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Syed Ameer Sohail
Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Borra Chandra Sekhar Reddy
Department of Agronomy, SHUATS, Allahabad, Uttar Pradesh, India

Gammingi Solomon Raj
Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Rajana Praveena
Department of Agronomy, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Correspondence
Syed Ameer Sohail
Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Effect of different priming methods on root nodulation in Kabuli chickpea (*Cicer Kabulim* L.) seeds

Syed Ameer Sohail, Borra Chandra Sekhar Reddy, Gammingi Solomon Raj and Rajana Praveena

Abstract
The experiment was conducted in Post Graduate Laboratory and Field Experimentation Centre of Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and sciences, Allahabad (U.P.)during Rabi 2017-18 in order to standardize the best method of priming viz., hydropriming, halopriming, osmopriming and organic priming evaluated by screening a range of duration concentrations viz., T0-Untreated (Control), T1-Distilled water(hydration), T2-Potassium chloride (kcl)1%,T3- Potassium chloride (kcl) 2%,T4- Calcium Chloride ($CaCl_2$) 1%, T5- polyethylene Glycol (PEG) 2%,and T6- Neem Leaf Extract 5% for 8hours.It was found that all the priming methods showed significance difference with the control and the field emergence percentage, number of nodules per plant at 30 DAS, number of nodules per plant at 45 DAS, nodules fresh weight at 30 DAS, nodules fresh weight at 45 DAS, nodules dry weight at 30 DAS, nodules dry weight at 45 DAS, plant height.

It was found that all the priming treatment showed significance difference with the control and the highest field emergence per cent and plant nodulation characters were observed for PEG6000 best priming. The Highest nodulation was observed in PEG and $CaCl_2$.Seed priming for 8 hours and then dried for 24 hours in shade. This study Showed that seed priming Could improve some root nodulation and plant height in Kabuli chickpea seed. In seed priming, its simplicity no requirements for extensive equipment and chemicals could be used method for overcoming problems related to a poor germination and seedling establishment and helps in sustaining agriculture and cost, growth and nodulation and with the help of seed priming treatments which are cost effective, economic, non-toxic and from eco-friendly sources.

Keywords: Kabuli chickpea (*Cicer Kabulium* L.), methods of priming, hydropriming, osmopriming, organic priming, PEG_{6000}, kcl, $cacl_2$, Neem leaf extract

1. Introduction

Pulses are also important for sustainable agriculture enriching the soil through biological nitrogen Kabuli Chickpea or white gram (*Cicer Kabulium* L.); In this group the colour of the seed is usually white. grains are bold and attractive. yield potential of this group is poor as compared to Desi or brown gram. plants are generally taller than the Desi gram stand more or less erect. The chromosome number is (2n=2x=16) Chickpea, (*Cicer kablium*), belongs to genus *Cicer*, tribe *cicereae*, and is a member of the legume, pea, or pulse family, "*Fabaceae*" and subfamily *Papilionacea* (Singh *et al*, 1997).

Pulses are also important for sustainable agriculture enriching the soil through biological nitrogen fixation, fixes about 40-50 kg of N/ha (Hariprasanna and Bhatt, 2002). It is an important nutritious pulse crop occupying unique position in Indian Agriculture, belongs to family Leguminosae with chromosome number 2n=2x=16 (Malik, 1994) [6].

Pulses maintain the soil fertility by fixing atmospheric nitrogen and improved soil structure. Pulses also play on important role in rainfed agriculture improving physical, chemical and biological properties of soils so considered excellent crop for natural resources management environmental crop diversification and consequently for viable agriculture. (Khan *et al.* 2006) [5]. A healthy crop of chickpea can fix up to 141kg nitrogen per hectare. Chickpea is the third most important pulse crop in the world after beans and peas. It is cultivated on an area of 12 million hectares with 8.9 million tones of annual production. Chickpea plays an important role to improve soil fertility by fixing atmospheric nitrogen with the help of root nodules. (Anabessa *et al.* 2006). Chickpea is native of south-eastern Turkey and Syria (Saxena and Singh, 1987).

Heydecker, (1973) [3] used different terms depending upon the method adopted for priming, namely (i) Hydropriming - soaking the seeds in water, (ii) Osmopriming - soaking the seeds in osmotic solution, (iii) Halopriming - soaking the seeds in salt solutions, (iv) Biopriming coating the seeds with biological agents like bacteria, fungi etc and (v) Solid matrix priming - mixing the seeds with an organic or inorganic carrier and water, for a specific period of time. Hydro priming technique can help to increase effectiveness of on farm priming. This is the simple technique, in which seeds are soaked in water before sowing and results in average yield increase, up to 30 percent in many crops. This method has been adopted by thousands of resource poor farmers for many crops in many countries, in both Asia and Africa

The general purpose of seed priming is to partially hydrate the seed to appoint where germination processes are begun but not completed. Treated seeds whit soaking in water (Hydropriming), Soaking in inorganic salt solutions (Halo priming) and different organic osmotic (Osmopriming) are usually redried before use, but they would exhibit rapid germination when re-imbibed under normal or stress conditions. Each treatment may havevarying effects depending upon plant species, stage of plant development, concentration/dose of priming agent, and incubation period (Ashraf *et al*., 2005) [1].

2. Materials and Methods

The experiment was conducted in Field Experimentation Centre, Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (U.P.) during rabi season 2017-18, in order to standardize the best method of priming specific to Kabuli chickpea (var. Ujjawal). The treatments used at different concentrations for priming were viz; TO-unprimed (control),T1-Distilled water hydration for 8 (hrs) T2- KCL 1% T3 -KCL 2%,T4-Calcium Chloride ($CaCl_2$) 1%,T5-Polyethylene Glycol (PEG) $_{600}$ 2%,T6-Neem leaf extract 5% for 8 hrs. After cleaning and grading, the seeds were soaked in respective priming solutions at different volume of seeds for twelve hours. Then the seeds were air dried under the shade to bring back to their original moisture content and used for sowing on field.

2.1 Preparation of Solutions

For the preparation of solution one gram of each chemical was taken in a beaker. These chemicals were added separately in 1000 ml. of distilled water with constant stirring. The volume of solution will finally constituted to one litter, then it becomes 1000 ppm stock solution of each chemical.

The flasks containing chemicals was covered with muslin cloth to avoid any contamination For the preparation of $cacl_2$1% solution 10(g), Kcl 2% solution 20(g), was taken in a measuring flask made up to 100 ml. Distilled water while for $CACL_2$ 1% solution 10(g). PEG 2% solution 20(g). Neem leaf extract (5%) solution 50 (ml) was taken in measuring flask and made up to 100 ml with distilled water.

2.2 Preparation of plant leaf extract

The fresh leaves of the Neem plants were collected separately and dried in shade. The shade dried leaves were powdered using mortar and pestle. Then exactly weight fifty gram of leaf powder using weighing balance and dissolved in 100 ml. of distilled water which was measured already in the beaker to make 5% leaf extract. The leaf extract was filtered by using muslin cloth to remove unwanted material and leaf debris.

Randomized Block Design (RBD**)** (Panse and Sukhatme, 1967) [7] with three replications was performed. Observations on field viz., field emergence and nodulation characters were worked out and the data was statistically analyzed using ANOVA.

2.3 Field Emergence

Number of seedling emerged on 15th days Field emergence (%) = ----------×100 Total no. of seeds sown One hundred seeds from each treatment in four replications were used for field emergence studies. The seeds were sown in well prepared soil at 3 cm deep. The field emergence count was taken on the 15th day after sowing and the emergence percentage was calculated taking into account the number of seedlings emerged three centimeters.

2.4 Number of nodules per plant

Ten plants from each treatment plot in three replication were uprooted 30 and 45 days after seeding (DAS), and the extent of nodulation was estimated by carefully washing the roots and detaching the nodules before counting (Khan *et al*., 2006) [5].

2.5 Nodules Fresh Weight

After washing the root nodules from the field the nodules were detached from the plant roots and weighed in an electronic weigh balance for fresh weight of root nodules expressed in milligrams (mg).

2.6 Nodules dry weight

After taking the fresh weight the root nodules they are kept in butter paper and kept in oven for drying at 80oC for 24 hrs and then weighed for dry weight of nodules expressed in milligrams (mg) (Khan *et al*., 2006) [5].

3. Results and Discussion

3.1 Analysis of Variance

Analysis of variance revealed that the differences among seven treatments were significant for growth and nodulation, *viz.*, field emergence percentage, number of nodules per plant at 30 DAS, number of nodules per plantat 45 DAS, nodules fresh weight at 30 DAS, nodules fresh weight at 45 DAS, nodules dry weight at 30 DAS, nodules dry weight at 45 DAS & plant height.

Table 1: Analysis of variance for 8Agronomic characters in Chickpea.

S. No	Characters	Mean sum of squares		
		Replications(df=2)	Treatments (df=6)	Error(df=12)
1.	Field emergence %	1.523	28.112*	0.783
2	No.of nodules per plant after 30 days	0.081	28.701**	0.101
3	No of nodules per plant after 45 days	0.172	4.763**	0.116
4.	Nodules fresh weight per plant30 days (gms)	0.000004	0.0045222**	0.00003
5.	Nodules fresh weight per plant 45 days(gms)	0.00002	0.000591**	0.00007
6.	Nodules dry weight per plant30 days(mg)	0.00003	0.000621**	0.0001
7.	Nodules dry weight per plant 45 days(mg)	0.00004	0.000872**	0.00005
8.	Plant height (cm)	1.201	60.113**	0.442

3.2 Mean performance

The data presented in the table showed mean performance and range of 7 treatments for 8 growth and nodule characters.

3.2.1 Field emergence (%)

The data of different seed priming treatment on field emergence are presented below The mean performance of field emergence ranged from 74.900 to 83.000 with mean value of 77.86 Maximum field mergence (83.00) was recorded by T_5 with Treatment of PEG 2% and it was followed by T_4 (80.367) with Treatment of $cacl_2$ 1%.Minimum field emergence was recorded by T_0 (74.900) with control. These are similar finding results reported by Umair Adrian ali *et al*., (2012) Singh *et al*.,(2006), Selvarani k and Umarani R (2011) [11], Meheta *et al*.,(2010), Bajpai *et al*., (2002), Sarmadi *et al*.,(2014).

3.2.2 No. of nodules per plant at 30 DAS

The data of different seed treatment on No. of nodules per plant are presented The mean performance of No. of nodules per plant ranged from 10.600 to 19.067 with mean value of 15.90 Maximum No. of nodules per plant (19.067) recorded by T_5 with Treatment of PEG 2% and it was followed by T_4 (18.333) with Treatment of $cacl_2$1%.Minimum No of nodules per plant was recorded by T_0 (10.600) with control. These are similar finding results reported by Premarthe and oertli (1994) [8, 10], Singh and Kataria (2012) [12, 13], George *et al*., (2014), Sarmadi *et al*., (2014).

3.2.3 No. of nodules per plant at 45 DAS

The data of different seed treatment on No of nodules per plant are presented below in The mean performance of No of nodules per plant ranged from 31.667 to 35.600 with mean value of 33.61 Maximum recorded No of nodules per plant (35.600) by T_5 with Treatment of PEG 2% and it was followed by T_4 (34.533) with Treatment of $cacl_2$1%minimum No. of nodules per plant was recorded by T_0 (31.667) with control. These are similar finding results reported by Premar the and oertli (1994) [8, 10], Singh and Kataria (2012) [12, 13], George *et al*., (2014), Sarmadi *et al*., (2014).

3.2.4 Nodules fresh weight per plant at 30 DAS

The data of different seed treatment on Nodules fresh weight are presented below The mean performance of Nodules fresh weight ranged from 0.057 to0.154 with mean value of 0.104 Maximum Nodules fresh weight (0.154) was recorded by T_5 with Treatment of PEG 2% and it was followed by T_4 (0.143) with Treatment of $cacl_2$ 1%.MinimumNodules fresh weight was recorded by T_0 (0.057) with control. These are similar finding results reported by Premarthe and oertli (1994) [8, 10], Singh and Kataria (2012) [12, 13], George *et al*., (2014), Sarmadi *et al*., (2014).

3.2.5 Nodules fresh weight per plant at 45 DAS

The data of different seed treatment on Nodules fresh weight are presented below The mean performance of Nodules fresh weight ranged from 0.743to0.784 with mean value of 0.765 Maximum Nodules fresh weight (0.784) was recorded by T_5 with Treatment of PEG 2% and it was followed by T_4 (0.776) with Treatment of $cacl_2$ 1%.Minimum Nodules fresh weight was recorded by T_0 (0.743) with control. These are similar finding results reported by Premarthe and oertli (1994) [8, 10], Singh and Kataria (2012) [12, 13], George *et al*., (2014), Sarmadi *et al*., (2014).

3.2.6 Nodules dry weight per plant at 30 DAS

The data of different seed treatment on Nodules dry weight are presented below The mean performance of Nodules dry weight ranged from 0.017 to 0.056 with mean value of 0.036 Maximum Nodules dry weight (0.056) was recorded by T_5 with Treatment of PEG 2% and it was followed by T_4 (0.051) with Treatment of $cacl_2$ 1%.MinimumNodules dry weight was recorded by T_0 (0.017) with control. These are similar finding results reported by Premarthe and oertli (1994) [8, 10], Singh and Kataria (2012) [12, 13], George *et al*.,(2014), Sarmadi *et al*., (2014).

3.2.7 Nodules dry weight per plant at 45 DAS

The data of different seed treatment on Nodules dry weight are presented below The mean performance of Nodules dry weight ranged from 0.124 to 0.171 with mean value of 0.151 Maximum Nodules dry weight (0.171) was recorded by T_5 with Treatment of PEG 2% and it was followed by T_4 (0.166) with Treatment of $cacl_2$1%.MinimumNodules dry weight was recorded by T_0 (0.124) with control. These are similar finding results reported by Premarthe and oertli (1994) [8, 10], Singh and Kataria (2012) [12, 13], George *et al*., (2014), Sarmadi *et al*., (2014).

3.2.8 Plant Height

The data of different seed priming treatment on Plant height are presented below The mean performance of Plant height ranged from 44.093 to 55.987 with mean value of 49.63 Maximum Plant height (55.987) was recorded by T_5 with Treatment of PEG 2% and it was followed by T_4 (53.840) with Treatment of $cacl_2$ 1%.Minimum plant height was recorded by T_0 (44.093) with control. These are similar finding results reported by Umair Adrian ali *et al*., (2012) Singh *et al*., (2006), Iqbal Hussain *et al*.,(2014), Selvarani k and Umarani R (2011) [11], Meheta *et al*., (2010), Bajpai *et al*.,(2002), Sarmadi *et al*.,(2014).

Table 2: Analysis of variance for 8Agronomic characters in Kabuli Chickpea.

S. No	Characters	Mean sum of squares		
		Replications (df=2)	Treatments (df=6)	Error(df=12)
1.	Field emergence %	1.523	28.112*	0.783
2.	No. of nodules per plant after 30 days	0.081	28.701**	0.101
3.	No. of nodules per plant after 45 days	0.172	4.763**	0.116
4.	Nodules fresh weight per plant30 days (gms)	0.000004	0.0045222**	0.00003
5.	Nodules fresh weight per plant 45 days (gms)	0.00002	0.000591**	0.00007
6	Nodules dry weight per plant30 days (mg)	0.00003	0.000621**	0.0001
7	Nodules dry weight per plant 45 days (mg)	0.00004	0.000872**	0.00005
8	Plant height	1.201	60.113**	0.442

Table 3: Mean performance of Kabuli chickpea for 8 agronomic characters

S. No	Treatments	Field emergence	No. of nodules 30 Das	No. of nodules 45 Das	Fresh weight 30 Das (mg)	Fresh weight 45 Das (mg)	Dry weight 30 Das (mg)	Dry weight 45 Das (mg)	Plant Height (cm)
1	T_0	74.900	10.600	31.667	0.057	0.743	0.017	0.124	44.093
2	T_1	75.377	13.133	32.900	0.068	0.752	0.024	0.134	45.253
3	T_2	75.610	15.533	33.100	0.078	0.761	0.028	0.147	47.563
4	T_3	76.033	16.433	33.467	0.093	0.766	0.031	0.154	48.480
5	T_4	80.367	18.333	34.533	0.143	0.776	0.051	0..166	53.840
6	T_5	83.000	19.067	35.600	0.154	0.784	0.056	0.171	55.987
7	T_6	78.500	18.200	34.000	0.132	0.772	0.044	0.158	52.217
Grand mean		77.86	15.90	33.61	0.104	0.765	0.036	0.151	49.63
C.D. (5%)		0.473	0.350	0.376	0.0018	0.0029	0.0012	0.0024	0.733
SEd(m)		0.976	0.170	0.182	0.0009	0.0014	0.0006	0.0011	0.355

4. Conclusion

On the basis of result obtained from the present experiment, following conclusions are drawn.

Among all the seed priming treatments, seed priming with PEG 6000 (osmo priming) was found to be the best priming treatment followed by Halopriming ($cacl_2$). Significantly increased the nodulation of Kabuli chickpea. Osmopriming with PEG showed maximum increase in nodulation. Priming of the Kabuli chickpea seeds for 8 hrs, in which PEG best result to enhanced nodulations in Kabuli chickpea.

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