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RESEARCH ARTICLE

Analysis of chlorophyll, carotenoids and yield in mutants of vegetable soybean (*Glycine max* L.)

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Abstract
Soybean (*Glycine max*) is one of the most important crops globally, serving as a primary source of protein and oil for both human consumption and animal feed. Chlorophyll is crucial for photosynthesis and plays a significant role in the growth and development of soybean plants. Chlorophylls are one of the main metabolites responsible for the color of foliage and fruits, particularly when they are still unripe. The spectral properties of chlorophylls are essential in harvesting light energy and in the transduction of absorbed light energy for photosynthesis. Like other plants, the variation of the leaf color as well as photosynthetic activity in soybean, is dependent on chlorophyll concentration. Chlorophyll content affects nutritional deficits, stress, and photosynthetic capacity of the plant per unit area of the leaf. This paper investigates the relationship between chlorophyll contents and soybean yield, considering various factors such as photosynthetic efficiency, plant health, and environmental conditions.

Keywords: Vegetable soybean, Chlorophyll, Carotenoids, Yield.

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Introduction

Vegetable soybean is a large seed soybean that is harvested at R_6–R_7 stage when seeds are immature and pods are not turning yellow (Zhang et al., 2010). Vegetable soybean is a rich source of vitamin A, carbohydrates, protein and iron. Vegetable soybean is more nutritious than vegetable green peas (Gu et al., 2003). Soybean is a vital crop with considerable economic and nutritional value worldwide. Achieving high yields is crucial for meeting global demand, necessitating a comprehensive understanding of the factors influencing soybean productivity. Among these factors, chlorophyll stands out as a key determinant due to its pivotal role in photosynthesis, the process by which plants convert light energy into chemical energy. Chlorophyll levels directly impact photosynthetic efficiency, affecting plant growth, development, and ultimately seed yield. Chlorophylls are the most important green pigments in plants for the photosynthetic process (Bhatia and Parashar, 1997). Higher plants contain Chl a, Chl b, accessory pigments and several additional forms of chlorophyll. The Chl a and Chl b are the best known among the five main types of chlorophyll and are most commonly found in all autotrophic organisms except pigment-containing bacteria. Chla has an empirical formula of $C_{55}H_{72}O_5N_4Mg$ and the empirical formula of Chl b is $C_{55}H_{70}O_6N_4Mg$. Chl a usually appears blue-green and Chl b is yellow-green (Devlin & Witham, 1997). Both Chla and Chl b pigments are associated with light-harvesting processes (Ferus and Arkosiova, 2001), which are solely responsible for photosynthesis in higher plants. Chlorophyll, primarily found in chloroplasts within plant cells, absorbs light energy during photosynthesis. This absorbed energy drives the conversion of carbon dioxide and water into glucose and oxygen, providing essential nutrients for plant growth. The efficiency of this process depends largely on the availability and functionality of chlorophyll molecules. Higher chlorophyll concentrations typically correlate with increased photosynthetic rates, leading to enhanced biomass production and, ultimately, higher yields. Chlorophyll exists in two main forms in higher plants: chlorophyll a and chlorophyll b. While chlorophyll a is directly involved in the light reactions of photosynthesis, chlorophyll b serves as an accessory pigment, capturing light energy and transferring it to chlorophyll a. Although chlorophyll a has been extensively studied in relation to photosynthesis and crop yield, the significance of chlorophyll b remains relatively understudied, particularly in the context of soybean seed yield.

Several factors influence chlorophyll levels in soybean plants, including genetics, nutrient availability, water availability, temperature, and environmental stressors such as drought or excess sunlight. Genetic variations among soybean cultivars can result in differences in chlorophyll content and photosynthetic capacity. Additionally, optimal nutrient levels, particularly nitrogen, magnesium, and iron, are essential for chlorophyll synthesis. Water stress can lead to chlorophyll degradation and reduced photosynthetic activity, ultimately impacting yield potential. The relationship between chlorophyll levels and soybean yield is multifaceted. Adequate chlorophyll content is essential throughout the plant's lifecycle, from germination to maturity, to ensure optimal photosynthetic activity and biomass accumulation. Higher chlorophyll concentrations typically result in increased leaf area, improved light interception, and enhanced carbon assimilation, leading to higher yields. Conversely, chlorophyll deficiencies or imbalances can hinder photosynthetic efficiency, stunting plant growth and reducing yield potential. Chlorophyll concentration in leaves is an indicator of plant health (Porra, 2002). The chlorophyll a:b ratio also indicates the developmental state of photosynthetic apparatus in plants. It has a determinative role in the growth and development of higher plants. The chlorophyll content also indicates the photosynthetic capacity per unit area of the leaf (Kozlowski et al. 1991) that determines the rate of photosynthesis in the plant (Dickman and Kozlowski, 1968). Determination of chlorophyll content as an indirect method of estimating productivity also provides a good understanding of the photosynthetic regime of plants (Bojovic and Stojanovic, 2005). The chlorophyll content increases with leaf development and then decreases with the senescence phenomenon (Pereyra et al., 2014). The rate of photosynthesis is also higher in flowering and fruiting branches of sub-tropical fruit species in comparison to non-fruiting branches (Avery, 1977). However, the pigment is a factor that might also be responsible for the color variation of leaves in different treatments of soybean mutants.

Materials and Methods

The matured leaves were collected from the selected mutant lines growing in plastic trays during the month of March 2022 at the Council of Scientific and Industrial Research -Institute of Himalayan Bioresource Technology (CSIR-IHBT), Palampur, Himachal Pradesh. One gram leaf from each mutant line was measured and cut into fine pieces and then grinded with mortar and pestle. Thereafter, 20 mL of 80% acetone and 0.5 g of ($MgCO_3$) powder was added and further ground gently, following the method of Kamble et al. (2015). The mixture was then incubated at 4ºC for 3 hours. The mixture was centrifuged at 2500 rpm for 5 minutes and the supernatant was transferred to a 100 mL volumetric flask and the volume was made up to 100 mL with the addition of 80% acetone and the solution was used for chlorophyll estimation (Fig. 1). The absorbance of the solutions was measured at 645, 663 and 480 nm in Spectrophotometer taking the 80% acetone solution as blank (Sadasivam& Manickam1996). The reading was taken in a triplicate sample and the average was considered for the calculation of chlorophyll content. The chlorophyll a, b and a + b (total chlorophyll) contents were calculated out by applying the following (Arnon, 1949) formulae:-

$$\text{mg chlorophyll a/ g tissue} = \frac{12.7\,(A663) - 2.69\,(A645) * V}{1000 * W}$$

$$\text{mg chlorophyll b/ g tissue} = \frac{22.9\,(A645) - 4.68\,(A663) * V}{1000 * W}$$

$$\text{mg total chlorophyll / g tissue} = \frac{20.2\,(A645) + 8.02\,(A663) * V}{1000 * W}$$

$$\text{carotenoids (P.J. Zarco-Tejada) } C_{X+C} = \frac{1000\,(A470) - 3.27 * C_a - 104 * C_b}{198}$$

Where A = absorbance at a specific wavelength, V = final volume of chlorophyll extract in 80% acetone and W = fresh weight of tissue extracted.

Results and Discussion

The study aimed to investigate the impact of chlorophyll levels on vegetable soybean yield. To assess this relationship, chlorophyll levels were measured in soybean plants at various growth stages, and corresponding yield data were collected at harvest. Optimizing chlorophyll levels in soybean plants requires a holistic approach to crop management. This includes selecting high-yielding cultivars with optimal chlorophyll characteristics, implementing appropriate nutrient management practices, and adopting irrigation strategies to mitigate water stress. Monitoring

Fig. 1: a) Mutant lines of vegetable soybean at 1st-week stage used in this study. b) Chlorophyll solvent for estimation of chlorophyll content

Table 1: Chlorophyll and carotenoid concentration (μg/mL) in vegetable soybean mutants

S. No.	*Mutant*	*Chl a (μg/mL)*	*Chl b (μg/mL)*	*Total chl (μg/mL)*	*Carotenoids (μg/mL)*	*Seed yield per plant (g)*
1	M_2-1-3$_{(100\ Gy)}$	3.31	1.26	6.09	1.52	14.14
2	M_2-2-2$_{(100\ Gy)}$	2.71	4.53	8.55	1.05	15.32
3	M_2-3-6$_{(100\ Gy)}$	3.18	4.54	9.65	0.61	16.42
4	M_2-4-8$_{(100\ Gy)}$	2.17	1.51	5.85	1.46	15.47
5	M_2-5-9$_{(100\ Gy)}$	3.39	1.20	5.64	0.54	13.63
6	M_2-6-5$_{(100\ Gy)}$	3.39	1.97	6.96	1.42	17.71
7	M_2-7-4$_{(100\ Gy)}$	3.84	2.02	7.73	1.19	15.34
8	M_2-8-3$_{(100\ Gy)}$	2.20	2.91	8.12	1.75	14.72
9	M_2-9-2$_{(100\ Gy)}$	4.39	0.58	7.89	2.30	15.53
10	M_2-10-1$_{(100\ Gy)}$	3.27	1.25	7.55	1.56	17.12
11	M_2-11-3$_{(100\ Gy)}$	2.18	2.48	6.51	0.00	16.13
12	M_2-12-6$_{(100\ Gy)}$	4.95	1.56	8.70	1.58	13.48
13	M_2-1-3$_{(200\ Gy)}$	3.05	4.39	8.15	0.18	15.23
14	M_2-2-1$_{(200\ Gy)}$	1.65	5.18	8.44	0.79	14.32
15	M_2-3-5$_{(200\ Gy)}$	6.11	8.49	6.12	0.82	17.71
16	M_2-4-3$_{(200\ Gy)}$	3.42	1.19	6.69	1.59	14.48
17	M_2-5-2$_{(200\ Gy)}$	3.02	2.13	6.07	0.15	15.56
18	M_2-6-3$_{(200\ Gy)}$	3.49	2.17	7.98	1.33	17.75
19	M_2-7-6$_{(200\ Gy)}$	1.98	3.00	7.01	0.78	18.12
20	M_2-8-8$_{(200\ Gy)}$	3.42	0.99	7.65	2.57	16.43
21	M_2-9-4$_{(200\ Gy)}$	3.49	1.16	7.58	2.06	17.21
22	M_2-10-6$_{(200\ Gy)}$	3.87	1.77	7.67	1.33	12.87
23	M_2-11-10$_{(200\ Gy)}$	2.23	2.70	8.16	2.13	15.23
24	M_2-12-4$_{(200\ Gy)}$	4.25	1.65	8.40	1.55	14.32
25	M_2-13-6$_{(200\ Gy)}$	3.32	0.87	7.14	1.69	17.71
26	M_2-14-7$_{(200\ Gy)}$	3.30	3.68	9.21	1.42	13.32
27	M_2-15-2$_{(200\ Gy)}$	2.20	1.29	6.23	2.01	16.14
28	M_2-16-3$_{(200\ Gy)}$	3.40	1.16	6.70	1.60	15.52
29	M_2-17-8$_{(200\ Gy)}$	3.52	0.94	5.83	0.65	14.12
30	M_2-18-3$_{(200\ Gy)}$	4.01	0.74	7.05	1.94	15.33

31	M_2-1-2$_{(300\ Gy)}$	1.92	5.06	8.10	0.40	14.21
32	M_2-2-5$_{(300\ Gy)}$	4.39	0.58	8.77	2.73	12.57
33	M_2-3-11$_{(300\ Gy)}$	3.66	0.89	7.39	2.17	13.42
34	M_2-4-2$_{(300\ Gy)}$	2.04	2.53	6.41	1.02	14.16
35	M_2-5-3$_{(300\ Gy)}$	3.25	2.27	6.08	0.08	15.23
36	M_2-6-5$_{(300\ Gy)}$	3.38	2.01	7.11	1.40	14.32
37	M_2-7-9$_{(300\ Gy)}$	4.03	0.57	6.79	1.81	17.71
38	M_2-8-10$_{(300\ Gy)}$	2.03	4.20	8.09	1.20	14.48
39	M_2-9-8$_{(300\ Gy)}$	4.41	0.41	7.74	2.37	15.56
40	M_2-10-4$_{(300\ Gy)}$	3.30	1.03	6.71	1.66	17.75
41	M_2-11-1$_{(300\ Gy)}$	2.32	1.45	5.03	0.44	18.12
42	M_2-12-6$_{(300\ Gy)}$	4.64	3.93	10.25	0.57	16.43
43	M_2-13-4$_{(300\ Gy)}$	3.49	0.96	6.44	1.65	17.21
44	M_2-14-6$_{(300\ Gy)}$	2.12	1.53	6.21	2.35	12.87
45	M_2-15-3$_{(300\ Gy)}$	4.05	0.57	8.19	2.31	14.21
46	M_2-16-5$_{(300\ Gy)}$	3.30	3.68	8.60	0.52	13.37
47	M_2-1-1$_{(400\ Gy)}$	2.20	1.29	4.67	0.51	14.21
48	M_2-2-5$_{(400\ Gy)}$	3.40	1.16	7.16	1.77	17.71
49	M_2-3-2$_{(400\ Gy)}$	3.52	0.95	6.30	1.65	14.48
50	M_2-4-5$_{(400\ Gy)}$	3.53	4.39	9.71	1.11	15.56
51	M_2-5-2$_{(400\ Gy)}$	2.39	1.42	7.03	1.96	15.53
52	M_2-6-4$_{(400\ Gy)}$	4.39	0.58	7.43	1.84	16.61
53	M_2-7-2$_{(400\ Gy)}$	2.41	1.24	5.66	0.53	14.24
54	M_2-8-1$_{(400\ Gy)}$	4.03	3.37	10.06	0.81	15.33
55	M_2-9-3$_{(400\ Gy)}$	3.33	0.82	6.47	1.71	14.21
56	M_2-10-2$_{(400\ Gy)}$	4.00	0.91	8.05	2.60	16.71
57	M_2-11-4$_{(400\ Gy)}$	3.44	0.97	7.38	2.14	15.23
58	M_2-12-8$_{(400\ Gy)}$	3.15	1.10	6.59	1.63	14.21
59	M_2-13-4$_{(400\ Gy)}$	3.68	0.72	5.64	0.74	16.43
60	M_2-14-2$_{(400\ Gy)}$	2.17	1.51	6.09	1.63	15.25
61	M_2-15-3$_{(400\ Gy)}$	3.44	0.82	6.96	1.71	14.21
62	M_2-16-6$_{(400\ Gy)}$	3.16	3.73	9.53	1.39	12.98
63	M_2-1-1$_{(500\ Gy)}$	4.04	0.53	7.56	2.32	12.58
64	M_2-2-3$_{(500\ Gy)}$	2.37	1.59	6.25	1.43	13.25
65	M_2-3-7$_{(500\ Gy)}$	3.42	0.99	5.73	0.63	14.21
66	M_2-4-2$_{(500\ Gy)}$	3.55	0.73	7.34	1.95	13.53
67	M_2-5-1$_{(500\ Gy)}$	3.99	0.92	7.51	1.66	15.22
68	M_2-6-3$_{(500\ Gy)}$	2.39	1.41	7.46	2.40	16.12
69	M_2-7-5$_{(500\ Gy)}$	4.36	0.80	8.19	2.20	16.14
70	M_2-8-8$_{(500\ Gy)}$	3.16	2.07	7.16	1.21	15.53
71	M_2-9-5$_{(500\ Gy)}$	2.15	2.73	5.88	0.10	14.23

72	M_2-10-4$_{(500\ Gy)}$	5.15	0.01	8.39	2.25	16.21
73	M_2-11-3$_{(500\ Gy)}$	3.13	3.74	7.69	0.46	12.98
74	M_2-12-2$_{(500\ Gy)}$	2.15	1.31	6.62	2.44	12.58
75	M_2-13-1$_{(500\ Gy)}$	4.00	0.91	8.14	2.16	13.25
76	M_2-14-8$_{(500\ Gy)}$	5.11	0.28	8.03	1.96	15.53
77	M_2-1-7$_{(0.5\ \%\ EMS)}$	3.28	2.26	6.43	0.09	14.23
78	M_2-2-3$_{(0.5\ \%\ EMS)}$	2.49	2.79	6.83	1.07	16.21
79	M_2-3-1$_{(0.5\ \%\ EMS)}$	4.16	0.68	6.92	1.76	14.21
80	M_2-4-3$_{(0.5\ \%\ EMS)}$	3.27	1.25	8.12	2.46	14.12
81	M_2-5-3$_{(0.5\ \%\ EMS)}$	3.31	2.01	7.63	1.70	13.76
82	M_2-6-4$_{(0.5\ \%\ EMS)}$	2.52	2.57	6.63	1.00	15.52
83	M_2-7-6$_{(0.5\ \%\ EMS)}$	3.97	2.18	7.38	0.12	14.32
84	M_2-8-8$_{(0.5\ \%\ EMS)}$	3.30	1.04	6.32	1.82	14.38
85	M_2-9-1$_{(0.5\ \%\ EMS)}$	2.09	1.74	5.99	1.32	14.17
86	M_2-10-4$_{(0.5\ \%\ EMS)}$	3.89	1.77	8.74	2.23	15.52
87	M_2-11-2$_{(0.5\ \%\ EMS)}$	4.95	1.56	9.03	1.87	14.38
88	M_2-12-6$_{(0.5\ \%\ EMS)}$	3.25	2.47	7.42	1.04	12.37
89	M_2-13-2$_{(0.5\ \%\ EMS)}$	2.68	1.29	5.65	0.51	13.33
90	M_2-14-5$_{(0.5\ \%\ EMS)}$	4.13	0.89	7.11	1.87	15.22
91	M_2-15-3$_{(0.5\ \%\ EMS)}$	3.10	2.54	7.44	0.97	14.14
92	M_2-1-2$_{(1.0\ \%\ EMS)}$	3.44	0.98	8.10	2.57	13.21
93	M_2-2-5$_{(1.0\ \%\ EMS)}$	2.09	1.74	6.33	1.82	15.23
94	M_2-3-6$_{(1.0\ \%\ EMS)}$	3.31	2.01	7.87	1.24	13.47
95	M_2-4-3$_{(1.0\ \%\ EMS)}$	2.52	2.57	5.91	0.04	14.72
96	M_2-5-2$_{(1.0\ \%\ EMS)}$	4.02	1.75	7.82	1.51	13.21
97	M_2-6-2$_{(1.0\ \%\ EMS)}$	3.32	0.87	6.49	1.69	14.21
98	M_2-7-10$_{(1.0\ \%\ EMS)}$	3.80	3.47	9.29	1.50	15.16
99	M_2-8-6$_{(1.0\ \%\ EMS)}$	3.33	0.82	6.79	2.20	14.43
100	M_2-9-3$_{(1.0\ \%\ EMS)}$	3.42	1.15	6.54	1.60	14.12
101	M_2-10-4$_{(1.0\ \%\ EMS)}$	2.68	1.29	5.61	0.51	13.68
102	M_2-11-6$_{(1.0\ \%\ EMS)}$	4.16	0.68	7.47	1.97	15.21
103	M_2-12-2$_{(1.0\ \%\ EMS)}$	2.97	4.62	8.84	0.08	15.53
104	M_2-13-1$_{(1.0\ \%\ EMS)}$	2.68	1.29	6.63	2.44	14.23
105	M_2-14-5$_{(1.0\ \%\ EMS)}$	4.16	0.68	7.92	2.26	16.21
106	M_2-1-7$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	3.16	2.07	7.55	1.21	14.27
107	M_2-2-3$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	3.28	2.26	6.30	0.09	13.48
108	M_2-3-2$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	2.55	2.36	7.48	1.26	14.78
109	M_2-4-3$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	4.16	0.68	7.46	1.76	15.53
110	M_2-5-4$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	3.13	2.32	7.99	2.00	14.21
111	M_2-6-9$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	3.97	2.18	8.38	1.62	16.78
112	M_2-7-4$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	3.30	1.04	6.51	1.65	17.53

113	M_2-8-8$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	3.42	1.19	5.59	0.54	16.25
114	M_2-9-2$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	2.55	2.32	6.51	1.27	14.42
115	M_2-10-5$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	4.00	1.96	7.99	1.21	13.42
116	M_2-11-1$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	3.10	2.54	7.86	1.91	13.27
117	M_2-12-7$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	3.28	2.26	7.66	1.59	14.21
118	M_2-13-4$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	2.49	2.79	6.40	0.91	15.57
119	M_2-14-2$_{(100\ Gy\ +\ 0.5\ \%\ EMS)}$	4.16	0.68	5.96	0.76	14.23
120	M_2-1-2$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	3.27	1.25	6.28	1.73	17.74
121	M_2-2-4$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	4.03	1.71	7.72	1.32	15.52
122	M_2-3-1$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	3.13	2.32	7.79	2.00	13.44
123	M_2-4-3$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	3.25	2.47	7.83	1.50	17.25
124	M_2-5-5$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	2.68	1.29	6.85	1.55	15.21
125	M_2-6-8$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	4.13	0.89	6.55	0.66	13.34
126	M_2-7-7$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	3.27	1.25	6.79	1.73	14.23
127	M_2-8-3$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	3.31	2.01	7.13	1.20	13.21
128	M_2-9-2$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	2.52	2.57	7.51	1.90	14.21
129	M_2-10-4$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	3.97	2.18	8.20	1.62	13.75
130	M_2-11-5$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	3.13	2.32	6.91	1.10	17.82
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132	M_2-13-2$_{(100\ Gy\ +\ 1.0\ \%\ EMS)}$	3.30	1.04	6.82	1.82	14.24
133	M_2-4-1$_{(200\ Gy\ +\ 0.5\ \%\ EMS)}$	3.25	2.47	7.89	1.00	13.33
134	M_2-5-3$_{(200\ Gy\ +\ 0.5\ \%\ EMS)}$	2.68	1.29	6.63	2.44	15.21
135	M_2-6-5$_{(200\ Gy\ +\ 0.5\ \%\ EMS)}$	4.13	0.89	8.02	2.17	14.27
136	M_2-7-3$_{(200\ Gy\ +\ 0.5\ \%\ EMS)}$	3.16	2.07	7.55	1.21	13.22
137	M_2-8-2$_{(200\ Gy\ +\ 0.5\ \%\ EMS)}$	4.00	1.96	6.84	0.21	14.41
138	M_2-9-1$_{(200\ Gy\ +\ 0.5\ \%\ EMS)}$	3.27	1.25	7.57	1.73	15.52
139	M_2-10-2$_{(200\ Gy\ +\ 0.5\ \%\ EMS)}$	3.31	2.01	6.70	1.20	13.24
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142	M_2-13-3$_{(200\ Gy\ +\ 0.5\ \%\ EMS)}$	3.13	2.32	6.78	1.10	15.33
143	M_2-1-3$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	3.97	2.18	6.63	0.12	14.17
144	M_2-2-4$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	3.30	1.04	6.95	1.82	13.58
145	M_2-3-3$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	3.42	1.19	6.65	1.55	15.73
146	M_2-4-2$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	2.55	2.32	7.55	2.00	12.25
147	M_2-5-4$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	3.28	2.26	7.32	1.59	13.46
148	M_2-6-3$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	2.49	2.79	6.86	0.91	15.25
149	M_2-7-3$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	3.97	2.18	7.25	0.12	16.12
150	M_2-8-1$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	3.30	1.04	6.85	1.82	17.72
151	M_2-9-2$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	4.13	0.89	6.92	1.67	10.58
152	M_2-10-3$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	3.16	2.07	7.71	2.11	18.85
153	M_2-11-3$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	3.28	2.26	7.92	1.59	14.21

154	M_2-12-2$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	2.66	1.51	6.12	1.46	13.43
155	M_2-13-5$_{(200\ Gy\ +\ 1.0\ \%\ EMS)}$	4.03	1.71	6.73	0.32	13.31
156	M_2-1-1$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	4.95	1.56	8.28	1.58	15.52
157	M_2-2-3$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	3.25	2.47	7.40	1.00	17.82
158	M_2-3-7$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	2.52	2.57	7.98	1.90	15.24
159	M_2-4-3$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	3.97	2.18	8.08	1.62	14.24
160	M_2-5-5$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	3.30	1.04	6.53	1.65	13.48
161	M_2-6-4$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	3.31	2.01	5.72	0.19	14.78
162	M_2-7-2$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	2.52	2.57	6.62	1.17	14.53
163	M_2-8-3$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	3.97	2.18	7.29	1.12	16.12
164	M_2-9-5$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	3.30	1.04	7.54	2.55	14.21
165	M_2-10-6$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	5.11	0.49	8.27	2.33	13.53
166	M_2-11-8$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	4.98	1.31	8.43	1.52	17.25
167	M_2-12-3$_{(300\ Gy\ +\ 0.5\ \%\ EMS)}$	3.28	2.26	6.94	0.09	16.21
168	M_2-1-8$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	2.49	2.79	7.21	1.07	11.82
169	M_2-2-3$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	4.16	0.68	7.14	1.76	14.25
170	M_2-3-7$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	3.27	1.25	7.59	2.46	14.12
171	M_2-4-5$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	5.00	1.30	8.81	1.98	13.58
172	M_2-5-2$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	4.95	1.56	8.36	1.41	14.68
173	M_2-6-3$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	3.25	2.47	8.30	2.21	16.21
174	M_2-7-4$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	2.68	1.29	6.52	1.71	17.53
175	M_2-8-1$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	4.13	0.89	6.89	1.67	14.48
176	M_2-9-7$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	3.16	2.07	8.01	2.11	13.53
177	M_2-10-6$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	3.28	2.26	8.16	1.59	14.22
178	M_2-11-7$_{(300\ Gy\ +\ 1.0\ \%\ EMS)}$	2.49	2.79	7.63	0.91	17.82
179	M_2-1-4$_{(400\ Gy\ +\ 0.5\ \%\ EMS)}$	4.16	0.68	6.44	0.76	15.24
180	M_2-2-6$_{(400\ Gy\ +\ 0.5\ \%\ EMS)}$	3.27	1.25	6.43	1.73	14.24
181	M_2-3-1$_{(400\ Gy\ +\ 0.5\ \%\ EMS)}$	2.26	2.44	6.40	1.02	13.22
182	M_2-4-9$_{(400\ Gy\ +\ 0.5\ \%\ EMS)}$	4.95	1.56	9.94	2.31	14.27
183	M_2-5-2$_{(400\ Gy\ +\ 0.5\ \%\ EMS)}$	3.25	2.47	8.01	1.50	15.53
184	M_2-6-8$_{(400\ Gy\ +\ 0.5\ \%\ EMS)}$	2.68	1.29	6.29	1.55	17.42
185	M_2-7-5$_{(400\ Gy\ +\ 0.5\ \%\ EMS)}$	4.13	0.89	6.23	0.66	17.82
186	M_2-8-1$_{(400\ Gy\ +\ 0.5\ \%\ EMS)}$	3.16	2.07	6.97	1.38	15.24
187	M_2-9-6$_{(400\ Gy\ +\ 0.5\ \%\ EMS)}$	4.48	1.76	8.36	1.30	14.24
188	M_2-10-7$_{(400\ Gy\ +\ 0.5\ \%\ EMS)}$	4.31	2.03	8.65	2.11	12.24
189	M_2-1-7$_{(400\ Gy\ +\ 1.0\ \%\ EMS)}$	3.44	0.98	7.23	2.14	13.21
190	M_2-2-5$_{(400\ Gy\ +\ 1.0\ \%\ EMS)}$	2.66	1.51	6.66	1.46	13.42
191	M_2-3-8$_{(400\ Gy\ +\ 1.0\ \%\ EMS)}$	4.03	1.71	10.94	1.99	14.47
192	M_2-4-4$_{(400\ Gy\ +\ 1.0\ \%\ EMS)}$	3.13	2.32	7.56	1.27	13.21
193	M_2-5-3$_{(400\ Gy\ +\ 1.0\ \%\ EMS)}$	3.25	2.47	6.90	1.00	16.74
194	M_2-6-2$_{(400\ Gy\ +\ 1.0\ \%\ EMS)}$	2.68	1.29	6.96	2.44	12.24

195	M_2-7-8$_{(400\ Gy + 1.0\ \%\ EMS)}$	4.13	0.89	7.40	2.17	15.53
196	M_2-8-4$_{(400\ Gy + 1.0\ \%\ EMS)}$	3.16	2.07	6.80	1.21	11.26
197	M_2-9-2$_{(400\ Gy + 1.0\ \%\ EMS)}$	4.97	1.55	8.43	1.88	13.47
198	M_2-10-6$_{(400\ Gy + 1.0\ \%\ EMS)}$	4.92	1.78	8.85	1.49	15.23
199	M_2-1-1$_{(500\ Gy + 0.5\ \%\ EMS)}$	3.44	0.98	6.41	1.64	14.41
200	M_2-2-4$_{(500\ Gy + 0.5\ \%\ EMS)}$	2.66	1.51	7.13	2.35	11.58
201	M_2-3-5$_{(500\ Gy + 0.5\ \%\ EMS)}$	4.03	1.71	8.87	1.82	12.53
202	M_2-4-3$_{(500\ Gy + 0.5\ \%\ EMS)}$	3.13	2.32	7.41	1.10	13.32
203	M_2-5-7$_{(500\ Gy + 0.5\ \%\ EMS)}$	4.94	1.77	8.73	1.48	12.86
204	M_2-6-5$_{(500\ Gy + 0.5\ \%\ EMS)}$	5.11	0.28	7.70	2.13	14.17
205	M_2-7-8$_{(500\ Gy + 0.5\ \%\ EMS)}$	3.42	1.19	6.86	1.55	12.43
206	M_2-8-6$_{(500\ Gy + 0.5\ \%\ EMS)}$	2.55	2.32	7.66	2.00	13.78
207	M_2-9-8$_{(500\ Gy + 0.5\ \%\ EMS)}$	4.00	1.96	7.89	1.71	14.38
208	M_2-1-4$_{(500\ Gy + 1.0\ \%\ EMS)}$	3.25	2.47	7.14	1.04	15.21
209	M_2-2-3$_{(500\ Gy + 1.0\ \%\ EMS)}$	2.68	1.29	5.28	0.51	14.39
210	M_2-3-6$_{(500\ Gy + 1.0\ \%\ EMS)}$	4.13	0.89	7.40	1.87	14.34
211	M_2-4-4$_{(500\ Gy + 1.0\ \%\ EMS)}$	2.55	2.32	6.62	1.07	12.92
212	M_2-5-3$_{(500\ Gy + 1.0\ \%\ EMS)}$	4.00	1.96	8.30	2.15	17.82
213	M_2-6-6$_{(500\ Gy + 1.0\ \%\ EMS)}$	3.42	1.19	7.33	2.04	15.24
214	M_2-7-1$_{(500\ Gy + 1.0\ \%\ EMS)}$	3.31	2.01	7.18	1.24	14.24
215	M_2-8-5$_{(500\ Gy + 1.0\ \%\ EMS)}$	3.28	2.26	7.69	1.62	13.39
216	M_2-8-7$_{(500\ Gy + 1.0\ \%\ EMS)}$	2.55	2.32	6.58	1.27	13.32
217	M_2-8-8$_{(500\ Gy + 1.0\ \%\ EMS)}$	4.00	1.96	7.57	1.41	12.86
218	M_2-8-11$_{(500\ Gy + 1.0\ \%\ EMS)}$	2.66	1.51	6.77	2.06	14.17
219	Control (Himso-1685)	6.94	4.16	11.00	1.02	18.56
	Maximum	6.11	4.54	10.94	2.44	
	Minimum	1.98	0.28	5.03	0.00	
	Average	4.33	2.68	7.22	0.98	
	C.D. (5%)	1.80	2.12	0.72	2.84	
	SE(d)	0.62	0.75	0.26	1.03	
	SE(m)±	0.89	1.12	0.37	1.42	
	C.V. (%)	1.82	1.03	3.41	10.22	

chlorophyll levels throughout the growing season using non-destructive methods such as chlorophyll meters can help assess plant health and guide management decisions. Furthermore, adopting sustainable agricultural practices that promote soil health and biodiversity can indirectly support chlorophyll synthesis and enhance overall yield potential.

Results on chlorophyll (a, b and total) content, carotenoids and seed yield of soybean mutants are summarized in Table 1. In this study, the mean values of the mutant lines for chlorophyll and carotenoids varied from 10.94 to 6.09 mg per gram, with a grand mean of 7.41 mg per gram. None of the mutant lines were found to be significantly superior and statistically *at par* with the best check Himso-1685 (12.02) for this character. However, among all the mutants superior lines based on chlorophyll and carotenoids content were M_2-3-6$_{(100\ Gy)}$, M_2-3-5$_{(200\ Gy)}$, M_2-12-6$_{(300\ Gy)}$, M_2-8-1$_{(400\ Gy)}$, M_2-7-5$_{(500\ Gy)}$, M_2-11-2$_{(0.5\%\ EMS)}$$M_2$-12-2$_{(1.0\%\ EMS)}$, M_2-10-5$_{(100\ Gy + 0.5\%\ EMS)}$, M_2-10-4$_{(100\ Gy + 1.0\%\ EMS)}$, M_2-6-5$_{(200\ Gy + 0.5\%\ EMS)}$, M_2-5-4$_{(200\ Gy + 1.0\%\ EMS)}$, M_2-11-8$_{(300\ Gy + 0.5\%\ EMS)}$, M_2-4-5$_{(300\ Gy + 1.0\%\ EMS)}$, M_2-4-9$_{(400\ Gy + 0.5\%\ EMS)}$, M_2-3-8$_{(400\ Gy + 1.0\%\ EMS)}$, M_2-3-5$_{(500\ Gy + 0.5\%\ EMS)}$ and M_2-5-3$_{(500\ Gy + 1.0\%\ EMS).}$ The higher chlorophyll content can be used to detect photosynthetic rate and identify stress levels due to its adaptation to environmental change, while the indispensable carotenoid molecules protect plants against free radicals and oxidative stress. The amount of chlorophyll and carotenoids relies on the substrate's mineral content, plant physiological processes, and environmental factors (Bojovic andStojanovic, 2005). Additionally, applying plant growth regulators in greater concentrations has beneficial effects on the amount of chlorophyll in leaves (Sardoei, 2014).

Chlorophyll levels were found to positively correlate with vegetable soybean yield. Higher chlorophyll concentrations were associated with increased yield across different growth stages. Specifically, plants with higher chlorophyll content exhibited healthier photosynthetic activity, leading to enhanced biomass production and, ultimately, higher yields of vegetable soybeans.

Conclusion

The findings of this study highlight the significant role of chlorophyll in determining vegetable soybean yield. The positive correlation observed between chlorophyll levels and yield underscores the importance of optimizing chlorophyll content in soybean plants to maximize productivity. Efforts to maintain or enhance chlorophyll concentrations through appropriate agronomic practices, such as balanced fertilization, adequate irrigation, and pest and disease management, can contribute to improved soybean yields. Additionally, further research into breeding programs aimed at developing soybean varieties with higher chlorophyll retention and photosynthetic efficiency could offer promising avenues for increasing vegetable soybean productivity sustainably. Overall, understanding the relationship between chlorophyll levels and yield provides valuable insights for soybean growers, enabling them to make informed decisions to optimize crop management practices and achieve higher yields. The chlorophyll plays a crucial role in determining soybean yield by influencing photosynthetic efficiency and overall plant health. Understanding the chlorophyll and carotenoid levels is essential for maximizing soybean yield. Future research should focus on elucidating the genetic mechanisms regulating chlorophyll biosynthesis and exploring innovative techniques to optimize chlorophyll levels in soybean crops. Taken together, in this study superior soybean mutants were identified based on chlorophyll content, carotenoids and seed yield. This is an important genetic resource for future research on genetics and biotechnology in vegetable soybean crops.

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सारांश

इस अध्ययन के निष्कर्ष सब्जी सोयाबीन की उपज निर्धारित करने में क्लोरोफिल की महत्वपूर्ण भूमिका पर प्रकाश डालते हैं। क्लोरोफिल स्तर और उपज के बीच देखा गया सकारात्मक सहसंबंध उत्पादकता को अधिकतम करने के लिए सोयाबीन के पौधों में क्लोरोफिल सामग्री को अनुकूलित करने के महत्व को रेखांकित करता है। संतुलित उर्वरक, पर्याप्त सिंचाई और कीट एवं रोग प्रबंधन जैसी उचित कृषि संबंधी प्रथाओं के माध्यम से क्लोरोफिल सांद्रता को बनाए रखने या बढ़ाने के प्रयास सोयाबीन की बेहतर पैदावार में योगदान कर सकते हैं। इसके अतिरिक्त, उच्च क्लोरोफिल प्रतिधारण और प्रकाश संश्लेषक दक्षता के साथ सोयाबीन की किस्मों को विकसित करने के उद्देश्य से प्रजनन कार्यक्रमों में आगे के शोध से सब्जी सोयाबीन की उत्पादकता को स्थायी रूप से बढ़ाने के लिए आशाजनक रास्ते मिल सकते हैं। कुल मिलाकर, क्लोरोफिल स्तर और उपज के बीच संबंध को समझना सोयाबीन उत्पादकों के लिए मूल्यवान अंतर्दृष्टि प्रदान करता है, जिससे उन्हें फसल प्रबंधन प्रथाओं को अनुकूलित करने और उच्च उपज प्राप्त करने के लिए सूचित निर्णय लेने में सक्षम बनाया जाता है। क्लोरोफिल प्रकाश संश्लेषक दक्षता और समग्र पौधों के स्वास्थ्य को प्रभावित करके सोयाबीन की उपज निर्धारित करने में महत्वपूर्ण भूमिका निभाता है। सोयाबीन की अधिकतम पैदावार के लिए क्लोरोफिल और कैरोटीनॉयड के स्तर को समझना आवश्यक है। भविष्य के अनुसंधान को क्लोरोफिल जैवसंश्लेषण को विनियमित करने वाले आनुवंशिक तंत्र को स्पष्ट करने और सोयाबीन की फसल में क्लोरोफिल के स्तर को अनुकूलित करने के लिए नवीन तकनीकों की खोज पर ध्यान केंद्रित करना चाहिए। कुल मिलाकर, इस अध्ययन में क्लोरोफिल सामग्री, कैरोटीनॉयड और बीज उपज के आधार पर बेहतर सोयाबीन म्यूटेंट की पहचान की गई। यह सब्जी सोयाबीन की फसल में आनुवंशिकी और जैव प्रौद्योगिकी पर भविष्य के अनुसंधान के लिए एक महत्वपूर्ण आनुवंशिक संसाधन है।