range of applications it may be used for; it can be used to make sauces, edible oil, animal feed, and even biodiesel. Moreover, Indian mustard is essential for improving both the economy and food security. The crop is quite profitable, but the crop is highly sensitive to salt stress. Studies have shown that salt stress in Indian mustard decreases a number of morpho-physio-seed-quality parameters, including root and shoot length, fresh weight, and seedling vigor (Kumawat et al., 2024). Moreover, mustard plants' growth characteristics, metabolites, and antioxidant defense system are adversely affected by salt stress, which results in oxidative damage and stunted growth. In order to augment salt tolerance, we can opt for seed treatment such as bioprimering. Biological priming, which involves treating seeds with different microorganisms, is a sustainable method of improving salt tolerance and stimulating development in *Brassica juncea*. Through improved antioxidant defenses, osmotic adjustment, gene expression modulation, and chloroplast integrity, these priming strategies have demonstrated promising outcomes in minimizing the detrimental effects of salinity stress, ultimately promoting plant development and productivity (Irshad et al., 2023). By improving growth, hydration status, and gas exchange management in plants, seed priming with biological agents not only activates the defense system against combined stress conditions but also increases stress resilience and production. In mustard plants, plant-growth-promoting rhizobacteria (PGPR) are essential for reducing salt stress. Research has demonstrated that applying PGPR strains to mustard cultivars can greatly improve photosynthesis and development while reducing ethylene and oxidative stress (Khan et al., 2023). Furthermore, it has

been shown that halotolerant PGPR strains, which were isolated from the rhizosphere of halophyte plants, can improve salt tolerance by stimulating certain growth metrics when exposed to salt stress (Rabhi et al., 2022). Plant growth and stress tolerance in salty soils are enhanced by PGPR through mechanisms that include osmotic adjustment, regulation of antioxidant systems, ion homeostasis, and phytohormonal balance (Giannelli et al., 2023). These results demonstrate the potential of PGPR as a long-term approach to reduce salt stress and increase mustard plant crop output. Indian mustard is a keystone of agricultural sustainability and economic progress since its productivity is critical to increasing the nation's total oilseed yield.

## MATERIALS AND METHOD

The experiment was conducted at Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh. In this experiment 15 genotypes of diverse geographic and genetic origin of Indian mustard were taken under consideration.

Table 1: List of Genotypes

Sl.No.	Genotypes
1. G1	RH-761
2. G2	P4-CS-54
3. G3	P21-KANTI
4. G4	P6-PBR-297
5. G5	P20-KRISHNA
6. G6	P28-ROHINI
7. G7	P28-NDYS-08
8. G8	P-8-PM-21
9. G9	P20-PUSATARAK
10. G10	P2-RB-50
11. G11	RGN-298
12. G12	PUSA VIJAY
13. G13	P-2-RH-406
14. G14	P-4-PATAN MUSTARD-67
15. G15	RLM-619

Table 2: Experimental Details

Season	Rabi 2021-22
Design	CRD
Total Number of Genotypes	15
Total Number of Replications	02
Number of treatments (PGPR)	03
Number of treatments (NaCl)	03
Date of sowing	01/12/2022
Number of pots	480

Biological priming, also called bio-priming, is a method of treating seeds that includes hydrating the seeds after applying helpful microorganisms, such as *Trichoderma*, *Bacillus*, and *Pseudomonas*, to promote seed germination and early seedling growth (Sood et al., 2021). Beneficial bacteria called Plant Growth-Promoting Rhizobacteria (PGPR) inhabit the rhizosphere of plants and stimulate growth by a number of processes, including the production of phytohormones, ion exchange, and antioxidant enzyme activation (Vaishnav & Patel, 2023). In this experiment, these fifteen genotypes were bio primed with NaCl at 20, 40 and 60 % and PGPR namely *Bacillus cereus*, *Aeromonas media* and *Rhizobium alamii*. These beneficial microorganisms used in biopriming serve as vital for enhancing plant health, nutrient cycling, stress tolerance, crop productivity, and seed germination. This practice also enhances soil fertility and health, demonstrating its potential for environmentally friendly agriculture (Srivastava et al., 2024). When it comes to Indian mustard, PGPR can be quite important in lowering salinity stress. Research has demonstrated that the application of PGPR can reduce the negative effects of salinity by promoting salt tolerance and enhancing stressed plant development. In order to maintain ion homeostasis, increase food availability, and produce Osmo protectants, these bacteria cause the creation of volatile organic compounds and plant growth hormones, which in turn promotes improved growth, development, and yield in plants under salt stress (Kotiyal & Sharma, 2024).

Table 3: List of Treatments

Treatment	Description
C	Control
S1	Control+S1 (20% NaCl)
S2	Control+S2 (40% NaCl)
S3	Control+S3 (60% NaCl)
T1	PGPR-1( <i>Bacillus cereus</i> )
T2	PGPR-2 ( <i>Aeromonas media</i> )
T3	PGPR-3 ( <i>Rhizobium alamii</i> )
T1S1	PGPR-1+S1
T1S2	PGPR-1+S2
T1S3	PGPR-1+S3
T2S1	PGPR-2+S1
T2S2	PGPR-2+S2
T2S3	PGPR-2+S3
T3S1	PGPR-3+S1
T3S2	PGPR-3+S2
T3S3	PGPR-3+S3

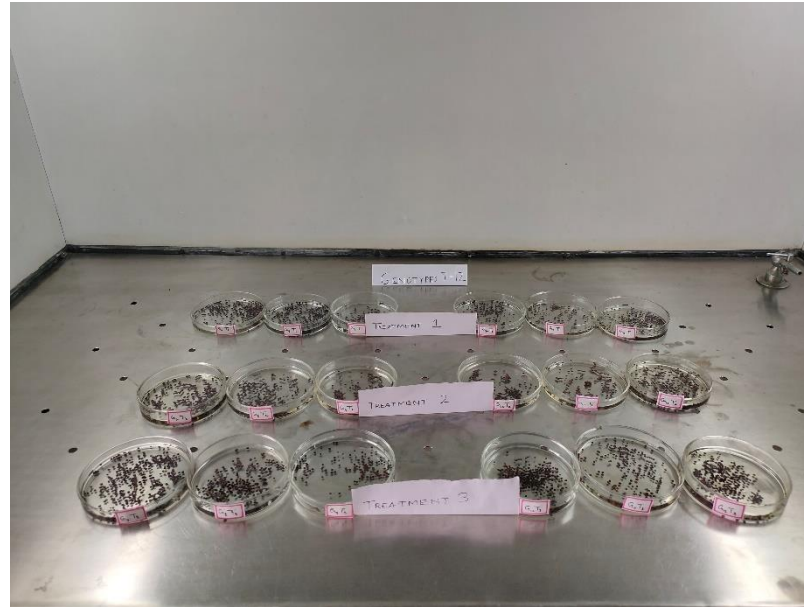


Figure 1: Seeds treated with various Biopriming agents

The various biochemical tests conducted in this experiment were:

### 1. Chlorophyll content (mg/g)

DMSO (Dimethyl sulfoxide): First 0.1 g of plant tissue was weighed and cut into smaller pieces and transferred to test tubes containing 10 ml of DMSO (Dimethyl sulfoxide) solvent. Test tubes were incubated in a water bath at 65°C for 37 1 hr until the 25 tissues became colourless. The test tubes were cooled for 30 min at room temperature and filtered. The absorption was measured at 665 nm and 648 nm using Thermo Scientific microplate reader. Blank determination was carried out with DMSO. The total chlorophyll concentration was expressed as mg/g fresh weight and determined by the following formula (Barnes et al., 1992)

Total chlorophyll (mg/g FW) = (7.49 A665+ 20.34 A648)

(Where: A665: Absorption value at 665 nm A648: Absorption value at 648 nm)

### 2. Protein (mg/g) By Lowerys Method

- Estimation of Protein Reagents:

Table 4: List of all the solutions used along with composition

Solution	Composition
Solution 'A'	4g of sodium hydroxide was dissolved in 800 ml of distilled water, then of 20 g sodium

	carbonate and 0.2g sodium potassium tartrate was added then make up the final volume 1000ml with distilled water and stored at room temperature.
Solution 'B'	0.5g CuSO <sub>4</sub> .5H <sub>2</sub> O dissolved in 80 ml of distilled water and make up the volume 100ml.
Solution 'C'	Mix 98 ml of solution 'A' with 2 ml of solution 'B' and make the volume of 100ml.
Solution 'D'	1 part of Folin- Ciocalteu reagent was diluted with distilled water to 1N.

- Preparation of Protein Standard - 100mg of BSA was dissolved in 100 ml of distilled water (for 1 mg/ml concentration).
- Extraction from Plant Sample For extraction of protein  
First 0.5g of plant leaves were taken and grind with distilled water using a mortar and pestle. The homogenate was centrifuged at 5000 rpm for 10 mins and the supernatant was used for protein estimation.
- Estimation of Protein  
Working standard solution was taken into a series of test tubes that is 0.02, 0.04, 0.06, 0.08, and 0.10ml using micropipette. 0.2ml of sample extract was pipette 38 out into separate test tube. The volume was made up to 1 ml with distilled water in all the tubes. A tube with 1 ml of distilled water served as blank. 5 ml of solution "C" was added to all tubes and mixed well and incubated at room temperature for 10 minutes. 0.5ml of solution 'D' was added, mixed well immediately and incubated at room temperature in dark for 30 minutes to develop blue color. O.D. was taken at 660 nm using Thermo Scientific microplate reader. The absorbance was read at 660 nm against the blank. Standard graph was drawn. The total amount of protein in the sample was calculated and expressed as the result as µg/ml sample (Wilson & Walker., 2007).

### 3. Proline content by Colorimetric Analysis.

Reagents:

- 3% Sulphosalicylic acid
- Acid Ninhydrin reagent – 0.625g of ninhydrin powder was dissolved in 15 ml glacial acetic acid and 10 ml 6M Phosphoric acid and agitated, until ninhydrin dissolves completely.
- Glacial acetic acid
- Toluene
- Preparation of Proline Standard - 100mg of proline was dissolved in 100 ml of distilled water (for 1 mg/ml concentration).

Procedure:

First 0.5 g of fresh plant shoot was weighed and then it was homogenized in 4 mL of 3% Sulphosalicylic acid using mortar and pestle. Then the homogenate was centrifuged for 10 min at 1000 rpm. The working standard solution was taken into a series of test tubes that is 0.02, 0.04, 0.06, 0.08, and 0.10ml using micropipette and

final volume was made up to 1 mL. In another test tube 1 mL of the supernatant was taken. After that 2 mL of acid Ninhydrin reagent and 2 mL of glacial acetic acid was added to each test tube. Then the mixture was incubated in a water bath at 100 °C for 60 min. After that the mixture was cooled suddenly in an ice bath. After cooling, 4 mL of toluene was added to the solution mixture and vortex. The chromophore containing toluene (upper layer) was transferred to a new test tube. Finally, the absorbance was read at 520 nm using Thermo Scientific microplate reader and toluene was used as a blank. The concentration of proline was determined using the standard curve and expressed as  $\mu\text{g mL}^{-1}$

Observation for 13 primary characteristics were noted in order to analyse the effect of various biochemical test against the salinity stress. The 13 characters traits under study are mentioned in table 5:

Table 5: List of all the Characters under study

Sl. No.	Characters / traits
1.	Days to 50% flowering
2.	Plant height (cm)
3.	Number of primary branches per plant
4.	Number of secondary branches per plant
5.	Number of siliquae per plant
6.	Number of seeds per siliqua
7.	Length of siliqua (cm)
8.	Seed yield (gm)
9.	Biological yield (g)
10.	Harvest Index (%)
11.	Chlorophyll content
12.	Proline
13.	Protein

## RESULT AND DISCUSSION

In order to evaluate the performance of the 15 genotypes and to compare the effect of the treatment on them we need to calculate the mean of the observation. The mean value of each character was worked out by dividing the totals by the corresponding number of observations.

Arithmetic mean  $X = \Sigma X / N$

(Where,  $\Sigma X$  = Sum of all the observations for each character in replication, N = Corresponding number of observations)

Table 6: Mean performance of mustard genotypes, PGPR treatments and interactions for yield and yield contributing traits

Treatments	Days to 50% flowering	Plant height (cm)	Number of primary branches per plant	Number of secondary branches per plant	Number of siliqua per plant	Number of seeds per siliqua	Length of siliqua (cm)	Seed yield (gm)	Biological yield (g)	Harvest Index (%)	Chlorophyll content	Proline	Protein
G1T1	56.25	94.50	2.50	2.25	91.00	12.00	4.98	15.50	48.75	31.80	0.98	15.27	20.79
G1T2	52.50	77.50	1.75	1.25	71.00	10.50	4.38	8.50	38.25	22.23	0.98	24.39	20.37
G1T3	50.50	75.75	1.25	1.00	68.25	10.50	3.75	7.25	36.25	20.01	1.35	25.39	20.67
G1T4	49.00	75.50	1.00	0.25	66.00	9.75	3.63	7.00	33.25	21.07	1.26	28.87	21.34
G1T5	57.50	100.75	2.75	2.75	94.80	13.00	5.48	19.50	51.25	38.07	1.01	17.95	23.48
G1T6	54.75	97.75	2.50	3.00	95.25	12.25	5.13	17.75	49.25	36.07	1.37	15.94	20.43
G1T7	53.75	99.25	3.00	3.50	99.00	12.75	5.40	19.75	52.25	37.80	1.18	17.05	21.83
G1T8	50.75	94.00	2.25	2.00	86.00	12.25	4.88	17.50	49.00	35.71	0.85	18.34	24.64
G1T9	54.38	91.00	2.00	1.75	78.00	11.75	4.75	17.25	48.00	35.94	0.57	20.27	26.57
G1T10	51.50	90.00	1.50	1.00	74.25	11.25	4.50	16.25	44.25	36.85	0.37	23.83	28.53
G1T11	49.75	92.25	2.25	2.50	85.00	12.50	5.08	18.00	47.00	38.32	1.06	17.59	21.75
G1T12	53.25	94.25	2.00	2.25	80.75	12.50	4.25	17.75	45.25	39.34	0.80	19.56	23.40
G1T13	56.13	91.00	1.25	1.25	78.25	11.25	4.13	16.50	43.25	38.18	0.54	22.17	25.59
G1T14	54.25	97.75	2.50	2.25	89.00	12.00	5.08	18.25	47.75	38.23	0.94	17.33	22.91
G1T15	52.25	95.75	1.25	1.75	86.00	11.75	4.95	18.00	46.50	38.73	0.63	19.63	24.12
G1T16	52.57	89.00	1.25	1.00	77.25	11.25	4.65	17.25	45.50	37.92	0.51	21.85	25.88
G2T1	57.75	88.50	2.63	2.75	82.00	11.25	4.20	7.75	37.50	20.67	1.51	13.80	19.48
G2T2	56.25	77.25	1.25	2.25	69.25	10.50	3.63	6.50	33.00	20.00	1.17	14.74	15.69

<b>G2T3</b>	52.50	75.00	1.25	1.75	67.50	10.00	3.25	6.63	32.25	20.54	0.98	16.13	16.73
<b>G2T4</b>	49.00	73.25	1.00	1.75	62.00	9.75	3.13	6.13	30.00	20.42	1.11	18.32	17.66
<b>G2T5</b>	48.00	98.00	2.75	3.25	91.25	13.50	4.93	8.50	43.25	19.64	1.70	11.53	20.80
<b>G2T6</b>	54.75	98.75	2.75	3.00	87.00	12.75	4.93	8.50	45.25	18.79	1.88	12.71	18.11
<b>G2T7</b>	54.00	98.25	2.88	2.75	89.00	12.50	4.65	9.75	46.00	21.21	1.81	13.62	19.85
<b>G2T8</b>	51.50	93.25	2.25	2.63	84.00	12.25	3.88	7.93	39.00	20.34	1.54	15.02	21.18
<b>G2T9</b>	57.00	93.00	2.00	2.38	80.00	11.50	3.88	7.88	43.00	18.33	1.20	16.23	23.12
<b>G2T10</b>	54.38	89.25	1.75	2.25	81.00	12.00	3.63	7.65	38.25	20.00	0.79	18.34	24.33
<b>G2T11</b>	50.75	96.75	2.50	2.88	83.25	12.50	4.50	8.13	43.25	18.80	1.63	13.25	18.93
<b>G2T12</b>	48.50	95.50	2.08	2.28	79.25	12.00	4.13	8.13	39.25	20.71	1.45	14.80	20.32
<b>G2T13</b>	51.38	93.25	2.00	2.00	79.25	11.25	4.00	7.88	39.25	20.07	0.91	16.19	22.14
<b>G2T14</b>	54.38	97.25	2.00	2.38	75.00	12.00	3.50	7.88	44.00	17.92	1.40	14.00	19.84
<b>G2T15</b>	52.75	93.25	1.88	2.00	74.25	11.25	3.50	7.75	41.25	18.81	1.35	15.06	21.82
<b>G2T16</b>	54.25	91.00	1.75	2.13	72.25	11.00	3.25	7.38	39.25	18.81	0.89	17.02	23.51
<b>G3T1</b>	56.25	81.00	2.70	3.13	79.00	12.50	4.68	11.25	43.25	26.03	1.15	14.26	21.45
<b>G3T2</b>	52.50	69.00	2.00	2.25	69.00	12.25	3.98	10.63	39.25	27.11	1.15	19.74	14.26
<b>G3T3</b>	49.00	67.25	1.75	2.25	69.00	12.00	3.78	10.00	37.25	26.81	0.86	21.46	15.92
<b>G3T4</b>	48.00	65.00	1.50	2.00	65.00	12.25	3.88	9.75	37.25	26.18	0.93	15.64	17.55
<b>G3T5</b>	57.00	89.25	3.00	4.50	87.00	13.00	5.58	12.98	51.25	25.34	1.20	17.89	19.24
<b>G3T6</b>	54.75	86.50	3.05	3.75	95.00	14.00	5.23	12.25	45.25	27.25	1.59	15.14	21.15
<b>G3T7</b>	53.75	88.00	3.13	4.00	97.00	13.00	4.95	12.63	45.25	27.95	1.39	16.24	21.71
<b>G3T8</b>	51.50	87.50	2.63	4.13	83.25	12.75	5.13	12.13	44.00	27.60	1.05	18.84	24.17

<b>G3T9</b>	54.38	83.00	2.50	4.13	82.00	11.75	4.95	11.95	46.00	26.03	0.93	19.89	25.64
<b>G3T10</b>	50.75	82.25	2.38	4.00	81.00	11.25	4.65	11.80	39.00	30.28	0.71	21.37	27.35
<b>G3T11</b>	48.50	82.00	3.00	3.25	91.00	12.75	5.00	12.13	44.20	27.49	1.30	15.66	21.27
<b>G3T12</b>	52.50	80.25	2.93	3.13	89.00	12.50	4.73	12.00	43.20	27.80	1.13	17.78	23.11
<b>G3T13</b>	55.88	79.25	2.68	3.13	82.00	12.25	4.38	11.50	41.00	28.04	0.81	19.92	24.97
<b>G3T14</b>	54.25	88.00	3.00	3.88	92.25	12.50	5.00	11.50	43.25	26.58	1.14	16.67	23.04
<b>G3T15</b>	52.63	87.25	2.85	3.75	88.00	12.00	4.75	11.13	42.50	26.18	0.95	18.88	23.67
<b>G3T16</b>	52.94	86.00	2.38	3.50	87.00	11.50	4.63	10.88	41.00	26.57	0.63	20.99	25.94
<b>G4T1</b>	56.17	84.00	2.25	2.75	73.00	12.00	4.40	10.25	35.00	29.33	1.40	13.68	20.25
<b>G4T2</b>	52.50	73.00	1.25	1.25	68.20	10.50	3.70	8.38	29.00	28.87	0.90	14.40	21.51
<b>G4T3</b>	49.00	72.20	1.00	1.25	64.00	10.75	3.55	8.13	27.50	29.60	1.17	16.12	21.52
<b>G4T4</b>	48.00	71.00	1.00	1.00	64.00	10.50	3.58	7.63	25.75	29.63	1.10	14.62	21.74
<b>G4T6</b>	54.75	89.00	2.25	3.25	79.25	13.25	5.08	11.25	39.95	28.20	1.74	12.86	21.94
<b>G4T7</b>	53.75	88.25	2.50	3.50	78.75	13.00	4.83	11.75	43.00	27.36	1.68	13.61	20.47
<b>G4T8</b>	51.50	88.25	2.38	3.13	74.00	11.75	4.38	11.50	41.00	28.04	1.45	15.10	22.40
<b>G4T9</b>	54.34	85.25	2.13	2.75	73.25	12.00	4.30	11.65	39.50	29.52	1.27	16.63	23.61
<b>G4T10</b>	50.75	84.00	2.00	2.38	73.00	11.50	4.33	11.28	38.00	29.67	0.71	18.78	25.02
<b>G4T11</b>	48.50	85.00	2.13	2.75	77.00	12.00	4.18	10.88	36.50	29.83	1.58	13.40	19.36
<b>G4T12</b>	52.63	81.00	2.25	2.75	75.00	11.50	4.75	10.38	34.00	30.56	1.34	14.35	20.83
<b>G4T13</b>	56.00	80.00	1.88	2.50	71.00	11.00	4.63	10.40	33.50	31.06	0.99	16.03	23.05
<b>G4T15</b>	52.63	81.00	2.00	2.50	72.50	10.75	4.13	11.40	38.25	29.81	1.20	15.51	22.35
<b>G4T16</b>	52.92	79.25	2.00	2.13	71.00	11.50	4.00	11.00	35.75	30.78	0.86	17.17	24.25

<b>G5T1</b>	56.25	72.50	2.13	2.25	71.00	12.00	4.78	10.50	37.00	28.44	0.88	15.47	21.42
<b>G5T2</b>	52.50	68.00	1.50	1.50	65.25	10.75	4.08	7.83	30.00	26.16	1.07	23.10	14.91
<b>G5T3</b>	49.00	63.00	1.50	1.25	62.00	10.75	3.90	7.65	27.25	28.17	0.93	18.05	16.52
<b>G5T4</b>	48.00	60.00	1.25	1.50	60.25	10.75	3.70	7.13	26.00	27.40	0.88	20.14	18.08
<b>G5T5</b>	57.00	79.00	2.50	3.00	83.50	13.00	5.38	12.25	43.25	28.34	1.04	18.08	19.84
<b>G5T6</b>	54.75	77.50	2.25	2.75	80.50	12.75	5.28	12.25	40.75	30.06	1.23	16.37	21.00
<b>G5T7</b>	53.75	76.00	2.50	2.75	77.75	12.50	5.28	11.75	43.00	27.36	1.12	17.25	22.41
<b>G5T8</b>	51.50	77.50	2.25	2.25	80.50	12.25	4.63	12.00	41.00	29.29	0.77	18.99	25.22
<b>G5T9</b>	54.38	73.25	2.13	2.50	80.25	11.75	4.38	11.13	39.25	28.37	0.48	21.20	25.66
<b>G5T10</b>	50.75	72.75	1.75	2.25	79.00	11.25	4.30	11.00	36.50	30.15	0.32	23.97	27.18
<b>G5T11</b>	48.50	75.00	2.00	2.25	75.00	11.75	4.18	12.00	37.25	32.23	1.20	18.81	21.93
<b>G5T12</b>	52.50	74.00	1.75	2.00	74.25	12.25	4.00	11.88	38.25	31.05	0.79	20.09	23.76
<b>G5T13</b>	55.88	73.50	1.88	1.75	73.00	11.75	3.98	11.25	36.25	31.04	0.69	22.97	25.43
<b>G5T14</b>	54.25	75.50	2.13	2.50	76.00	11.25	4.63	11.50	37.50	30.69	0.89	17.99	23.04
<b>G5T15</b>	52.63	73.25	1.75	2.25	74.25	10.50	4.40	11.00	37.00	29.73	0.67	20.04	25.00
<b>G5T16</b>	52.94	71.00	1.50	2.00	73.50	10.25	4.10	10.75	35.00	30.76	0.52	22.36	26.47
<b>G6T1</b>	56.25	68.25	2.00	2.75	59.00	11.75	4.68	10.75	37.00	29.06	1.20	15.47	27.46
<b>G6T2</b>	52.50	63.75	1.00	1.00	55.05	11.25	3.80	7.75	29.50	26.30	0.70	17.77	21.23
<b>G6T3</b>	49.00	60.75	1.00	0.75	52.50	11.00	3.63	7.25	29.25	24.83	1.07	23.11	26.48
<b>G6T4</b>	48.00	63.25	1.00	0.75	52.00	10.50	3.55	7.25	28.25	25.66	0.77	15.50	25.80
<b>G6T5</b>	57.00	74.50	2.75	3.50	70.75	13.00	5.28	11.75	41.50	28.31	0.65	18.56	23.76
<b>G6T6</b>	54.75	75.50	2.75	3.75	73.00	13.00	5.10	11.25	41.25	27.28	0.92	20.11	23.57

<b>G6T7</b>	53.75	73.75	2.25	3.25	71.00	12.50	5.05	11.50	42.25	27.23	0.78	22.90	26.77
<b>G6T8</b>	51.50	73.00	2.50	3.25	66.50	11.75	4.75	10.75	40.00	26.88	0.96	15.12	27.52
<b>G6T9</b>	54.38	71.25	2.25	2.50	65.00	11.50	4.90	10.25	39.25	26.11	0.89	21.15	23.64
<b>G6T10</b>	50.75	69.75	1.75	2.50	63.50	10.50	4.75	10.50	38.25	27.46	0.94	18.28	24.29
<b>G6T11</b>	48.50	73.25	2.25	2.75	68.00	12.50	4.75	10.75	40.50	26.53	0.98	15.93	21.71
<b>G6T12</b>	52.50	73.75	2.00	2.50	67.00	12.00	4.63	11.00	38.75	28.39	0.91	15.30	22.39
<b>G6T13</b>	55.88	71.00	1.75	2.25	66.25	11.50	4.40	10.25	37.75	27.16	0.66	23.71	27.26
<b>G6T14</b>	54.25	72.00	1.75	2.75	69.00	11.25	5.00	10.88	37.50	28.98	0.69	22.87	26.67
<b>G6T15</b>	52.63	70.75	1.50	2.63	70.00	11.50	4.88	10.75	36.75	29.23	0.86	23.28	26.48
<b>G6T16</b>	52.94	70.00	1.50	2.25	68.00	11.00	4.63	10.13	36.25	27.93	1.03	23.01	22.57
<b>G7T1</b>	56.25	69.50	2.25	2.25	67.00	11.75	4.03	9.25	37.00	25.04	1.11	15.20	20.64
<b>G7T2</b>	52.50	58.25	1.50	1.50	56.50	11.50	3.93	7.75	31.00	25.06	0.89	23.35	27.56
<b>G7T3</b>	49.00	54.75	1.25	1.00	53.25	11.00	3.75	7.00	28.25	24.78	0.97	15.18	21.69
<b>G7T4</b>	48.00	54.25	1.00	0.75	51.25	10.75	3.70	6.75	28.00	24.17	0.69	15.96	20.50
<b>G7T5</b>	57.00	76.25	2.75	3.50	74.25	13.00	4.28	10.98	41.50	26.49	0.81	16.74	23.02
<b>G7T6</b>	54.75	77.00	2.75	3.25	74.20	13.25	4.38	11.50	41.00	28.04	0.97	15.03	20.36
<b>G7T7</b>	53.75	72.75	2.50	3.00	72.00	12.25	4.25	10.75	41.75	25.74	1.17	15.81	21.73
<b>G7T8</b>	51.50	73.50	2.63	2.75	68.00	12.00	4.08	10.25	39.75	25.87	1.12	17.40	23.82
<b>G7T9</b>	54.38	72.50	2.50	2.50	64.00	12.50	3.95	10.00	39.00	25.66	0.82	19.17	24.90
<b>G7T10</b>	50.75	74.00	2.00	2.25	62.00	11.50	3.85	9.50	37.00	25.70	1.16	21.35	26.96
<b>G7T11</b>	48.50	74.25	2.50	3.00	69.50	12.50	4.08	11.25	40.00	28.13	0.99	14.95	21.09
<b>G7T12</b>	52.50	73.25	2.25	2.50	67.00	11.75	4.00	10.75	38.25	28.11	1.12	17.72	22.37

<b>G7T13</b>	55.88	71.00	2.00	2.25	67.50	11.25	4.00	10.50	37.00	28.44	1.14	19.35	24.61
<b>G7T14</b>	54.25	70.75	2.25	2.25	68.00	11.50	4.08	11.25	39.00	28.88	0.81	16.16	22.27
<b>G7T15</b>	52.63	70.50	2.00	2.25	66.00	11.75	3.83	10.75	37.00	29.02	0.88	18.53	22.98
<b>G7T16</b>	52.94	68.75	1.75	2.00	64.00	11.00	3.70	10.50	37.00	28.41	0.81	20.28	24.95
<b>G8T1</b>	57.75	68.50	2.25	2.75	70.50	11.50	4.05	9.75	32.00	30.54	1.14	17.50	24.11
<b>G8T2</b>	56.25	57.50	1.25	1.00	56.00	11.00	3.65	8.25	27.00	30.63	1.14	17.38	24.76
<b>G8T3</b>	52.50	55.25	1.00	0.75	55.00	10.75	3.55	7.40	25.25	29.36	0.98	15.48	24.49
<b>G8T4</b>	49.00	53.25	1.00	0.75	54.00	11.00	3.30	7.35	24.25	30.52	0.75	18.19	22.93
<b>G8T5</b>	48.00	72.75	2.75	3.50	77.25	12.25	4.63	11.75	39.00	30.13	1.19	16.34	23.04
<b>G8T6</b>	54.75	75.00	2.50	3.25	76.00	12.25	4.43	12.50	37.25	33.55	0.83	19.70	22.73
<b>G8T7</b>	53.75	71.75	2.50	3.50	78.00	12.00	4.50	11.50	37.25	30.86	0.82	19.75	25.87
<b>G8T8</b>	51.50	69.50	2.25	3.25	73.00	12.25	4.38	10.75	36.00	29.87	0.94	17.86	26.82
<b>G8T9</b>	57.00	70.00	2.25	2.75	72.00	12.00	4.10	10.50	33.00	31.80	1.12	15.93	24.31
<b>G8T10</b>	54.38	67.00	2.00	2.50	71.00	11.75	3.73	10.00	32.00	31.37	1.05	17.97	23.25
<b>G8T11</b>	50.75	73.25	2.00	3.00	70.00	12.00	4.13	11.75	35.25	33.40	0.82	21.47	27.08
<b>G8T12</b>	48.50	73.00	2.00	2.75	69.00	11.75	3.98	11.50	34.25	33.62	0.82	23.02	20.67
<b>G8T13</b>	51.38	71.00	1.75	2.63	69.00	11.00	3.85	10.50	32.50	32.29	1.06	19.17	23.25
<b>G8T14</b>	54.25	67.50	2.00	2.75	76.00	12.00	4.20	9.63	35.50	27.11	0.73	17.77	27.35
<b>G8T15</b>	52.63	66.00	1.75	2.75	75.00	12.00	4.10	9.50	33.75	28.15	0.90	22.29	26.53
<b>G8T16</b>	54.25	65.00	1.50	2.25	72.00	11.25	3.98	9.25	32.00	28.91	1.02	21.89	25.11
<b>G9T1</b>	56.00	67.50	2.00	2.50	67.00	12.50	4.70	9.75	30.75	31.66	0.66	15.41	20.64
<b>G9T2</b>	52.50	59.75	0.75	1.00	59.00	11.75	4.15	7.78	26.25	29.62	0.73	16.63	25.45

<b>G9T3</b>	49.00	58.00	1.00	1.00	57.00	11.50	4.00	7.24	22.50	32.15	0.99	22.14	21.68
<b>G9T4</b>	48.00	54.00	0.75	0.75	54.75	11.25	3.95	7.05	23.25	30.42	0.74	16.30	23.75
<b>G9T5</b>	57.75	78.25	2.75	3.25	80.50	12.75	5.05	11.75	38.25	30.77	1.13	18.41	25.85
<b>G9T6</b>	54.75	80.00	2.50	3.25	84.25	13.50	5.05	11.25	35.50	31.67	0.70	22.46	26.16
<b>G9T7</b>	53.75	78.00	2.75	3.00	80.00	13.25	5.05	10.75	36.00	29.83	0.96	17.76	23.09
<b>G9T8</b>	51.50	76.25	2.25	2.75	80.00	11.50	4.75	10.75	36.25	29.69	0.76	17.86	25.56
<b>G9T9</b>	54.25	74.00	2.50	3.00	78.00	12.25	4.73	10.75	34.75	31.06	0.83	22.37	24.51
<b>G9T10</b>	50.75	72.50	2.25	2.25	76.00	11.50	4.60	10.25	32.75	31.33	0.83	16.72	21.47
<b>G9T11</b>	48.50	78.00	2.25	2.50	82.00	12.00	4.88	11.50	34.75	33.11	1.20	16.20	23.84
<b>G9T12</b>	52.88	76.25	2.00	2.50	79.00	12.25	4.80	11.25	33.25	33.83	0.79	15.92	26.69
<b>G9T13</b>	56.25	72.00	1.75	2.25	74.00	11.50	4.63	10.75	34.50	31.16	1.11	20.21	25.35
<b>G9T14</b>	54.25	76.25	1.75	2.75	78.00	12.00	4.95	10.50	32.50	32.34	0.92	16.99	28.09
<b>G9T15</b>	52.63	74.00	1.50	2.50	75.00	11.50	5.00	10.50	33.25	31.63	0.94	15.51	23.47
<b>G9T16</b>	52.88	73.75	1.50	2.25	74.00	12.25	4.65	10.25	29.75	34.46	0.70	16.18	23.31
<b>G10T1</b>	56.25	83.50	2.25	3.00	68.00	12.00	4.98	11.00	40.00	27.64	0.77	18.05	27.66
<b>G10T2</b>	52.50	66.00	1.00	1.75	42.25	11.75	3.93	7.68	35.75	21.47	1.01	19.15	24.25
<b>G10T3</b>	49.00	64.00	1.00	1.50	36.50	11.50	3.80	7.00	33.25	21.07	1.16	21.02	22.00
<b>G10T4</b>	48.00	63.25	1.00	1.25	33.25	11.00	3.78	7.10	32.25	22.09	1.05	19.43	27.58
<b>G10T5</b>	57.75	95.50	2.75	4.25	74.75	13.25	5.50	12.83	54.25	23.65	0.68	17.02	27.92
<b>G10T6</b>	54.75	92.50	3.00	4.25	80.75	13.25	5.40	12.03	51.25	23.49	0.98	15.92	23.13
<b>G10T7</b>	53.75	96.50	2.75	4.75	85.00	12.75	4.93	12.00	48.78	24.63	1.20	21.12	26.74
<b>G10T8</b>	51.50	87.75	2.50	3.75	66.75	12.75	5.38	11.90	45.38	26.23	0.83	16.49	25.13

<b>G10T9</b>	54.38	86.50	2.25	3.50	64.00	12.00	4.95	11.95	45.25	26.42	0.77	18.48	20.70
<b>G10T10</b>	50.75	86.00	2.00	2.75	60.00	12.50	4.38	11.65	43.25	26.95	1.04	19.87	20.80
<b>G10T11</b>	48.50	94.00	2.75	3.50	74.25	13.00	4.98	11.00	44.25	24.85	0.87	16.84	26.65
<b>G10T12</b>	52.88	90.50	2.50	3.50	69.75	12.75	4.75	11.15	43.36	25.62	0.78	17.14	23.30
<b>G10T13</b>	56.25	90.50	2.25	3.00	69.00	11.50	4.38	11.75	47.00	25.00	1.11	21.29	22.51
<b>G10T14</b>	54.25	84.25	2.25	4.25	72.50	12.25	4.75	11.75	43.25	27.19	1.02	21.55	23.75
<b>G10T15</b>	52.63	85.00	2.25	3.75	70.25	12.25	4.40	11.75	43.25	27.21	0.66	23.01	20.82
<b>G10T16</b>	52.94	80.75	2.00	3.50	67.25	10.50	4.12	10.75	41.75	25.72	0.80	15.78	23.27
<b>G11T1</b>	56.25	86.00	2.00	2.75	67.00	12.00	4.20	7.19	33.00	21.79	0.97	18.43	24.45
<b>G11T2</b>	52.50	64.00	1.00	1.00	59.00	11.50	3.63	6.90	28.50	24.27	0.96	19.21	20.91
<b>G11T3</b>	49.00	59.00	1.00	1.00	53.00	11.50	3.63	6.65	25.50	26.10	0.74	20.61	26.65
<b>G11T4</b>	48.00	57.00	1.00	0.75	51.00	11.50	3.50	6.57	25.00	26.26	1.17	16.68	26.82
<b>G11T5</b>	57.00	88.00	2.75	4.00	71.00	14.50	5.15	8.05	40.00	20.16	0.78	16.64	21.38
<b>G11T6</b>	54.75	94.00	3.00	4.50	79.00	15.00	5.10	10.75	43.00	25.00	1.08	20.83	27.73
<b>G11T7</b>	53.75	95.50	3.25	4.75	84.00	13.00	5.30	9.65	39.50	24.43	0.90	15.71	21.08
<b>G11T8</b>	51.50	84.00	2.50	3.25	69.00	13.25	4.55	7.57	36.00	21.04	1.10	21.82	24.78
<b>G11T9</b>	54.38	80.50	2.25	2.75	69.00	12.50	4.25	7.74	35.00	22.10	0.85	18.14	27.39
<b>G11T10</b>	50.75	77.00	1.75	2.25	67.00	12.50	4.20	7.40	32.50	22.78	0.67	19.24	21.81
<b>G11T11</b>	48.50	87.50	2.50	3.25	74.00	13.50	4.50	8.53	39.00	21.86	1.06	18.97	24.24
<b>G11T12</b>	52.50	81.50	2.25	2.50	71.50	13.25	4.65	7.85	36.00	21.82	0.87	21.90	26.02
<b>G11T13</b>	55.88	67.00	2.00	2.25	70.50	12.50	4.35	7.28	32.00	22.77	0.76	20.06	23.96
<b>G11T14</b>	54.25	86.00	2.50	3.50	81.00	13.50	4.75	7.25	34.00	21.34	1.05	16.50	22.82

<b>G11T15</b>	52.63	85.00	2.00	3.25	80.00	12.00	4.70	7.10	29.50	24.08	0.92	15.81	22.40
<b>G11T16</b>	52.94	79.50	2.00	2.50	69.00	12.00	4.25	7.04	29.50	23.90	0.78	22.06	20.51
<b>G12T1</b>	56.25	70.50	2.25	2.75	85.00	12.25	4.35	10.13	35.25	28.72	1.08	17.87	27.71
<b>G12T2</b>	52.50	63.00	1.00	1.75	66.50	10.50	3.80	8.20	29.50	27.83	0.82	23.01	28.04
<b>G12T3</b>	49.00	64.00	1.00	1.50	64.00	10.50	3.90	7.85	28.00	28.05	0.85	18.86	24.81
<b>G12T4</b>	48.00	54.00	1.00	1.50	61.00	10.50	3.40	7.58	26.00	29.13	1.11	14.94	21.19
<b>G12T5</b>	57.00	90.50	2.75	4.25	94.00	12.75	5.25	11.88	40.00	29.70	0.74	15.31	26.34
<b>G12T6</b>	54.75	85.50	2.75	4.50	100.00	13.25	5.65	12.25	42.50	28.84	1.01	21.41	26.71
<b>G12T7</b>	53.75	87.50	3.00	4.75	95.00	13.00	5.35	11.33	38.00	29.82	0.79	23.75	22.16
<b>G12T8</b>	51.50	82.50	2.25	2.75	89.50	12.25	4.63	11.35	39.25	28.92	0.68	18.07	23.92
<b>G12T9</b>	54.38	77.00	2.00	2.50	87.50	12.00	4.50	10.98	37.75	29.08	0.99	18.36	22.79
<b>G12T10</b>	50.75	75.50	1.75	2.50	86.50	11.50	4.35	10.88	35.50	30.65	0.79	22.19	22.68
<b>G12T11</b>	48.50	83.50	2.50	3.25	93.50	12.75	5.28	11.35	36.50	31.11	1.13	20.96	26.63
<b>G12T12</b>	52.50	82.50	2.25	3.00	86.50	12.75	5.00	10.63	34.00	31.25	1.14	20.70	27.65
<b>G12T13</b>	55.88	77.00	1.75	2.75	84.00	12.25	4.63	10.45	33.75	30.96	0.92	21.58	20.73
<b>G12T14</b>	54.25	82.50	2.75	3.50	86.50	13.50	5.38	11.20	39.00	28.72	0.91	19.40	27.15
<b>G12T15</b>	52.63	80.00	2.25	3.25	84.00	13.00	5.00	11.33	37.75	30.00	0.80	19.28	27.13
<b>G12T16</b>	52.94	80.00	1.75	2.75	82.50	12.50	4.50	11.33	36.50	31.03	0.92	21.70	23.46
<b>G13T1</b>	57.00	70.50	1.75	2.75	61.00	12.25	4.13	10.08	32.50	31.06	0.70	23.70	24.09
<b>G13T2</b>	56.25	58.50	1.00	1.50	46.50	10.50	3.88	8.20	27.50	29.84	0.66	22.48	22.14
<b>G13T3</b>	52.50	56.00	1.00	1.25	43.00	11.00	3.65	7.46	25.25	29.60	1.08	21.58	22.88
<b>G13T4</b>	49.00	51.00	1.00	1.00	39.00	11.25	3.13	7.20	23.58	30.55	1.15	18.36	21.66

<b>G13T5</b>	48.00	76.50	2.75	3.75	69.00	14.75	5.30	11.95	39.00	30.65	0.76	15.45	20.89
<b>G13T6</b>	54.75	80.50	2.50	3.50	72.50	13.50	5.13	12.28	39.25	31.28	0.75	23.73	19.89
<b>G13T7</b>	53.75	80.50	2.75	4.00	70.00	14.50	5.10	11.58	38.00	30.45	0.96	18.28	23.35
<b>G13T8</b>	51.50	69.50	2.25	2.75	65.00	12.25	5.13	11.70	37.50	31.21	1.11	21.38	21.53
<b>G13T9</b>	56.63	68.00	1.75	2.75	59.50	12.00	4.63	10.20	33.25	30.69	1.17	21.15	21.17
<b>G13T10</b>	54.38	66.00	1.50	2.50	55.00	11.75	4.25	10.48	31.50	32.33	1.20	22.28	21.14
<b>G13T11</b>	50.75	71.00	2.25	3.13	67.00	12.50	4.85	11.10	35.00	31.73	1.07	21.53	25.47
<b>G13T12</b>	48.50	70.50	2.00	2.88	59.00	12.75	4.38	10.85	34.50	31.47	0.69	18.21	20.33
<b>G13T13</b>	51.38	70.00	1.75	2.25	54.00	11.50	4.50	10.48	33.00	31.75	1.16	19.76	20.59
<b>G13T14</b>	54.25	71.00	2.50	3.38	64.00	12.00	4.98	10.13	32.00	31.66	0.80	22.66	27.90
<b>G13T15</b>	52.63	69.50	2.25	3.25	62.00	12.50	5.10	11.75	34.50	34.03	1.01	15.18	27.19
<b>G13T16</b>	54.07	66.00	2.00	2.25	59.00	11.75	4.50	10.33	32.00	32.27	1.12	17.41	24.21
<b>G14T1</b>	56.25	70.50	1.75	3.25	68.50	12.50	4.25	10.35	34.83	29.72	0.82	16.57	23.85
<b>G14T2</b>	52.50	59.50	1.00	1.75	51.50	11.50	3.60	8.55	29.25	29.22	1.09	21.29	24.43
<b>G14T3</b>	49.00	58.50	1.00	1.50	48.50	12.00	3.48	8.05	28.75	28.04	1.07	17.07	24.54
<b>G14T4</b>	48.00	56.50	1.00	1.50	48.00	11.50	3.38	7.44	25.83	28.80	1.21	15.45	26.82
<b>G14T5</b>	57.75	84.50	2.75	4.25	73.50	15.50	4.95	11.35	39.25	28.92	1.14	21.28	24.62
<b>G14T6</b>	54.75	79.00	2.50	4.50	76.50	14.50	5.25	11.85	42.00	28.23	0.84	18.41	28.13
<b>G14T7</b>	53.75	77.50	2.75	4.50	81.50	14.50	5.05	11.65	40.23	28.98	1.12	18.17	25.89
<b>G14T8</b>	51.50	80.50	2.00	3.13	67.50	12.50	4.55	11.50	38.50	29.84	1.18	21.63	21.05
<b>G14T9</b>	54.38	71.00	1.50	3.13	67.50	13.00	4.70	11.20	37.08	30.21	0.91	19.14	27.39
<b>G14T10</b>	50.75	73.00	1.50	2.50	59.50	12.50	4.38	9.98	34.08	29.30	1.13	19.54	20.15

<b>G14T11</b>	48.50	73.50	2.25	3.00	70.50	12.50	4.88	11.33	41.33	27.42	1.15	23.85	26.36
<b>G14T12</b>	52.88	72.00	1.75	3.00	67.00	12.00	4.75	10.88	37.05	29.37	1.06	17.32	25.67
<b>G14T13</b>	56.25	70.00	1.75	2.88	65.00	11.50	4.38	10.35	34.08	30.43	1.02	15.68	22.84
<b>G14T14</b>	54.25	73.50	2.50	3.88	70.50	13.25	4.38	9.88	36.08	27.39	0.91	20.78	25.89
<b>G14T15</b>	52.63	73.00	2.25	3.25	70.00	12.50	4.13	9.75	33.50	29.15	1.12	18.88	26.66
<b>G14T16</b>	52.94	70.00	1.75	2.75	64.00	12.25	3.95	9.28	32.25	28.78	1.10	18.57	25.24
<b>G15T1</b>	56.25	86.50	1.75	3.50	69.00	12.50	4.40	16.55	48.25	34.31	1.06	23.11	23.52
<b>G15T2</b>	52.50	66.50	1.00	2.25	57.50	11.50	3.93	9.05	37.88	23.90	0.72	20.46	23.54
<b>G15T3</b>	49.00	66.00	1.00	2.25	55.00	11.00	4.05	8.40	35.75	23.50	0.90	22.85	25.28
<b>G15T4</b>	48.00	62.50	1.00	2.00	51.00	10.00	3.50	8.25	33.25	24.83	0.97	21.86	25.29
<b>G15T5</b>	57.00	99.50	2.75	4.75	95.00	13.50	5.38	19.05	52.25	36.46	0.82	19.95	26.06
<b>G15T6</b>	54.75	90.00	2.50	4.63	90.00	12.50	5.08	18.70	50.25	37.22	0.76	21.18	21.37
<b>G15T7</b>	53.75	94.00	2.75	4.88	88.50	14.50	5.10	19.08	52.38	36.42	0.73	21.18	26.94
<b>G15T8</b>	51.50	85.00	2.00	3.88	71.00	12.50	4.66	18.35	49.75	36.89	0.89	15.54	26.24
<b>G15T9</b>	54.38	80.50	2.00	3.25	70.00	13.00	4.45	18.05	48.75	37.03	0.72	21.77	22.43
<b>G15T10</b>	50.75	79.50	1.75	2.75	68.00	12.50	4.25	17.65	48.28	36.57	0.76	21.42	23.21
<b>G15T11</b>	48.50	84.00	2.25	3.13	76.50	12.50	4.85	17.45	47.00	37.13	0.90	19.97	24.65
<b>G15T12</b>	52.50	82.50	2.25	2.75	72.50	12.75	4.75	17.23	45.50	37.86	0.67	18.71	25.18
<b>G15T13</b>	55.88	79.50	1.75	2.25	69.50	12.50	5.15	17.08	42.83	39.88	1.05	18.02	21.45
<b>G15T14</b>	54.25	79.00	2.00	3.00	73.50	12.50	4.50	18.20	47.75	38.12	0.87	15.15	20.28
<b>G15T15</b>	52.63	76.50	1.75	2.75	68.50	12.00	4.50	18.03	45.33	39.77	1.06	16.85	20.50
<b>G15T16</b>	52.94	74.00	1.50	2.25	70.50	11.50	4.13	17.85	44.85	39.81	0.86	15.36	21.65

<b>S.Em ±</b>	<b>1.39</b>	<b>1.38</b>	<b>0.21</b>	<b>0.27</b>	<b>1.36</b>	<b>0.54</b>	<b>0.16</b>	<b>0.33</b>	<b>1.04</b>	<b>1.06</b>	<b>0.02</b>	<b>0.29</b>	<b>0.38</b>
<b>C.D. 5%</b>	<b>3.87</b>	<b>3.85</b>	<b>0.58</b>	<b>0.76</b>	<b>3.80</b>	<b>1.49</b>	<b>0.44</b>	<b>0.93</b>	<b>2.91</b>	<b>2.94</b>	<b>0.05</b>	<b>0.82</b>	<b>1.07</b>
<b>G1</b>	53.07	91.00	1.94	1.86	82.49	11.71	4.69	15.75	45.36	34.14	0.90	20.34	23.27
<b>G2</b>	52.95	90.72	2.05	2.40	78.52	11.63	3.94	7.77	39.61	19.69	1.33	15.05	20.22
<b>G3</b>	52.79	81.35	2.60	3.43	83.54	12.40	4.71	11.53	42.69	27.08	1.06	18.15	21.90
<b>G4</b>	52.81	82.35	2.00	2.49	72.88	11.66	4.36	10.60	36.36	29.25	1.27	15.11	21.92
<b>G5</b>	52.79	72.61	1.93	2.18	74.13	11.60	4.44	10.75	36.58	29.33	0.84	19.68	22.37
<b>G6</b>	52.79	70.29	1.88	2.45	64.79	11.66	4.61	10.18	37.13	27.34	0.88	19.51	24.85
<b>G7</b>	52.79	69.46	2.12	2.32	65.28	11.83	4.00	9.92	37.04	26.72	0.97	17.64	23.09
<b>G8</b>	52.92	67.27	1.93	2.51	69.61	11.68	4.04	10.12	32.90	30.76	0.96	18.86	24.52
<b>G9</b>	52.86	71.79	1.90	2.35	73.66	12.08	4.69	10.13	32.15	31.55	0.88	17.94	24.31
<b>G10</b>	52.88	84.16	2.16	3.27	64.65	12.19	4.65	10.83	43.27	24.95	0.92	18.89	24.14
<b>G11</b>	52.79	79.47	2.11	2.77	69.69	12.75	4.42	7.72	33.63	23.11	0.92	18.92	23.94
<b>G12</b>	52.79	77.22	2.07	2.96	84.13	12.21	4.69	10.55	35.58	29.62	0.92	19.84	24.95
<b>G13</b>	52.84	68.44	1.94	2.68	59.10	12.30	4.54	10.36	33.02	31.29	0.96	20.20	22.78
<b>G14</b>	52.88	71.41	1.88	3.05	65.60	12.75	4.38	10.21	35.26	28.99	1.06	18.98	24.97
<b>G15</b>	52.79	80.35	1.88	3.15	71.63	12.33	4.55	16.19	45.63	34.98	0.86	19.59	23.60
<b>S.Em ±</b>	<b>0.35</b>	<b>0.35</b>	<b>0.05</b>	<b>0.07</b>	<b>0.34</b>	<b>0.13</b>	<b>0.04</b>	<b>0.08</b>	<b>0.26</b>	<b>0.26</b>	<b>0.00</b>	<b>0.07</b>	<b>0.10</b>
<b>C.D. 5%</b>	<b>0.97</b>	<b>0.96</b>	<b>0.15</b>	<b>0.19</b>	<b>0.95</b>	<b>0.37</b>	<b>0.11</b>	<b>0.23</b>	<b>0.73</b>	<b>0.74</b>	<b>0.01</b>	<b>0.20</b>	<b>0.27</b>
<b>T1</b>	56.48	77.45	2.17	2.76	71.87	12.05	4.46	10.67	37.48	28.39	1.03	16.92	23.17
<b>T2</b>	53.25	65.44	1.22	1.54	59.50	11.15	3.87	8.13	31.45	26.17	0.93	19.81	21.94
<b>T3</b>	49.80	63.30	1.14	1.34	56.57	11.05	3.71	7.60	29.49	26.18	1.01	19.67	22.12

<b>T4</b>	48.27	60.92	1.04	1.17	54.17	10.82	3.54	7.35	28.13	26.48	0.99	18.02	22.58
<b>T5</b>	55.40	86.44	2.74	3.72	81.11	13.42	5.18	12.46	43.94	28.26	1.01	17.07	23.21
<b>T6</b>	54.75	85.24	2.64	3.68	82.88	13.27	5.08	12.43	42.92	29.00	1.11	18.12	22.83
<b>T7</b>	53.77	85.17	2.75	3.80	83.10	13.07	4.99	12.36	42.91	28.67	1.11	18.15	23.33
<b>T8</b>	51.45	81.47	2.33	3.05	74.94	12.29	4.65	11.73	40.83	28.50	1.02	17.97	24.27
<b>T9</b>	54.87	78.45	2.14	2.81	72.67	12.10	4.50	11.44	39.93	28.56	0.90	19.33	24.26
<b>T10</b>	51.53	77.24	1.85	2.45	70.45	11.72	4.28	11.09	37.41	29.43	0.83	20.34	23.88
<b>T11</b>	49.04	81.55	2.36	2.95	77.10	12.49	4.68	11.81	40.12	29.46	1.13	17.96	23.40
<b>T12</b>	51.84	80.05	2.15	2.69	73.77	12.32	4.51	11.55	38.33	30.06	0.96	18.17	23.45
<b>T13</b>	55.08	77.07	1.88	2.36	71.49	11.64	4.36	11.13	37.18	29.88	0.93	19.74	23.58
<b>T14</b>	54.26	80.29	2.29	3.05	76.22	12.17	4.63	11.41	39.28	28.92	0.96	18.03	24.11
<b>T15</b>	52.61	78.72	1.95	2.80	74.39	11.82	4.49	11.37	38.01	29.70	0.93	18.52	24.07
<b>T16</b>	53.16	76.27	1.75	2.37	71.42	11.44	4.21	10.93	36.56	29.74	0.84	19.44	24.02
<b>S.Em ±</b>	<b>0.36</b>	<b>0.36</b>	<b>0.05</b>	<b>0.07</b>	<b>0.35</b>	<b>0.14</b>	<b>0.04</b>	<b>0.09</b>	<b>0.27</b>	<b>0.27</b>	<b>0.00</b>	<b>0.08</b>	<b>0.10</b>
<b>C.D. 5%</b>	<b>1.00</b>	<b>0.99</b>	<b>0.15</b>	<b>0.20</b>	<b>0.98</b>	<b>0.39</b>	<b>0.11</b>	<b>0.24</b>	<b>0.75</b>	<b>0.76</b>	<b>0.01</b>	<b>0.21</b>	<b>0.28</b>
<b>C.V. %</b>	<b>3.72</b>	<b>2.54</b>	<b>14.64</b>	<b>14.51</b>	<b>2.68</b>	<b>6.29</b>	<b>4.98</b>	<b>4.36</b>	<b>3.91</b>	<b>5.23</b>	<b>2.39</b>	<b>2.24</b>	<b>2.33</b>

## 1. Quantitative character

### a. Seed yield per plant (g):

Among the treatments, PGPR-1 (12.46 g) showed significantly highest seed yield per plant followed by PGPR-2 (12.43 g) and PGPR-3 (12.36 g). While, Control+S1 (8.13 g), Control+S2 (7.60 g) and Control+S3 (7.35 g) were recorded lowest seed yield per plant. The seed yield per plant was significantly highest in RLM-619 (16.19 g) followed by RH- 761 (15.75 g) and P21- KANTI (11.53 g) whereas, P4-CS-54 (7.77 g) and RGN- 298 (7.72 g) recorded lowest seed yield per 59 plants among the selected genotypes. Among the genotypes and treatment interaction, the genotype RH-761 with PGPR-3 (19.75 g), RLM-619 with PGPR-3 (19.08 g) and RH-761 with PGPR-1 (19.50 g) showed significantly highest seed yield per plant, whereas, lowest seed yield per plant were noticed in P4-CS-54 with Control+S3 (6.13 g) and with Control+S1 (6.50 g).

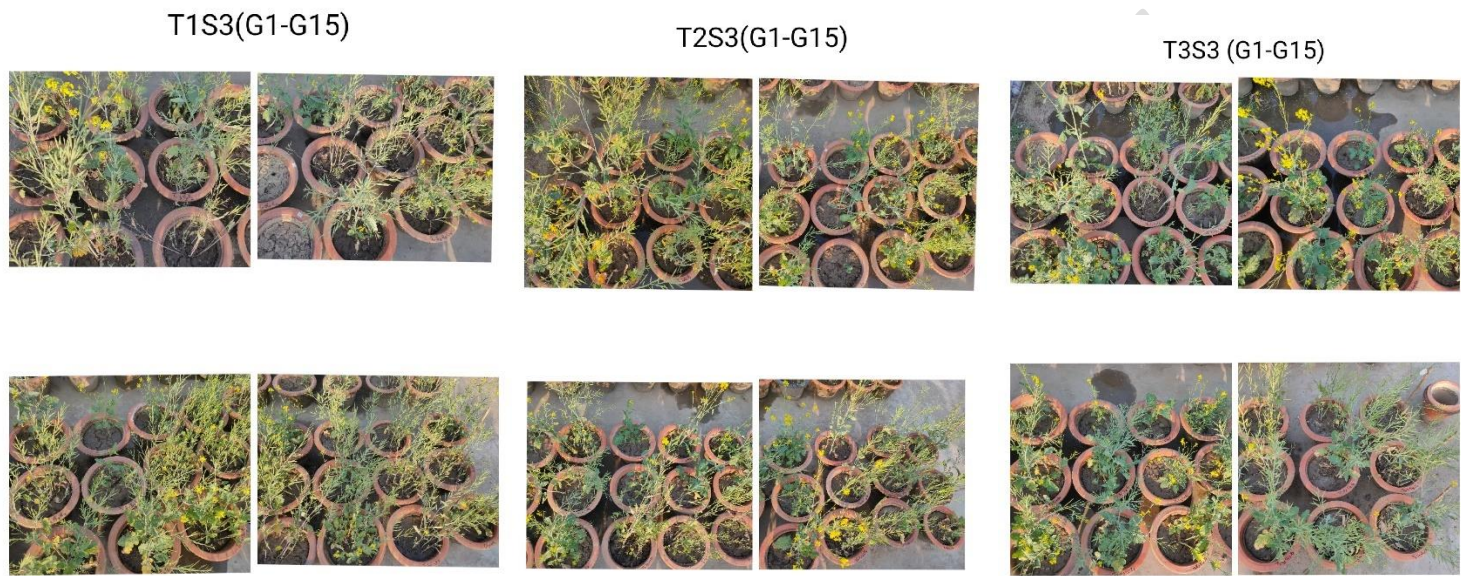


Figure 2: Seed Yield in various genotype after treatment application

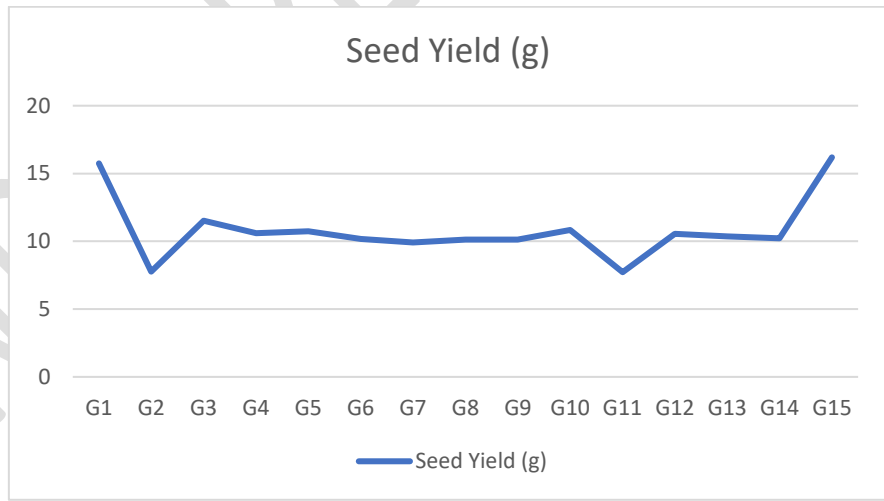


Figure 3: Seed Yield on the basis of Genotypes

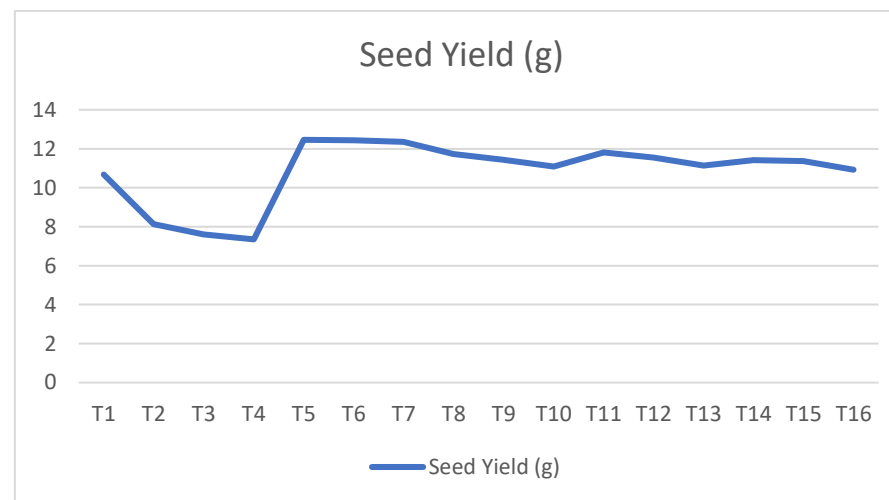


Figure 4: Seed Yield on the basis of Treatments

## 2. Qualitative characters

### a. Chlorophyll content (%):

Among the treatments, S2+PGPR-1 (1.13 %) showed significantly highest chlorophyll content followed PGPR-2 and PGPR-3 (1.11 %). While, S1+PGPR-3 (0.83 %) S3+PGPR-3 (0.84 %) were recorded lowest chlorophyll content. The chlorophyll content was significantly highest in P4-CS-54 (1.33 %) followed by P6-PBR-297 (1.27 %) P-4-PATAN MUSTRAD- 67 and P21- KANTI (1.06 %) whereas, P20 - Krishna (0.84 %) and RLM-619 (0.86 %) recorded lowest chlorophyll content among the selected genotypes. Among the genotypes and treatment interaction, the genotype P4-CS-54 (1.88 %) and P6-PBR-297 (1.74 %) with PGPR-2 and P4-CS-54 with PGPR-3 (1.81 %) showed significantly highest chlorophyll content, whereas, lowest chlorophyll content was noticed in P20- Krishna (0.32 %) and RH-761 (0.37 %) with S1+PGPR-3.

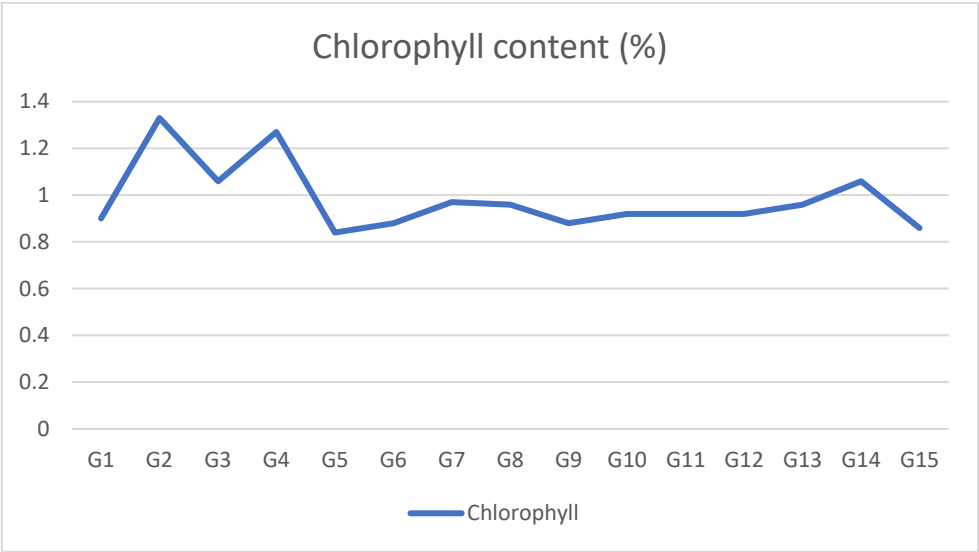


Figure 5: chlorophyll content on the basis of Genotypes

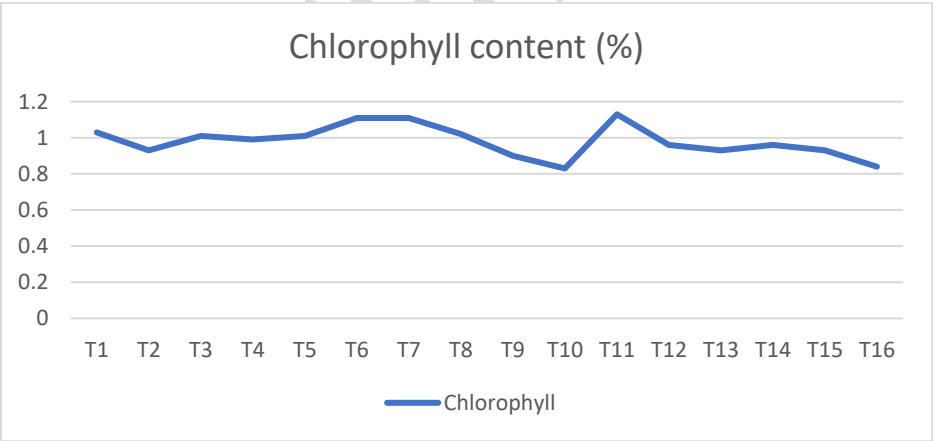


Figure 6: chlorophyll content on the basis of Treatments

b. Proline content (%)

Among the treatments, S1+PGPR-3 (20.34 %) showed significantly highest proline content followed Control+S1 (19.81 %) and S2+PGPR-3 (19.74 %). While, PGPR-1 (17.07 %), S2+PGPR-1 (17.96 %) were recorded lowest proline content. The proline content was significantly highest in RH- 761 (20.34 %) followed by P-2-RH-406 (20.20 %) and PUSA VIJAY (19.84 %) whereas, P4-CS-54 (15.05 %) and P6-PBR-297 (15.11 %) recorded lowest proline content among the selected genotypes. Among the genotypes and treatment interaction, the genotype RH-761 with Control+S3 (28.87 %) with Control+S2 (25.39 %) and with Control+S1 (24.39 %) showed significantly highest proline content, whereas, lowest proline content was noticed in P4-CS-54 with PGPR-1 (11.53 %) and PGPR-2 (12.71 %).

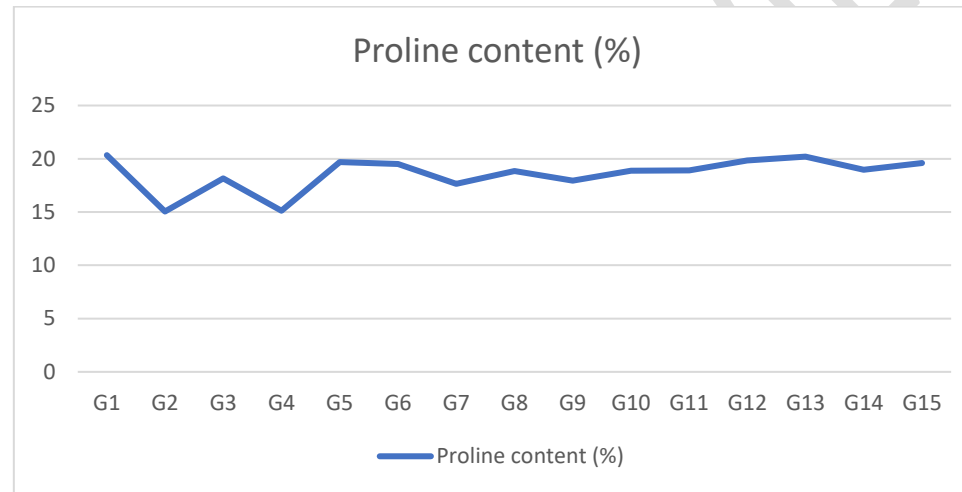


Figure 7: Proline content on the basis of Genotypes

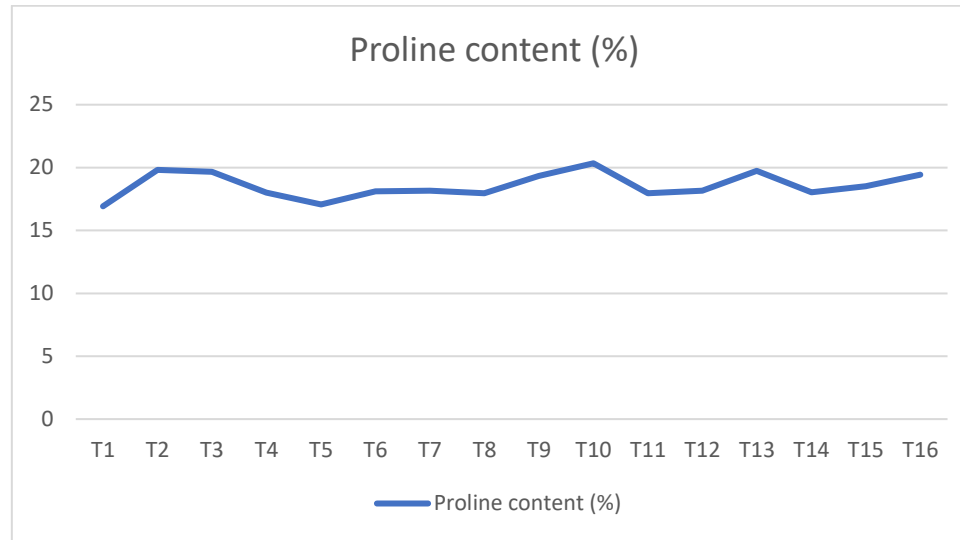


Figure 8: Proline content on the basis of Treatments

c. Protein (%)

Among the treatments, S1+PGPR-1 (24.27 %) showed significantly highest protein content followed by S1+PGPR-2 (24.26 %) and S3+PGPR-1 (24.11 %). While, Control+S1 (21.94 %) and Control+S2 (22.12 %) were recorded lowest protein content. The protein content was significantly highest in P-4-PATAN MISTRAD- 67 (24.97 %) followed by PUSA VIJAY (24.95 %) and P28-Roshini (24.85 %) whereas, P4-CS-54 (20.22 %) and P21- KANTI (21.90 %) recorded lowest protein content among the selected genotypes. Among the genotypes and 61 treatment interaction, the genotype RH-761 with S1+PGPR-3 (28.53 %) and P-4- PATAN MISTRAD-67 with PGPR-2 (28.13 %) showed significantly highest protein content, whereas, lowest protein content were noticed in P21-KANTI (14.26 %) P20-Krishna (14.91 %) with Control+S1.

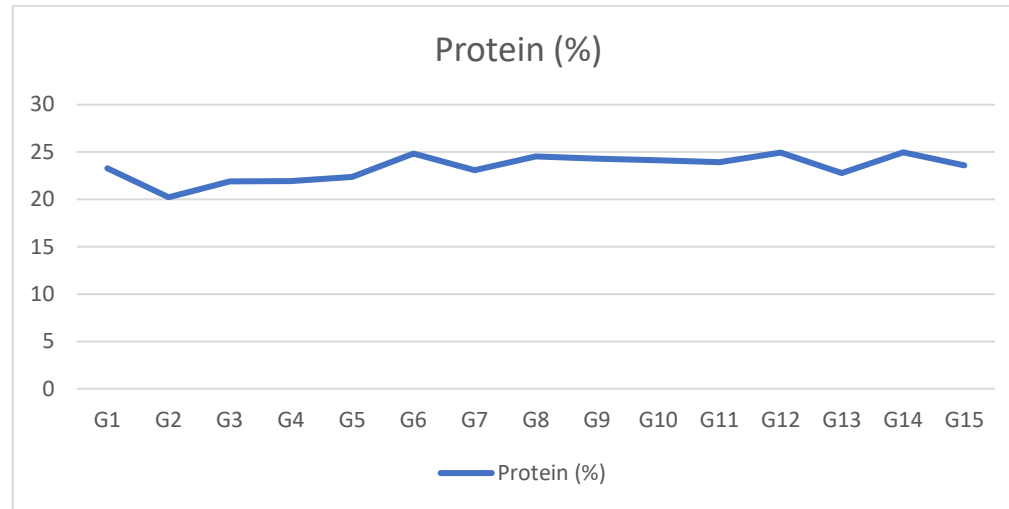


Figure 9: Protein (%) on the basis of Genotypes

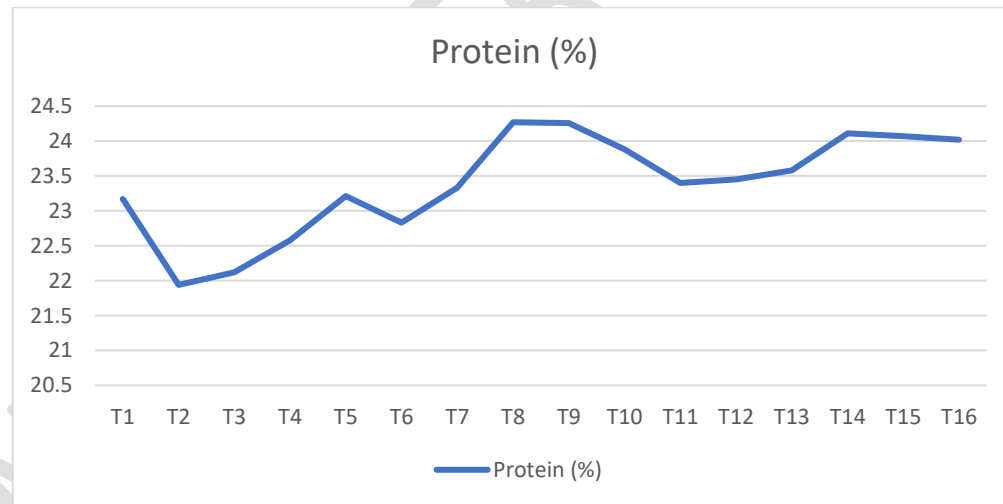


Figure 10: Protein (%) on the basis of Treatments

### 3. Genetic Parameters:

A fundamental requirement of every crop improvement program is the presence of sufficient genetic variety. To guarantee appropriate genotype selection, it is essential to consider the heritability and genetic progression of genotypes. The degree of genetic variability within the population under study is measured by the genotypic coefficient of variation (GCV) and the phenotypic coefficient of variation (PCV). The percentage of variability that can be inherited is represented by heritability ( $h^2$ ). Estimating genetic gain (GAM) also aids in forecasting the beneficial gains that can be attained by selection. PCV values typically exceed GCV values, suggesting that environmental variables as well as genotypes contribute to variation. The GCV and PCV were categorized as low (less than 10%), moderate (10-20%) and high (more than 20%) (Burton & Devane, 1953). Analysis of the genetic parameters in mustard genotypes- GCV, PCV,  $h^2$  (Broad Sense), GA and GAM.

#### a. Coefficient of variation

Genotypic and phenotypic coefficients of variation were computed based on the estimate of genotypic and phenotypic variances as follows.

$$GCV = \sqrt{GV \bar{x}} \times 100$$

$$PCV = \sqrt{PV \bar{x}} \times 100$$

(Where, GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation; GV = Genotypic variance; PV = Phenotypic variance; X = General mean of character).

Further, the PCV and GCV were classified (Sivasubramaniam & Menon, 1973):

0-10%	Low
10 – 20%	Moderate
More than 20%	High

#### b. Heritability

Heritability in broad sense refers to the proportion of genetic variance to the total observed variance in the population. It has been estimated as per the formula given by Lush (1940).

$$h^2 \text{ (broad sense)} = \frac{\text{Genotypic variance } (\sigma^2_g)}{\text{Phenotypic variance } (\sigma^2_p)} \times 100$$

$$\text{Phenotypic variance } (\sigma^2_p) \times 100$$

(Where,  $\sigma^2_g$  and  $\sigma^2_p$  are the genotypic and phenotypic variances).

Further, the range of heritability in broad sense was classified (Johnson et al., 1955):

Less than 30%	Low
30 – 60%	Moderate
More than 60%	High

c. *Genetic Advance (GA)*

The expected genetic gain or advance for each character was estimated by using the following method (Johnson et al., 1955).

$$GA = k \times \sigma_{2g}$$

(Where  $\sigma_{2g}$  = Genotypic variance  $\sigma_{2p}$  = Phenotypic variance  $k$  = Selection differential at 5 per cent selection intensity i.e. 2.06).

d. *Genetic Advance as per cent Mean (GAM)*

Genetic advance as per cent mean was worked out for each character adopting the formula (Johnson et al., 1955).

$$GAM = GA \times 100 \times X$$

(Where, GA = Genetic advance X = General mean).

The range of genetic advance as per cent of mean was classified:

Less than 10%	Low
10 – 20%	Moderate
More than 20%	High

Table 7: List of Genetic Parameters under consideration

	GCV	PCV	h <sup>2</sup> (Broad Sense)	GA	GAM
<b>Chlorophyll content</b>	25.44	25.55	99.10	0.51	52.17
<b>Proline</b>	15.72	15.88	98.00	5.96	32.07
<b>Protein</b>	11.62	11.85	96.10	5.49	23.47

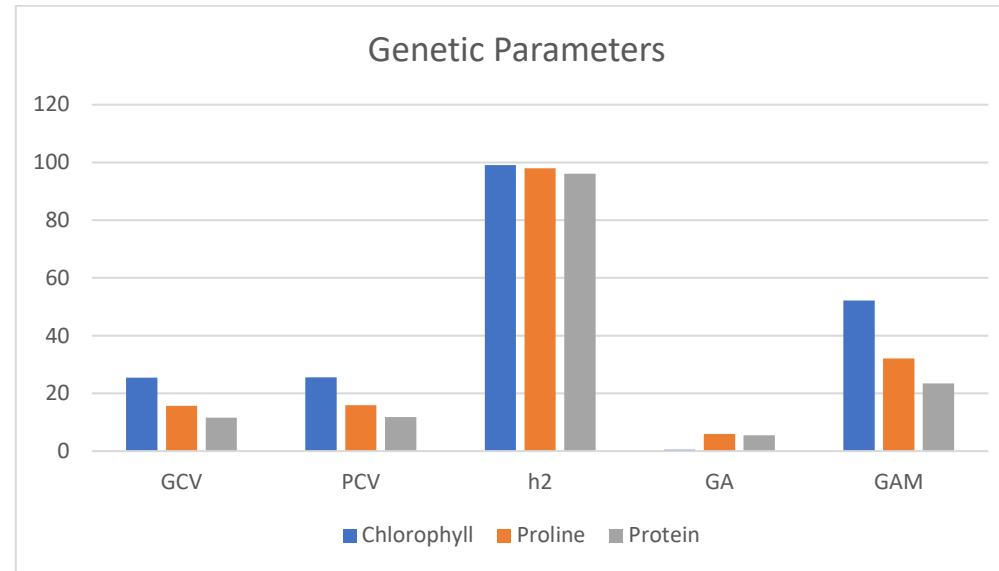


Figure 11: Comparison among all the Genetic Parameters

## CONCLUSION

This study analysed the chlorophyll content, proline content, and protein content of various genotypes and treatments. Among the treatments, the highest chlorophyll content was observed in S2+PGPR-1, PGPR-2 and PGPR-3, however, S1+PGPR-3 and S3+PGPR-3 were recorded lowest chlorophyll content. The chlorophyll content was significantly highest in P4-CS-54 P6- PBR-297, P-4-PATAN MUSTRAD- 67 and P21- KANTI, whereas, P20 - Krishna and RLM-619 recorded lowest chlorophyll content among the selected genotypes. Among the genotypes and treatment interaction, the genotype P4- CS-54 and P6-PBR-297 with PGPR-2 and P4-CS-54 with PGPR-3 showed significantly highest chlorophyll content, whereas, lowest chlorophyll content was noticed in P20-Krishna and RH-761 with S1+PGPR-3. The study also reported that S1+PGPR-3, Control+S1 and S2+PGPR-3 had the highest proline content, whereas The lowest proline content was recorded in PGPR-1 and S2+PGPR-1. The highest proline content was found in RH-761, P-2-RH-406 and PUSA VIJAY. The highest proline content was observed in RH-761 with Control+S3 and Control+S1. Among the treatments, S1+PGPR-1, S1+PGPR-2 and S3+PGPR-1 showed significantly highest protein content. While, Control+S1 and Control+S2 were recorded lowest protein content. The protein content was significantly highest in P-4-PATAN MUSTRAD- 67, PUSA VIJAY (24.95 %) and P28-Roshini (24.85 %) whereas, P4-CS-54 and P21- KANTI recorded lowest protein content among the selected genotypes. Among the genotypes and treatment interaction, the genotype RH-761 with S1+PGPR-3 and P-4-PATAN MUSTRAD-67 with PGPR-2 showed significantly highest protein content, whereas, lowest protein content was noticed in P21-KANTI P20-Krishna with Control+S1. According to variability studies of the present investigation, PCV was higher than the GCV, high PCV and GCV estimates in traits like chlorophyll content, moderate, proline and protein. The study found high estimates of heritability in traits such as chlorophyll content, proline, and protein. The low estimates of genetic advancement were recorded in proline, protein and chlorophyll content. Hence, RLM-619, RH-761, and P21-KANTI genotypes with PGPR-1, PGPR-2, and PGPR-3 treatments are most effective for increasing seed

yield per plant, using strategies like plant breeding, genetic engineering, and seed biopriming for improving the tolerance against the salinity. The study demonstrated that the biological priming remarkably enhanced physiological and biochemical responses to salinity stress of Indian mustard (*Brassica juncea*) through a complicated mechanism. The involvement of beneficial microorganisms and bioactive compounds in the biological priming of seeds enhances advance defense systems in plants. This enzymatic activation decreases the oxidative stress and restores the intracellular equilibrium arising from saline stress. Altogether, these physiological and biochemical changes revealed the level of salinity stress tolerance in *Brassica juncea* after biological priming, and suggested that the biological priming could be a feasible, effective and environment friendly biotechnological management intervention for enhancing the productivity of crops under salt affected field conditions.

**Disclaimer (Artificial intelligence)**

Option 1:

I, Aishmita, hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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