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RESEARCH ARTICLE

EFFECT OF SEED ENHANCEMENT TREATMENT USING LEAF EXTRACT ON PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF GREEN GRAM (*Vigna Radiate L.*) SEED ADT 3

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ABSTRACT

Green gram (*Vigna radiate L.*) is also known as mung bean or golden bean belongs to the Family Leguminosae. Green gram is a short duration crop and mainly cultivated as a rainfed crop under Rice fallow condition and irrigated crop. The low productivity under rainfed condition is due to soil moisture deficit, uneven rainfall, low soil fertility and poor crop management. To overcome this problem, seed researchers like Henckel as early as 1964 recommended the seed hardening techniques to alleviate the moisture stress condition. The present study will emphasize on the effect of seed hardening using various leaf extract like prosopis, pungam, nochi, neem,umathai, aduthoda, nerium, papaya, bittergourd etc. on seed and seedling characteristics like germination per cent, speed of germination, accumulated speed of germination, mean daily germination, root length, shoot length, seedling length, DMP, SV-I and SV-II of green gram ADT3. From the present study, it could be concluded that green gram seeds should be hardened with 1% prosopis leaf extract for 3 hours @ 1/3 rd volume of solution to enhance the seed and seedling quality characteristics under adverse environment conditions. In addition, green gram seeds may also be hardened with 1% pungam leaf extract to get the similar results.

INTRODUCTION

Green gram (*Vigna radiate L.*) is also known as mung bean or golden bean belongs to the Family Leguminosae. Green gram is a native of India and Central Asia. It is the third important pulse crop cultivated throughout India. Green gram is a short duration crop and mainly cultivated as a rainfed crop under Rice fallow condition and irrigated crop. In India, Green gram was cultivated over an area of 3 million hectare with annual grain production of one million tonnes. The six major green gram producing states are Maharashtra, Rajasthan, Bihar, Andhra Pradesh, Karnataka and Tamilnadu. Green gram is consumed in several ways in southern India as whole grain or broken cotyledon. Green gram is used to prepare Kichadi and Pongal in Tamilnadu. It is an important dietary protein food to humans. In addition to protein (23.86 g), it supplies fibre (16.3 g), fat (1.15 g), vitamins like A, C, B, Niacin, Minerals like calcium, magnesium, potassium, phosphorus, sodium, sugars (6.6 g) and carbohydrates (62.62 g) per 100 g of grain (source: USDA Nutrient Database).Sprouts grain contain high quality protein which are easily digestible and relatively rich in aminoacids like Lysine, Leucine, Phenylalanine, Valine and Isoleucine. Green gram does not cause flatulence that many other legumes may cause. The Net availability of total pulse is estimated to be 31.6 g/day during 2010 was less when compared against 37.0 g/day during 2009 (Directorate of Economics and Statistics, Department of Agriculture and Co-operation). Green gram is grown on a variety of soil ranging from sandy loam to heavy black cotton soils. Green gram crop cannot withstand water logging during major growth stages. A well-drained soil with pH ranging from 5.0 to 7.5 is ideal for its seed production and cultivation. Problematic soils like Saline, Alkali and Acidic are not suitable for seed production. The yield potential of green gram in research plot is 10 – 12 quintals per hectare as against 8 – 9 quintals per hectare in farmer's field. The National average yield is still low at 4 – 5 quintals per hectare. This yield gap needs to be addressed by improving seed production packages and supply of good quality seeds to the farmers. The supply of certified/ quality seed is 1.76 lakhs quintal during the year 2010 – 2011. In India about 70 per cent of cultivated land is under rainfed condition. The low productivity under rainfed condition is due to soil moisture deficit, uneven rainfall, low soil fertility and poor crop management.

One of the ways to improve the production or covering the yield gap to some extend is adopting new or advanced seed invigoration techniques that may help to overcome the adverse soil environment during initial crop growth and development. Moisture stress is one of the abiotic stresses which affect the productivity by intensive flowering dropping, poor pod formation, poor pod filling and low dry matter accumulation (Singh *et al*., 1991). The reason for low productivity of green gram may be due to inadequate pre-sowing seed treatment techniques to cope with moisture stress problem. To overcome this problem, seed researchers like Henckel (1964) recommended the seed hardening techniques to alleviate the moisture stress condition. Seed hardening has been reported to

Table 1. Observation Recorded and their formula

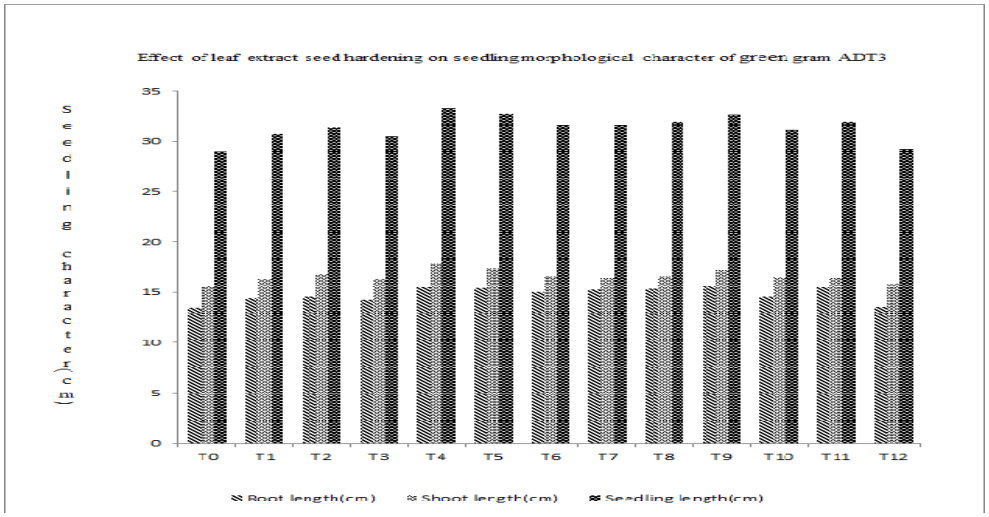
S.No.	Parameter	Formula	Unit	Ref
1	Germination Percentage	$\frac{\text{Total number of seeds germinated on final day}}{\text{Total number of seeds used}} \times 100$	%	ISTA Rules,2010
2	Speed of Germination	N1/1+N2-N1/2+……+Nn-N_{n-1} /Final day where N1= Total Number of seeds germinated on day1; N2 = Total Number of seeds germinated on day2 and so on till Nn = Total number of seeds germinated on final day.	-	Maguire,1962
3	Accumulated Speed of Germination	N1/1+ N2/2+……..+Nn/Final day where N1= Total Number of seeds germinated on day1; N2 = Total Number of seeds germinated on day2 and so on till Nn = Total number of seeds germinated on final day.	-	Bradbeer (1988), Wardle *et al*. (1991), Haugland and Brandsaeter (1996)
4	Mean Daily Germination	FGP/D where FGP = Final germination percentage and D = Experiment period	% /day	Scott et.al., 1984
5	Germination Value	∑DGS/NxGPx10 where DGS = Daily germination speed, N= Total number of daily count, GP = Germination per cent.	-	Djavanshir and Pourbeik,1976
6	Emergence index	*EI*=Σ*TiNi/S,* Where *Ti* is the number of days after sowing, *Ni* is the number of seeds germinated on *i*th day, and *S* is the total number of seeds used.	Seed day	Scott et.al., 1984
7	Germination index	N/D where N=Number of total emerged seed; D = Experiment period	Seed / day	Abdul-Baki and Anderson,1973
8	Root length	Mean value of the length between collar region to tip of the primary root of ten seedlings	cm	ISTA Rules,2010
9	Shoot length	Mean value of the length between the collar region to tip of the primary shoot of the same ten seedlings used for root measurement	cm	ISTA Rules,2010
10	Dry matter production	Ten normal seedlings used for growth measurements were placed in a paper cover and dried under shade for 24h and then in the hot air oven maintained at 100ºC for 24h.The dried seedlings were cooled in a desiccator for 30 minutes and the dry weight was recorded 10 per seedlings and was expressed in milligram.	Gram per 10 seedlings	ISTA Rules,2010
11	Seedling Vigour I	Total seedling length (cm) x Germination Per cent (%)	-	Abdul-Baki and Anderson,1973
12	Seedling VigourII	Dry matter production (gram /10 seedlings) x Germination Per cent (%)	-	Abdul-Baki and Anderson,1973

Table 2. Effect of leaf extract seed hardening on seed physiology characters of Greengram ADT3

S. No	Treatment	Speed of Germination	Accumulated Speed of Germination	Germination (%)	Mean Daily Germination	Germination Value
1	T_0	13.39	47.49	80(63.47)	10.00	1339.53
2	T_1	14.28	50.82	86(67.66)	10.69	1526.02
3	T_2	14.50	51.61	87(68.88)	10.88	1576.76
4	T_3	13.27	47.39	81(64.19)	10.13	1343.88
5	T_4	16.25	57.76	96(82.63)	12.00	1950.31
6	T_5	15.98	56.80	94(80.13)	11.75	1878.01
7	T_6	14.56	51.80	88(69.76)	11.00	1602.19
8	T_7	14.11	50.18	85(67.23)	10.63	1498.79
9	T_8	14.40	51.24	87(68.92)	10.88	1565.91
10	T_9	15.33	54.38	93(74.70)	11.63	1781.47
11	T_{10}	14.58	51.69	90(71.65)	11.25	1640.75
12	T_{11}	15.19	53.98	92(73.64)	11.50	1747.35
13	T_{12}	13.57	48.17	82(64.92)	10.25	1390.92
14	C.D(P=0.05)	0.448	1.562	2.616	0.336	93.73
15	SE.D	0.221	0.772	1.291	0.158	46.33

Table 3. Effect of leaf extract seed hardening on physiological and morphological qualities of seed and seedling of Greengram ADT3

S.No.	Treatment	Dry matter productior (mg/seedlings^{-10})	Seedling vigour I	Seedling vigour II
1	T_0	0.1655	2325.15	13.2446
2	T_1	0.1787	2628.87	15.2889
3	T_2	0.1900	2731.83	16.5369
4	T_3	0.1679	2474.69	13.6066
5	T_4	0.2210	3196.75	21.2216
6	T_5	0.2124	3076.38	19.7262
7	T_6	0.1949	2808.64	17.1541
8	T_7	0.1918	2688.24	16.3084
9	T_8	0.1983	2779.66	17.2479
10	T_9	0.2064	3043.33	19.2010
11	T_{10}	0.1932	2800.37	17.3848
12	T_{11}	0.2028	2942.19	18.6632
13	T_{12}	0.1760	2398.87	14.4295
14	C.D(P=0.05)	0.001	100.118	0.707
15	SE.D	0.8028	49.49	0.3486



induce drought resistance in plants and such seeds have the capacity to withstand dehydration and overheating. Other beneficial effects of hardening are inducing better root growth, higher rate of photosynthesis and dry matter accumulation (Henckel, 1964). Presowing seed hardening is the method that results in modifying the physiological and biochemical processes of seed to mitigate the adverse environment. Hardening of seeds resulted in the absorption of more water due to increase in the elasticity of cell wall and development of a stronger and efficient root system (Krishnasamy and Srimathi, 2001). Seed hardening can be done by using organic and inorganic products. In the present study, to protect the soil from pollution, we are using organic products like leaf extract obtained from various herbal and multi – purpose plants available around us for seed hardening. The present study will emphasize on the effect of seed hardening with various leaf extract on seed and seedling characteristics of green gram ADT3.

MATERIALS AND METHODS

The present investigation was carried using genetically pure seeds of green gram cv. ADT3 obtained from the Tamilnadu Rice Research Institute, Aduthurai, Tamilnadu. The bulk seeds were manually cleaned to remove unwanted material from the lot and was graded using BSS 8 x 8 sieve for uniformity. Experiment was conducted in the Seed Testing laboratory, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Tamilnadu. After cleaning and grading, seeds were preconditioned by keeping the seeds in between the layers of moistened gunny bags to avoid soaking injury for one hour. After preconditioning, the conditioned seeds were soaked in the respective leaf extract solution at $1/3^{rd}$ volume of seeds for three hours. Then the seeds were air dried under the shade to bring back to their original moisture content and used for sowing.

Preparation of plant leaf extract

The fresh leaves of the concerned plants were collected separately and dried under shade. The shade dried leaves were powdered using mortar and pestle. Then exactly weigh one gram of leaf powder using weighing balance and dissolved in 100 ml of distilled water which was measured already in the beaker to make 1% leaf extract. The leaf extract was filtered by using muslin cloth to remove unwanted material and leaf debris.

Treatment details

T_o - Control
T_1 - 1% Perungondraii (*Delonix elata*)
T_2 - 1% Bittergourd (Momordica *charantia*)
T_3 - 1% Papaya (*Carica papaya*)
T_4 - 1% Prosopis (*Prosopis juliflora*)
T_5 -1% Pungam (*Pongamia pinnata*)
T_6 -1% Neem (*Azadirachta indica*)
T_7 -1% Nerium (*Nerium oleander*)
T_8 -1% Aduthoda ilai (*Adutoda vasica*)
T_9 -1% Nochi (*Vitex nigundo*)
T_{10} -1% Kuppameni (*Acalypha indica*)
T_{11} -1% Umathai (*Datura metel*)
T_{12} -1% Keelanelli (Phyllanthus *niruri*)

Germination test was conducted in a completely randomised block design with three replications. From each treatment, randomly selected 50 seeds per replication were put for germination in a sterilized sand media. Daily count on the number of germinated seeds was recorded separately for each treatment and replications till the final count (8^{th} day). The trays were incubated at normal light at room temperature. Observations on germination percentage, speed of germination, Accumulated speed of germination, Mean daily germination, Germination value, Germination index, Emergence index, Root length, shoot length, Dry matter production and seedling vigour were worked out. The data were statistically analysed using ANOVA.

RESULT AND DISCUSSION

Seed hardening is one of the presowing seed enhancement techniques which had a significantly positive effect on different aspects of seed and seedling quality characteristics under laboratory and field condition. In this present laboratory study, seeds were evaluated for their physiological quality and Morphological qualities. The germination per cent was ranged from 99% to 80% which were significantly different over the various seed hardening treatment. The highest germination was observed in T_4 (99%) followed by T5 (97%) whereas the lowest per cent was recorded by T_0 (untreated seeds). Seed hardening treatment had increased the germination per cent of hardened seed over non hardened seeds. This increase in germination per cent may be due to the modification of physiological and biochemical nature of seed embryo and its associated structures, i.e. pre –enlargement of the embryo (Austin *et al*.,1969) and biochemical changes like

enzyme activation, Gibberellins like substances(Lee *et al*.,1998: Lee and Kim,2000: Basra *et al*.,2005) were released during the II phase of germination which triggers the synthesis of hydrolytic enzymes that causes the early availability of high energy compounds and vital biomolecules to the germinating seedling (Renugadevi and Vijayageetha,2006). The beneficial influence on higher germination (99%), Germination Index (12.44) and Emergence Index (3.205) in T_4 may be due to the presence of growth promoting substance present in 1% prosopis leaf extract migrates into the seed, might have brought this positive effect on seed germination and other germination indices. Early germination may be due to the greater hydration of colloids and higher viscosity of protoplasm and cell membrane that allows the early entrance of moisture that activates the early hydrolysis of reserve food materials in the seed when compared to untreated seeds. Prosopis leaf extract contains plant mineral nutrient like nitrogen (5.6%), phosphorus (P_2O_5- 0.9%), Potassium (K_2O – 3.11%) and Calcium (CaO – 1.0%) (Nadeem Binzia, 1992). The higher germination might be due to the role of calcium as an enzyme cofactor in germination process by increasing protein synthesis as reported by Christansen and Foy, 1979.

The beneficial effect of T_5 may be due to the manurial value of pongmia pinnata leaves which contains 1.16% Nitrogen, 0.14% Phosphorus, 0.49% Potash and 1.54% Lime (Duke, 1983; Singh,1982) and it contains various alkaloids like pinnalin, pongamol, Saponin, β-sitosterol and tannins (Savita sangwan *et al*.,2010). The presence of saponin, tannins, flavonoids glycosides and phenolic compounds in prosopis and pungam leaf extracts, would have triggers the germination process earlier thereby utilize the available nutrients. Saponins are readily soluble in water which is present in the pongamia leaf extract may enhanced the nutrient absorption and also protect the seedling against pathogens as reported by Satish *et al*., 2007 and anti- oxidant activity of prosopis leaf extract (Napar *et al*., 2012; Manisathiya and Muthuchelian,2010). The beneficial effect of T_4 and T_5 seed hardening may also due to free radical quenching property and counteracts the free radicals and reduce the damage effect by autoxidation. The increases in germination rate by T_4 and T_5 have been interpreted as the repair of accumulated damage that occurs during hydration cycle of seed hardening process (Burgass and Powell, 1984).

In addition to saponins and other nutrients, prosopis and pungam also had anti-oxidant properties which scavenges the free radical activity thereby improves the germination. The seedlings from untreated seeds failed to mobilize the reserves from the seeds during germination in the initial period may be the reason for poor germination but the hardened seeds made up the loss by using the improved synthesis of secondary metabolites and synthesizing the biomass through other physiological processes. Similarly like germination per cent, speed of germination and accumulated speed of germination are significantly different over other hardening treatment. T_4 treatment recorded higher speed of germination (16.42) and accumulated speed of germination (58.42) which indicates their earliness in germination which could be due to cell wall elasticity that paved the way for easy radicle emergence out of seed coat and the mobilization of food reserves to the growing seedlings. The Mean Daily germination was significantly more in T_4 (12.44) followed by T5 (12.13). The lowest mean daily germination was observed in control which indicates the poor daily germination. The highest value of mean daily germination in T_4 may due to the hardening process which causes earlier emergence of radicle and availability of various nutrients when compared to control. The shoot length and the seedling length were significantly different over other treatments. The Maximum shoot length (17.83cm) and maximum seedling length (33.30 cm) was recorded in treatment T_4 followed by T_5 (17.34 cm and 32.72 cm respectively). The small seedling (29.06 cm) with short shoot (15.56 cm) was observed in T_0. The stimulatory effect on germination and the growth of seedlings of hardened seed (T_4) 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 increased seedling growth and dry weight observed in T_4 treatment might be due to greater early vigour and higher percentage of germination because of which the seedling had reached autotorpic stage well in advance than control .The increase in dry weight was claimed to be due to enhanced lipid utilization and enzyme activity due to the presence of bioactive substances like auxin in prosopis leaf extract (Rathinavel and Dharmalingam, 1999) and development of seedling to reach autotropic stage and enabling them to produce relatively more quantity of drymatter which discerning the cause for the hike in vigour index by hardening treatment. T_4 (2042) hardened seeds increased germination value by 52% over untreated seedsT_0 (1339).

This increase in germination value may be due to the cumulative increase in germination and speed of germination by T_4 over T_0. The treatments were significantly different in case of seedling vigour I, seedling vigour II and Dry matter production. The highest seedling vigour I (3296.7), seedling vigour II (21.99) and more biomass production (0.2210 g / 10 seedlings) was recorded in T4 followed by T5 whereas low value was recorded in T_0. This may be due to the beneficial effect of prosopis leaf extract seed hardening which activates the growth promoting substances and translocations of secondary metabolites to the growing seedling. Physiologically active substances might have activated the embryo and other associated structures which resulted in the absorption of more water due to cell wall elasticity and development of stronger and efficient root system and that would have ultimately resulted in higher vigour index (Rangaswamy *et al*., 1993). Many researchers also reported the benefits of seed hardening with prosopis and pungam leaf extract to overcome the adverse condition (Rathinavel and Dharmalingam, 2000 in uppam cotton; Khab Bahadar Marwat and Muhammed Azim khan, 2006 in Wheat and Renugadevi *et al*., 2008 in Cluster bean). Thus from the present study, it could be concluded that green gram seeds should be hardened with 1% prosopis leaf extract for 3 hours @ 1/3 rd volume of solution to enhance the seed and seedling quality characteristics under adverse environment conditions. In addition, green gram seeds may also be hardened with 1% pungam leaf extract to get the similar results.

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