Mysore J. Agric. Sci., 56 (2) : 39-48 (2022)

Evaluation of Hard Seedness and Methods to Overcome in Green Gram

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ABSTRACT

The study was carried out to evaluate the hard seededness in the three popular varieties of greengram *viz*., KKM 3, WGG 42 and TRCRM 147 and laboratory study was conducted to break hard seededness with different methods *viz.,* hot water, acid and mechanical scarification, treatment with liquid nitrogen and freezing treatments and experiment was laid out with FCRD with three replications. Results revealed that, among the three varieties, WGG 42 having significantly lower number of hard seeds (16.15 %) compared to KKM 3 (18.46 %) and TRCRM 147 (19.92 %). Among the treatments, the seeds treated with Conc. H_2SO_4 for 2 min break the hard seededness and performs better results in seed quality parameters like germination (96.0 %), root length (11.71 cm), shoot length (26.35 cm), mean seedling length (38.06 cm), mean seedling dry weight (21.58 mg), seedling vigor index I (3652) and seedling vigor index II (207) compared to untreated control in seed quality parameters (32.44%, 9.94 cm, 16.31 cm, 26.25 cm, 19.58 mg, 841 and 64, respectively). WGG 42 reported less hard seed percentage and improve in 267 per cent of germination and Conc. H_2SO_4 is the better method to break the hard seeds in green gram.

Keywords : Green gram, Hard seeds, Scarification, WGG 42, TRCRM 147, KKM 3, Seed coat

GREEN GRAM, also called mung bean (*Vigna radiata* L.) is a major pulse crop in the agriculture sector. It can be consumed in a variety of ways such as whole grain, dal and sprouted. It is an excellent source of high quality protein with high digestibility. The seeds contain 24-25 per cent protein, 56 per cent carbohydrate, 1.3 per cent fat, 124 mg / 100 g calcium, and 326 mg / 100 g phosphorus. However, the acceptance and popularity of mung bean cultivation is often limited by the low germination of the immediate past year's seeds. The low seed germination is mainly attributed to the high level of hardseededness in mung bean, which reduces its plantability in next season. The seed germinability is reported to progressively decline with the increase in the proportion of hard seeds in the seed lot (Paul *et al*., 2020).

Hard seed (that is the presence of a hard seed coat that blocks the germination process by not allowing water to pass to embryo) is the main cause of dormancy in most species of *Leguminosae*. Seeds of different species may differ in the site(s) of water uptake during imbibition. The cell layer in the seed lens area is thin and is likely to be the water-gap (Channakeshava, 1999 and Baskin, 2003). Most legume crop plants produce hard seeds in varying percentages. Most workers have found this trait to be highly heritable (Jayasuriya *et al.,* 2013). Hard seededness in mung beans creates problems in testing for germinability under laboratory conditions. Due to this state of affair, there is a great problem under field condition in securing uniform germination and good crop stand for maximum crop production.

This problem can be overcome by application of the several pretreatments available, *viz*., seed treatment include mechanical scarification (Pandrangi *et al*., 2003) and acid treatment (Can *et al*., 2009) with sulfuric acid, mechanical scarification with sandpaper or heat treatments (Pandita *et al*., 1999), hot water (McNair, 1917), liquid nitrogen (Pritchard *et al*., 1988). Liquid nitrogen exposure influences the structure / integrity of the hilar region, cuticle and macrosclereids in relation to seed imbibition rate and germination (Singh, 2005).

This attribute is of ecological importance, but it also poses practical problems. The large numbers of hard seeds in a given lot limits germination under laboratory conditions and field emergence, creating difficulties for seed testing. To ensure optimum plant population size in the field it is essential to know the amount of hard seeds in various cultivars of greengram so that corrective measures for over coming this can be taken. The presence of seed dormancy seriously interferes with seed management and uses. In view of the above, a research programme has been undertaken to evaluate the methods to overcome hardseededness in mung bean.

Material and Methods

The freshly harvested seeds of popular green gram varieties *viz*., KKM 3 (V_1), WGG 42 (V_2) and TRCRM 147 (V_3) were collected from the field and used for this study. Germination test was carried out with four replications under controlled conditions as per the ISTA (Anonymous, 2008). The study was carried out in FCRD. Seeds were subjected to the following treatments before germination test *viz*.,

- T_1 : Immersion in hot water @ 100^{0}C for 2 minutes
- T_2 : Immersion in hot water @ 100^{0}C for 3 minutes
- T_3 : Chemical scarification in Conc. Sulphuric acid for 1 minute
- T_4 : Chemical scarification in Conc. Sulphuric acid for 2 minutes
- T_5 : Chemical scarification in Conc. Nitric acid for 1 minute
- T_6 : Chemical scarification in Conc. Nitric acid for 2 minutes
- T_7 : Sand paper scarification for 2 minutes
- T_8 : Sand paper scarification for 4 minutes
- T_9 : Treatment with Liquid nitrogen for 5 minutes
- T_{10} : Treatment with Liquid nitrogen for 10 minutes
- T_{11} : Treatment with Liquid nitrogen for 20 minutes
- T_{12} : Seeds exposed with - 18^0 C for 24h
- T_{13} : Control

With the above treatments selected, we studied the effect of different breaking methods on seed quality parameters like germination (%), root length (cm), shoot length (cm), mean seedling length (cm), mean seedling dry weight (mg / seedling), seedling vigour index I and seedling vigour index II at Department of Seed Science and Technology, GKVK, Bengaluru.

Results and Discussion

The data obtained in the experiment is presented and discussed here. Seeds which were collected from field were studied to get the hard seeds for experimental purposes. Significant difference was observed among three varieties in hard seedness percentage and the given treatments were also significantly worked in breaking the hard seeds of three green gram varieties.

The significant difference was observed in three varieties and thirteen treatments on all the seed quality parameters. Interaction studies also significantly differed. Germination has significantly differed among three varieties and the variety WGG 42 showed the highest germination (79.31 %) followed by KKM 3 (75.64 %) and the lowest was in TRCRM 147 (72.82 %) (Fig.1). It represents that WGG 42 performs better in breaking methods of hard seeds. Removal of hard seededness involves disturbing of the water-gap structures in response to environmental factors causing the seeds to become water permeable.

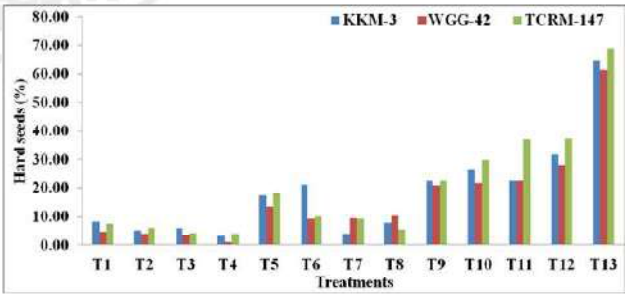


Fig.1 : Effect of hard seed breaking methods on hard seeds (%) in three greengram varieties (KKM 3, WGG 42, TRCRM 147)

Among different treatments studied seed treated with Conc. sulphuric acid for 2 min (96.00 %) showed the highest germination than other treatments and followed by Conc. sulphuric acid for 1 min (94.22 %) and hot water treatment for 2 and 3 min (91.67 % and 93.63 %, respectively) and sandpaper

TABLE 1

Effect of hard seed breaking methods on seed germination (%), hard seeds (%), abnormal seedlings (%) in three greengram varieties

Varieties (V)	Germination (%)	Hard seeds (%)	Abnormal seedlings (%)
V_1 : KKM-3	75.64	18.46	5.90
V_2 : WGG-42	79.31	16.15	4.54
V_3 : TRCRM-147	72.82	19.92	7.26
Mean	75.92	18.18	5.90
SEm±	0.58	0.57	0.36
CD (P = 0.05)	1.63	1.61	1.01
Treatments (T)			
T_1 : Hot water treatment @100^{0}C for 2 min	91.67	6.67	1.67
T_2 : Hot water treatment @100^{0}C for 3 min	93.33	4.89	1.78
T_3 : Treatment with Conc. H_2SO_4 for 1 min	94.22	4.33	1.44
T_4 : Treatment with Conc. H_2SO_4 for 2 min	96.00	2.56	1.44
T_5 : Treatment with Conc. HNO_3 for 1 min	78.11	16.33	5.56
T_6 : Treatment with Conc. HNO_3 for 2 min	80.00	13.56	6.44
T_7 : Sand paper scarification for 2 min	86.11	7.56	6.33
T_8 : Sand paper scarification for 4 min	88.56	7.78	3.67
T_9 : Liq Nitrogen for 5 min	67.56	22.11	10.33
T_{10} : Liq Nitrogen for 10 min	62.78	25.89	11.33
T_{11} : Liq Nitrogen for 20 min	59.89	27.44	12.67
T_{12} : -18º C	56.33	32.33	11.33
T_{13} : Control	32.44	64.89	2.67
Mean	75.92	18.18	5.90
SEm±	1.21	1.19	0.75
CD (P = 0.05)	3.40	3.35	2.10
Varieties (V) * Treatments (T)			
V_1T_1	90.00	8.33	1.67
V_1T_2	92.67	5.00	2.33
V_1T_3	93.00	5.67	1.33
V_1T_4	95.33	3.00	1.67
V_1T_5	77.33	17.33	5.33
V_1T_6	73.67	21.33	5.00
V_1T_7	91.33	3.67	5.00
V_1T_8	89.00	7.67	3.33
V_1T_9	68.67	22.67	8.67
V_1T_{10}	61.00	26.33	12.67
V_1T_{11}	63.33	22.67	14.00
V_1T_{12}	56.00	31.67	12.33
V_1T_{13}	32.00	64.67	3.33

Varieties (V)	Germination (%)	Hard seeds (%)	Abnormal seedlings (%)
V_2T_1	94.33	4.33	1.33
V_2T_2	95.00	3.67	1.33
V_2T_3	96.00	3.33	0.67
V_2T_4	97.33	1.00	1.67
V_2T_5	84.33	13.67	2.00
V_2T_6	88.33	9.33	2.33
V_2T_7	88.00	9.67	2.33
V_2T_8	87.00	10.33	2.67
V_2T_9	69.67	21.00	9.33
V_2T_{10}	67.33	21.67	11.00
V_2T_{11}	64.00	22.67	13.33
V_2T_{12}	63.33	28.00	8.67
V_2T_{13}	36.33	61.33	2.33
V_3T_1	90.67	7.33	2.00
V_3T_2	92.33	6.00	1.67
V_3T_3	93.67	4.00	2.33
V_3T_4	95.33	3.67	1.00
V_3T_5	72.67	18.00	9.33
V_3T_6	78.00	10.00	12.00
V_3T_7	79.00	9.33	11.67
V_3T_8	89.67	5.33	5.00
V_3T_9	64.33	22.67	13.00
V_3T_{10}	60.00	29.67	10.33
V_3T_{11}	52.33	37.00	10.67
V_3T_{12}	49.67	37.33	13.00
V_3T_{13}	29.00	68.67	2.33
SEm±	2.09	2.06	1.29
CD (P = 0.05)	5.88	5.80	3.64
CV (%)	4.77	19.61	37.95

scarification for 2 and 4 minutes (86.11 % and 88.56 %, respectively) (Table 1 and Plate 1). Sulphuric acid disrupts the seed coat and expose the lumens of the macrosclereids cells, permitting imbibition of water, which triggers seed germination (Amusa, 2011). This was consistent with results from other studies that reported prompt and uniform germination in hard water-impermeable seed coated seeds soaked in sulphuric acid (McDonald and Omoruyi, 2003; Keshtkar *et al.,* 2008; Likoswe *et al*., 2008 and Aref *et al.,* 2011). Seeds treated with hot water for 3 min (93.63 %) and Conc. sulphuric acid for 1 min (94.22

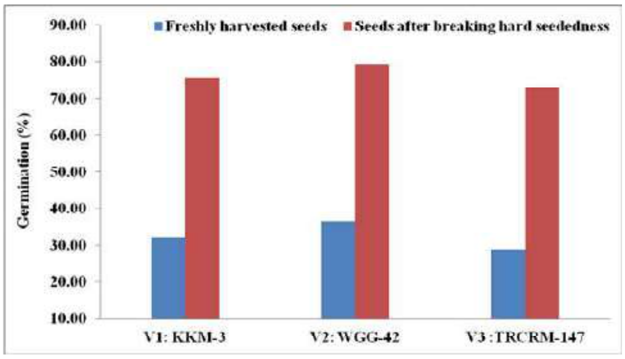


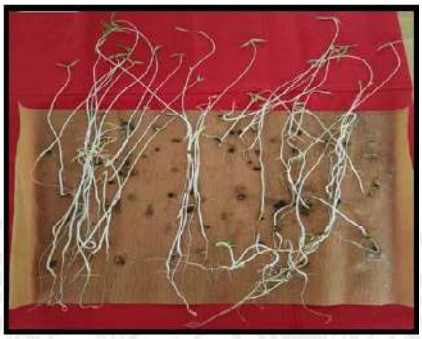
Fig. 2 : Evaluation of germination (%) in three greengram varieties of freshly harvested and seeds exposed after different treatments

T_4 : Seeds treated with Conc. H_2SO_4

T_2 : Seeds treated with Hot water

T_8 : Seeds treated with sand paper

T_6 : Seeds treated with Conc. HNO_3



T_9 : Seeds treated with liq N_2

T_6 : Seeds treated with Conc. HNO_3

Plate 1 : Effect of hard seed breaking methods on seed germination

%) on par with each other in showing germination percentage (Table 1). Temperature changes cause tensions between the different tissues that make up the seeds, which generate expansion and contraction that cause cracks in the seed coat. These cracks, in the seeds, are associated with the entry of water and with this the beginning of germination. In seeds treated with liquid nitrogen for 5 min (67.56 %) showed the better when compared with 10 min and 20 min (62.89 and 59.89 %, respectively), also seed exposed with -18^0C also showed less germination (56.33 %), which were less than acceptable level minimum germination as per certification standards. In control only 32.44 per cent germination was observed (Table 1 and Plate 1 D-F).

Interaction studies also significantly differed among treatments and varieties, the highest germination was found in V_2T_4 (97.33 %) and the lowest was found in V_3T_{13} (29.00 %). Since seed coat rupturing implies water absorption, these results indicated that, regardless of other factors such as fungal contamination, the most suitable techniques to break down hard seededness. This could be due to seed coat properties like water impermeable palisade cell structure and composition which are influenced by genotype and environment during production.

Hard seed percentage significantly differed among three varieties; WGG 42 showed the least hard seed (16.15 %) than KKM 3 (18.46 %) and TRCRM 147 (19.92 %). It represents that WGG 42 variety performs better to the breaking methods of hard seeds. Among different treatments studied seed treated with Conc. sulphuric acid for 2 min (2.56 %) showed the lowest hard percentage than other treatments and Conc. sulphuric acid for 1 min (4.33 %) followed by hot water treatment for 2 and 3 min (6.67 % and 4.89 %, respectively). Seed treated with sandpaper scarification for 2 and 4 min (7.56 and 7.78 %, respectively), seeds treated with hot water for 3 min (4.89 %) and Conc. sulphuric acid for 1 minute (4.33 %) on par with each other in showing hard seededness (Table 1, Fig. 1). Immersion in hot water is a effective in overcoming hard seededness when compared with other methods like mechanical and chemical scarification. In seeds treated with liquid nitrogen for 5 min (22.11 %) showed the less hard seed when

TABLE 2

Effect of hard seed breaking methods on mean seedling length (cm), mean seedling dry weight (mg), seed vigour index-I and seed vigour index-II

Varieties (V)	Mean seedling length (cm)	Mean seedling dry weight (mg)	Seed vigour index -I	Seed vigour index -II
V_1 : KKM-3	30.71	21.04	2419	159
V_2 : WGG-42	32.60	22.27	2666	177
V_3 : TRCRM-147	31.86	21.76	2412	159
Mean	31.73	21.69	2498	165
SEm±	0.19	0.17	22.88	1.89
CD (P = 0.05)	0.54	0.47	64.0	5.0
Treatments (T)				
T_1 : Hot water treatment @100^{0}C for 2 min	35.94	21.94	3295	201
T_2 : Hot water treatment @100^{0}C for 3 min	37.08	20.08	3462	188
T_3 : Treatment with Conc. H_2SO_4 for 1 min	37.25	20.53	3511	193
T_4 : Treatment with Conc. H_2SO_4 for 2 min	38.06	21.58	3652	207
T_5 : Treatment with Conc. HNO_3 for 1 min	33.55	22.48	2623	176
T_6 : Treatment with Conc. HNO_3 for 2 min	34.40	23.47	2753	187
T_7 : Sand paper scarification for 2 min	35.28	22.49	3033	194
T_8 : Sand paper scarification for 4 min	34.71	22.56	3074	199
T_9 : Liq Nitrogen for 5 min	30.06	21.82	2040	148
T_{10} : Liq Nitrogen for 10 min	25.52	21.59	1619	135
T_{11} : Liq Nitrogen for 20 min	22.33	22.57	1342	135
T_{12} : -18^o C	22.03	21.28	1242	120
T_{13} : Control	26.25	19.58	841	64
Mean	31.73	21.69	2498.97	165.19
SEm±	0.40	0.35	47.64	3.94
CD (P = 0.05)	1.12	0.97	134	11
Va rieties (V) * Treatments (T)				
V_1T_1	34.48	21.67	3103	195
V_1T_2	35.42	20.50	3286	190
V_1T_3	36.23	20.80	3370	193
V_1T_4	36.84	20.60	3511	196
V_1T_5	33.07	22.00	2556	170
V_1T_6	33.95	21.67	2503	160
V_1T_7	34.22	20.30	3126	186
V_1T_8	33.78	20.83	3006	185
V_1T_9	32.65	20.33	2245	140
V_1T_{10}	23.23	21.33	1420	130
V_1T_{11}	22.80	22.07	1446	140
V_1T_{12}	21.08	20.53	1180	115

Varieties (V)	Mean seedling length (cm)	Mean seedling dry weight (mg)	Seed vigour index -I	Seed vigour index -II
V_1T_{13}	21.54	20.83	689	67
V_2T_1	36.17	22.30	3412	210
V_2T_2	38.03	20.37	3609	194
V_2T_3	37.67	19.97	3616	192
V_2T_4	38.48	21.90	3745	213
V_2T_5	33.85	23.03	2856	194
V_2T_6	34.42	21.27	3038	188
V_2T_7	35.93	27.03	3154	237
V_2T_8	33.31	26.73	2896	232
V_2T_9	33.76	22.13	2348	154
V_2T_{10}	32.86	20.77	2215	140
V_2T_{11}	23.07	22.30	1477	143
V_2T_{12}	22.55	20.97	1428	133
V_2T_{13}	23.78	20.73	864	75
V_3T_1	37.16	21.87	3369	198
V_3T_2	37.80	19.37	3490	179
V_3T_3	37.84	20.83	3546	195
V_3T_4	38.87	22.23	3700	212
V_3T_5	33.74	22.40	2456	163
V_3T_6	34.83	27.47	2719	214
V_3T_7	35.70	20.13	2820	159
V_3T_8	37.03	20.10	3321	180
V_3T_9	23.77	23.00	1527	149
V_3T_{10}	20.47	22.67	1222	136
V_3T_{11}	21.13	23.33	1104	122
V_3T_{12}	22.47	22.33	1117	111
V_3T_{13}	33.43	17.17	970	50
SEm±	0.69	0.60	82.51	6.82
CD (P = 0.05)	1.94	1.69	232.31	19.19
CV (%)	3.76	4.78	5.72	7.15

compared with 10 min and 20 min (25.89 and 27.44 %, respectively), also seed exposed with -18^{0}C shown more (32.33 %) hard seededness. In control 88.33 per cent hard seed percentage was observed.

Interaction studies also significantly differed among treatments and varieties. The lowest hard seeds was found in V_2T_4 (1.00 %) and found highest in V_3T_{13} (68.67 %). Seed surface morphology reveals that hard and impermeable testa is the main barrier for imbibitions and consequently retards germination. Similar hard seed-coated dormancy is common in other legumes also. Extreme hard seed coat in the mature, dry state is generally due to the presence of heavily thickened galactomannan or mannan polymers on the walls of the endosperm cells (Mojeremane *et al*., 2017).

In KKM 3 variety, effect of different hard seed breaking methods was studied. Among studied methods on quality parameters, the highest shoot length (25.10

cm), root length (11.74 cm), mean seedling length (36.84 cm), mean seedling dry weight (20.60 mg), seedling vigor index I and II (3511 and 196) was observed in seeds treated with Conc. sulphuric acid for 2 minutes, which is followed by hot water treatment for 2 and 3 min and sandpaper scarification for 2 and 4 min. Mechanical scarification, however, may be very time consuming, especially if a large number of seeds are required (Fig. 3). Seeds treated with Conc. sulphuric acid for 1 min and hot water treatment for 3 min shown on par in root length (11.38 and 11.32 cm), shoot length (24.85 and 24.10 cm), mean seedling dry weight (20.80 and 20.50 mg), seedling vigor index II (193 and 190 compared to control (11.49 cm, 10.05 cm, 21.54 cm, 20.83 mg, 1154 and 111, respectively) in all quality parameters (Table 1 and 2, Plate 1). From the results, it can be concluded that Conc. sulphuric acid was the best treatment to break hard seeds in KKM 3 variety.

The macrosclereid cell layer, consisting of very rigid dead and lignified cells, often breaks during these contractions. In contrast, the tissues that make up the embryonic axis are in a more flexible vitreous or gummy state (Bewley *et al.,* 2013). During cooling and rewarming they do not undergo permanent (deleterious) anatomical modifications. The results obtained in the present investigation confirms the finding of Lambat *et al.* (2020) and Cherian *et al.* (2011) in pigeon pea.

In WGG-42 variety, studies on quality parameters Conc. sulphuric acid for 2 minutes showed highest shoot length, root length, mean seedling length, mean seedling dry weight, seedling vigor index I and II (26.53 cm, 11.57 cm, 37.67 cm, 19.97 mg, 3745, 213, respectively) (Fig. 3). Seeds treated with Conc. sulphuric acid for 2 min and hot water treatment for 3 min shown on par in root length (11.93, 11.57 cm) and mean seedling length (38.03, 38.48 cm). Also, seeds treated with Conc. sulphuric acid for 1 min and hot water treatment for 3 min shown on par in shoot length (26.53, 26.10 cm), mean seedling dry weight (19.97, 20.37 mg), seedling vigor index I (3616, 3609) and seedling vigor index II (192, 194), respectively. Seeds treated with liquid N_2 shown lowest in all parameters. Compared to all treatments control shown lowest (13.71 cm, 10.06 cm, 23.78 cm, 20.73 mg, 1356, 117, respectively) (Table 1 and 2) in all quality parameters.

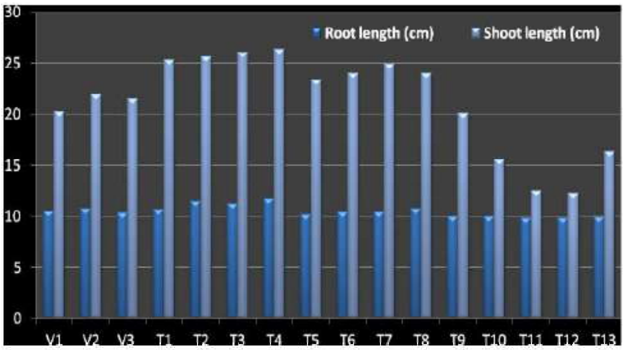


Fig. 3 : Effect of various hard seed breaking methods on root and shoot length in green gram

Varieties :

V_1: KKM-3
V_2: WGG-42
V_3: TRCRM-147

Treatments :

T_1: Immersion in hot water @ 100^{0}C for 2 minutes
T_2: Immersion in hot water @ 100^{0}C for 3 minutes
T_3: Chemical scarification in Conc. Sulphuric acid for 1 minute
T_4: Chemical scarification in Conc. Sulphuric acid for 2 minutes
T_5: Chemical scarification in Conc. Nitric acid for 1 minute
T_6: Chemical scarification in Conc. Nitric acid for 2 minutes
T_7: Sand paper scarification for 2 minutes
T_8: Sand paper scarification for 4 minutes
T_9: Treatment with Liquid nitrogen for 5 minutes
T_{10}: Treatment with Liquid nitrogen for 10 minutes
T_{11}: Treatment with Liquid nitrogen for 20 minutes
T_{12}: Seeds exposed with -18^{0}C for 24h
T_{13}: Control

A seed with a permeable testa absorbs water quickly (within minutes to hours), while an impermeable seed does not absorb water even after several days or weeks. From the results, it can be concluded that Conc. sulphuric acid is the best treatment to break hard seeds in WGG 42 variety. The results are confirmed in black gram with the findings of Gangaraju and Balakrishna (2016) and Guma (2010).

TRCRM 147, reported Conc. sulphuric acid for 2 min shown highest shoot length, root length, mean seedling length, mean seedling dry weight, seedling vigor index I and II (27.03 cm, 11.83 cm, 38.87 cm, 22.23 mg, 3546, 195) (Fig. 3) and it can be concluded that it was the rapid desiccation produced by chemical and not its hydrolytic capacity which seems to cause fragmentation of integuments and thus allowing the passage of water to the embryo. Seeds treated with Conc. sulphuric acid for 1 min and hot water treatment for 3 min shown on par in root length (11.13, 11.10 cm), shoot length (26.71, 26.70 cm), mean seedling length (37.84, 37.80 cm). Seeds treated with liquid N_2 (Plate 1) and -18^{0}C was shown lowest in all parameters.

The opening of the hilar region and the cracks observed in the cuticle and the macrosclereid cell layer in the seeds exposed to the LN **is** the result of contractions brought about by the cooling and subsequent rewarming of the seeds. This is mainly due to differences in the cooling rate, which is influenced by their respective chemical composition. These differences in cooling rates cause contractions between tissues that in turn cause different coefficients of expansion. From the results, it is clear that Conc. sulphuric acid was the best treatment to break hard seeds in TRCRM 147 variety (Table 1 and 2). These results were similar in performed treatments with sulfuric acid induced increases and uniformity in germination (De Morais *et al*., 2014 and Kumar, 2020).

In the present experiment, the untreated seeds were not germinated and remained hard throughout the germination test. In contrast, all methods used in this study to overcome hard seededness improved seed germination. Mechanical scarification and immersion for 2 minutes in sulphuric acid were the most effective treatments, resulting in higher final germination than other treatments. Interestingly, boiling water treatment improved germination significantly than other treatments. Implications for such relatively simple and cheap methods require little skill and can be practically applied for large-scale seedling production and restoration measures. However, acid scarification is considered a potentially risky treatment for seeds and those carrying out the treatments and it is not recommended as a general operation for removing hard seededness. Acid scarification can be used an alternate method when no other methods are available, with a careful preliminary verification. The effectiveness of boiling water observed in this work could probably be attributed to the softening of the hard seed coat, which allowed entrance of water and air into the seed that subsequently triggered germination in green gram.

The presence of hard seeds in the green gram is mainly due to their hard seed coat covering. The acid scarification, hot water of hard seeds effectively enhanced the germination of all three varieties. Among these, WGG 42 responded better with the Conc. sulphuric acid for 2 minutes treatment and germination increased with reduction in the hard seeds compared to KKM 3 and TRCRM 147.

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(*Received :* August 2021 *Accepted :* February 2022)