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Impact of selenium spray on monocarpic senescence of soybean (*Glycine Max* L.)

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Abstract

An experiment was conducted to study the effect of selenium on senescence phenomenon in soybean. Selenium was applied both as seed treatment at 5 ppm and as foliar spray at 50 and 100 ppm on 45, 60 and 75 DAS. The impact of selenium on delaying senescence was well established by increased activity of antioxidant enzymes such as, catalase and peroxidase and more numbers of leaves. Improved source strength by retaining more leaf number and area with better partitioning efficiency was considered as the contributing factor for significant yield improvement in selenium-treated plants.

Key words: Selenium, oxidative stress, senescence, antioxidant enzymes.

Introduction

Senescence, an endogenously controlled deteriorative change, causes natural death of cells, tissues, organs or organisms. Senescence occurs as an orderly loss of functions and structures comprising an array of biochemical and physiological processes resulting in the removal of nutrients from the decaying tissues[1]. Among the factors that are found to induce senescence, the dominant one is the accumulation of deleterious free radicals and free radical induced lipid peroxides[2]. Though the cells possess a well organized antioxidative defense system comprising of relevant enzymes and vitamins, as the leaves mature, an imbalance between the generation of free radicals and the removal by antioxidants results in degenerative changes and ultimately cell death[3].

Selenium, one of the most beneficial elements, exists in the functional part of the active centre of four types of selenium dependent glutathione-peroxidases, which are found in the liquid portion of the cell[4]. This group of enzymes prevents the formation of free radicals and also destroys or counteracts any lipid peroxides that are present in the cell[5]. Trelease and Trelease[6] observed an increase in biomass production when the selenium accumulator was treated with selenium. Selenium dependent enzymes have been identified, in which, an integral selenocysteine is inserted in the catalytic site[7].

Monocarpic senescence of soybean has been studied extensively. But no information is available on the impact of selenium on delaying senescence of soybean. In the present work, an attempt had been made to study the morphological changes and some biochemical events *viz*., changes in level of pigments, proteins, antioxidative enzymes, nitrate reductase activity and total free amino acids that are associated with senescence.

Materials and Methods

A pot culture experiment was conducted in Tamil Nadu Agricultural University, Coimbatore (11°N; 77°E; 426.7m MSL), India to study the response of soybean to selenium spray. The experimental soil is well drained clay loam in texture with pH 7.6. The soil is low in available N (195.6 kg ha^{-1}), medium in available P (6.3 kg ha^{-1}) and high in available K (386.4 kg ha^{-1}). Soybean variety CO2 with field duration of 85 days was employed in the study. The crop was sown during April, 2002 and harvested during June, 2002.

Seeds of CO2 soybean, pretreated with fungicide (Thiram @ 2 g kg^{-1} seeds) and rhizobium (200 g kg^{-1} seeds), were sown in earthern pots (30 cm x 40 cm) containing 10 kg of soil. Fertilizers were applied to the soil NPK @ 20:80:40 kg ha^{-1} at the time of sowing. A population of three plants per pot was maintained. The six treatments replicated four times in a randomized complete block design were as follows:

T_1: Seed treatment of selenium @ 5 ppm
T_2: T_1 + Foliar spray of selenium @ 50 ppm
T_3: T_1 + Foliar spray of selenium @ 100 ppm
T_4: Foliar spray of selenium @ 50 ppm
T_5: Foliar spray of selenium @ 100 ppm
T_6: Control
Selenium as sodium selenate was sprayed thrice at 45, 60 and 75 DAS.

Observations on morphological and biochemical characters were recorded at vegetative, flowering and maturity stages, and yield and yield attributes at harvest. The height of the plant was measured from the ground level to the tip of the highest leaf and expressed as cm. The maximum root length was measured from stem base and expressed as cm. Leaf area was determined by using Leaf Area Meter (LICOR model LI 3100) and expressed as cm^2. For total dry matter production, the plant samples were dried in a hot air oven at 70°C, weighed and expressed as g $plant^{-1}$.

Chlorophyll was extracted in 80 per cent acetone and the conte was estimated by the method of Arnon[8]. The total protein content wasquantified as per Lowry et al.[9] The activities of nitrate reductase and peroxidase were measured according to Nicholas et al.[10] and Putter[11] respectively. The catalase activity was determined by measuring the rate of reduction of hydrogen peroxide as per the method of Chance and Machly[12]. The total

free amino acid was quantified by using ninhydrin as suggested by Sadasivam and Manickam[13]. The data were analyzed statistically according to Gomez and Gomez[14] for its significance at 5% level.

Results and Discussion

A significant improvement in plant height, leaf number and leaf area was observed due to seed treatment of selenium 5 ppm combined with foliar spray of selenium 100 ppm given at 45, 60 and 75 DAS (S_3T_3). This treatment resulted in the production of 69 per cent more leaves with 60 per cent increase in leaf area over control. The total dry matter production of soybean varied from 16.53 to 18.73 g plant^{-1} irrespective of the treatments. The highest dry matter production of 18.73 g was recorded by S_3T_3 with a 13 per cent increase over control. Stomatal CO_2 flux decreased stomatal resistance, and thus raised net photosynthetic rate which directly influenced the total dry matter production of selenium treated early rice cultivars as observed by Kegin et al.[15] and Feng et al.[16].

Soybean had maximum total chlorophyll content during vegetative stage and the content started declining at the time of flowering, indicating the ageing induced differential rate of degradation of leaf pigments. According to Panigrahi and Biswal[17], the content of total chlorophyll declined after the leaf reached full expansion. The decline in chlorophyll content may be partially due to lipid peroxidation of chloroplast membranes[18] or due to the formation of hydroperoxides of fatty acids[19]. That selenium sprayed leaves (S_3T_3) could maintain the maximum total chlorophyll content even upto maturity (Table 2) indicated the protective role of selenium thereby delaying pigment degradation. Selenium is also involved in increasing chlorophyll content by altering its biosynthetic pathway[16].

The data on leaf soluble protein content and nitrate reductase activity revealed an increasing trend from vegetative to flowering stages and declined at maturity. A decline in total protein content in senescent leaves of soybean was also observed by Thimann and Martin[20], which was attributed to the rise in activities of specific degradative enzymes during leaf senescence[21] or to disruption of cell organelles[22]. The study indicated that selenium-treated plants (S_3T_3) could maintain higher soluble protein content than that of control, thereby sustaining high metabolic rate even at the time of maturity. High rate of nitrate reductase activity (Table 3) and enhanced total free amino acid content are considered to be the contributing factors for high soluble protein content as

Table 1. Effect of foliar spray of selenium on morphological attributes.

Treatment	Morphological attributes				
	Plant height (cm)	Root length (cm)	No. of leaves	Leaf area (cm^2 plant^{-1})	TDMP (g plant^{-1})
T_1	48.04	22.27	41.86	680.33	16.74
S_1T_2	50.76	23.81	50.24	780.94	17.16
S_1T_3	52.33	24.33	50.24	835.49	17.47
S_1T_4	50.55	22.90	48.14	805.43	17.16
S_1T_5	52.43	23.80	51.28	837.52	17.37
T_6	46.15	21.50	40.82	628.00	16.53
S_2T_2	57.04	24.50	53.38	904.42	17.27
S_2T_3	58.82	25.18	60.70	931.82	17.79
S_2T_4	57.46	24.25	55.47	920.56	17.16
S_2T_5	58.61	25.18	59.66	937.33	17.47
S_3T_2	59.76	24.25	62.80	963.22	18.21
S_3T_3	61.43	25.08	69.08	1005.07	18.73
S_3T_4	57.77	24.05	64.89	953.06	17.79
S_3T_5	58.73	25.36	68.03	973.52	18.42
CD (P=0.05)					
S	0.613	0.264	0.625	9.537	0.203
T	0.867	0.374	0.884	13.488	0.287
SxT	1.502	0.648	1.531	23.363	0.498

S_1 – 45 DAS; S_2 – 60 DAS; S_3 - 75 DAS

Table 2. Effect of foliar spray of selenium on total chlorophyll and soluble protein contents.

Treatment	Total chlorophyll (mg g^{-1})			Soluble protein (mg g^{-1})		
	Vegetative	Flowering	Maturity	Vegetative	Flowering	Maturity
T_1	1.48	1.20	1.00	11.61	13.60	11.51
S_1T_2	1.86	1.74	1.33	12.56	14.97	12.35
S_1T_3	1.94	1.76	1.43	12.87	16.45	12.45
S_1T_4	1.85	1.74	1.36	12.50	14.86	12.14
S_1T_5	1.92	1.75	1.40	12.82	16.43	12.24
T_6	1.36	1.04	1.00	11.09	13.34	10.46
S_2T_2	1.87	1.75	1.38	12.56	16.01	12.14
S_2T_3	1.97	1.80	1.45	12.97	17.69	12.66
S_2T_4	1.86	1.72	1.37	12.56	15.80	11.93
S_2T_5	1.94	1.77	1.41	12.76	17.58	12.56
S_3T_2	1.86	1.76	1.45	12.66	16.11	12.76
S_3T_3	1.95	1.80	1.50	12.87	18.00	13.08
S_3T_4	1.85	1.74	1.43	12.45	15.70	12.56
S_3T_5	1.93	1.79	1.46	12.76	17.58	13.18
CD (P=0.05)						
S	0.019	0.017	0.014	0.141	0.178	0.137
T	0.028	0.025	0.020	0.199	0.252	0.194
SxT	0.048	0.044	0.036	0.346	0.436	0.337

S_1 – 45 DAS; S_2 – 60 DAS; S_3 - 75 DAS

Table 3. Effect of foliar spray of selenium on nitrate reductase activity and total free amino acid in soybean.

Treatment	Nitrate reductase activity ($\mu g\ NO_2\ g^{-1}\ hr^{-1}$)			Total free amino acid ($\mu g\ g^{-1}$)		
	Vegetative	Flowering	Maturity	Vegetative	Flowering	Maturity
T_1	6.15	11.30	8.26	20.32	25.39	21.37
S_1T_2	6.59	11.72	8.47	23.92	26.73	24.71
S_1T_3	7.22	13.39	8.68	29.05	32.99	30.88
S_1T_4	6.28	11.51	8.37	22.23	28.91	23.50
S_1T_5	6.90	12.50	8.68	26.02	31.92	27.73
T_6	5.23	10.57	8.16	19.90	24.24	21.05
S_2T_2	7.01	12.24	8.58	23.95	34.70	25.28
S_2T_3	7.43	14.13	9.31	29.22	38.43	31.00
S_2T_4	6.75	11.88	8.37	23.13	34.54	26.08
S_2T_5	7.32	13.10	9.10	26.16	36.80	27.02
S_3T_2	7.01	12.14	9.21	23.94	35.31	27.55
S_3T_3	7.22	13.60	9.52	29.12	38.53	32.69
S_3T_4	6.90	11.93	8.68	22.84	35.29	26.82
S_3T_5	7.11	12.66	9.31	26.09	37.54	31.40
CD (P=0.05)						
S	0.141	0.178	0.137	0.277	0.368	0.302
T	0.199	0.252	0.194	0.392	0.521	0.427
SxT	0.346	0.436	0.337	0.680	0.903	0.741

S_1 – 45 DAS; S_2 – 60 DAS; S_3 - 75 DAS

Table 4. Effect of foliar spray of selenium on catalase and peroxidase activity in soybean.

Treatment	Catalase (enzyme unit)			Peroxidase (enzyme unit)		
	Vegetative	Flowering	Maturity	Vegetative	Flowering	Maturity
T_1	4.32	4.09	2.97	6.50	5.23	3.91
S_1T_2	4.56	4.16	3.15	6.65	5.35	4.04
S_1T_3	5.08	4.31	3.36	6.86	5.48	4.35
S_1T_4	4.31	4.14	3.02	6.59	5.33	4.18
S_1T_5	4.95	4.18	3.30	6.70	5.44	4.29
T_6	4.21	3.90	2.80	6.28	5.10	3.72
S_2T_2	4.58	4.31	3.39	6.64	5.47	4.16
S_2T_3	5.08	4.49	3.56	6.93	5.71	4.38
S_2T_4	4.32	4.27	3.15	6.59	5.44	4.35
S_2T_5	4.95	4.42	3.51	6.69	5.52	4.37
S_3T_2	4.56	4.35	3.43	6.65	5.47	4.40
S_3T_3	5.06	4.50	3.62	6.89	5.71	4.59
S_3T_4	4.31	4.29	3.33	6.61	5.45	4.37
S_3T_5	4.97	4.45	3.55	6.70	5.54	4.56
CD (P=0.05)						
S	0.053	0.048	0.036	0.077	0.062	0.048
T	0.075	0.068	0.052	0.109	0.089	0.067
SxT	0.131	0.117	0.090	0.189	0.154	0.117

S_1 – 45 DAS; S_2 – 60 DAS; S_3 - 75 DAS

evidenced from the present study (Table 2). Selenium may increase nitrate reduction by inducing the enzyme synthesis[23] or replace sulphur enabling seleno amino acids to be incorporated with proteins[24]. Though this substitution appears to be small, it may have a significant effect on the properties of selenium-substituted proteins[25].

Catalase is one of the most important controlling factors with protective mechanism against toxic oxygen species, which also participates in several electron transfer reactions of normal cell metabolism[26]. Catalase showed a significant decline in its activity in control plants compared to selenium treated plants (Table 4). This decline could result in greater availability of free radicals, increase in lipid peroxidation and membrane deterioration[27]. Similarly, the activity of peroxidase also decreased significantly in control plants. As reported by Wei et al.[28] the rate of production of superoxide anion and hydrogen peroxide increase with age and the activity of free radical scavenging enzymes decreases during ageing. These events lead to an ageing-dependent increase of lipid peroxides, which react with lipids, proteins and nucleic acids, and overwhelm the oxidative enzymes leading to oxidative damage to vital biomolecules, changes in membrane permeability and cellular metabolic functions. As selenium is a component of ascorbate peroxidase, it is directly involved in the dismutation of free radicals which are produced during senescence[29].

The increased yield in selenium treated plants (Table 5) may be attributed to maintaining a good source-sink relationship and increased photosynthetic rate[30]. The yield increase to the extent of 11 per cent over control due to selenium treatment, S_3T_3, was significant over other treatments. The postponement of leaf senescence by selenium spray and with high rate of photosynthesis could be the possible reasons for the improved source strength. The better partitioning efficiency, as evidenced by high harvest index coupled with more number of pods per plant, seeds per pod and seed weight, have also contributed for yield improvement in selenium treated plants. Effective dismutation of reactive oxygen species leading to decreased senescence by selenium also paved way to increased yield[31]. These results were in conformity with the finding of Ashok Bhattacharyya et al.[32] in rice.

Conclusions

Application of selenium as seed treatment (5 ppm) and foliar spray (100 ppm) during 45, 60 and 75 DAS significantly improved the source activity coupled with increased antioxidant enzymes (catalase and peroxidase) and may have contributed to the delayed leaf senescence and improved yield in selenium-treated plants.

Table 5. Effect of foliar spray of selenium on yield and yield components in soybean.

Treatment	Clusters per plant	Pods per plant	100 grain weight (g)	Seeds per pod	Grain yield (g plant^{-1})	TDMP (g plant^{-1})	HI (%)
T_1	5.75	29.30	14.00	2.62	3.87	14.13	41.34
S_1T_2	5.86	29.93	14.14	2.82	3.95	14.33	42.39
S_1T_3	6.38	30.87	14.25	2.86	4.08	14.75	42.59
S_1T_4	6.07	29.72	14.16	2.75	3.91	14.33	42.28
S_1T_5	6.28	30.66	14.26	2.85	3.98	14.65	42.28
T_6	5.75	29.20	14.01	2.60	3.86	13.92	41.23
S_2T_2	5.96	30.03	14.18	2.86	3.95	14.33	42.18
S_2T_3	6.48	30.87	14.29	2.90	4.18	15.17	42.70
S_2T_4	6.07	29.62	14.20	2.83	3.93	14.23	42.28
S_2T_5	6.28	30.56	14.24	2.88	4.08	14.96	42.49
S_3T_2	6.38	31.60	14.28	2.90	4.03	14.54	43.33
S_3T_3	6.48	34.33	14.36	2.94	4.29	15.49	44.27
S_3T_4	6.28	32.02	14.26	2.86	3.97	14.23	42.70
S_3T_5	6.48	33.49	14.30	2.93	4.18	15.28	43.96
CD (P=0.05)							
S	0.071	0.358	0.168	0.033	0.047	0.171	0.499
T	0.100	0.506	0.238	0.047	0.066	0.242	0.706
SxT	0.174	0.877	0.412	0.082	0.115	0.419	1.223

S_1 – 45 DAS; S_2 – 60 DAS; S_3 - 75 DAS

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