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Effect of chitosan by seed priming and foliar application on growth and yield of Wal (*Lablab purpureus* L. Sweet) under water stress

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Abstract
The present investigation entitled "Effect of Chitosan by seed priming and foliar application on growth and yield of Wal (*Lablab purpureus* L. Sweet) under water stress" was carried out at Research and Education farm, Department of Agricultural Botany, College of Agriculture, during the *rabi* 2021-22. Observations were recorded in context with phenological, morphological, physiological, biochemical and yield contributing parameters. There was a broad range of variability for the different morpho-physiological and biochemical characteristics among all treatments of chitosan under moisture stress. Among all interaction treatments P_2F_2 was found to be significantly superior reporting better performance in context with plant height, leaf area index, total chlorophyll content, number of pods and seed yield when compared with rest of the treatments. Thus, the current study revealed that the use of foliar spray of chitosan (200 ppm) along with seed priming with chitosan (2%) enhanced the yield of lablab bean grown under water stress condition.

Keywords: Chitosan, priming, foliar, *Lablab purpureus* L.

Introduction
Among pulses Lablab bean (*Lablab purpureus* L.), also known as the Hyacinth bean, Bonavist (sem), Chicharas, Chink, Pavta, Kadva, Auri, Field bean, Sem, Indian bean, and Country bean, is an important pulse crop that is cultivated throughout the country. Lablab bean is a long-cultivated legume crop that is a member of the Fabaceae family. Lablab bean is adaptable to a wide range of climatic conditions (Kimani *et al*. 2012) [9], including arid, semi-arid, humid regions and subtropical climates with temperatures ranging from 22 °C to 35 °C and pH levels ranging from 4.4 to 7.8. It can flourish in locations with an average annual rainfall of 25-120 mm and can endure drought or shaded conditions (Cook *et al*. 2005) [5].
In the Konkan region of Maharashtra, Lablab bean is widely grown during *rabi* season especially on residual moisture mainly for grain purposes. Farmers cultivate the highest-priced pulse crop immediately after harvesting paddy. There are two varieties of Lablab bean released by DBSKKV, Dapoli *viz*.: Konkan Lablab bean-1 and Konkan Lablab bean-2. Farmers adopt two methods of sowing in Lablab bean for growing on residual moisture. Sometimes Lablab bean seed are dibbled before the harvest of paddy in standing crops. Some times without land preparation (after harvest of paddy) the lablab bean seed are sown depending on residual moisture present in the field. Severe water stress inhibits plant growth by altering a wide range of physiological and biochemical activities, including photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth stimulants (Abdalla 2011, Azadeh *et al*. 2014) [1, 3].
Chitosan (CHT) is a poly (1,4)-2-amino-2-deoxy-β-D glucose that is a deacetylation derivative of chitin and is present in the exoskeletons of arthropods such as insects, crustaceans such as lobsters, shrimp and crabs, molluscan radulae, fish and squid, as well as the scales of lissamphibians (Kurtia, K. 2006) [10]. The agricultural and horticultural uses for chitosan, primarily for plant defence and yield increase, are based on how this glucosamine polymer influences the biochemistry and molecular biology of the plant cell. Foliar application of chitosan at a concentration of 250 mg/L in cowpea improved growth and yield parameters in both drought stress and non-stress conditions (Farouk and Amany 2012) [6]. When compared to chitosan-untreated plants, foliar-applied chitosan, specifically 200 mg/l, boosted plant growth, yield, and quality, as well as physiological elements in plant shoot under stressed or non-stressed conditions (Abu-Muriefah 2013) [2].

Chitosan seed priming enhanced seed germination and seedling growth in wheat under osmotic stress, resulting in improved tolerance (Hameed *et al*. 2014) [8].

Although there has been some research about effects of chitosan application on different plants under drought stress, but rare information is available about how chitosan seed priming and foliar application impact on growth trend and yield of pulses cultivated under residual moisture. To the best of our knowledge, there is no previous study on effect of chitosan application on various physiological parameters and yield contributing characters of Lablab bean. Considering the above facts and importance, the need of the present investigation entitled "Effect of Chitosan by seed priming and foliar application on growth and yield of Wal (*Lablab purpureus* L. Sweet) under water stress" was undertaken with the following objective 1. To study the impact of seed priming and foliar application of chitosan on growth and yield of Wal.

2. Material and Methods

The present investigation was carried out at Research and Education farm, Department of Agricultural Botany, College of Agriculture, Dapoli, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli during the *rabi* 2021-22. The experiment was laid out in Factorial Randomized Block Design (FRBD) with 3 replications provided with 18 (9 Irrigated + 9 Non irrigated) treatments and konkan wal 2 variety was used.

Experimental details

Factor 1: Seed primed with chitosan at the time of sowing		Factor 2: Foliar Application of chitosan	
P_0	0.0%	F_0	00 ppm
P_1	1.0%	F_1	100 ppm
P_2	2.0%	F_2	200 ppm

Seed priming: Seed priming was done one day prior to sowing. Seeds were submerged in the chitosan solution (1% and 2%) for 8 hours, after that these seeds were air dried in the shed for 2 hours before sowing.

Foliar application: Foliar spray of chitosan was done at 30, 60 and 90 DAS with the help of hand sprayer.

Treatment details: Number of treatments: 18 (9 Irrigated + 9 Non irrigated).

P_0F_0	**Control**
P_0F_1	No seed priming, 100 ppm foliar spray of chitosan.
P_0F_2	No seed priming, 200 ppm foliar spray of chitosan.
P_1F_0	Seed primed with 1% chitosan, No foliar application.
P_1F_1	Seed primed with 1% chitosan, 100 ppm foliar spray of chitosan.
P_1F_2	Seed primed with 1% chitosan, 200 ppm foliar spray of chitosan.
P_2F_0	Seed primed with 2% chitosan, No foliar application.
P_2F_1	Seed primed with 2% chitosan, 100 ppm foliar spray of chitosan.
P_2F_2	Seed primed with 2% chitosan, 200 ppm foliar spray of chitosan.

Observations recorded

Following observations were taken during the course of experimentation.

Plant height (cm)

The plant height was measured from ground level to the highest growing point of main stem with meter scale and expressed in centimetre (cm). The height of the plant was recorded at 30, 60, 90 days after sowing and at harvest.

Total chlorophyll content of leaves (mg/g)

The chlorophyll content (chlorophyll a, b, total chlorophyll and chlorophyll stability index) of the leaves was analyzed by using the method of Barnes *et al.* (1992) [4]. Fully open mature leaf was taken as the experimental sample for chlorophyll estimation. For this, 0.5 g of clean leaf sample was immersed in 10 ml of dimethyl sulphoxide (DMSO). The sample was incubated at 80 °C for four hours in hot air oven. After incubation, it was taken out and 1 ml of the solution was diluted to 5 ml DMSO and the absorbance was recorded in spectrophotometer (HALO DB-20S UV-VIS double beam) at 645, 652 and 663 nm wavelengths, using pure DMSO as blank. The results were expressed as mg/g fresh weight.

Leaf area index (LAI)

Leaf area index was determined by taking a statistically significant sample of foliage from a plant canopy, measuring the leaf area per sample plot and dividing it by the plot land surface area. Leaf area index (LAI) was calculated as per the formula given by Watson (1958) [13].

Number of pods per plant

Number of pods per plant was counted from pods harvested from five randomly selected plants from each treatment plot at the time of harvest. Later average was worked out to record the observations.

Seed yield (g/plant)

All the pods of the individual selected plants from each treatment were harvested, threshed and dried. These seeds were separately weighed on weighing balance and then seed yield per plant was recorded in grams.

Statistical analysis

The data collected were subjected to the statistical analysis for Factorial Randomized block design. The statistical analysis of the data was done by the standard method known as "Analysis of Variance" described by Panse and Sukhatme (1967) [11]. The standard error (SE) of mean and critical difference (CD) at 5 per cent level was worked out, wherever the results were significant.

Results and Discussion

The results obtained in the present investigation are presented and discussed in the tables given below.

Plant height (cm): The statistical analysis of the data on plant height revealed that significant variation found among treatments and the data was presented in the Table 1.

Table 1: Influence of chitosan by seed priming and foliar application on plant height (cm) of Lablab bean grown under residual moisture

30 DAS					**60 DAS**				
P/F	F_0	F_1	F_2	Mean	P/F	F_0	F_1	F_2	Mean
P_0	10.47	10.61	10.72	10.60	P_0	36.21	37.20	38.46	37.29
P_1	10.93	11.05	11.21	11.06	P_1	36.40	37.87	39.99	38.08
P_2	10.47	10.65	10.74	10.62	P_2	36.55	37.33	38.57	37.48
Mean	10.62	10.77	10.89	10.76	Mean	36.39	37.46	39.01	37.62
	P	F	PF			P	F	PF	
SE±	0.05	0.05	0.08		SE±	0.09	0.09	0.16	
CD @ 5%	0.136	0.136	0.235		CD @ 5%	0.275	0.275	0.477	
90 DAS					**At harvest**				
P/F	F_0	F_1	F_2	Mean	P/F	F_0	F_1	F_2	Mean
P_0	91.01	91.99	94.98	92.66	P_0	93.68	95.43	97.29	95.47
P_1	88.21	91.39	92.45	90.68	P_1	91.30	94.43	95.79	93.84
P_2	86.61	88.93	90.73	88.76	P_2	90.00	92.20	93.87	92.02
Mean	88.61	90.77	92.72	90.70	Mean	91.66	94.02	95.65	93.78
	P	F	PF			P	F	PF	
SE±	0.16	0.16	0.28		SE±	0.18	0.18	0.30	
CD @ 5%	0.487	0.487	0.844		CD @ 5%	0.526	0.526	0.911	

One of the most important morphological characteristics is plant height, which is measured as stem length. It is an essential component of plant ecological strategy. It has a strong relationship with life duration and time to maturity. The stem bears branches and leaves that are exposed to sunlight. It also bears pods, forming an essential link between source and sink. In the present study, plant height increased continuously up to the harvest in all treatments under moisture stress condition as well as irrigated condition. Treatments differed significantly at all the stages. The maximum plant height may be correlated to the maximum duration to complete life cycle. At harvest, under water stress condition minimum plant height was recorded at treatment P_2F_0 (90.00 cm) and maximum plant height was observed at treatment P_0F_2 (97.29 cm). These results are parallel with the research done by Farouk and Amany (2012) [6] in cowpea.

Total chlorophyll content (mg g^{-1})

It was observed that there was significant variation in total chlorophyll content between treatments under water stress condition and the data was presented in the Table 2.

Table 2: Influence of chitosan by seed priming and foliar application on total chlorophyll content (mg g^{-1}) of Lablab bean grown under residual moisture

30 DAS					**60 DAS**				
P/F	F_0	F_1	F_2	Mean	P/F	F_0	F_1	F_2	Mean
P_0	0.6263	0.6570	0.6710	0.6514	P_0	1.1380	1.2350	1.3410	1.2713
P_1	0.7186	0.7360	0.7470	0.7339	P_1	1.2230	1.3073	1.3450	1.2884
P_2	0.7630	0.7740	0.7820	0.7730	P_2	1.2580	1.3020	1.3790	1.2930
Mean	0.7027	0.7223	0.7333	0.7194	Mean	1.2030	1.2748	1.3550	1.2843
	P	F	PF			P	F	PF	
SE±	0.003	0.003	0.006		SE±	0.021	0.021	0.037	
CD @ 5%	0.010	0.010	0.018		CD @ 5%	0.064	0.064	0.111	
90 DAS					**At Harvest**				
P/F	F0	F1	F2	Mean	P/F	F0	F1	F2	Mean
P_0	1.0340	1.1380	1.2160	1.1627	P0	0.5870	0.6140	0.6390	0.6133
P_1	1.1063	1.2090	1.2270	1.2141	P1	0.6030	0.6230	0.6440	0.6233
P_2	1.1540	1.2040	1.2400	1.2050	P2	0.6170	0.6380	0.6590	0.6413
Mean	1.0981	1.1937	1.2300	1.2073	Mean	0.6090	0.6217	0.6473	0.6327
	P	F	PF			P	F	PF	
SE±	0.037	0.037	0.063		SE±	0.022	0.022	0.038	
CD @ 5%	0.110	0.110	0.190		CD @ 5%	0.066	0.066	0.114	

To determine the plant's photosynthetic efficiency, the amount of chlorophyll contained in the leaf tissue must be known. The current study found that chlorophyll concentration increased with crop age and decreased throughout the maturity period. A reason for the decrease in chlorophyll content triggered by a water deficit is that moisture stress, which produces reactive oxygen species (ROS) such as O_2 and H_2O_2, can lead to lipid peroxidation and thus chlorophyll destruction. Additionally, as chlorophyll content decreases due to the change in colour of the leaf from green to yellow, the reflectance of incident radiation increases. The drop in total chlorophyll concentration could be attributed to a fall in the plant's leaf water status.

It was observed that there was significant variation in total chlorophyll content between treatments and it was highest at the 60 days of crop growth and thereafter began to decline. Maximum total chlorophyll content was recorded in treatment P_2F_2 (1.379 mg g^{-1}) whereas minimum total chlorophyll content was recorded in treatment P_0F_0 (1.138 mg g^{-1}) at 60 DAS, under water stress condition. Results are in confirmation with the results of Farouk and Amany (2012) [6] in cowpea.

Leaf area index: The statistical analysis of the data on leaf area index revealed a significant variation among treatments and among interaction effects under water stress condition and the data was presented in the Table 3.

Table 3: Influence of chitosan by seed priming and foliar application on leaf area index of Lablab bean grown under residual moisture

30 DAS					60 DAS				
P/F	F_0	F_1	F_2	Mean	P/F	F_0	F_1	F_2	Mean
P_0	0.0997	0.0995	0.1004	0.0999	P_0	0.1570	0.1796	0.1906	0.1757
P_1	0.1020	0.1014	0.1012	0.1015	P_1	0.1747	0.2029	0.2013	0.1930
P_2	0.1022	0.1004	0.1008	0.1011	P_2	0.1705	0.2039	0.2076	0.1940
Mean	0.1013	0.1004	0.1008	0.1008	Mean	0.1674	0.1955	0.1998	0.1876
	P	F	PF			P	F	PF	
SE±	0.00002	0.00002	0.00004		SE±	0.002	0.002	0.004	
CD @ 5%	0.00006	0.00006	0.00011		CD @ 5%	0.007	0.007	0.012	
90 DAS					At Harvest				
P/F	F0	F1	F2	Mean	P/F	F0	F1	F2	Mean
P_0	0.3386	0.3785	0.4148	0.3773	P_0	0.2695	0.3270	0.3730	0.3231
P_1	0.3609	0.4297	0.4482	0.4129	P_1	0.2799	0.3850	0.4093	0.3581
P_2	0.3617	0.4759	0.4929	0.4435	P_2	0.2903	0.4339	0.4511	0.3918
Mean	0.3537	0.4280	0.4520	0.4112	Mean	0.2799	0.3820	0.4111	0.3577
	P	F	PF			P	F	PF	
SE±	0.003	0.003	0.005		SE±	0.003	0.003	0.005	
CD @ 5%	0.008	0.008	0.014		CD @ 5%	0.009	0.009	0.015	

The leaf area index (LAI) expresses the ratio of leaf surface (one side only) to the ground area occupied by the crop. The assimilatory surface area of a crop stand and its growth influence the quantity of solar energy intercepted by the canopy and represent the crop's productive capability. Differences in LAI owing to chitosan application are obvious from the start. A progressive pattern of LAI accumulation was seen with crop age advancement up to 90 DAS, after which it began to fall.

In present study it was noted that there was a significant difference among treatments for leaf area index. Leaf area index increased upto 90 days of crop growth and later declined. Under water stress condition leaf area index was maximum in treatment P_2F_2 (0.4929) while leaf area index was noted minimum in treatment P_0F_0 (0.3386) at 90 DAS. Reddy (2002) stated that the growth parameters such as LAI, AGR and RGR increase significantly with application of plant growth regulators in chickpea.

Number of pods per plant

Number of pods per plant recorded significant differences among treatments and interaction effects between seed priming and foliar application was found significant at harvest. The data regarding number of pods per plant is presented in table 4.

Table 4: Influence of chitosan by seed priming and foliar application on Number of pods per plant of Lablab bean grown under residual moisture

P/F	F_0	F_1	F_2	Mean
P_0	11.30	12.87	14.23	12.80
P_1	11.71	13.85	17.45	14.34
P_2	12.99	15.35	17.80	15.38
Mean	12.00	14.02	16.49	14.17
	P	F	PF	
SE±	0.24	0.24	0.41	
CD @ 5%	0.710	0.710	1.229	

The number of pods per plant is an important trait that has the most direct influence on pod yield per plant. In the current study, it was discovered that the quantity of pods per plant varied greatly between treatments. Increase in number of pods per plant may be due to plant height and number branches per plant. Number of pods per plant recorded significant difference among treatments at harvest. Maximum number of pods was recorded in treatment P_2F_2 (17.80) which was at with P_1F_2 (17.45) over rest of the treatments and minimum number of pods was found in treatment P_0F_0 (11.30), under water stress condition. Similar results were reported by Farouk and Qados (2013) [7] in cowpea.

Seed yield per plant (g)

Seed yield per plant found significant differences among treatments and interaction effects between seed priming and foliar application was found significant at harvest. The data regarding seed yield per plant is presented in table 5.

Table 5: Influence of chitosan by seed priming and foliar application on Seed yield per plant (g) of Lablab bean grown under residual moisture

P/F	F_0	F_1	F_2	Mean
P_0	7.89	9.30	10.13	9.11
P_1	8.57	10.34	11.31	10.07
P_2	8.83	10.47	11.60	10.30
Mean	8.43	10.04	11.01	9.83
	P	F	PF	
SE±	0.22	0.22	0.39	
CD @ 5%	0.670	0.670	1.161	

Seed is the economic part of the total dry matter. It is the net economic gain from the source and sink capacity, according to plant physiologists. In present investigation, under water stress condition maximum seed yield per plant was produced in treatment P_2F_2 (11.60 g) which was at par with P_1F_2 and P_2F_1 over other treatments, minimum seed yield per plant was found in treatment P_0F_0 (7.89 g). Similar findings were reported by Zeng *et al*. (2012) [14] in soyabean.

Conclusion

In the current study, there was a broad range of variability for the different morpho-physiological and biochemical characteristics among all treatments of chitosan under moisture stress conditions. Decrease in yield and yield components was recorded under water stress in treatment P_0F_0 (control). Among all treatments P_2F_2 (seed priming with chitosan 2% + foliar spray of chitosan @ 200 ppm) was found to be significantly superior followed by treatment F_2 (foliar spray of chitosan @ 200 ppm) reporting better performance in context with all the parameters studied. Thus, the current study revealed that the use of foliar spray of chitosan (200 ppm) along with seed priming with chitosan (2%) enhanced the yield of lablab bean grown under water stress condition.

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