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Effects of Hardening Methods on Seedling Characters, Germination and Nodulation in Greengram (*Vigna radiata* L.)

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A B S T R A C T

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.), in order to standardize the suitable hardening chemicals (organic or Inorganic) for Greengram seeds (var. Samrat). Four different Organic and Inorganic Solutions viz., T_0-Untreated (Control), T_1-Hardened with Distilled water, T_2-Hardenedwith KNO_3 20%, T_3–Hardenedwith NaCl 1%, T_4–Hardened with $CaCl_2$ 1%, T_5-Hardened with Tulsi leaf extract and T_6-Hardened with Neem Leaf Extract 5% were taken and in their solutions seeds hydrated for 12 hours and then dried for 24 hours in shade. It was found that among all the Hardening treatments showed significance difference with the control while highest germination percentage, seedling length and weight, vigour index, and nodulation were observed for seeds treated with KNO_3(20% Solution). This study also showed that Seed Hardening with neem leaves extracts, Tulsi leaf extract, $CaCl_2$ and distilled water were found to increase the seedling character, growth and yield. The study helps to improve the seedling character, growth and nodulation with the help of seed Hardening treatments which are cost effective, economic, non-toxic and from eco-friendly sources.

Introduction

Pulses constitute an important ingredient in predominantly vegetarian diet and are important source of protein that nutritionally balances the protein requirement of vegetarian population. They supply minerals and vitamins and provide an abundance of food energy. Pulses provide a cheaper source of nutrients/ proteins as they generally contain nearly twice as much as protein as that of cereals and hence correctly called poor man's meat. 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).

When nitrogen is supplied either through inorganic or organic source to the crop, the increase in chlorophyll occurs (Austin *et al.*, 1973). Greengram is one of the important pulse crops in India. Greengram (*Vigna radiata* L.) (2n = 22) is a self-pollinated legume crop originated in South Asia. More than 70% of world's green gram production comes from India and accounts for about 10 to 12% of total pulse production in the country (Ministry of Agriculture, Government of India, 2014). It is also commonly known as mungbean. It is quite versatile crop grown for seeds, green manure and forage and it is also

considered as "Golden Bean" because of its nutritive values and suitability for increasing the soil, by the way of addition of nitrogen to the soil.

Green revolution made our nation self-sufficient in cereals but we are still deficit in pulse production. India still imports Green Gram from countries like Myanmar, Australia and Africa to satisfy the pulse requirement. This impose great burden on our economy. Poor crop establishment is a major constraint for mungbean production (Naseem *et al.*, 1997) (Rahmianna *et al.*, 2000). Usually seeds with low vigour produce weak and unproductive plants (Olisa *et al.*, 2010) and high yields can be associated with early vigor (Kumar *et al.*, 2002). However rapid germination of seedlings could emerge and produce deep roots before the upper layers of the soil are dried and crusted, which may result in better crop establishment and higher crop yield (Ashraf *et al.*, 2005). The successful establishment of crop mainly depends upon good quality seed. To provide higher quality seeds, scientists have developed new technologies called "Seed Enhancement Techniques". The main objective of this technique is to optimize the application of seed treatment products by improving the technical quality of seeds. The two important enhancement technologies are seed priming and seed hardening that have been employed successfully for many crops.

It is reported that seed hardening is one of the most important developments to help rapid and uniform germination and emergence of seeds and to increase seed tolerance to adverse environmental conditions (Heydecker *et al.*, 1973, 1975; Harris *et al.*, 1999). Seed hardening has presented promising, and even surprising results, for many seeds including the cereal seeds. The root system of mung bean mainly located in the upper 20-25 cm depth which, under dry conditions, transformed into short tuberized roots, unable to absorb proper moisture and nutrition for growing plants. Water stress at any stage of growth may causes changes in plant morphology, physiology and consequently affects crop growth. To overcome this stress condition seed hardening is done. Physiological seed treatments that enhance the performance of seed are based primarily on seed hydration and dehydration (May *et al.*, 1962). Seed priming/hardening is a common practice followed to enhance seed performance with respect to rate and uniformity of germination (De Lespinay *et al.*, 2010). In addition to better establishment, hardened crops grew more vigorously, flowered earlier and yielded higher (Farooq *et al.*, 2008).

Materials and Methods

The present evaluation entitled "Effect of hardening on seedling characters, germination and nodulation in greengram (*Vigna radiata* L.) var. Samat." was conducted during Kharif 2016, in Post Graduate Laboratory of Seed Science and Field Experimentation Centre of the Department of Genetics And Plant Breeding, Sam Higginbottom, University of Agriculture, Technology and Sciences, Allahabad (U.P.). The treatments used at different concentrations for hardeaning were T0-Unhardened seeds (Control), T1-Distilled water, T2 -KNO_3 (20%), T3-NaCl (1%), T4-$CaCl_2$ (1%), T5-Tulsi leaves extract (5%) and T6-Neem leaves extracts (5%).

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 constitute to one litter, then it became 1000 ppm stock solution of each chemical. The flasks containing chemicals was covered with muslin cloth to avoid any contamination. For

the preparation of KNO_3 solution (KNO_3-20%) 200 gm of potassium nitrte was taken and mixed with 1000 L of distilled water. For the preparation of Sodium chloride (NaCl-1%) solution 10 (gm) NaCl was taken in a measuring flask and made up to 1000 ml. distilled water, while for (1%) Calcium chloride ($CaCl_{2)}$ solution 10 (gm) $CaCl_2$ salt was taken in a measuring flask and made up to 1000 ml with distilled water and for Tulsi leaves extract solution 1 kg of tulsi leaves was taken and then grinded after this the matter is mixed with water and then water was strained out in a measuring flask, 50 ml of tulsi leaves extract solution was taken and made up to 1000 ml with distilled. By using the same procedure neem leaf extract solution was prepared, 50 ml solution of Neem leaf extract was taken in a measuring flask and made up to 1000 ml distilled water.

After preparation of solution of Potassium nitrate (KNO3), Sodium chloride (NaCl), Calcium chloride ($CaCl_2$), Tulsi leaf extract, and Neem Leaf Extract, Greengram seeds were soaked in required solution for 12 hour at 25^0C temperature. After 12 hrs of soaking the solution was drained out from the beaker and pre-soaked seeds air dried or shade dried to original weight and then placed for germination in laboratory under controlled condition incompletely randomized design in between the paper (CRD) and in the field under RBD(randomized block design) where we observed nodulations.

The observations of the characters *viz* Germination percentage (ISTA 2004), Root length (cm), shoot length (cm), Seedling length (cm), Seedling fresh weight (gm), seedling dry weight (gm), Vigour index I, Vigour index II (Baki and Anderson,1973) were recorded. The experimental data recorded were subjected to analysis of variance, range, mean, and coefficient of variation (Fischer, 1936).

Result and Discussion

According to the results, all studied traits were affected by the treatments and there was completely significant difference between control (unhardened seeds) and Hardened seeds (Tables 1, 2 & 3). Seed hardening means alternate drying and wetting of seeds (Pen aloza and Eira, 1993). More than one pre-sowing treatment causing an increase in seed weight and it was reported by Vijaya (1996) in cowpea and blackgram, Maheshwari (1996)

The mean performance of germination percentage ranged from 76.16% to 86.01%with mean value of 81.50%. Significantly highest percentage of germination (86.01%) was reported in the seeds hardened with T_2 KNO_3 20% and it was followed by T_6 Neem leaf extract solution *i.e.,* 85.20% and seeds hardened with calcium chloride ($CaCl_2$) 1% shows 81.04% germination. Minimum germination percentage was recorded by T_0 seeds *i.e.,* 76.16% with unhardened control.

It has been reported that hardened seeds showed better germination pattern and higher vigour level than non- hardened seeds (Ruan *et al.,* 2002). The stimulatory effect on germination and the growth of seedlings of hardened seed could be due to the fertilizing effect resulting from the nutrient release from damaged or decayed tissue of storage organ by hydrolysis (Orr *et al.,* 2005).

The mean performance of seedling root length ranged from 12.21cm to 16.06 cm with mean value of 13.93 cm. Maximum root length (16.06cm) was recorded by T_2 hardened with KNO_3 20% and it was followed by T_6 (15.18 cm) hardened with Neem leaf extract 5% followed by $CaCl_2$ *i.e.,* 14.02cm and Minimum root length was recorded by T_0 *i.e.,* 12.21 cm that is unhardened seeds.

The mean performance of seedling shoot length ranged from 19.7 cm to 24.01 cm with mean value of 21.70 cm. Maximum shoot length (24.01 cm) was recorded by T_2 hardened with KNO_3 20% and it was followed by T_6 (23.96 cm) hardened with Neem leaf extract 5%. The shortest shoot length was found in T_0unhardened control (19.7 cm).

The mean performance of seedling length ranged from 32.56cm to 39.91cmwith mean value of 35.59cm. Maximum seedling length 39.91cm was recorded by T_2hardened with KNO_3 20% and it was followed by T_6 39.24cmhardened with Neem leaf extract 5% and shortest seedling length was recorded in T_0 unhardened control *i.e.,* 32.56cm.

The higher seedling length in $KNO_3$is due to cumulative positive effect of H_2O and K on root and shoot growth. There are reports that hydration of seeds that equals, but does not exceed, the lag phase of hardening permits early DNA replication, increased RNA and protein synthesis, greater ATP availability, faster embryo growth, repair of deteriorated seed parts (Karssen *et al.,* 1989; Saha *et al.,* 1990), and reduced leakage of metabolites than checks (Giri and Schillinger, 2003).

All these causes increased in seed root and shoot length which ultimately increased the total seedling length. The mean performance of seedling fresh weight ranged from 2.18gm to 3.38gmwith mean value of 2.61gm.

Table.1 Analysis of variance for seedling characters in greengram

S. No.	Characters	Mean sum of squares	
		Treatments (df=6)	Error (df=21)
1.	Germination Percentage	40.13*	0.40
2.	Root Length	6.74**	0.68
3.	Shoot Length	12.36*	1.47
4.	Seedling Length	39.49**	3.52
5.	Seedling Fresh Weight	0.75**	0.12
6.	Seedling Dry Weight	0.048*	0.007
7..	Seed Vigour Index I^{st}	540744.64**	64512.0009
8.	Seed Vigour Index II^{nd}	445.55**	63.001

* And ** significant at 5% and 1% level of significance, respectively

Table.2 Mean performance of Greengram for agronomic characters

S. No.	Treatments	No. of nodulation (45 DAS)
1	**T_0**	15.80
2	**T_1**	17.60
3	**T_2**	19.26
4	**T_3**	16.33
5	**T_4**	18.46
6	**T_5**	18.06
7	**T_6**	18.5
Grand Mean		**17.12**
C.D. (5%)		1.983
SE(m)		0.643
RANGE	**MIN**	**14.83**
	MAX	**19.26**

Table.3 Mean performance of greengram for 8 seedling characters

S.No.	Treatments	Germination %	Root Length (cm)	Shoot Length (cm)	Seedling Length (cm)	Fresh Weight of Seedling (gm)	Dry Weight of Seedling (gm)	Seed Vigour Index Ist	Seed Vigour Index IInd
1	T_0	76.16	12.21	19.7	32.56	2.18	0.37	2431.87	28.15
2	T_1	79.61	13.01	20.3	33.2	2.28	0.46	2656.25	36.73
3	T_2	86.01	16.06	24.01	39.91	3.38	0.68	3451.73	58.96
4	T_3	80.28	13.67	20.33	34.9	2.37	0.59	2732.31	47.81
5	T_4	81.04	14.02	21.31	33.81	2.48	0.58	2869.37	47.4
6	T_5	82.19	14.48	22.02	35.5	2.54	0.59	3006.24	48.71
7	T_6	85.20	15.18	23.96	39.24	3.02	0.65	3339.09	55.82
Grand Mean		**81.50**	**13.93**	**21.70**	**35.59**	**2.61**	**0.56**	**2897.59**	**46.28**
C.D.(5%)		4.54	1.21	1.78	0.90	0.52	0.12	373.49	11.67
SE(m)		1.54	0.41	0.60	0.30	0.17	0.04	126.99	3.96
RANGE	**MIN**	**76.6**	**12.21**	**19.7**	**32.56**	**2.18**	**0.37**	**2431.87**	**28.15**
	MAX	**86.01**	**16.06**	**24.01**	**39.91**	**3.38**	**0.68**	**3451.73**	**58.96**

Maximum seedling fresh weight (3.38gm) was recorded by T_2 hardened with KNO_3 20% and it was followed by T_6 (3.02gm) hardened with Neem leaf extract 5% and Lowest value of seedling fresh weight was found in T_0 unhardened control (2.18gm). The cumulative positive effect of the hardening on seed root length and shoot length causes to increase in fresh weight of the seedling.

The mean performance of seedling dry weight ranged from 0.37gm to 0.68gmwith mean value of 0.56gm. Maximum seedling dry weight (0.68gm) was recorded by T_2 hardened with KNO_3 20% and it was followed by T_6 (0.65gm) hardened with Neem leaf extract 5% and Lowest value of seedling dry weight was found in T_0 unhardened control (0.37gm).

The mean performance of seedling vigour index Ist ranged from 2431.87 to 3451.73with mean value of 2897.59.

Maximum seedling vigour index Ist (3451.73) was recorded by T_2primed with KNO_3 20%and it was followed by T_6 (3339.09) hardened with neem leaf extracts 5% and Minimum seedling vigour index Ist was recorded by T_0 unprimed (2431.87) in control (Unhardened seeds).

The mean performance of seedling vigour index IInd ranged from 28.15 to 58.96 with mean value of 46.28. Maximum seedling vigour index IInd (58.96) was recorded by T_2primed withKNO_3 20%and it was followed by T_6 (55.82) hardened with Neem leaf extract 5% Minimum seedling vigour index IInd was recorded by unhardened T_0 (28.15) in control.

Field observation

The mean performance of number of nodulations per plant ranged from 15.80 to 19.26with mean value of 17.12. Maximum number of nodulations per plant (19.26) was recorded by T_2 hardened with KNO_3 20% and it was followed by T_6 (18.50) hardened with Neem leaf extract 5% and Minimum number of nodulations per plant was recorded by T_0 (15.80) unhardened seeds.

Hardening with KNO_3 20% solution recorded significantly higher values for growth and yield attributing characters, *viz.*, nodulation at 45 days after sowing (DAS), respectively, number of pods per plant (19.26), in compared with other treatments. Neem leaf extract 5% also shows at par results when compared to other treatments. Hardening with

KNO_3 20% solution recorded significantly higher values for seedling characters, *viz.*, seed germination percentage (86.01%), shoot length (24.01cm), root length (13.01cm), seedling length (33.31cm), seed vigour index I^{st} (3451.73), seed vigour index II^{nd} (58.96), seedling fresh weight (3.38gm) and seedling dry weight (0..68gm) in compared with other treatments. Neem leaf extract 5% also shows at par results when compared to other treatments.

Soaking of seed with Neem solution is also advantageous to obtain healthy seedlings. The root system of mung bean mainly located in the upper 20-25 cm depth (Kjellstrom, 1991) which, under dry conditions, transformed into short tuberized roots, unable to extract proper moisture and nutrition for growing plants. Water stress at any stage of growth may causes changes in plant morphology, physiology and consequently affects crop growth (Hashem *et al.*, 1998). To overcome this stress condition seed hardening is done.

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