

Pre-treatment effect on seed germination of *Calopogonium mucunoides*: A promising cover crop for forest land restoration and climate resilience

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Abstract

Calopogonium mucunoides have been widely used as a pasture legume, cover crop and nitrogen fixing plant in tropical and sub-tropical regions. It reduces soil erosion, controls wind and water erosion; improve soil properties and adapts to wide climatic conditions. The present study investigated the effect of different pre-treatment on germination percent (GP), mean germination time (MGT), germination value (GV), peak value (PV) and germination index (GI) of *C. mucunoides*. The experiment was conducted at Forest Research Institute, India. Treatment used were hot water soaking for 6 hrs at 40, 60, 80 & 88 °C; acid scarification using H₂SO₄ in 1, 3 & 5% concentration; sand scarification, GA₃ treatment at 50 & 100ppm; KNO₃ at 0.1, 1 & 3%. Germination parameters such as GP (ISTA, 2010); MGT (Orchard, 1977); GV (Djavanshir & Pourbeik, 1976); PV (Czabator, 1962), and GI (Timson, 1965) of the species were measured as per the standard methods. Experiment was conducted using CRD in a seed germinator at 25±1 °C. ANOVA was performed, and significant treatment means were separated by Duncan's new multiple range tests. Highest GP was observed as 97% at 88 °C followed by 87, 86 & 83% at 80, 60 & 40 °C hot water treatment respectively. Sand scarification method exhibited the GP of 80%. Lowest MGT was recorded in GA₃ (50 & 100ppm) and hot water (88 °C). Maximum GV, PV and GI were recorded in the seed treated with 88 °C hot water. The result of ANOVA showed a significant difference (P<0.05) in the effect of GP, MGT and GV using different pre-treatment. Pre-treatment had a significant role in the germination parameters of *C. mucunoides*. In laboratory, untreated seed produce the GP of 56% while sand scarification and hot water soaking had increase the GP ranges from 80-97% appears to be more promising, cost effective and safest method for large scale cultivation of these cover crops to prevent soil erosion and restore the soil fertility of wastelands of tropical regions of the world.

Keywords: Cover crop, pre-treatment, dormancy, germination percent, mean germination time.

Introduction, scope and main objectives

Cover crops have a long tradition of use in agriculture, horticulture and environmental engineering (Wistrom et al., 2018) and are considered as the tool for improving soil quality, soil fertility and nutrient holding ability of soil. It reduces soil erosion, control wind and water erosion (Naderman, 1991, Doran and Smith, 1987; Power, 1990; Hargrove, 1991). It improves soil physical, chemical, biological properties and improves water infiltration rate (Unger and Vigil 1998), nitrogen recycling and enrichment thus, reducing the N fertilizers requirement for the succeeding crop (Decker et al., 1994; Singh, et al., 2004). It promotes the growth of the arbuscular mycorrhizal fungi that can enhance water and nutrient uptake by the plants (Zak, et al., 1998). Cover crops have the potential to increase soil carbon sequestration, mitigate emission of greenhouse gas, provide benefits to soil health, feed for livestock, bio fuel production and farm economics. Cover crops can also improve soil hydraulic properties such as water infiltration, water retention capacity, and saturated hydraulic conductivity through increased soil aggregation. Runoff losses can decrease up to 80% and sediment loss from 40 to 96% with cover crops (Canqui et al., 2015). Poeplau and Don (2015) estimated that cover crops can sequester about $0.32 \pm 0.08 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ of carbon to 22cm soil depth. Planting cover crops is a proactive and crucial step to sound soil management and accumulate benefits for long-term soil stewardship. Long term use of cover crop can increase yields, save on nitrogen costs over time and lead to more profitable system. The genetic resources of cover crops have vast and often untapped potential.

C. mucunoides have been widely introduced as a forage and pasture legume, green manure, cover crop and nitrogen fixing herbaceous plant in tropical and sub-tropical regions (Cook et al., 2015). The species adapt to a wide range of soil textures and pH ranges and is tolerant to wide climatic conditions and nodulates well in very wet conditions (Skerman, 1977). The species is well tolerant to shade and have excellent compatibility with grasses and other legumes and suppresses the growth of weeds. Bermudez et al., (1968) recorded 16.7% crude protein in the dry matter in the species. Vergara (1967) recorded an average phosphorous content of 0.25% and 1% calcium in dry matter (Skerman, 1977). *Calopogonium* grows well in acid soil which fixes 3.8 mg N₂ per day per plant and 87 % of fixed N₂ is transferred to the top soil (Trivedi, 2002).

Calopogonium mucunoides is one of the promising cover crop for improving the soil characteristics and restoration of land. Root of *C. mucunoides* can improve the soil fertility and can reduce the surface runoff and nutrient leaching by increasing the soil porosity and infiltration rate. Introduction of this species can improve the wasteland, fallow lands and forest land facing extreme soil related problems. Physical dormancy associated with hardseedness in *Calopogonium mucunoides* causes poor germination percent and uneven germination rate are the major problems associated with the species. Seed dormancy is common in Fabaceae (subfamilies *Caesalpiinoideae*, *Mimosoideae* and *Papilionoideae*) due to hard and impermeable seed coat. (Rolston, 1978; Rusdy, 2017). The hard seed coat prevents imbibitions and gaseous exchange that leads to physical dormancy and subsequently, it is the foremost cause of poor and erratic germination (Sharma et al., 2008). Mechanical scarification, acid scarification, and hot water treatment are recommended for breaking physical dormancy of tropical and sub tropical herbaceous and tree legumes (Doran et al., 1983; Prasad and Nautiyal, 1996; Veasey and Freitas 2002; Kavita et al., 2015; Rusdy 2015).

The present study investigated the effect of different pre-treatment on germination percent (GP), mean germination time (MGT), germination value (GV), peak value (PV) and germination index (GI) of *C. mucunoides*.

Methodology/approach

Experimental site

Seeds of *C. mucunoides* were obtained from Thrissur, Kerala. The experiment was conducted at Forest Tree Seed Laboratory, Forest Research Institute, Dehradun, India to evaluate the effect of different pre treatments on seed germination of *C. mucunoides*.

Experimental design

Experiment was conducted in a Complete Randomized Design with four replications of 25 seeds for each treatment and control. Each Petri dish contained 25 seeds representing an experimental unit (25 seeds × 4 replicates × control/hot water/acid scarification/sand scarification/growth promoters=52 experimental units). Seed were placed on Whatman filter paper in a petri dish and kept in a seed germinator at 25±1°C with 24 hour photoperiod and high relative humidity (RH > 90%). Seed germination was recorded on a daily basis from the day of sowing. Emergence of radicle from the seed was counted as germinated. Seed germination experiment was extended up to 21 days from sowing.

Seed pre treatment

Seeds were subjected to different pre-treatments viz., Hot water soaking at 40°C, 60°C, 80°C, 88°C; acid scarification using 98% conc. H₂SO₄ (1% (v/v), 3% (v/v), 5% (v/v)); sand scarification, GA₃ treatment (500ppm and 1000ppm); KNO₃ treatment (0.1% (w/v), 1% (w/v), 3% (w/v)).

Hot water treatment

In hot water treatment, 100ml of distilled water was preheated at four temperatures 40°C, 60°C, 80°C, 88°C. 100 Seeds were soaked in hot water at 40°C, 60°C, 80°C and 88°C and allowed to cool to room temperature and left in the water for 6 hours (25 seed × 4 replicates × 4 temperature level= 16 experimental units). Treated seeds were dried before germination test.

Acid scarification

Conc. H₂SO₄(98%) at 1% (v/v), 3% (v/v), 5% (v/v) was used for acid scarification. Standard stock solutions were prepared in a volumetric flask by dissolving 1 ml, 3 ml and 5 ml conc. H₂SO₄ in 100 ml of distilled water. The sample containing 100 seeds were soaked for 5 minutes in the different concentrations of standard solutions of H₂SO₄ (25 seed × 4 replicates × 1% (v/v)/3% (v/v)/ 5% (v/v) conc. H₂SO₄ = 12 experimental units). Seeds were washed thoroughly in running tap water, allowed to dry for a few minutes and plated in a petri dish.

Sand scarification

Sand scarification was performed by using sterilized quartzite sand which weighs half the weight of 100 seeds. Sand along with seeds were shaken well in a bottle for 15 minutes (25 seed × 4 replicates × sand scarification = 4 experimental units). The seeds scarified with sand were washed in tap water allowed to dry and plated on a petri dish.

Gibberellic acid treatment

Standard solution of 500 ppm and 1000 ppm Gibberellic acid were prepared in two volumetric flasks by dissolving 50 mg and 100 mg gibberellic acid in 100 ml water. The sample containing 100 seeds were soaked for 24 hours in different concentrations of standard solutions of GA₃ (25 seed × 4 replicates × 500ppm /1000ppm GA₃= 8 experimental units). Treated seed were dried before germination test.

Potassium nitrate treatment

0.1% (w/v), 1% (w/v), 3% (w/v) KNO₃ standard solutions were prepared in three volumetric flasks by dissolving 0.1 g, 1 g, and 3 g KNO₃ salt in 100 ml of water. The sample containing 100 seeds were soaked for 24 hours in different concentrations of standard solutions of KNO₃ (25 seed × 4 replicates × 0.1% (w/v)/ 1% (w/v)/ 3% (w/v) KNO₃ = 12 experimental units). Treated seed were dried before germination test.

Control

In control, seeds were not subjected to any treatment before germination test (25 seed × 4 replicates × control = 4 experimental units).

Statistical analysis

Statistical analysis of germination data was performed with SPSS 16.0 software package. The data was subjected to analysis of variance (ANOVA), Duncan, Dunnett t (2-sided) post hoc test was used for testing the significance of different pre-treatments against control treatment as standard reference on GP. Tukey HSD method was used for checking the significance level of MGT and GV between treatments.

Germination parameters such as germination percent (ISTA, 2010); Mean Germination Time (Orchard, 1977); Germination value (Djavanshir and Pourbeik, 1976); Peak value (Czabator, 1962), and Germination Index (Timson, 1965) of the species were measured as per the standard methods. Germination percent of seed was expressed as; Germination percentage (G) = total number of seed germinated at end of germination test/total number of seeds taken for germination test. Mean germination time (MGT) = $\sum Fx/\sum F$; where F is the number of seeds germinated on day x. Timson germination index = $\sum G/T$, where G is the percentage of seed germinated per day, and T is the germination period. Germination value was expressed as $\sum DGS/N \times$ (Final cumulative Germination Percent/10); where DGS is daily germination speed which is calculated by dividing cumulative germination percent by the number of days since beginning the test, N is number of counts and 10 is constant through germination test.

Results

The present study investigated the effect of different pre-treatments on germination percent, mean germination time, germination value, and peak value and germination index of *C. mucunoides*.

Seeds of *Calopogonium mucunoides* exhibited highest germination percent in hot water treatments. Highest germination percent was observed in hot water treatment at 88°C (97%) followed by 80°C (87%), 60°C (86%), 40°C (83%) and sand scarification (80%). Seed treatment with 1% (v/v), 3% (v/v), and 5% (v/v) H₂SO₄ exhibited 53%, 64% and 58% of germination percent, respectively. Seed soaked in 500 ppm and 1000 ppm, GA₃ recorded the germination percent of 74.5% and 67.5% respectively. 0.1% (w/v), 1% (w/v) and 3% (w/v) KNO₃ exhibited the germination percent of 58.5%, 67.5% and 64% respectively. Species exhibited poor germination percent (56%) in control (Figure 1).

Mean germination time of seeds varies with different pre treatments (Figure1). Lowest mean germination time was recorded in 0.1% (w/v) KNO₃ (3.32 days) followed by 1% (w/v) KNO₃ (3.57 days), 500 ppm GA₃ (4.68 days), 3% (w/v) KNO₃ (4.76 days), 1000 ppm GA₃ (4.82 days) and 88°C hot water (5.58 days). Highest mean germination time was observed in 5% (v/v) H₂SO₄ (10.47 days) followed by 1% (v/v) H₂SO₄ (8.78 days) (Figure1).

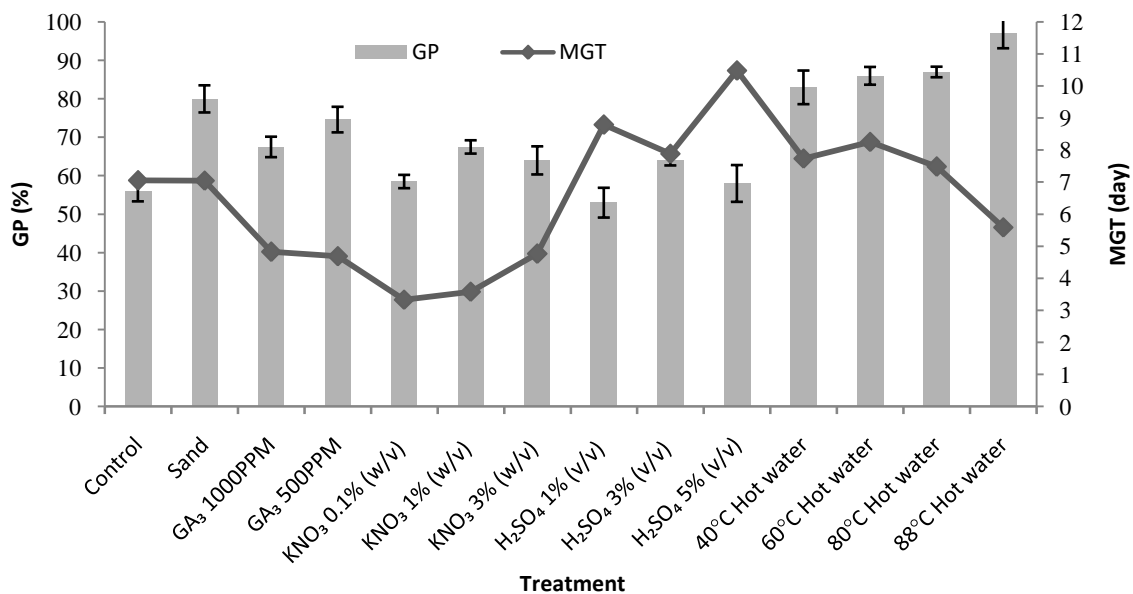


Fig 1: Effect of seed pre-treatments on germination percent and mean germination time in *Calopogonium mucunoides*. Vertical error bars represent the standard deviation.

Germination value ranged between 12.33 to 61.18 in *C. mucunoides* subjected to different pre-treatments. Highest GV was observed in 88°C hot water (61.18) followed by 500 ppm GA₃ (47.23). In addition, GV>35 was observed in 60°C hot water (38.52), 40°C hot water (37.66), sand (36.83), 80°C hot water (36.04) and 1% (w/v)

KNO₃ (35.66). Lowest GV was observed in 0.1% (w/v) KNO₃ (12.33) followed by 1% (v/v) H₂SO₄ (12.91) and control 500 ppm GA₃ (16.96) (Figure 2).

In *C. mucunoides*, peak value ranged from 3.69 to 14.75 by using different pre treatments. Highest peak value was observed in the seeds treated with 88°C hot water (14.75) while lowest peak value was observed in 5% (v/v) H₂SO₄ (3.69) (Figure 2).

Germination index of the species ranged from 1.46 to 21.18. Highest GI was observed in the seeds treated with 500 ppm GA₃ (21.18) followed by 1000 ppm GA₃ (11.85) while lowest GI was observed in 5% (v/v) H₂SO₄ (3.69) followed by 1% (v/v) H₂SO₄ (1.55) (Figure 2).

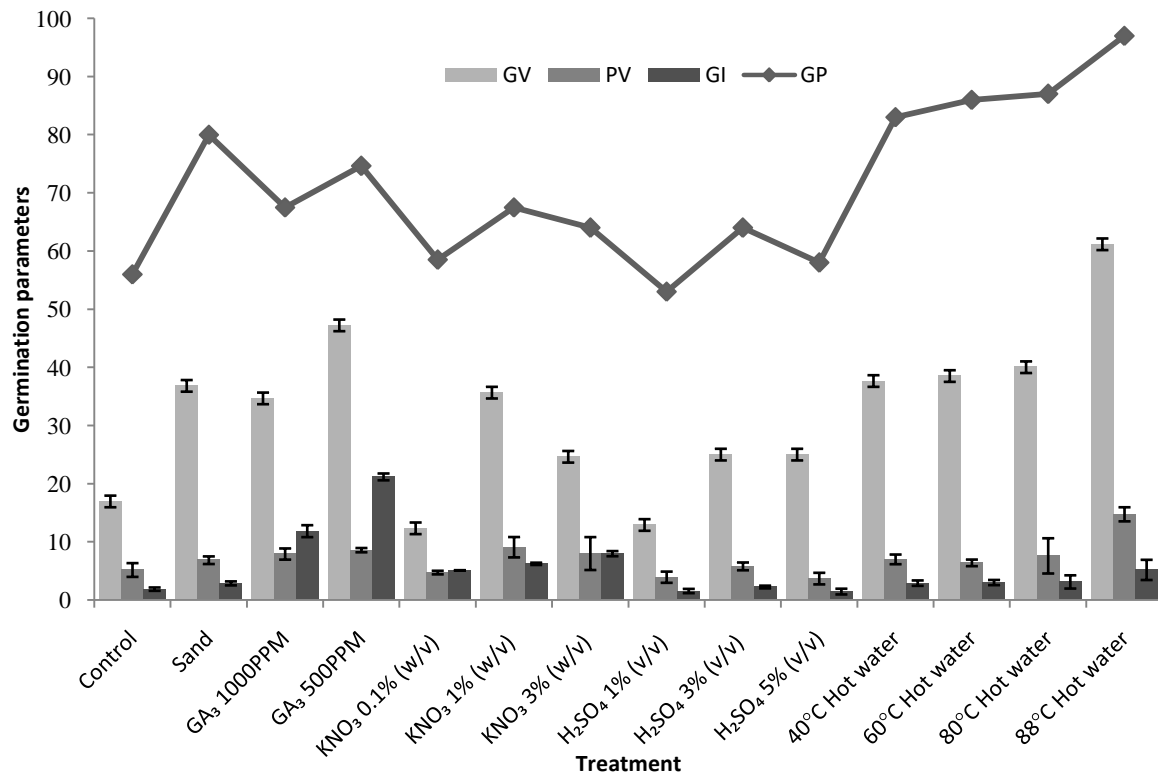


Fig 2: Effect of seed pre-treatments on germination value, peak value, germination index along with germination percent in *Calopogonium mucunoides*. Vertical error bars represent the standard deviation.

Significance of different pre-treatments on germination percent of *C. mucunoides* were compared with GP of control treatment taken as standard. Dunnett t (2-sided) statistical analysis was used for testing the significance level of different pre treatment (Table 1). As per statistical analysis, pretreatment with sand, 500ppm GA₃, Hot water treatment at 40°C, 60°C, 80°C and 88°C have significant effect ($p \leq 0.05$) on GP as compared to standard control treatment while seed pre treated with 1000 ppm GA₃, 0.1% (w/v) KNO₃, 1% (w/v) KNO₃, 3% (w/v) KNO₃, 1% (v/v) H₂SO₄, 3% (v/v) H₂SO₄ and 5% (v/v) H₂SO₄ are not significant ($p \geq 0.05$) with standard control treatment. Post hoc tukey test analysis exhibited lowest MGT in 0.1% (w/v) KNO₃ and 1% (w/v) KNO₃ which are significantly different ($p \leq 0.05$) from other pretreatment. Similarly, highest germination value was recorded in hot water treatment at 88°C which are significantly different ($p \leq 0.05$) from other pre treatments (Table 1).

Treatment	GP	MGT	GV
Control	56(2.82)	7.04 (0.47034) ^{bcd}	16.96(2.18915) ^{ab}
Sand	80 (3.26599) ^a	7.04(0.18501) ^{bcd}	36.83(3.74367) ^{bcd}
GA ₃ 1000PPM	67.5(4.33013) ^b	4.82(0.63148) ^{ab}	34.68(0.04768) ^{bcd}
GA ₃ 500PPM	74.5(2.59808) ^a	4.68(0.07961) ^{ab}	47.23(2.69316) ^{de}
KNO ₃ (0.1%)	58.5(0.86603) ^b	3.32(0.08172) ^a	12.33(0.37072) ^a
KNO ₃ (1%)	67.5(0.86603) ^b	3.57(0.04393) ^a	35.66(5.04007) ^{bcd}
KNO ₃ (3%)	64(2.30940) ^b	4.76(0.47773) ^{ab}	24.63(2.00658) ^{abc}
H ₂ SO ₄ (1ml)	53(4.43471) ^b	8.78(0.53301) ^{de}	12.91(2.44711) ^a
H ₂ SO ₄ (3ml)	64 (5.65685) ^b	7.88(0.36798) ^{cde}	25.01(3.09068) ^{abc}
H ₂ SO ₄ (5ml)	58(7.39369) ^b	10.47(0.61184) ^e	25.01(3.09068) ^{abc}
Hot water 40°C	83(7.18795) ^a	7.73(0.41376) ^{cde}	37.66(5.06743) ^{cd}
Hot water 60°C	86(1.15470) ^a	8.25(0.37866) ^{cde}	38.52(3.24884) ^{cd}
Hot water 80°C	87(7.18795) ^a	7.48(0.81607) ^{bcd}	36.04(9.28377) ^{bcd}
Hot water 88°C	97(2.03467) ^a	5.58(1.37066) ^{abc}	61.18(4.31311) ^e
F	9.691	13.653	11.396
P	^a ≤0.05 ^b ≥0.05	<0.05	<0.05

Table 1. ANOVA and post hoc results for effect of pretreatments on GP, MGT and GV of *C. mucunoides* seeds

Values in parenthesis are standard error of mean. Dunnett t (2-sided) analysis used for testing the significance of GP against control as standard, Tukey HSD method used for testing the significance of MGT and GV between treatments. ($p \leq 0.05$) significant, ($p \geq 0.05$) non significant.

Discussion

The results of present study revealed that pre treatment with sand, 500 ppm GA₃, Hot water treatment at 40°C, 60°C, 80°C and 88°C significantly ($p \leq 0.05$) affect germination percent in *C. mucunoides*. Hard seed coat is one of the notable characteristic in *C. mucunoides* which causes seed dormancy, delay in germination and loss of viability due to prolonged time required for breaking dormancy in natural conditions. In laboratory conditions, untreated seed produced the germination percent of 56% while sand scarification and hot water soaking had increase the germination percent from 80% to 97%.

Rusdy (2017) reported that seed dormancy in most of the forage tropical legumes is physical dormancy. Impermeability of the seed coat is caused by the presence of one or more palisade layers of lignified Malphigian cells (macrosclereids) tightly packed together and impregnated with water-repellant chemicals. Seeds of such legume species will not germinate quickly when subjected to favourable conditions because their hard seed coat prevents water and gases entering the seeds. Under natural conditions, this impermeability gradually decreases, so that a certain percentage of seeds germinate in each period (Morais et al., 2014; Rusdy, 2017).

Hot water treatment and sand scarification were most effective for improving the germination percent in *C. mucunoides* than control treatment, acid scarification (H₂SO₄), GA₃ and KNO₃. Sand scarification mechanically alters the seed coat properties and makes it permeable to water and gases and enhances the germination percent in *C. mucunoides*. Hot water soaking at 40°C, 60°C, 80°C and 88°C for 6 hours softens seed coat thereby improving permeability and causing rapid physiological changes in the seed which attribute high germination percent in *C. mucunoides*. Hot-water treatments have been used successfully in large number of tropical and sub-tropical seeds (Doussi and Thanos, 1994; Prasad and Nautiyal, 1996; Doran et al., 1983). Hot water seed

treatment has the beneficial effect of priming seeds, resulting in faster germination than untreated seeds. Fast and uniform germination percent has been earlier reported in seeds of *Albizia lebbek*, *A. procera*, *Peltophorum pterocarpum*, *Acacia auriculiformis* and *L. leucocephala* soaked in 100°C hot water (Sharma et al., 2008). Previous studies in *C. mucunoides* reported that treatment with concentrated sulphuric acid for 20 minutes, hot water at 50°C to 70°C for several hours, glycerine at 50°C for one hour can break dormancy and enhance the seed germination (Prondonoff, 1968; Skerman, 1977). Sulphuric acid disrupt the seed coat and expose the lumens of the macrosclereids cells, permitting imbibition of water (Nikoleave, 1977) which triggers germination. Morais et al., (2014) reported highest germination percent in sand paper scarification followed by immersion in conc. H₂SO₄ and hot water soaking at 60°C in *C. mucunoides*. Seed treatment with 1% (v/v), 3% (v/v), and 5% (v/v) H₂SO₄ exhibited poor germination, low germination value, peak value and germination index in *C. mucunoides*. In addition, highest mean germination time was recorded in seed treated with acid scarification (5% (v/v) H₂SO₄ (10.47 days) followed by 1% (v/v) H₂SO₄ (8.78 days). The results indicate that acid scarification using H₂SO₄ has deleterious effect on seed embryo and other tissues which could be the reason for low germination parameters. Kumar et al., (2020) conducted seed germination studies in five legume species viz., *Albizia thompsonii*, *Calopogonium mucunoides*, *Crotalaria micans*, *Tephrosia candida* and *Albizia procera*. Seeds were pre-treated in control (no treatment), mechanical scarification (seed coat cutting), H₂SO₄ (97%) for different durations (30 sec, 5 min, 15 min and 30 min) and heat scarification 60°C (9, 19 and 24 hours) and 80°C (4, 19 and 24 hours). Study reported that pre treatment with Con. H₂SO₄ and mechanical scarification provided significant germination percent in these legume species. In the present study growth promoters significantly shorten the MGT as compared to other treatment. Matthews and Khajeh-Hosseini (2007) reported that MGT is the mean of the lag period from the start of imbibition to physiological germination (radicle protrusion). Lowest MGT was reported in the seed treated with KNO₃ and GA₃. GA₃ can activate synthesis of protein and other metabolites that are required by the embryo for germination (Golmohammadzadeh and Rezvani 2015). However this treatment alone could not be a substitute to scarification methods due to dominant physical dormancy. As per the results of present study, breaking of hard seed coat using sand scarification or hot water methods appear to be promising and safest method as compared to acid scarification and treatment with growth promoters.

Conclusion

The present study concluded that physical dormancy is prevalent in *C. mucunoides* due to hard seed coat. The effective treatment for breaking physical dormancy recommended for the species are hot water at 88°C, 80°C, 60°C, 40°C and sand scarification. These pre-treatments are promising, cost effective and safest method for large scale plantation of this cover crop which has the potential to improve soil fertility and land restoration of wastelands, fallow lands and forest land of tropical regions of the world.

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