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B Lohitha
Ph.D., Scholar, Department of Seed Science and Technology Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

A Dutta
Associate Professor, Department of Seed Science and Technology Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Corresponding Author:
B Lohitha
Ph.D., Scholar, Department of Seed Science and Technology Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Biopriming in chickpea (*Cicer arietinum* L.): An eco-friendly approach for quality seed production

B Lohitha and A Dutta

Abstract
An experiment was conducted during rabi season of 2019-20 and 2020-21 with two varieties of chickpea (*Cicer arietinum* L.) *viz.* JG-14 and Bidisha to study the response of genotypes towards seed biopriming. The design of experiment was split plot with two factor analysis comprising varieties as first factor and biopriming treatments [*viz.* T_1- Rhizobium, T_2-PSB, T_3-Azotobacter, T_4-Rhizobium+PSB, T_5-PSB+Azotobacter, T_6- Rhizobium+PSB+Azotobacter, T_7-Control (water soaking of seeds)] as second factor. Highest seed yield (228.37 g m^{-2}) was recorded in V_2T_4 (Bidisha with Rhizobium + PSB) followed by V_2T_6 (Bidisha with Rhizobium + PSB + Azotobacter). The two varieties and different treatments had significant effect on various characters. Among the treatments, T_6 (Rhizobium + PSB + Azotobacter) recorded highest seed yield followed by T_4 (Rhizobium + PSB) with 206.02 and 205.57 g.sq.m^{-1} respectively. Between the varieties, Bidisha produced higher seed yield as compared to JG-14 (171.03 and 153.22 g sq.m^{-1} respectively). Pattern of pod development revealed that fresh pod weight and fresh seed weight increased up to 104 days after sowing irrespective of treatments and varieties, there after declined at 111 days after sowing. Therefore, it can be concluded that for getting higher seed yield, chickpea seeds should be primed with Rhizobium + PSB or Rhizobium + PSB + Azotobacter before sowing and harvesting should be done at 104 days after sowing for both Bidisha and JG-14 varieties.

Keywords: Bio-priming, chickpea, seed development, yield

1. Introduction
Chickpea (*Cicer arietinum* L.) belongs to the family Fabaceae, subfamily Faboideae. It is commonly known by other names like gram or Bengal gram, Egyptian pea, garbanzo bean or garbanzo. Chickpea have 34 perennial and 8 annual species [15]. Generally, there are two types of chickpeas: 'Kabuli' (large ram-shaped, cream coloured, no anthocyanin pigment present) and 'Desi' (small angular and dark coloured, with anthocyanin pigment on one or other parts) [16]. It is believed that the chickpea originated in south east Turkey and spread south and west afterwards. For chickpea, Central Asia, Mediterranean Region, India (with Ethiopia as a secondary center of diversity) and the North East were the centers of diversity. In India, Madhya Pradesh ranks first in area with 3.43 million hectares, followed by Rajasthan with 1.60 million hectares (DAC&FW, GOI, 2019). India is the largest importer of chickpea in world and also the world's largest producer accounting to 75% (9.44 million tonnes) of total chickpea production in 2017 [9], where central zone has largest chickpea area occupying more than 55% total production area and in 2018 chickpea world production has increased by 16% rising to 17.2 million tonnes, making India's production accounting to 66% (11.4 million tons) to global chickpea production, while Australia, United States and Turkey were secondary producers (FAOSTAT of United Nations, 2020). Chickpea is a good source of energy, minerals, vitamins, fiber, and also contains potentially health- beneficial phytochemicals [18]. Moreover, the grain legume crop has health benefits, which include reducing diabetic, cancer and cardiovascular risks. Chickpea, similar to other legumes, fixes atmospheric nitrogen up to 140 kg/ha through biological nitrogen fixation and improves soil fertility with help of symbiotic bacteria in nodules. Apart from being a food crop for human consumption, gram is an excellent concentrate for horses and an energy source in animal feeding. Chickpea crop faces moisture stress mostly at reproductive stage as the moisture preserved, depletes progressively with the growth of crop and is mostly susceptible to fusarium wilt. Along with these insect pests, drought and extreme temperatures also cause yield loss of the crop. Therefore, to ensure proper and uniform germination, reduce dormancy and to maintain vigour and viability of seeds even in unfavourable conditions priming offers one of the best solutions than using pesticides which offer temporary solution. Moreover, it is environmentally friendly and cost effective process.

Seed priming is a controlled process of hydration where seeds are exposed to low water potential that permits some physiological and pre germinative changes but restricts germination [8]. Primed seeds show faster germination rate upon rehydration, along with uniform emergence. Among all the different priming methods, the recent modern technique is bio priming which is a combination of beneficial microorganisms application on surface of seed and seed hydration [14] that integrates both biological and physiological methods to improve growth of plant, along with controlling diseases. Beneficial microorganisms like Pseudomonas and Rhizobium improve plant growth by colonizing on root surface, aiding in enhanced growth of plant.

In contrast to Rhizobium species, Azotobacter species are free living bacteria which fix atmospheric nitrogen without any symbiotic association with plants, although some species have association. The phosphate solubilizing bacteria (PSB) produces siderophores, several acids (organic), carboxyl and hydroxyl groups by solubilizing Ca, Fe, and Al inorganic phosphates present in the soil and chelating them to the available calcium and to the bound phosphates [12]. In the present study, the effect of different bio priming methodology treatments on chickpea genotypes and their effects on seedling parameters and pod development were studied in detail to find out the best priming treatment.

2. Material and Methods

The present experiment was conducted during the *rabi* season, 2019-20 and 2020-21 at Jaguli Instructional farm, Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur, Nadia (23.66^0N latitude and 88.24^0E longitude in the Gangetic Alluvial Zone) of West Bengal, India. The bioagents were collected from Nodule Research Laboratory, BCKV, Mohanpur, West Bengal and seeds collected from NSP (National Seed Project), BCKV, Mohanpur, West Bengal. During the experiment different plant growth parameters were recorded at frequent intervals, from sowing to harvest and also harvested products have been studied. The soil in the experimental site has sandy loam texture with pH 6.2, organic carbon 0.54%, along with available Nitrogen 125.44 kg ha^{-1}, Phosphorus (P_2O_5) 554.67 kg ha^{-1} and Potassium (K_2O) 128.48 kg ha^{-1}. The experiment was laid in split plot with two factor analysis comprising varieties [*viz.* JG-14 and Bidisha] as first factor and biopriming treatments [*viz.* T_1- Rhizobium @50g per 100 ml of water, T_2-PSB @50g per 100 ml of water, T_3-Azotobacter @50g per 100 ml of water, T_4-Rhizobium+PSB @25g each per 100 ml of water, T_5-PSB+Azotobacter@25g each per 100 ml of water, T_6- Rhizobium+PSB+Azotobacter @16 g each per 100ml of water, T_7-Control (water soaking of seeds)] as second factor.

The chickpea seeds were soaked for six hours replication wise as well as treatment wise and shade dried for one hour. Seeds were sown in the field on November 20th, 2019 and November 10th, 2020 with spacing 30 cm row to row and 10 cm plant to plant. Plot size was 3m x 1.8 m. A basal dose of fertilizer N, P_2O_5 and K_2O @ 20:40:20 kg ha^{-1} in the form of Urea, SSP and MOP was applied prior to sowing of seeds. Plant protection measures were followed as per necessity. Five random plants from each treatment with three replications each were tagged and observed for morphological and other yield attributing parameters like plant height, days to flowering and maturity, number of primary and secondary branches, number of pods per plant, seeds per pod,100 seed weight and seed yield. The seed quality characters like fresh and dry pod weight, seed weight, pod wall weight, nodule number per plant were studied in the laboratory of Department of Seed Science and Technology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya. Pooled data over two years, comprising field and laboratory parameters were analyzed statistically as per Split Plot Design (two factor analysis) by using OPSTAT computer programming [13] developed by CCS HAU, Hisar.

3. Results and Discussion

3.1 Seed yield and its attributes

Varieties had significant effect for the character plant height (Table 1). The variety JG-14 had higher plant height as compared to variety Bidisha. Among all the treatments, T_6 (Rhizobium+PSB+Azotobacter) had maximum effect (21.23cm) for plant height at 30 days after sowing. T_4 recorded maximum at both 60 days after sowing and 90 days after sowing (36.73 and 38.67 cm respectively). Similar study was conducted by Warwate *et. al.* [17], where a combination of bioagents likes PSB, Azotobacter and other bioagents rather alone treatment influenced the shoot growth parameter. The results studied here are close to findings by Negi *et. al.* [10], where biopriming of French bean seeds using combination of bioagents provided higher emergence and plant growth in field, due to the interaction of different microorganisms in the root soil interface that encourages more nitrogen fixation in the plant, which is subsequently reflected in a higher growth rate. For both primary and secondary branches per plant, treatments had significant influence while varieties showed non-significant effect which is quite opposite to plant height character. The interactions between treatment and varieties, showed non-significant influence for the character plant height. Although, the number of primary branches were more in JG-14 (3.51) than Bidisha (3.28), the number secondary of branches were more in Bidisha (9.15), as compared to JG-14(8.97). This may be due to long primary branch in Bidisha, which makes space for more number of secondary branches. Among the treatments, T_4 (Rhizobium+PSB) had maximum effect on both primary and secondary branches per plant (4.0 and 10.43 respectively). Chaurasia and Bineeta [2], reported similar findings where number of primary branches per plant were increased due to biopriming in seeds. The above results were in conformity with findings of Glick *et. al.* [5] where seed treatments with PGPR enhanced growth of the several crops.

Flowers play an important role in all seed crops and yield is dependent largely upon the basic reproductive units. Higher yield varieties tend to show high ratio of productive flowers to total open flowers, thus resulting in great pod fertilization efficiency that in turn leads to greater yield. Also, the duration of flowering is also linked with performance of crop, lesser the duration, earlier the pod formation.

Treatments had shown significant influence on duration of flowering as so varieties, except in 100 percent flowering where varieties had non-significant effect, while their interaction had non-significant influence. Among treatments, T_6(Rhizobium+PSB+Azotobacter) recorded earliest days to first flowering (73.0 days) and T_3(Azotobacter) recorded maximum days to flowering. Among varieties JG-14 was earliest to first flowering (72.81 days) as compared to Bidisha (75.85 days). Among the treatments, T_4 (Rhizobium+PSB) recorded earliest days to 50 percent flowering (76.75 days). Among varieties JG-14 was earliest to 50 percent flowering (77.61days) as compared to Bidisha (79.93 days). Among the treatments, T_4 (Rhizobium+PSB) recorded earliest days to 100

percent flowering (80.93days). Among varieties Bidisha was earliest to 100 percent flowering (83.31 days) as compared to JG-14(84.04 days).

Earliest maturity is a desirable character for chickpea varieties. Among treatments, T_4 (Rhizobium+PSB) recorded minimum days to maturity (115.50days). Among varieties JG-14 (115.5) was earlier as compared to Bidisha(122.93days). Number of pods per plant is an important character that is directly related to seed yield. The treatments and varieties had significant effect except in 100 percent flowering, where varieties showed non significant effect, while their interaction showed non-significant effect. But, in case of number of seeds per pod, treatments had non-significant effect while varieties and interaction between treatment and varieties showed significant effect. The number of pods per plant were higher in Bidisha (58.65) when compared to JG-14(55.24).

Table 1: Varietal response towards biopriming on growth and yield attributing characters in chickpea

Treatments	Plant Height			Number of branches/Plant		Days to Flowering				Number of pods per plant	Number of seeds per pod	100 seed weight (g)	Seed Yield (gm^{-2})
	At 30 DAS	At 60 DAS	At 90 DAS	P.B	S.B	First	50%	100%	Maturity				
T_1	19.63	35.08	37.25	3.83	8.07	74.08	77.92	83.17	118.67	63.36	1.38	18.75	179.15
T_2	19.80	34.20	37.67	2.90	8.93	74.63	78.13	83.00	117.42	57.45	1.36	18.98	157.27
T_3	20.67	35.03	36.17	3.33	8.77	75.28	79.42	85.17	119.67	44.22	1.35	21.42	127.03
T_4	20.77	36.73	38.67	4.00	10.43	73.45	76.75	80.93	115.50	72.13	1.43	19.09	205.57
T_5	20.77	33.20	35.17	3.40	8.50	74.90	80.33	84.50	120.25	49.01	1.32	17.12	148.88
T_6	21.23	32.30	35.50	3.50	10.23	73.00	78.33	83.33	120.25	72.71	1.41	20.02	206.02
T_7	20.77	33.67	35.67	2.80	8.47	74.93	80.50	85.67	122.75	39.72	1.27	17.27	111.02
S.Em(±)	0.40	1.12	1.12	0.21	0.31	0.38	0.54	0.89	0.84	2.37	0.03	0.59	3.57
CD(P=0.05)	NS	N S	N S	0.65	0.98	1.18	1.69	2.75	2.62	7.39	N S	1.83	11.13
Varieties													
JG-14(V1)	29.06	37.78	39.83	3.51	8.96	72.81	77.61	84.05	115.50	55.24	1.13	23.90	153.22
Bidisha(V2)	11.98	30.86	33.33	3.28	9.15	75.85	79.93	83.32	122.93	58.65	1.59	14.01	171.03
S.Em(±)	0.14	0.62	0.62	0.11	0.19	0.55	0.37	0.40	1.00	0.78	0.02	0.27	3.84
CD(P=0.05)	0.42	1.89	1.89	NS	N S	1.70	1.13	NS	3.05	2.37	0.07	0.81	11.74
Interaction(V) × (T)													
S.Em(±)	0.47	0.47	1.61	0.30	0.47	1.10	0.88	1.16	2.05	2.78	0.05	0.77	8.01
CD(P=0.05)	NS	N S	N S	NS	N S	NS	N S	NS	NS	NS	0.16	2.38	NS

T1 – Rhizobium.T2 – PSB, T3 – Azotobacter, T4 - Rhizobium + PSB, T5 - PSB+Azotobacter, T6 - Rhizobium+PSB+Azotobacter and T7 -Control, DAS-days after sowing, P.B-primary branches, S.B-secondary branches, V-Variety, T-Treatment.

Since the number of secondary branches per plant in Bidisha were more as compared to JG-14, that in turn resulted in a greater number of pods per plant in Bidisha and T_6(Rhizobium+PSB+Azotobacter) had shown maximum influence (72.71). Similar work was reported by Sharma *et al.* [11], who concluded yield enhancement due to early floral initiation, more flowers and pods per plant in salicylic acid primed seed. Highest number of seed per pod (1.43 and 1.41 respectively) was observed in T_4 (Rhizobium+PSB) followed by T_6 (Rhizobium+PSB+Azotobacter). Between varieties Bidisha had higher number of seeds per pod (1.59) as compared to JG-14(1.13).

The reason for higher number of seeds per pod in Bidisha was due to its small size, a greater number of seeds fitted in one pod in comparison to JG-14 which had large sized seed. Maximum value for 100 seed weight (21.42 g) was recorded in T_3 (Azotobacter) followed by T_6 (Rhizobium+PSB+Azotobacter), whereas T_6 recorded higher seed yield (206.02 g/sq.m) followed by T_4 (205.57 g/sq.m). Higher seed yield (g/sq.m) was recorded in Bidisha as compared to JG-14 (171.03 and 153.22 g/sq.m respectively). This is due to greater number of secondary branches which resulted in a greater number of pods and seeds in Bidisha. Field study conducted by Gan *et al.* [4] revealed that the yield and number of fertile pods increased by 15% and 53% more in small sized chickpea seeds when compared to large sized chickpea seeds. Owing to its small seed size, Bidisha, showed higher seed yield when compared to JG-14. Harris *et al.*, [7] further reported that the yield of primed seeds were higher when compared to non-primed seeds.

3.2 Pod and Seed developmental characters

Tracing the sequence of pod and seed development and fixing the time for maturity have more practical utility in getting higher quantity of quality seeds. For identification of seed maturation period, the study of pod and seed development pattern is essential to obtain maximum yield with best quality seed. The parameters taken in variety JG-14 was recorded from 90 days after sowing up to 111 days after sowing at 7 days interval and for variety Bidisha from 97 days after sowing to 111 days after sowing owing to its late pod production.

Table 2: Pattern of pod development in Chickpea as influenced by biopriming

Treatments	Fresh pod weight (g per pod)		Fresh seed weight (g per pod)		Fresh pod wall weight (g per pod)		Dry pod weight (mg per pod)		Dry seed weight (mg per pod)		Number of nodules per plant		
	JG-14	Bidisha	JG-14	Bidisha	JG-14	Bidisha	JG-14	Bidisha	JG-14	Bidisha	Treatments	JG-14	Bidisha
T_1	3.04	1.34	0.97	1.34	2.08	0.93	477.3	202.0	152.0	61.5	T_1	12.08	15.08
T_2	3.09	1.16	0.57	1.16	2.52	0.83	484.6	173.0	89.3	37.6	T_2	11.75	14.81
T_3	3.21	1.34	0.73	1.34	2.49	0.93	503.4	203.3	114.5	50.8	T_3	9.58	12.03
T_4	3.75	1.82	0.90	1.82	2.85	1.20	587.7	417.3	140.8	93.6	T_4	15.67	19.66
T_5	3.24	1.25	0.71	1.25	2.53	0.82	490.9	189.3	111.8	65.6	T_5	10.92	13.71
T_6	3.35	1.68	1.20	1.68	3.06	1.32	482.8	199.8	128.0	57.7	T_6	15.83	19.93
T_7	2.88	1.04	0.55	1.04	3.27	0.82	550.7	300.6	130.1	84.6	T_7	10.67	13.43
S.Em(±)	0.04	0.06	0.03	0.06	0.06	0.05	7.40	22.7	4.5	8.6	SEm(±)	0.40	0.40
CD(P=0.05)	0.14	0.18	0.09	0.18	0.17	0.14	23.0	70.6	14.1	26.9	CD(P=0.05)	1.23	1.23
Stages(S)													
90 DAS	1.70	0.00	0.42	0.00	1.83	0.00	302.6	41.9	79.4	11.7	70 DAS	8.29	10.31
97 DAS	3.36	0.61	0.74	0.61	2.88	0.42	574.8	132.2	114.2	40.5	80 DAS	18.14	22.80
104 DAS	4.07	2.49	1.14	2.49	3.05	1.74	612.60	358.3	159.3	117.0	90 DAS	13.67	17.22
111 DAS	3.78	2.40	0.93	2.40	3.00	1.77	554.2	349.7	142.3	88.7	100 DAS	9.33	11.75
S.Em(±)	0.05	0.28	0.02	0.03	0.04	0.04	8.30	5.6	2.8	5.4	S.Em(±)	0.40	0.56
CD(P=0.05)	0.13	0.80	0.06	0.08	0.11	0.10	23.84	16.1	8.0	15.6	CD(P=0.05)	1.14	1.59
Interaction (T) ×(S)													
S.Em(±)	0.12	0.09	0.06	0.09	0.10	0.09	20.5	26.1	7.9	15.1	S.Em(±)	0.10	1.38
CD(P=0.05)	0.34	0.23	0.17	0.26	0.30	0.27	59.3	79.6	23.0	44.6	CD(P=0.05)	2.90	4.01

T_1 – Rhizobium.T_2 – PSB, T_3 – Azotobacter, T_4 - Rhizobium + PSB, T_5 - PSB+Azotobacter, T_6 - Rhizobium+PSB+Azotobacter and T_7 -Control

Parameters like fresh and dry pod weight, seed and pod wall weight was observed to be maximum at 104 days after sowing, after which gradually declined at 111days after sowing. The treatment, stage of crop and interaction between treatment and stage of crop as depicted in table 2, had significant effect on different parameters like fresh and dry pod weight, seed weight, pod wall weight and nodule number for both the varieties. Among treatments, maximum effect was recorded in T_4 followed by T_6 in both the varieties. Similar trend was observed for fresh seed weight in JG-14 and Bidisha. In case of JG-14, the fresh pod wall weight was maximum under T_7 (Untreated dry seed) with 3.27 g pod^{-1} followed by T_6 (3.06 g pod^{-1}). In case of Bidisha, T_6(Rhizobium + PSB+ Azotobacter) recorded maximum fresh pod wall weight (1.32 mg pod^{-1}) followed by T_4. The dry pod weight (587.7 and 417.3 mg pod^{-1} for JG-14 and Bidisha respectively) and the dry seed weight (140.8 and 93.6 mg pod^{-1} for JG-14 and Bidisha respectively) was maximum under T_4. Before the seed growth actually begun, the pod wall grows to a large extent by maximum accumulation of dry matter, then gradually with seed growth, pod wall weight reduces slightly with increase in dry matter accumulation in seed, which increase linearly followed by decrease in growth rate at the end of seed filling completion (Davies *et al.*) [3].

Among all treatments maximum nodules were recorded in T_6 (15.83) followed by T_4 (15.67). Similarly, Bidisha had maximum number of nodules (19.93) in T_6 followed by T_4 (19.66). The number of nodules per plant increased gradually from 70 days after sowing to 80 days after sowing and then gradually reduced at 90 days after sowing and then slightly reduced at 100 days after sowing. Similar trend was observed in case of Bidisha also for number of nodules per plant, since nitrogen requirement was found to be more during early growth in chickpea, nodule number was high then after in advanced stages senescence occurs not only in leaves but also in nodules reducing the nodule number. Highest number of nodules recorded at 80days after sowing in both the varieties. The results were in conformity with findings by Ali and Bano [1], who had showed that the coincidence of leaf and nodule senescence is correlated with degradation of protein and chlorophyll contents, along with reduction in sugar assimilation (in older leaves as well as nodules) except in pods and seeds, leading to reduced nitrogenase activity. Also, some reports had shown that during reproductive growth, the stage of pod filling coincides with senescence (Grover *et al.*) [6].

4. Conclusion

The results obtained in the present study have great relevance to the improvement of chickpea seed yield. Characters like number of primary branches, number of secondary branches, 100 seed weight (g), yield (g) per sq.m had influence due to different seed priming treatments. T_4(Rhizobium+PSB) followed by T_6 (Rhizobium+PSB+Azotobacter) showed maximum influence in both the varieties. Varieties recorded significant effect, among which Bidisha had higher value as compared to JG-14. Likewise, among treatments, T_4(Rhizobium+PSB) followed by T_6 (Rhizobium+PSB+Azotobacter) also showed highest number of seeds per pod. Bidisha produced higher seed yield (g/sq.m) as compared to JG-14. Pattern of pod development revealed that, both the varieties showed increased fresh pod and seed weight up to 104 days after sowing irrespective of treatments, there after declined at 111 days after sowing. In both the varieties number of nodules recorded highest at 80days after sowing. So, chickpea seed treated with Rhizobium+PSB would be useful for improvement of seed yield and the varieties gained physiological maturity at 104 days after sowing.

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