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Chlorophyll a and b content along with carotinoids in M_3 generation of Him Palam Hara Soya 1 variety of soybean [*Glycine max* (L.) Merrill]

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Abstract
Green plants produce a wide range of primary and secondary metabolites in different quantity. 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 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 leaf. The study of chlorophyll content in soybean mutants growing in different treatments at Palampur location district Kangra of Himachal Pradesh has shown some variability among them as an individual character.

Keywords: Soybean leaves, pigment, photosynthesis, metabolite, productivity

Introduction
Chlorophylls are the most important green pigments in plants for the photosynthetic process (Bhatia & Parashar 1997) [3]. 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 five main types of chlorophyll and are most commonly found in all autotrophic organisms except pigment containing bacteria. Chl a has an empirical formula of C55H72O5N4Mg and the empirical formula of Chl b is C55H70O6N4Mg. Chl a usually appears blue-green and Chl b is yellow-green (Devlin & Witham 1997) [5]. Both Chl a and Chl b pigments are associated with light harvesting processes (Ferus & Arkosiova 2001) [7], which are solely responsible for photosynthesis in higher plants.

Chlorophyll concentration in leaves is an indicator of plant health (Porra 2002) [11]. The chlorophyll a:b ratio also indicates the developmental state of photosynthetic apparatus in plants. It has a determinative role in growth and development of higher plants. The chlorophyll content also indicates the photosynthetic capacity per unit area of the leaf (Kozlowski *et al*. 1991) [9] that determines the rate of photosynthesis in the plant (Dickman & Kozlowski 1968) [6]. Determination of chlorophyll content as an indirect method of estimating the productivity also provides a good understanding of the photosynthetic regime of plants (Bojovic & Stojanovic 2005) [4]. The chlorophyll content increases with leaf development and then decreases with the senescence phenomenon (Pereyra *et al*. 2014) [10]. 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) [2]. However, the pigment is a factor that might also be responsible for the colour variation of leaf in different treatments of soybean mutants. The present study was undertaken for estimation of chlorophyll content in the mature leaf of soybean mutants of variety Himso 1685 growing in Palampur, Kangra district Himachal Pradesh.

Materials and Method
The matured leaves were collected from the selected mutant lines growing in plastic trays during the month of march 2022 at CSIR-IHBT, Palampur, Himachal Pradesh. One gram leaf from each mutant lines is 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 grinded gently following the method of Kamble *et al*. (2015) [8]. The mixture was then incubated at 4 ºC for 3 hours.

The mixture was centrifuged at 2500 rpm for 5 min and the supernatant was transferred to a 100 ml volumetric flask and the volume was made up to 100ml 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 nm, 663nm and 480 nm in Spectrophotometer taking the 80% acetone solution as blank (Sadasivam & Manickam1996) [12]. The reading was taken in a triplicate sample and average was considered for calculation of chlorophyll content. The chlorophyll a, b and a + b (total chlorophyll) contents were calculated out by applying the following (Arnon 1949) [1] formulae:-

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

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

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

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

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

Fig 1: Mutants lines of Soybean at week stage

Fig 2: Chlorophyll solvent for estimation

Results and discussion

Table 1: Chlorophyll and carotenoid concentration (µg/mL) in 219 mutants along with check

Sr. no.	Mutant	OD readings		Chl a (µg/mL)	Chl b (µg/mL)	Total chl (µg/mL)	OD readings	Carotenoids (µg/mL)
		A645	A663				A470	
1	M3-1-1	0.1664	0.3081	3.31	1.26	3.11	0.4141	1.52
2	M3-1-2	0.2781	0.2845	2.71	4.53	7.24	0.5987	1.05
3	M3-1-3	0.2882	0.3256	3.18	4.54	7.72	0.5124	0.61
4	M3-1-4	0.1186	0.2045	2.17	1.51	3.68	0.4215	1.46
5	M3-1-5	0.1286	0.3061	3.39	1.20	4.59	0.2151	0.54
6	M3-1-6	0.1664	0.3145	3.39	1.97	5.36	0.4544	1.42
7	M3-1-7	0.1781	0.3542	3.84	2.02	5.86	0.4141	1.19
8	M3-1-8	0.1882	0.2222	2.20	2.91	5.11	0.5987	1.75
9	M3-1-9	0.1186	0.3854	4.39	0.58	4.97	0.5124	2.30
10	M3-1-10	0.1286	0.2962	3.27	1.25	4.52	0.4215	1.56
11	M3-1-11	0.1664	0.2161	2.18	2.48	4.66	0.2151	0.00
12	M3-1-12	0.1781	0.4445	4.95	1.56	6.51	0.4544	1.58
13	M3-2-1	0.2781	0.3121	3.05	4.39	7.43	0.4141	0.18
14	M3-2-2	0.2882	0.2002	1.65	5.18	6.82	0.5987	0.79
15	M3-2-3	0.5423	0.6221	6.11	8.49	14.59	0.8945	0.82
16	M3-2-4	0.1286	0.3081	3.42	1.19	4.61	0.4215	1.59
17	M3-2-5	0.1664	0.2845	3.02	2.13	5.15	0.2151	0.15
18	M3-2-6	0.1781	0.3256	3.49	2.17	5.66	0.4544	1.33
19	M3-2-7	0.1882	0.2045	1.98	3.00	4.98	0.4141	0.78
20	M3-2-8	0.1186	0.3061	3.42	0.99	4.41	0.5987	2.57
21	M3-2-9	0.1286	0.3145	3.49	1.16	4.65	0.5124	2.06
22	M3-2-10	0.1664	0.3542	3.87	1.77	5.65	0.4215	1.33
23	M3-2-11	0.1781	0.2222	2.23	2.70	4.92	0.6541	2.13
24	M3-2-12	0.1682	0.3854	4.25	1.65	5.90	0.4544	1.55
25	M3-2-13	0.1106	0.2962	3.32	0.87	4.19	0.4141	1.69
26	M3-2-14	0.2482	0.3256	3.30	3.68	6.97	0.5987	1.42
27	M3-2-15	0.1086	0.2045	2.20	1.29	3.49	0.5124	2.01
28	M3-2-16	0.1266	0.3061	3.40	1.16	4.56	0.4215	1.60
29	M3-2-17	0.1182	0.3145	3.52	0.94	4.46	0.2151	0.65
30	M3-2-18	0.1186	0.3542	4.01	0.74	4.75	0.4544	1.94
31	M3-3-1	0.2882	0.2222	1.92	5.06	6.98	0.5124	0.40
32	M3-3-2	0.1186	0.3854	4.39	0.58	4.97	0.5987	2.73
33	M3-3-3	0.1186	0.3256	3.66	0.89	4.55	0.5124	2.17
34	M3-3-4	0.1664	0.2045	2.04	2.53	4.58	0.4215	1.02
35	M3-3-5	0.1781	0.3061	3.25	2.27	5.52	0.2151	0.08

36	M3-3-6	0.1682	0.3145	3.38	2.01	5.40	0.4544	1.40
37	M3-3-7	0.1106	0.3542	4.03	0.57	4.60	0.4141	1.81
38	M3-3-8	0.2482	0.2222	2.03	4.20	6.23	0.5987	1.20
39	M3-3-9	0.1106	0.3854	4.41	0.41	4.82	0.5124	2.37
40	M3-3-10	0.1182	0.2962	3.30	1.03	4.33	0.4215	1.66
41	M3-3-11	0.1186	0.2161	2.32	1.45	3.76	0.2151	0.44
42	M3-3-12	0.2882	0.4445	4.64	3.93	8.57	0.4544	0.57
43	M3-3-13	0.1186	0.3121	3.49	0.96	4.45	0.4141	1.65
44	M3-3-14	0.1186	0.2002	2.12	1.53	3.65	0.5987	2.35
45	M3-3-15	0.1106	0.3554	4.05	0.57	4.61	0.5124	2.31
46	M3-3-16	0.2482	0.3256	3.30	3.68	6.97	0.4215	0.52
47	M3-4-1	0.1086	0.2045	2.20	1.29	3.49	0.2151	0.51
48	M3-4-2	0.1266	0.3061	3.40	1.16	4.56	0.4544	1.77
49	M3-4-3	0.1186	0.3145	3.52	0.95	4.47	0.4141	1.65
50	M3-4-4	0.2882	0.3542	3.53	4.39	7.92	0.5987	1.11
51	M3-4-5	0.1186	0.2222	2.39	1.42	3.81	0.5124	1.96
52	M3-4-6	0.1186	0.3854	4.39	0.58	4.97	0.4215	1.84
53	M3-4-7	0.1106	0.2222	2.41	1.24	3.66	0.2151	0.53
54	M3-4-8	0.2482	0.3854	4.03	3.37	7.40	0.4544	0.81
55	M3-4-9	0.1086	0.2962	3.33	0.82	4.15	0.4141	1.71
56	M3-4-10	0.1266	0.3554	4.00	0.91	4.91	0.5987	2.60
57	M3-4-11	0.1182	0.3081	3.44	0.97	4.41	0.5124	2.14
58	M3-4-12	0.1186	0.2845	3.15	1.10	4.25	0.4215	1.63
59	M3-4-13	0.1106	0.3256	3.68	0.72	4.40	0.2151	0.74
60	M3-4-14	0.1186	0.2045	2.17	1.51	3.68	0.4544	1.63
61	M3-4-15	0.1106	0.3061	3.44	0.82	4.26	0.4141	1.71
62	M3-4-16	0.2482	0.3145	3.16	3.73	6.89	0.5987	1.39
63	M3-5-1	0.1086	0.3542	4.04	0.53	4.56	0.5124	2.32
64	M3-5-2	0.1266	0.2222	2.37	1.59	3.96	0.4215	1.43
65	M3-5-3	0.1186	0.3061	3.42	0.99	4.41	0.2151	0.63
66	M3-5-4	0.1086	0.3145	3.55	0.73	4.28	0.4544	1.95
67	M3-5-5	0.1266	0.3542	3.99	0.92	4.90	0.4141	1.66
68	M3-5-6	0.1182	0.2222	2.39	1.41	3.80	0.5987	2.40
69	M3-5-7	0.1286	0.3854	4.36	0.80	5.16	0.5124	2.20
70	M3-5-8	0.1664	0.2962	3.16	2.07	5.23	0.4215	1.21
71	M3-5-9	0.1781	0.2161	2.15	2.73	4.88	0.2151	-0.10
72	M3-5-10	0.105	0.4445	5.15	-0.01	5.14	0.4544	2.25
73	M3-5-11	0.2482	0.3121	3.13	3.74	6.88	0.4141	0.46
74	M3-5-12	0.1086	0.2002	2.15	1.31	3.46	0.5987	2.44
75	M3-5-13	0.1266	0.3554	4.00	0.91	4.91	0.5124	2.16
76	M3-5-14	0.1186	0.4445	5.11	0.28	5.40	0.4215	1.96
77	M3-6-1	0.1781	0.3081	3.28	2.26	5.54	0.2151	0.09
78	M3-6-2	0.1882	0.2461	2.49	2.79	5.28	0.4544	1.07
79	M3-6-3	0.1186	0.3667	4.16	0.68	4.84	0.4141	1.76
80	M3-6-4	0.1286	0.2962	3.27	1.25	4.52	0.5987	2.46
81	M3-6-5	0.1664	0.3081	3.31	2.01	5.32	0.5124	1.70
82	M3-6-6	0.1781	0.2461	2.52	2.57	5.09	0.4215	1.00
83	M3-6-7	0.1882	0.3667	3.97	2.18	6.14	0.2151	0.12
84	M3-6-8	0.1186	0.2962	3.30	1.04	4.34	0.4544	1.82
85	M3-6-9	0.1286	0.2002	2.09	1.74	3.84	0.4141	1.32
86	M3-6-10	0.1664	0.3554	3.89	1.77	5.65	0.5987	2.23
87	M3-6-11	0.1781	0.4445	4.95	1.56	6.51	0.5124	1.87
88	M3-6-12	0.1882	0.3081	3.25	2.47	5.72	0.4215	1.04
89	M3-6-13	0.1186	0.2461	2.68	1.29	3.98	0.2151	0.51
90	M3-6-14	0.1286	0.3667	4.13	0.89	5.03	0.4544	1.87
91	M3-6-15	0.1882	0.2962	3.10	2.54	5.64	0.4141	0.97
92	M3-7-1	0.1186	0.3081	3.44	0.98	4.42	0.5987	2.57
93	M3-7-2	0.1286	0.2002	2.09	1.74	3.84	0.5124	1.82
94	M3-7-3	0.1664	0.3081	3.31	2.01	5.32	0.4215	1.24
95	M3-7-4	0.1781	0.2461	2.52	2.57	5.09	0.2151	-0.04
96	M3-7-5	0.1682	0.3667	4.02	1.75	5.77	0.4544	1.51
97	M3-7-6	0.1106	0.2962	3.32	0.87	4.19	0.4141	1.69
98	M3-7-7	0.2482	0.3667	3.80	3.47	7.27	0.5987	1.50
99	M3-7-8	0.1086	0.2962	3.33	0.82	4.15	0.5124	2.20
100	M3-7-9	0.1266	0.3081	3.42	1.15	4.57	0.4215	1.60
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106	M3-8-1	0.1664	0.2962	3.16	2.07	5.23	0.4215	1.21
107	M3-8-2	0.1781	0.3081	3.28	2.26	5.54	0.2151	0.09
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135	M3-10-3	0.1286	0.3667	4.13	0.89	5.03	0.5124	2.17
136	M3-10-4	0.1664	0.2962	3.16	2.07	5.23	0.4215	1.21
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151	M3-11-6	0.1286	0.3667	4.13	0.89	5.03	0.4141	1.67
152	M3-11-7	0.1664	0.2962	3.16	2.07	5.23	0.5987	2.11
153	M3-11-8	0.1781	0.3081	3.28	2.26	5.54	0.5124	1.59
154	M3-11-9	0.1286	0.2461	2.66	1.51	4.17	0.4215	1.46
155	M3-11-10	0.1664	0.3667	4.03	1.71	5.74	0.2151	0.32
156	M3-11-11	0.1781	0.4445	4.95	1.56	6.51	0.4544	1.58
157	M3-11-12	0.1882	0.3081	3.25	2.47	5.72	0.4141	1.00
158	M3-11-13	0.1781	0.2461	2.52	2.57	5.09	0.5987	1.90
159	M3-12-1	0.1882	0.3667	3.97	2.18	6.14	0.5124	1.62
160	M3-12-2	0.1186	0.2962	3.30	1.04	4.34	0.4215	1.65
161	M3-12-3	0.1664	0.3081	3.31	2.01	5.32	0.2151	0.19
162	M3-12-4	0.1781	0.2461	2.52	2.57	5.09	0.4544	1.17
163	M3-12-5	0.1882	0.3667	3.97	2.18	6.14	0.4141	1.12
164	M3-12-6	0.1186	0.2962	3.30	1.04	4.34	0.5987	2.55
165	M3-12-7	0.1286	0.4461	5.11	0.49	5.60	0.5124	2.33
166	M3-12-8	0.1664	0.4445	4.98	1.31	6.29	0.4215	1.52
167	M3-12-9	0.1781	0.3081	3.28	2.26	5.54	0.2151	0.09
168	M3-12-10	0.1882	0.2461	2.49	2.79	5.28	0.4544	1.07
169	M3-12-11	0.1186	0.3667	4.16	0.68	4.84	0.4141	1.76

170	M3-12-12	0.1286	0.2962	3.27	1.25	4.52	0.5987	2.46
171	M3-13-1	0.1664	0.4461	5.00	1.30	6.30	0.5124	1.98
172	M3-13-2	0.1781	0.4445	4.95	1.56	6.51	0.4215	1.41
173	M3-13-3	0.1882	0.3081	3.25	2.47	5.72	0.6541	2.21
174	M3-13-4	0.1186	0.2461	2.68	1.29	3.98	0.4544	1.71
175	M3-13-5	0.1286	0.3667	4.13	0.89	5.03	0.4141	1.67
176	M3-13-6	0.1664	0.2962	3.16	2.07	5.23	0.5987	2.11
177	M3-13-7	0.1781	0.3081	3.28	2.26	5.54	0.5124	1.59
178	M3-13-8	0.1882	0.2461	2.49	2.79	5.28	0.4215	0.91
179	M3-13-9	0.1186	0.3667	4.16	0.68	4.84	0.2151	0.76
180	M3-13-10	0.1286	0.2962	3.27	1.25	4.52	0.4544	1.73
181	M3-13-11	0.1664	0.2221	2.26	2.44	4.70	0.4141	1.02
182	M3-14-1	0.1781	0.4445	4.95	1.56	6.51	0.5987	2.31
183	M3-14-2	0.1882	0.3081	3.25	2.47	5.72	0.5124	1.50
184	M3-14-3	0.1186	0.2461	2.68	1.29	3.98	0.4215	1.55
185	M3-14-4	0.1286	0.3667	4.13	0.89	5.03	0.2151	0.66
186	M3-14-5	0.1664	0.2962	3.16	2.07	5.23	0.4544	1.38
187	M3-14-6	0.1781	0.4061	4.48	1.76	6.24	0.4141	1.30
188	M3-14-7	0.1882	0.3945	4.31	2.03	6.34	0.5987	2.11
189	M3-14-8	0.1186	0.3081	3.44	0.98	4.42	0.5124	2.14
190	M3-14-9	0.1286	0.2461	2.66	1.51	4.17	0.4215	1.46
191	M3-14-10	0.1664	0.3667	4.03	1.71	5.74	0.5462	1.99
192	M3-15-1	0.1781	0.2962	3.13	2.32	5.45	0.4544	1.27
193	M3-15-2	0.1882	0.3081	3.25	2.47	5.72	0.4141	1.00
194	M3-15-3	0.1186	0.2461	2.68	1.29	3.98	0.5987	2.44
195	M3-15-4	0.1286	0.3667	4.13	0.89	5.03	0.5124	2.17
196	M3-15-5	0.1664	0.2962	3.16	2.07	5.23	0.4215	1.21
197	M3-15-6	0.1781	0.4461	4.97	1.55	6.52	0.5135	1.88
198	M3-15-7	0.1882	0.4445	4.92	1.78	6.70	0.4544	1.49
199	M3-15-8	0.1186	0.3081	3.44	0.98	4.42	0.4141	1.64
200	M3-15-9	0.1286	0.2461	2.66	1.51	4.17	0.5987	2.35
201	M3-15-10	0.1664	0.3667	4.03	1.71	5.74	0.5124	1.82
202	M3-16-1	0.1781	0.2962	3.13	2.32	5.45	0.4215	1.10
203	M3-16-2	0.1882	0.4461	4.94	1.77	6.71	0.4522	1.48
204	M3-16-3	0.1186	0.4445	5.11	0.28	5.40	0.4544	2.13
205	M3-16-4	0.1286	0.3081	3.42	1.19	4.61	0.4141	1.55
206	M3-16-5	0.1664	0.2461	2.55	2.32	4.87	0.5987	2.00
207	M3-16-6	0.1781	0.3667	4.00	1.96	5.95	0.5124	1.71
208	M3-16-7	0.1882	0.3081	3.25	2.47	5.72	0.4215	1.04
209	M3-16-8	0.1186	0.2461	2.68	1.29	3.98	0.2151	0.51
210	M3-16-9	0.1286	0.3667	4.13	0.89	5.03	0.4544	1.87
211	M3-17-1	0.1664	0.2461	2.55	2.32	4.87	0.4141	1.07
212	M3-17-2	0.1781	0.3667	4.00	1.96	5.95	0.5987	2.15
213	M3-17-3	0.1286	0.3081	3.42	1.19	4.61	0.5124	2.04
214	M3-17-4	0.1664	0.3081	3.31	2.01	5.32	0.4215	1.24
215	M3-17-5	0.1781	0.3081	3.28	2.26	5.54	0.5185	1.62
216	M3-17-6	0.1664	0.2461	2.55	2.32	4.87	0.4544	1.27
217	M3-17-7	0.1781	0.3667	4.00	1.96	5.95	0.4521	1.41
218	M3-17-8	0.1286	0.2461	2.66	1.51	4.17	0.5417	2.06
219	Control	0.1153	0.5924	6.94	1.16	6.39	0.4141	

According to the study, Chl a concentrations ranged from 1.65 to 6.11 g/mL, Chl b concentrations from -0.01 to 8.49 g/mL, total chlorophyll concentrations ranged from 3.11 to 14.59 g/mL, and the carotenoid content was -0.01 to 2.73 g/mL. Among 218 mutants, M3-2-3 has highest Chl a (6.11 g/mL) while M3-2-2 had the lowest (1.65 g/mL); the mutant M3-5-10 had least (-0.01 g/mL) and M3-2-3 (8.49 g/mL) had maximum concentration of chl b however, M3-3-2 had max (2.73 g/mL) and M3-5-9 (-0.10 g/mL) had minimum quantity of carotenoid content (Table 1). The higher chlorophyll content can be used to detect photosynthetic rate and identify stress level sue to its adaptation to environmental change (Shibghatallah 2013) [14] while, the indispensable carotenoid molecules protect plant 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 & Stojanovic 2005) [4]. Additionally, applying plant growth regulators in greater concentrations has beneficial effects on the amount of chlorophyll in leaves (Sardoei 2014) [13].

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