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Effect of Ag-nanoparticle in biochemical and physiological changes during progress of seed development and maturation in green gram

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Abstract

Seed development and maturation comprises of a series of events involving cell division/ histo-differentiation, retains deposition and desiccation. Development of green gram seed has been divided into eight consecutive stages; 5, 10, 15, 20, 25, 30, 35, 40 days after anthesis (DAA). During seed development, chlorophyll was declined, while carbohydrate and protein contents were increased, suggesting their supportive role in germination and early seedling growth. As seeds developed upto 30 days after anthesis did not germinate at all, germination potential and seedling parameters including vigour index were considered for the last two stages of development (35 and 40 DAA) only. The highest germination potential was recorded as 81.24 % and 80.99 %, for seeds developed at the stage of 40 DAA in first and second year respectively. Significantly highest vigour was observed at 40 DAA, when average was made over genotypes and treatments. Seeds of Samrat produced after seed treatment with Ag-NP were of highest vigour status when harvested at 40 DAA. So, for seed purpose ideal harvesting time for greengram is 40 DAA as at that time seed vigour maximum.

Keywords: Ag-nanoparticle, carbohydrate, germination, greengram, protein, vigour, seed development

Introduction

Seed development within the life cycle of any higher plant may be a crucial process by providing the link between two different sporophytic generations (Baud *et al.* 2002). Generally, seed development involves an orchestrate programme of pattern, which has formation, reserves deposition and desiccation. Formation of a seed is initiated after double fertilization that takes place in an embryo sac, so repeated organic process occurs so as to determine the seed structures (Baud *et al.* 2002). After that, seed development or seed filling phase starts during which the water content reduces and storage reserves accumulate and proceed to cell expansion. Carbohydrates and storage proteins are chief storage reserves generally accumulated in most of the seeds. Seed filling is extremely complex process, as many genes and variety of enzymes are involved to manage storage of every component (Weber *et al.* 2005). For the method of seed filling, the monomers of assorted macromolecules viz., amino acids, fatty acids and monosaccharides are translocated through sieve elements of phloem to synthesize their long chain polymers (Weber *et al.* 2005). Next, the seed enters into maturation drying phase where changes within the seed size and big fall in moisture content occurs (Berjak and Pammenter 2008). Maturation drying phase is totally absent in recalcitrants therefore, these seeds possess high amount of water (30-70%) at the time of shedding from mother plant (Berjak and Pammenter 2008).

Process of seed development is genetically programmed, and developing seed converts the precursors of carbon and nitrogen, into stable reserves required later for various processes (Weber *et al.* 2005). During seed development, as a by-product of metabolic reactions reactive oxygen species (ROS) are generated, which are shown to play dual functions either cytotoxicity (when exists in high amount) or playing a task in development, dormancy breakage and defense against biotic and abiotic stresses (Berjak and Pammenter 2008). These reactive oxygen species (ROS) are popularly known to react with all types of macromolecules, pigments and other cellular components, leading their degradation (Parkhey *et al.* 2014). Plant cells are having tremendous potency to forestall themselves from such oxidative injury by keeping the reactive oxygen species (ROS) in restraint via an efficient and highly redundant network of antioxidants (Chandra *et al.* 2015). Enzyme (SOD), catalase (CAT), guaiacol peroxidase (POX) and ascorbate peroxidase (APX) constitute the most important enzymatic systems by which cells catabolize free radicals, thus minimizes the severity of oxidative damage (Chandra *et al.* 2015). SOD plays key role in conversion of superoxide into oxygen and peroxide. While CAT, POX and APX catalyzes the conversion of

oxide into water and molecular oxygen and so prevent further generation of free radicals (Chandra *et al.* 2015). Very limited reports are available concerning to the function of antioxidants during seed development however; nobody has yet attempted to explore them in developing green gram seeds.

The physiological and biochemical investigations conducted by He and Gao (2008) and Kok *et al.* (2013) on developing seeds of shrub and *Elaeis guineensis*, respectively, provided comprehensive knowledge regarding seed metabolism. Certainly, information regarding developmental biochemical changes is an imperative base in understanding the regulatory set- up coordinating seed development and metabolism. Additionally, in-depth knowledge regarding pattern and mechanisms of reserves accumulation in seed is pre-requisite in any plant-breeding programme (Kok *et al.* 2013). The metabolic changes going down during seed development are studied for several species (Baud *et al.* 2002, Silveira *et al.* 2004, Saldivar *et al.* 2011, and Pavithra *et al.* 2014), however there's an absolute lack of data for green gram.

Green gram seeds are sensitive towards desiccation and coldness hence got damaged. There is no information regarding biochemical changes going down during seed developmental stages of green gram seeds. From the above view, the present experiment was conducted to determine the physiological (germination percentage of seed, seedling length (cm), fresh and dry weight (g) of seedlings; and vigour index) and biochemical (contents of chlorophyll (mg g^{-1}) of whole pod, protein and carbohydrate of seed) changes during seed developmental stages.

Materials and methods

Three green gram genotypes namely Pusa Vishal, Samrat, SML-1822; were sown for making study on seed developmental pattern at Kalyani D block Farm, BCKV, West Bengal, India during 2019 and 2020. Pre-sowing seed priming of three individual genotypes was made with distilled water and 20 ppm Silver Nanoparticle (Chakraborty and Bordolui, 2021). Plant population grown after distilled water soaking and Ag-NP plots were utilized to assess the influence of Ag-NP on seed development. Approximately 200 flowers of each of distilled water and Ag-NP treated plots in each replication were tagged on a single date of anthesis. 20 pods developed from tagged flowers were harvested from each plot at 5 days after anthesis (DAA) and 5 days interval thereafter till 40 DAA, thus leading to harvesting at 8 different stages of development in the experimental plots with three replications following Randomised Block Design.

The observations on physiological and biochemical characters were recorded during different growth stages of the plant. The physiological characters like germination percentage of seed, seedling length (cm), fresh and dry weight (g) of seedlings; and vigour index were recorded at 5 days interval thereafter till 40 DAA. Biochemical characters i.e., chlorophyll content (mg g^{-1}) of whole pod (Barua *et al*., 2016), soluble protein by Lowry's method (mg g^{-1} fresh weight) (Lowry *et al*., 1951) and total carbohydrate content by Anthrone's method (mg g^{-1} fresh weight) (Hedge *et al*., 1962) of seed were observed during the respective developmental stages.

At first two stages of development stages i.e., at 5 DAA and 10 DAA, protein and carbohydrate were estimated for entire pod, as seeds could not be separated well, and were too small and insufficient for analysis irrespective of the genotypes over the years. While only seeds were used for estimation of both the biochemical parameters from 15 DAA to 40 DAA.

Results

Changes in average chlorophyll content (mg g^{-1}) of pods at different post-fertilization stages

It could be revealed through Table 1 that chlorophyll content of developing pods varied significantly among the genotypes and for different stages of development as well as significant influence of seed treatment with Ag-NP over untreated control was also noticed in both the years. Chlorophyll content was found to be increased at any stages of development over the previous stages till 30 DAA, after which it decline in progressive way till 40 DAA, may be due to the stepping towards maturity after attainment at 30 DAA. Average chlorophyll content was estimated as maximum (6.601 and 6.735 mg g^{-1}) of pods irrespective of years) for G_1 (Pusa Vishal) followed by that of G_2 (Samrat) and G_3 (SML-1822). Non-significant enhancement in chlorophyll content with varying rate for individual genotypes irrespective of seed treatment over the years would be recorded till 30 DAA and then declined.

Table 1: Average chlorophyll content (mg g^{-1}) at different post-fertilization stages

Genotype	Treatment	Developmental Stages (Days after Anthesis)								Mean GXT	Mean G	Mean T	Mean SXG			
		5DAA	10DAA	15DAA	20DAA	25DAA	30DAA	35DAA	40DAA				Stages	G_1	G_2	G_3
2019																
G_1	T_0	3.830	4.803	5.687	6.740	8.243	8.977	6.607	5.803	6.336	6.601	5.828 (T_0)	S_1	4.299	3.814	3.737
	T_1	4.767	5.490	5.860	6.960	8.557	9.283	7.647	6.360	6.865			S_2	5.147	4.667	4.425
G_2	T_0	3.600	4.313	5.117	6.347	7.283	8.003	6.567	5.103	5.791	6.086		S_3	5.774	5.459	4.874
	T_1	4.027	5.020	5.800	6.823	7.623	8.980	7.583	5.183	6.379		6.365 (T_1)	S_4	6.850	6.585	6.115
G_3	T_0	3.563	4.307	4.667	6.020	6.587	7.523	6.027	4.153	5.355	5.601		S_5	8.400	7.453	6.769
	T_1	3.910	4.543	5.080	6.210	6.950	8.790	6.350	4.943	5.847			S_6	9.130	8.492	8.157
Mean S		3.950	4.746	5.369	6.517	7.541	8.593	6.797	5.258				S_7	7.127	7.075	6.189
Mean SXT	T_0	3.664	4.474	5.157	6.369	7.371	8.168	6.400	5.020				S_8	6.082	5.143	4.548
	T_1	4.235	5.018	5.580	6.664	7.710	9.018	7.193	5.495							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**G X T**	**S X G X T**								
SEm (±)		**0.156**	**0.096**	**0.078**	**0.271**	**0.221**	**0.135**	**0.383**								
LSD (0.05%)		**0.439**	**0.269**	**0.220**	**NS**	**NS**	**NS**	**NS**								
2020																
G_1	T_0	3.820	4.813	5.793	7.847	8.550	8.977	6.823	5.800	6.552	6.735	5.979 (T_0)	S_1	4.299	3.827	3.737
	T_1	4.777	5.500	5.880	7.167	8.617	9.243	7.873	6.273	6.913			S_2	5.157	4.687	4.449
G_2	T_0	3.623	4.327	5.120	6.350	7.507	8.250	6.650	5.113	5.862	6.102		S_3	5.837	5.485	4.934
	T_1	4.030	5.047	5.850	6.910	7.687	8.333	7.667	5.170	6.333		6.391 (T_1)	S_4	7.507	6.630	6.182
G_3	T_0	3.567	4.337	4.730	6.110	7.013	7.577	6.563	4.213	5.509	5.716		S_5	8.584	7.597	7.083
	T_1	3.907	4.560	5.137	6.253	7.153	8.753	6.613	4.967	5.916			S_6	9.110	8.292	8.165
Mean S		3.954	4.764	5.418	6.773	7.755	8.522	7.032	5.256				S_7	7.348	7.159	6.588
Mean SXT	T_0	3.670	4.492	5.214	6.769	7.690	8.268	6.679	5.042				S_8	6.037	5.142	4.590
	T_1	4.238	5.036	5.622	6.777	7.819	8.776	7.384	5.470							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**G X T**	**S X G X T**								
SEm (±)		**0.146**	**0.090**	**0.073**	**0.254**	**0.207**	**0.127**	**0.359**								
LSD (0.05%)		**0.411**	**0.252**	**0.206**	**NS**	**NS**	**NS**	**NS**								

G= Genotypes, G_1= Pusa Vishal, G_2= Samrat, G_3= SML-1822; T= Treatments, T_0= Control, T_1= Ag-Nanoparticle @ 20 ppm.
S= Stages of Seed Development; S_1= 5 DAA, S_2= 10 DAA, S_3= 15 DAA, S_4= 20 DAA, S_5= 25 DAA, S_6= 30 DAA, S_7= 35 DAA, S_8= 40 DAA.

Changes in average total soluble protein content (mg g^{-1}) of seeds/pods at different post-fertilization stages

Total soluble protein content was estimated for whole pods at first two stages and then it was done for separated developing seeds at all other six stages. Significant variation among the genotypes was recorded for its soluble protein content over the years and it was of highest magnitude (175.087 and 175.235 mg g^{-1} in 2019 and 2020 respectively) for G_1 (Pusa Vishal) followed by G_2 (Samrat) & G_3 (SML-1822). Pre-sowing seed treatment with Ag-NP was noted to exert significant influence on average protein content of seed could be noticed over untreated control in both the years. Average protein content estimated at different stages of development was also found to be varied significantly; enhancement in protein content could be recorded till 35 DAA in both the years and then declined at 40 DAA, may be due to deterioration of this sensible biochemical parameter. Soluble protein content of seed enhanced with increasing rate till 30 DAA from 15 DAA then rate of enhancement declined drastically at 35 DAA from 30 DAA. Performance of individual genotypes over stages of development, when average was made over treatments, exhibited that protein content significantly enhanced with increasing rate till 30 DAA irrespective of the genotypes in both the years, then enhancement occurred at slower rate from 30 to 35 DAA after which it declined; varying rate of enhancement could be noticed among the genotypes over the years. When average was made over the genotypes, enhancement, in protein content could be recorded till 35 DAA in both the years, after which it declined, weather pre-sowing seed treatment was made or not.

Table 2: Average total soluble protein content (mg g^{-1}) at different post-fertilization stages

Genotype	Treatment	Developmental Stages (Days after Anthesis)								Mean GXT	Mean G	Mean T	Mean SXG			
		5DAA	10DAA	15DAA	20DAA	25DAA	30DAA	35DAA	40DAA				Stages	G_1	G_2	G_3
2019																
G_1	T_0	74.883	100.328	129.041	152.244	191.419	240.335	244.890	225.997	169.892	175.087	166.887 (T_0)	S_1	83.184	76.291	71.817
	T_1	91.484	106.642	135.810	159.963	200.885	252.583	257.370	237.513	180.281			S_2	103.485	102.062	82.681
G_2	T_0	68.611	100.182	126.112	151.921	189.353	240.661	245.222	226.303	168.546	173.380		S_3	132.426	130.890	126.829
	T_1	83.971	103.941	135.667	156.244	200.138	252.005	256.781	236.970	178.215		177.692 (T_1)	S_4	156.104	154.083	152.519
G_3	T_0	60.428	77.027	122.227	150.355	189.131	236.078	240.552	221.993	162.224	168.402		S_5	196.152	194.746	193.555
	T_1	83.205	88.334	131.431	154.682	197.978	250.402	255.148	235.463	174.58			S_6	246.459	246.333	243.240
Mean S		77.097	96.076	130.048	154.235	194.817	245.344	249.994	230.707				S_7	251.130	251.002	247.850
Mean SXT	T_0	67.974	92.512	125.793	151.507	189.968	239.025	243.555	224.764				S_8	231.755	231.637	228.728
	T_1	86.220	99.639	134.303	156.963	199.667	251.663	256.433	236.649							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**G X T**	**S X G X T**								
SEm (±)		**1.125**	**0.689**	**0.562**	**1.948**	**1.591**	**0.974**	**2.755**								
LSD (0.05%)		**3.158**	**1.934**	**1.579**	**5.470**	**4.467**	**NS**	**NS**								
2020																
G_1	T_0	74.571	100.805	129.034	152.954	192.204	240.404	244.985	226.063	170.128	175.235	167.084 (T_0)	S_1	83.122	75.912	71.795
	T_1	91.673	107.101	135.577	161.575	201.129	251.970	256.772	236.940	180.342			S_2	103.953	102.204	83.062
G_2	T_0	68.260	100.245	127.253	151.808	191.674	240.237	244.815	225.907	168.775	173.262		S_3	132.306	131.068	127.543
	T_1	83.563	104.163	134.883	155.982	200.093	251.166	255.952	236.183	177.748		177.640 (T_1)	S_4	157.265	153.895	152.814
G_3	T_0	60.935	77.211	123.121	151.388	188.181	235.848	240.343	221.780	162.351	168.591		S_5	196.667	195.884	193.250
	T_1	82.654	88.912	131.964	154.239	198.319	250.914	255.695	235.947	174.831			S_6	246.187	245.702	243.381
Mean S		76.943	96.406	130.305	154.658	195.267	245.0898	249.760	230.470				S_7	250.879	250.384	248.019
Mean SXT	T_0	67.922	92.754	126.469	152.050	190.686	238.830	243.381	224.583				S_8	231.502	231.045	228.864
	T_1	85.963	100.059	134.141	157.265	199.847	251.350	256.140	236.357							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**G X T**	**S X G X T**								
SEm (±)		**1.130**	**0.692**	**0.565**	**1.957**	**1.598**	**0.979**	**2.768**								
LSD (0.05%)		**3.172**	**1.943**	**1.586**	**5.495**	**4.486**	**NS**	**NS**								

G= Genotypes, G_1= Pusa Vishal, G_2= Samrat, G_3= SML-1822; T= Treatments, T_0= Control, T_1= Ag-Nanoparticle @ 20 ppm.
S= Stages of Seed Development; S_1= 5 DAA, S_2= 10 DAA, S_3= 15 DAA, S_4= 20 DAA, S_5= 25 DAA, S_6= 30 DAA, S_7= 35 DAA, S_8= 40 DAA.

Changes in average carbohydrate content (mg g^{-1}) at different post-fertilization stages

Similar to soluble protein content, ccarbohydrate content was also determined from whole pods at initial two stages and from separate seeds for other developmental stages. Though non-significant, higher carbohydrate content was estimated for G_1 (Pusa Vishal) followed by that of G_2 (Samrat) and G_3 (SML-1822). Overall influence of Ag-NP on enhancement was recorded over untreated control in both the years (Table 3). Enhancement in carbohydrate content could be recorded till 30 DAA in both the years, on an average, and then it was declined at the other two developmental stages; may be due to degradation on attainment of physiological maturity. Similar scenario for individual genotypes over seed treatment procedures could be noticed, though non-significant, with the progress in seed development.

Table 3: Average carbohydrate content (mg g^{-1}) at different post-fertilization stages

Genotype	Treatment	Developmental Stages (Days after Anthesis)								Mean GXT	Mean G	Mean T	Mean SXG			
		5DAA	10DAA	15DAA	20DAA	25DAA	30DAA	35DAA	40DAA				Stages	G_1	G_2	G_3
2019																
G_1	T_0	174.667	243.487	313.057	382.530	499.928	681.752	648.824	637.850	447.762	453.709	447.111 (T_0)	S_1	178.254	177.357	176.612
	T_1	181.840	249.233	322.117	393.983	514.897	702.165	668.250	644.760	459.656			S_2	246.360	245.737	244.489
G_2	T_0	173.550	242.817	312.790	382.380	499.732	681.485	648.570	638.063	447.423	452.981		S_3	317.587	317.402	316.884
	T_1	181.163	248.657	322.013	392.710	513.233	699.895	666.090	644.540	458.538		458.706 (T_1)	S_4	388.257	387.545	386.979
G_3	T_0	173.450	240.870	312.077	381.450	498.517	679.827	646.992	635.987	446.146	452.036		S_5	507.413	506.483	505.742
	T_1	179.773	248.107	321.690	392.507	512.967	699.533	665.745	643.080	457.925			S_6	691.959	690.690	689.680
Mean S		177.407	245.529	317.291	387.593	506.546	690.776	657.412	640.713				S_7	658.537	657.330	656.369
Mean SXT	T_0	173.889	242.391	312.641	382.120	499.392	681.021	648.129	637.300				S_8	641.305	641.302	639.534
	T1	180.925	248.666	321.940	393.067	513.699	700.531	666.695	644.127							
Effects		S	G	T	S X G	S X T	G X T	S X G X T								
SEm (±)		0.903	0.553	0.452	1.564	1.277	0.782	2.212								
LSD (0.05%)		2.536	NS	1.268	NS	3.586	NS	NS								
2020																
G_1	T_0	175.573	243.757	314.533	383.917	501.817	684.278	651.159	637.710	449.093	454.934	448.509 (T_0)	S_1	178.858	178.194	177.012
	T_1	182.143	250.310	324.290	394.923	516.204	703.896	669.828	644.610	460.776			S_2	247.034	246.155	244.955
G_2	T_0	174.860	243.130	314.017	383.820	501.691	684.106	650.995	637.583	448.775	454.345		S_3	319.412	318.702	317.477
	T_1	181.527	249.180	323.387	394.320	515.416	702.821	668.804	643.870	459.916		460.043 (T_1)	S_4	389.420	389.070	388.742
G_3	T_0	173.773	241.620	311.870	383.397	501.138	683.351	650.277	635.853	447.660	453.549		S_5	509.011	508.554	508.125
	T_1	180.250	248.290	323.083	394.087	515.111	702.405	668.409	643.877	459.439			S_6	694.087	693.464	692.878
Mean S		178.021	246.048	318.530	389.077	508.563	693.476	659.912	640.584				S_7	660.494	659.900	659.343
Mean SXT	T_0	174.735	242.836	313.473	383.711	501.519	683.912	650.810	637.049				S_8	641.160	640.727	639.865
	T_1	181.307	249.260	323.587	394.443	515.577	703.041	669.014	644.119							
Effects		S	G	T	S X G	S X T	G X T	S X G X T								
SEm (±)		0.771	0.472	0.385	1.335	1.090	0.667	1.888								
LSD (0.05%)		2.164	NS	1.082	NS	3.060	NS	NS								

G= Genotypes, G_1= Pusa Vishal, G_2= Samrat, G_3= SML-1822; T= Treatments, T_0= Control, T_1= Ag-Nanoparticle @ 20 ppm.
S= Stages of Seed Development; S_1= 5 DAA, S_2= 10 DAA, S_3= 15 DAA, S_4= 20 DAA, S_5= 25 DAA, S_6= 30 DAA, S_7= 35 DAA, S_8= 40 DAA.

Changes in germination potential and other quality parameters of developing seeds

As seeds developed upto 30 days after anthesis did not germinate at all for the three genotypes studied herein, germination potential and seedling parameters including vigour index were considered for the last two stages of development (35 and 40 DAA) only. Average germination potential of the developing seeds varied significantly for the stages of development only in both the years; significant variation among the genotypes and between the stages of development could be noticed for average seedling length along with the significant influence of pre-sowing seed treatment with Ag-NP over that of untreated control, the first order interactions i.e., SXG, SXT and GXT also varied significantly; for average seedling fresh weight, significant variation among the genotypes between the stages of development and treatments were observed along with the first order interactions in both the years; while for seedling dry weight, average genotypic performance varied significantly along with the stages of development and treatment effects only; and clear variation among the genotypes and stages of development were found for average vigour index along with significant influence of Ag-NP over untreated control, first order interactions as well as second order interactions were also significant for this important determined seed parameter.

Significantly greater germination potential could be noticed at 40 DAA over that of 35 DAA in both the years, when average was made over genotypes and treatment; but the same could not satisfy the requirement after minimum seed certification standard (MSCS) specified for this crop at 35 DAA (Table 4). It was of highest magnitude for G_2 (Samrat) over other two genotypes, though non-significant. Average maximum germination potential was recorded as 81.24% and 80.99%, both values were greater than the value specified in MSCS for this crop, for seeds developed at the stage of 40 DAA in first and second year respectively.

Significantly longest seedlings were recovered for G_2 (Samrat) in both the years, on an average followed by that of G_1 (Pusa Vishal) and G_3 (SML-1822). It was of almost similar length for seeds developed at 40 DAA over the years and greater than that of seeds developed at 35 DAA. Significant influence of pre-sowing seed treatment with Ag-NP over untreated control was also noticed in both the years. When average was made over treatments, it was of significantly longest for seeds developed at 40 DAA for G_2 (Samrat) followed by that of G_1 (Pusa Vishal) developed at 40 DAA. Seeds developed after seed treatment with Ag-NP for individual genotypes were also noted to produce longer seedlings than that of its counterpart produced after untreated control in both the years.

It could be revealed from both the Tables 6 and 7 that both average fresh and dry weight of seedlings were recorded as maximum for G_2 (Samrat) followed by that of G_1 (Pusa Vishal) and G_3 (SML-1822) in both the years; both the parameters were of significantly higher magnitudes for seeds developed at 40 DAA than those of seeds produced at 35 DAA in both the years; and significant influence of pre-sowing seed treatment with Ag-NP, on an average, could be noticed over untreated control. Significant influence of Ag-NP over untreated control could also be noticed for individual genotypes, when average was made over stages of development, in both the years. On average made over treatments, seed produced for G_2 (Samrat) at 40 DAA exhibited significantly higher magnitudes of both fresh and dry weight of seedlings irrespective of the year of study.

Seeds produced for G_2 (Samrat) exhibited significantly highest vigour potential, on an average, irrespective of the years followed by that of G_1 (Pusa Vishal) and G_3 (SML-1822), sequence in vigour potential of seeds of the genotypes may be due to the determining procedure of this important seed parameter. It was of significantly higher magnitude for seeds developed at 40 DAA than that of seeds produced at 35 DAA, when average was made over genotypes and treatments. Clear significant influence of pre-sowing seed treatment with Ag-NP over untreated control could be noticed over the years, on an average; similar trend could also be noticed for vigour status of seeds of individual genotypes produced after treatment with Ag-NP in both the years. Seeds developed at 40 DAA were of higher vigour potential for individual genotypes, when average as made over treatment (Table 8). It is to be noted that seeds of G_2 (Samrat) produced after seed treatment with Ag-NP were of highest vigour status when harvested at 40 DAA followed by that of G_1 (Pusa Vishal), also produced after Ag-NP, and it was of lowest vigour status for G_3 (SML-1822) produced from untreated control. Variation among these three genotypes with respect to its seed vigour status irrespective of seed treatment and year of study may be due to its strict genetic potential.

Table 4: Average germination percentage (transform value) of seeds at different post-fertilization stages

Genotype	Treatment	Developmental Stages (Days after Anthesis)								
		35DAA	40DAA	Mean GXT	Mean G	Mean T	Mean SXG			
							Stages	G_1	G_2	G_3
2019										
G_1	T_0	46.08 (42.74)	81.25 (64.32)	63.665 (53.53)	63.81 (53.62)	63.58 (48.65) (T_0)	S_7	46.34 (42.89)	46.60 (43.03)	45.59 (42.46)
	T_1	46.60 (43.03)	81.30 (64.37)	63.95 (53.70)			S_8	81.28 (64.35)	81.79 (64.72)	80.66 (63.90)
G_2	T_0	46.53 (42.99)	81.59 (64.57)	64.06 (53.78)	64.20 (53.87)					
	T_1	46.67 (43.07)	81.99 (64.86)	64.33 (53.97)		63.84 (48.80) (T_1)				
G_3	T_0	45.50 (42.40)	80.54 (63.81)	63.02 (53.11)	63.13 (53.18)					
	T_1	45.68 (42.51)	80.78 (63.98)	63.23 (53.25)						
Mean S		46.18 (42.79)	81.24 (64.32)							
Mean SXT	T_0	46.04 (42.71)	81.13 (64.23)							
	T_1	46.32 (42.87)	81.36 (64.40)							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**G X T**	**S X G X T**		
SEm (±)		**0.154**	**0.188**	**0.154**	**0.266**	**0.217**	**0.266**	**0.376**		
LSD (0.05%)		**0.449**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**		
2020										
G_1	T_0	46.36 (42.90)	81.14 (64.24)	63.75 (53.57)	63.77 (53.59)	63.47 (53.39) (T_0)	S_7	46.40 (42.92)	46.47 (42.96)	45.42 (42.36)
	T_1	46.44 (42.94)	81.15 (64.26)	63.80 (53.60)			S_8	81.15 (64.25)	81.40 (64.43)	80.44 (63.73)
G_2	T_0	46.44 (42.94)	81.36 (64.40)	63.90 (53.67)	63.93 (53.70)					
	T_1	46.50 (42.98)	81.43 (64.46)	63.97 (53.72)		63.47 (53.49) (T_1)				
G_3	T_0	45.25 (42.26)	80.28 (63.61)	62.77 (52.94)	62.93 (53.04)					
	T_1	45.58 (42.45)	80.59 (63.85)	63.09 (53.15)						
Mean S		46.10 (42.75)	80.99 (64.14)							
Mean SXT	T_0	46.02 (42.70)	80.93 (64.08)							
	T_1	46.17 (42.79)	81.06 (64.19)							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**GXT**	**S X G X T**		
SEm (±)		**0.172**	**0.211**	**0.172**	**0.299**	**0.244**	**0.299**	**0.422**		
LSD (0.05%)		**0.503**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**		

(Figures in parenthesis are arc-sin transformed values)
G= Genotypes, G_1= Pusa Vishal, G_2= Samrat, G_3= SML-1822.
T= Treatments, T_0= Control, T_1= Ag-Nanoparticle @ 20 ppm.
S= Stages of Seed Development; S_7= 35 DAA, S_8= 40 DAA.

Table 5: Average seedling length (cm) at different post-fertilization stages

Genotype	Treatment	Developmental Stages (Days after Anthesis)								
		35DAA	40DAA	Mean GXT	Mean G	Mean T	Mean SXG			
							Stages	G_1	G_2	G_3
2019										
G_1	T_0	16.42	17.34	16.88	19.68	17.50	S_7	18.76	20.17	15.41
	T_1	21.09	23.87	22.48			S_8	20.61	22.48	17.18
G_2	T_0	19.29	20.93	20.11	21.33					
	T_1	21.06	24.03	22.54		20.70				
G_3	T_0	15.01	15.99	15.50	16.29					
	T_1	15.81	18.37	17.09						
Mean S		18.11	20.09							
Mean SXT	T_0	16.91	18.09							
	T_1	19.32	22.09							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**G X T**	**S X G X T**		
SEm (±)		**0.042**	**0.052**	**0.042**	**0.073**	**0.060**	**0.073**	**0.103**		
LSD (0.05%)		**0.123**	**0.150**	**0.123**	**0.213**	**0.174**	**0.213**	**NS**		
2020										
G_1	T_0	16.55	17.46	17.01	19.70	17.53	S_7	18.78	20.45	15.21
	T_1	21.00	23.77	22.39			S_8	20.62	22.64	16.92
G_2	T_0	19.53	20.96	20.25	21.54					
	T_1	21.36	24.31	22.84		20.67				
G_3	T_0	14.74	15.93	15.34	16.07					
	T_1	15.68	17.91	16.80						
Mean S		18.14	20.06							
Mean SXT	T_0	16.94	18.12							
	T_1	19.35	22.00							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**GXT**	**S X G X T**		
SEm (±)		**0.050**	**0.062**	**0.050**	**0.087**	**0.071**	**0.087**	**0.123**		
LSD (0.05%)		**0.147**	**0.180**	**0.147**	**0.255**	**0.208**	**0.255**	**NS**		

G= Genotypes, G_1= Pusa Vishal, G_2= Samrat, G_3= SML-1822:
T= Treatments, T_0= Control, T_1= Ag-Nanoparticle @ 20 ppm.
S= Stages of Seed Development; S_7= 35 DAA, S_8= 40 DAA.

Table 6: Average fresh weight (g) at different post-fertilization stages

Genotype	Treatment	Developmental Stages (Days after Anthesis)								
		35DAA	40DAA	Mean GXT	Mean G	Mean T	Mean SXG			
							Stages	G_1	G_2	G_3
2019										
G_1	T_0	1.450	1.860	1.655	1.883	1.713	S_7	1.710	1.922	1.617
	T_1	1.970	2.253	2.112			S_8	2.057	2.194	1.912
G_2	T_0	1.850	2.077	1.964	2.058					
	T_1	1.993	2.310	2.152		2.090				
G_3	T_0	1.400	1.640	1.520						
	T_1	1.833	2.183	2.008	1.764					
Mean S		1.749	2.054							
Mean SXT	T_0	1.567	1.859							
	T_1	1.932	2.249							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**G X T**	**S X G X T**		
SEm (±)		**0.003**	**0.004**	**0.003**	**0.005**	**0.004**	**0.005**	**0.008**		
LSD (0.05%)		**0.009**	**0.011**	**0.009**	**0.016**	**0.013**	**0.016**	**0.022**		
2020										
G_1	T_0	1.790	1.907	1.849	1.988	1.845	S_7	1.880	1.959	1.830
	T_1	1.970	2.283	2.127			S_8	2.095	2.202	1.990
G_2	T_0	1.867	2.027	1.947	2.080					
	T_1	2.050	2.377	2.214		2.141				
G_3	T_0	1.703	1.773	1.738	1.910					
	T_1	1.957	2.207	2.082						
Mean S		1.890	2.096							
Mean SXT	T_0	1.787	1.902							
	T_1	1.992	2.289							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**GXT**	**S X G X T**		
SEm (±)		**0.005**	**0.006**	**0.005**	**0.008**	**0.006**	**0.008**	**0.011**		
LSD (0.05%)		**0.013**	**0.016**	**0.013**	**0.023**	**0.019**	**0.023**	**NS**		

G= Genotypes, G_1= Pusa Vishal, G_2= Samrat, G_3= SML-1822:
T= Treatments, T_0= Control, T_1= Ag-Nanoparticle @ 20 ppm.
S= Stages of Seed Development; S_7= 35 DAA, S_8= 40 DAA.

Table 7: Average dry weight (g) at different post-fertilization stages

Genotype	Treatment	Developmental Stages (Days after Anthesis)								
		35DAA	40DAA	Mean GXT	Mean G	Mean T	Mean SXG			
							Stages	G_1	G_2	G_3
2019										
G_1	T_0	0.153	0.170	0.162	0.194	0.165	S_7	0.178	0.202	0.168
	T_1	0.203	0.250	0.227			S_8	0.210	0.234	0.185
G_2	T_0	0.163	0.200	0.182	0.2175					
	T_1	0.240	0.267	0.254		0.227				
G_3	T_0	0.143	0.163	0.153						
	T_1	0.193	0.207	0.200	0.1765					
Mean S		0.183	0.210							
Mean SXT	T_0	0.153	0.178							
	T_1	0.212	0.241							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**G X T**	**S X G X T**		
SEm (±)		**0.003**	**0.004**	**0.003**	**0.005**	**0.004**	**0.005**	**0.008**		
LSD (0.05%)		**0.009**	**0.011**	**0.009**	**NS**	**NS**	**NS**	**NS**		
2020										
G_1	T_0	0.153	0.167	0.160	0.183	0.161	S_7	0.172	0.185	0.152
	T_1	0.190	0.223	0.207			S_8	0.195	0.222	0.183
G_2	T_0	0.163	0.200	0.182	0.203					
	T_1	0.207	0.243	0.225		0.209				
G_3	T_0	0.127	0.153	0.140	0.168					
	T_1	0.177	0.213	0.195						
Mean S		0.170	0.200							
Mean SXT	T_0	0.148	0.173							
	T_1	0.191	0.226							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**GXT**	**S X G X T**		
SEm (±)		**0.004**	**0.005**	**0.004**	**0.007**	**0.006**	**0.007**	**0.010**		
LSD (0.05%)		**0.012**	**0.014**	**0.012**	**NS**	**NS**	**NS**	**NS**		

G= Genotypes, G_1= Pusa Vishal, G_2= Samrat, G_3= SML-1822:
T= Treatments, T_0= Control, T_1= Ag-Nanoparticle @ 20 ppm.
S= Stages of Seed Development; S_7= 35 DAA, S_8= 40 DAA.

Table 8: Average vigour index of seeds at different post-fertilization stages

Genotype	Treatment	Developmental Stages (Days after Anthesis)								
		35DAA	40DAA	Mean GXT	Mean G	Mean T	Mean SXG			
							Stages	G_1	G_2	G_3
2019										
G_1	T_0	756.79	1408.66	1082.725	1272.21	1123.51 (T_0)	S_7	869.81	940.00	702.43
	T_1	982.83	1940.55	1461.69			S_8	1674.61	1838.72	1385.73
G_2	T_0	897.31	1707.59	1302.45	1389.36					
	T_1	982.69	1969.85	1476.27		1346.92 (T_1)				
G_3	T_0	682.79	1287.90	985.35	1044.08					
	T_1	722.06	1483.55	1102.81						
Mean S		837.41	1633.02							
Mean SXT	T_0	778.96	1468.05							
	T_1	895.86	1797.98							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**G X T**	**S X G X T**		
SEm (±)		**3.737**	**4.577**	**3.737**	**6.473**	**5.285**	**6.473**	**9.154**		
LSD (0.05%)		**10.911**	**13.363**	**10.911**	**18.898**	**15.431**	**18.898**	**26.726**		
2020										
G_1	T_0	767.39	1416.85	1092.12	1271.98	1123.88 (T_0)	S_7	871.245	949.99	690.88
	T_1	975.10	1928.57	1451.84			S_8	1672.71	1842.61	1361.18
G_2	T_0	906.97	1706.09	1306.53	1396.30					
	T_1	993.01	1979.13	1486.07		1338.99 (T_1)				
G_3	T_0	667.13	1278.84	972.99	1026.03					
	T_1	714.63	1443.52	1079.08						
Mean S		837.37	1625.50							
Mean SXT	T_0	780.50	1467.26							
	T_1	894.25	1783.74							
Effects		**S**	**G**	**T**	**S X G**	**S X T**	**GXT**	**S X G X T**		
SEm (±)		**4.709**	**5.767**	**4.709**	**8.155**	**6.659**	**8.155**	**11.534**		
LSD (0.05%)		**13.747**	**16.837**	**13.747**	**23.811**	**19.441**	**23.811**	**33.673**		

G= Genotypes, G_1= Pusa Vishal, G_2= Samrat, G_3= SML-1822: T= Treatments, T_0= Control, T_1= Ag-Nanoparticle @ 20 ppm.
S= Stages of Seed Development; S_7= 35 DAA, S_8= 40 DAA.

Discussion

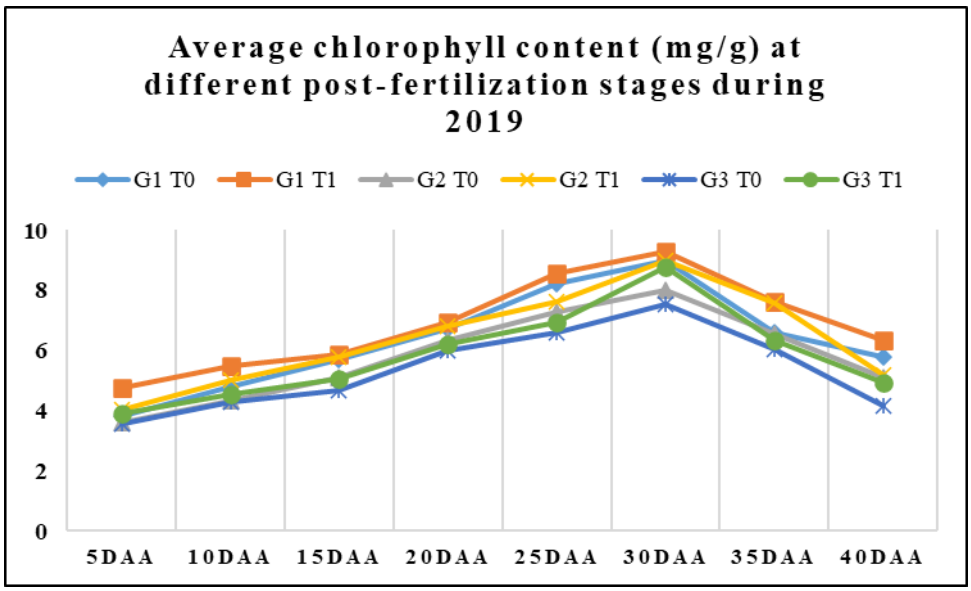
Chlorophyll content increased upto 30 DAA but then it decreased until 40 DAA. Same pattern was recorded by Pandita and Nagarajan (2006). They found rapid decline in chlorophyll content with the advancement of onion seed development. Razzaq *et al.* (2016) studied the effect of Ag-NPs on wheat and found that it has the potential to significantly increase chlorophyll contents. Reduction in seed protein content with progress in maturity may be due to its denaturation through release in moisture content leading to desiccation. Varied trend in protein accumulation of individual genotypes over the years may indicate strict expression of inherent potential for this character. Bollini and Chrispeels (1979) reported that a decrease in protein synthesis at maturity in pea and bean (*Phaseolus vulgaris*) seeds was due to the limited availability of mRNA. Krishnaraj *et al.* (2012) found that Ag-NPs showed a significant effect on synthesis of protein and carbohydrate contents of *Bacopa monnieri*. In case of carbohydrate content, Mehmood and Murtaza, (2017) in *Pisum sativum*, found that seed carbohydrate contents were increased after application of Ag-NPs. Latif *et al.* (2017) reported that the effect Ag-NPs on the chemical attributes of *Triticum aestivum* significantly increased chlorophyll, carbohydrate and protein content. Prażak *et al.* (2020) reported that concentrations of Ag-NPs had an immediate beneficial effect, resulting in fast and uniform germination in laboratory as well as a positive effect in the later stages of seedling development, manifested as an increase in the average seedling height, fresh and dry weight.

Conclusion

Pre-sowing seed treatment with Ag-NP significantly enhanced all the parameters over untreated control. Chlorophyll content of developing pods varied significantly among the genotypes and for different stages of development as well as significant influence of seed treatment with Ag-NP over untreated control was also noticed. Chlorophyll content was increased between two consecutive stages of development till 30 DAA, after which it declined in progressive way till 40 DAA. Seed priming with Ag-NP exerted significant influence on average protein content of seed could be noticed over untreated control. Enhancement in protein content could be recorded till 35 DAA and then declined at 40 DAA. Carbohydrate enhancement could be recorded till 30 DAA and then it was declined at the other two developmental stages. As seeds developed upto 30 days after anthesis did not germinate at all, germination potential and seedling parameters including vigour index were considered for the last two stages of development (35 and 40 DAA) only. Average maximum

germination potential was recorded as 81.24 % and 80.99 %, for seeds developed at the stage of 40 DAA in first and second year respectively. Vigour was of significantly higher magnitude for seeds developed at 40 DAA than that of seeds produced at 35 DAA, when average was made over genotypes and treatments. Seeds of G_2 (Samrat) produced after seed treatment with Ag-NP were of highest vigour status when harvested at 40 DAA followed by that of G_1 (Pusa Vishal), also produced after Ag-NP, and it was of lowest vigour status for G_3 (SML-1822) produced from untreated control.

Figure 1: Average chlorophyll content (mg g^{-1}) at different post-fertilization stages



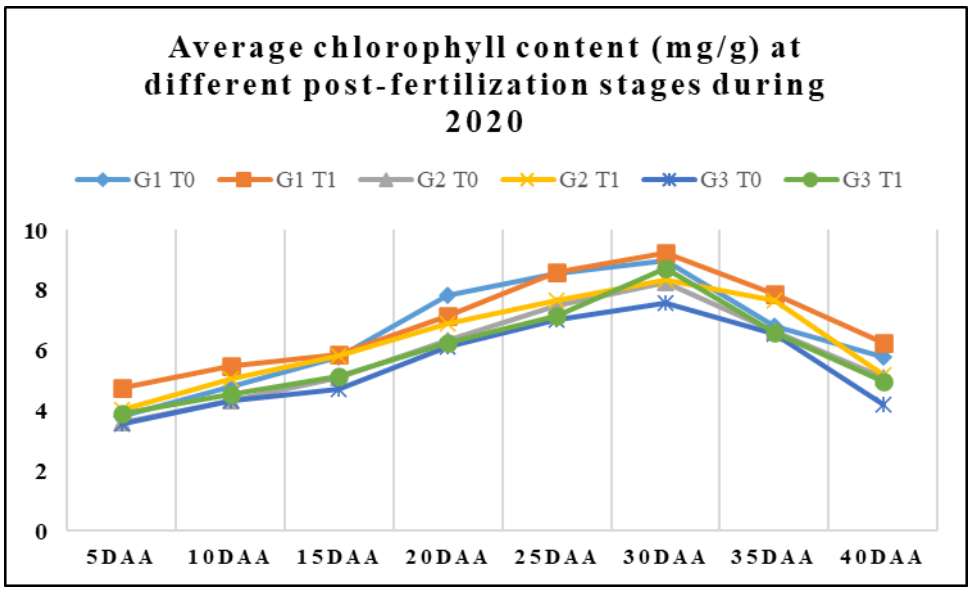


Figure 2: Average total soluble protein content (mg g^{-1}) at different post-fertilization stages

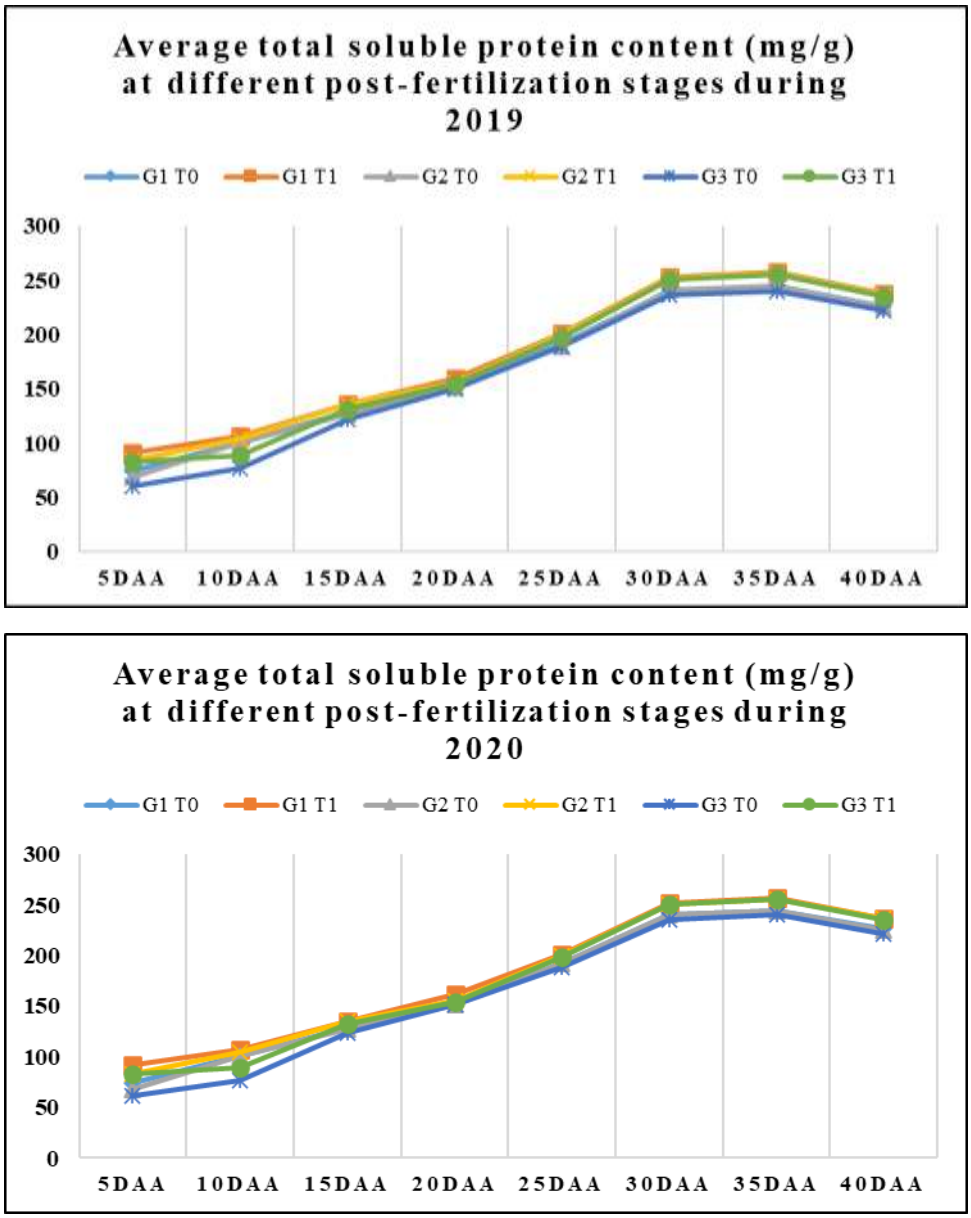
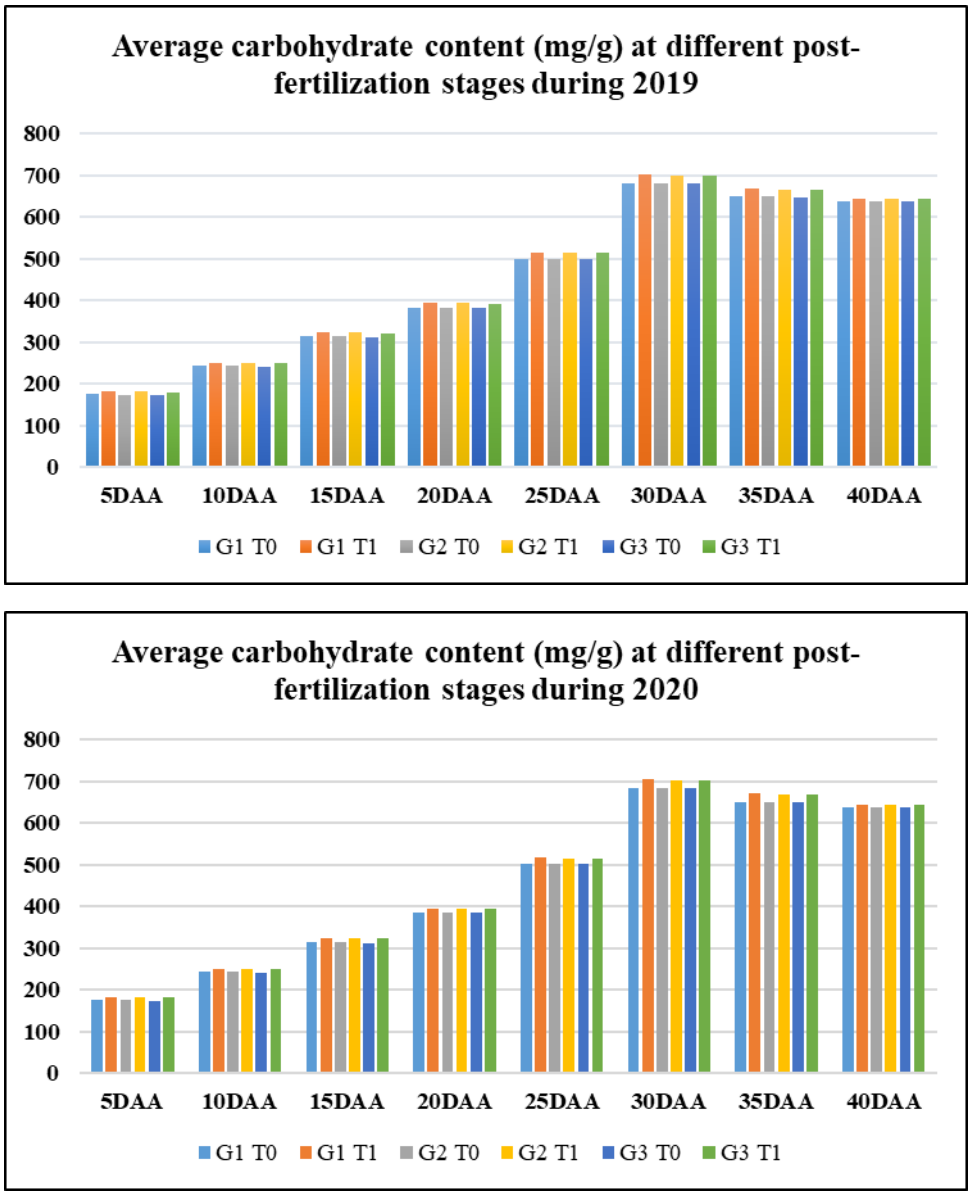


Figure 3: Average carbohydrate content (mg g^{-1}) at different post-fertilization stages



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