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GA_3 ASSISTED SEED DORMANCY BREAKING IN *ABELMOSCHUS MOSCHATUS* MEDIK. SUBSP. *MOSCHATUS*

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ABSTRACT. *Abelmoschus moschatus* Medik. subsp. *moschatus* is a wild uncultivated variety of common lady's finger (*Abelmoschus esculentus*) possessing a high degree of seed dormancy. Methods of dormancy breaking in the seed of the plant were investigated through different physical and chemical methods. Different preconditioning treatments including hot water, dry heat, physical scarification and chemical treatments including exogenous GA_3 were applied to explore the initiation in germination. Seeds were germinated under the controlled photoperiod and temperature. The viability of the test seeds was estimated by topographical 2, 3, 5-Triphenyltetrazolium chloride (TTC) solution test. Highest germination percentage was obtained in the seeds treated with the exothermic reaction of H_2SO_4 and H_2O, followed by exposure to gibberellic acid (GA_3). The exposure time of exogenously applied GA_3 had a significant influence on the germination response. The optimum germination temperature was found to be 30 ± 0.5°C. Treatment with 0.75% of 2, 3, 5-Triphenyltetrazolium chloride solution for 4 h at 35 ± 0.5°C enabled to correlate the viability of the seeds with the germinative values. Maximum germination was induced through this technique and dormancy of the seed can be attributed due to hard impermeable seed coat and endogenous physiological factor.

Keywords: dormancy; scarification; seed coat; germination; GA_3.

INTRODUCTION

Abelmoschus moschatus Medik. subsp. *moschatus,* commonly known as musk mallow (English), is an annual plant native to India, China, Tropical Asia and some parts of Pacific islands. It is a shrub from the *Malvaceae* family growing up to 3 m high with variably lobed alternate leaves. The flowers are bright yellow during the blooming time and the

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fruits are greenish capsule, comparatively shorter than the cultivated lady's finger (*Abelmoschus esculentus* L. Moench). The plant finds importance both as an industrial and vegetable crop. The plant is mainly cultivated for the raw material of perfumery industries due to the characteristics aromatic property of their seeds (Borssum, 1966). Different soups and dishes are prepared from fruits and leaves for which it is also considered as a food plant (De La Ripelle, 2006). The plant is also ethnomedicinally important and used in various traditional and complementary medicine. Seeds are an effective aphrodisiac, antispasmodic and used in tonics (Cravo *et al.*, 1992). Leaves are used to check vomiting and are useful in treating intestinal disorders, urinary discharge, nervous disorders, hysteria, skin diseases etc. Seeds possess emollient and demulcent property. Flower infusions are used as a contraceptive. Myricelin, a bioactive compound found in the plant, also proves to enhance insulin sensitivity by increasing post receptor insulin signalling and the compound is under investigation for the development of novel antidiabetic compound (Mukesh and Namita, 2013).

Abelmoschus moschatus Medik. subsp. *moschatus* is mainly propagated through seeds and clonal propagation needs longer time for acclimatization, which may delay the fruit production. However, seeds reported form North Eastern Region of India in our study exhibits a high degree of dormancy and very less germination percentage was obtained through conventional method. Dormancy is the inability of viable seeds to germinate in favourable environmental condition and physiologically it can be called as a resting phase (Gill *et al.*, 2014). Limited information is available regarding the nature and factors of seed dormancy in this plant. Various endogenous and physical factors can be responsible for the dormancy of seeds. Dormancy can be overcome through hydration by appropriate scarification methods (Fang *et al.*, 2006). The present work is undertaken to standardize a scarification and preconditioning technique to break the dormancy and to investigate the nature and cause of the same for rapid cultivation and to ensure its sustainable utilization.

MATERIALS AND METHODS

Seed source. Mature ripe fruit pods of *Abelmoschus moschatus* Medik. subsp. *moschatus* were collected from Dibrugarh University campus [Lat: N 27°29'06.8" Long: E 094°42'35.7" (Garmin GPS 72)]. The seeds were separated from the pods and the immature and damaged seeds were removed. Prior to scarification, the seeds were washed in distilled water with a few drops of Tween-20 and then surface sterilized with 1% sodium hypochlorite (NaClO) solution, for 5 min, followed by washing in sterile distilled water.

Mechanical and physical treatment. The seed coats were removed through a mechanical procedure. Various physical treatments including hot air, warm water at varying temperature range

and exposure time were employed to explore its effect on germination.

Chemical treatments. Chemical scarification including soaking the seeds in Nitric acid (HNO_3), dilute and concentrated Sulphuric acid (H_2SO_4) and Ethanol (EtOH: 96% v/v) for 5 and 10 minutes were employed. The seeds were then washed thoroughly with distilled water before incubated for germination.

GA$_3$ treatment. Moist chilling in gibberellic acid (GA_3) at different concentrations was employed for both scarified and non-scarified seeds. The effect of continuous exposure to exogenous GA_3 on the rate of seedlings development was evaluated through the addition of GA_3 in the stratification medium.

Design of experiment and germination condition. Experiments were conducted with three replicates through one way factorial ANOVA. The effect of incubation time in different GA_3 concentration on germination percentage was evaluated through a complete randomized design arranged in 4*3 factorial scheme (four concentration and three incubation time). The preconditioned seeds were placed in Whatman no. 1 filter paper moistened with 5 ml of sterile distilled water in sterilized 90 mm Petri dishes (Gorai *et al.*, 2011). The seeds were kept for germination in culture room maintaining a constant temperature and photoperiod. Seeds were considered as germinated when the radical length reached at least half of the seed length (Fang *et al.*, 2006). The seeds were kept in the culture room for 15 days and data were recorded after every 24 h.

Seed viability testing. Topographical 2, 3, 5-Triphenyltetrazolium chloride (TTC) solution test was employed to determine the viability of the seeds. Different TTC solution at concentration 0.1-1% was prepared in phosphate buffer (pH 6.5-7.5). Imbibed seeds were cut longitudinally into two equal halves and incubated in TTC solution for 4 h at 35°C. The dyed seeds were considered as viable. The experiment was conducted following the guidelines of International Rules for Seed Testing 2015-ISTA.

Data analysis. The germinating rate was calculated using germination rate index defined by Chu *et al.* (1978); Germination rate $= \sum_{n=1}^{15} \frac{G}{n}$, where G is number germinating since n-1 and n is the total experimental period (in days). Percentage of germination was arcsine, transformed before subjected to statistical analysis. Data were represented as mean ± SE. After analysis of variance, data were subjected to Tukey's Multiple Comparison Test at α = 0.05 (95% confidence interval) to determine the significant difference between the various experimental group. Statistical analysis was done in GraphPad Prism 5, and Minitab 17 was used to create analytical graphs.

RESULTS

Seeds without scarification did not germinate up to 30 days of incubation. All the non-scarified seeds failed to imbibe even after prolong soaking in water, which suggests that the seeds possess impermeable hard seed coat, which prevents the entry of water to the embryo. Mechanical removal of the seed coat did not initiate germination, due to intact attachment of the seed coat to the embryo and, probably, got mechanical injury. Seeds subjected to hot water

treatment showed very less germination percentage. Only 25% germination was obtained in seeds treated with hot water (80°C for ½ h). Similarly, seeds treated with dry heat (100°C for 1 h), followed by soaking in distilled water also showed only 17% germination (*Table 1*). Among chemical scarification, seeds treated with concentrated H_2SO_4, followed by addition of equal volume of distilled water to the solution showed maximum germination of 70%. Treatment time also influenced the rate of germination; highest germination was obtained when the seeds were soaked for 10 min. However, treatment above 15 min shows a gradual degradation of the seeds. Among chemical scarification, HNO_3 and EtOH treatment also broke the dormancy of the seeds, but the percentage of germination was not very high. The initiation of germination in the seeds treated with various chemicals further proves the presence of hard seed coat, which is one of the major causes of seed dormancy in the seeds of musk mallow. Regardless of the chemical treatment, a strong positive correlation was found between the germination rate and germination percentage at $r^2 = 0.967$, $p = 0.05$ (r^2 = correlation coefficient and p = calculated probability)

Table 1 - Different scarification treatment and germination responses

Treatments	Germination rate	Germination percentage
Hot water (40 °C), ½ h	0	0
Hot water (60 °C), ½ h	0	0
Hot water (80 °C), ½ h	0.30^{a}	25^{a}
Dry heat (100 °C), 1 h	0.21^{d}	17^{a}
Dry heat (120 °C), 1 h	0.19^{d}	15^{a}
Mechanical seed coat removal	0	0
Cold water treatment (4 °C), 1 h	0	0
Soaking in distilled water, 12 h	0	0
Soaking in distilled water, 24 h	0	0
HNO_3, 10 min	0.39^{ae}	30^{a}
HNO_3, 15 min	0.32^{e}	25^{a}
Dilute H_2SO_4, ½ h	0.08^{b}	7^{b}
Conc. H_2SO_4, 10 min	0.46^{c}	35^{c}
Conc. H_2SO_4 + H_2O, 10 min	0.52^{c}	40^{c}
(Conc. H_2SO_4 + H_2O, 10 min) + GA_3 (250 ppm), 6 h	0.92	70
EtOH (95%) - 15 min	0.04^{b}	8^{b}
GA_3 (100-250 ppm), 1 h	0	0
GA_3 (100-250 ppm), 24 h	0	0
LSD (5 %)	0.11	9.44

Values are mean of three replicates and mean values in the same column, followed by the same letter are not significantly different at $p<0.05$ in accordance with Tukey's multiple comparison tests. Percentage of germination is reported through germination tolerance calculator v 0.3 ISTA.

Soaking the seeds in different concentrations of GA_3, as a scarification agent, was unable to break the dormancy of the seeds. However, scarified seeds soaked in GA_3 showed enhanced germination percentage. Highest germination percentage was obtained when the seeds were soaked in GA_3, after scarification with H_2SO_4 + H_2O treatment. The concentration and soaking time greatly affected the germination percentage. The germination percentage of H_2SO_4 + H_2O scarified seeds increased by 75% after preconditioning by moist chilling in GA_3 for 6 h at 250 ppm. Higher concentration and longer moist chilling showed greater germination percentage (*Fig. 1*). However, a lower development rate was observed in the seedlings when the GA_3 was added to the stratification medium, compared to seeds germinated with no GA_3 in the stratification medium.

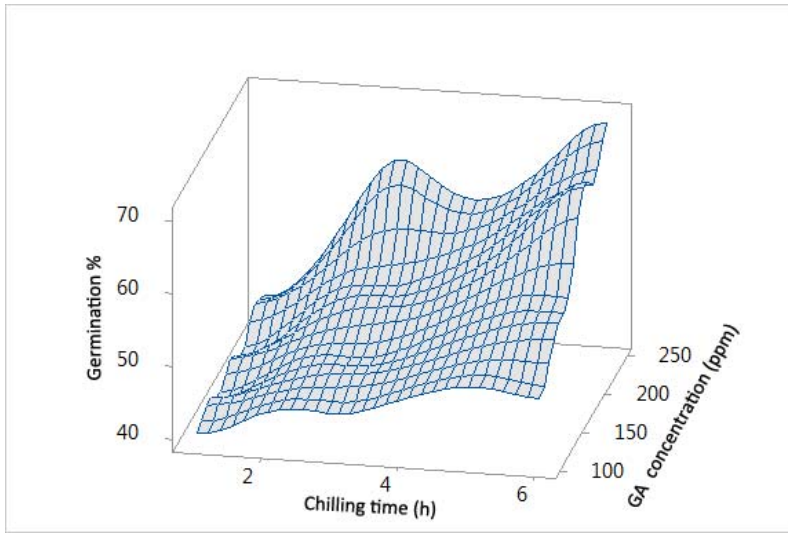

Figure 1 - Effect of percentage of germination of scarified seeds of *Abelmoschus moschatus* subsp. *moschatus* on GA$_3$ concentration (ppm) and chilling time (h)

The viability of seeds evaluated through TTC solution test showed 81.67 ± 6.0 % viable seeds; 0.75% of TTC in phosphate buffer, incubated for 4 h at 35°C, showed optimum distinguishable staining of the embryos. Pearson correlations analysis of viability with the percentage of germination after scarification and preconditioning of seeds collected from different plants of musk mallow showed a strong correlation (r^2 = 0.711, p = 0.05) (*Fig. 2*). This finding confirms that maximum germination percentage was obtained through this scarification technique in terms of the viability of seeds.

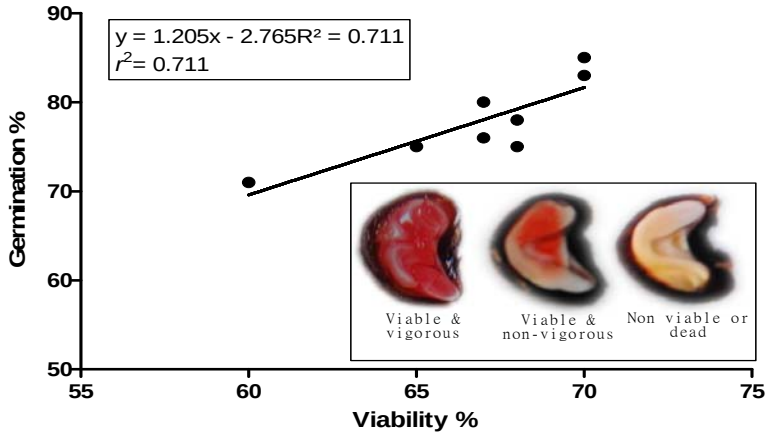

Figure 2 - Correlation plot of germination percentage with viability of samples collected from different musk mallow plants. Values (*n* = 20) from three replicates.

DISCUSSION

Seed coats are often a determining factor for seed dormancy in the majority of plants. Production of chemical inhibitor, impermeably to water, prevention of oxygen uptake and physical constrains are various inhibitory effects towards germination (Taiz and Zeiger, 2002). Presences of intact seed coat even after prolong soaking in water and immediate imbibition of the scarified seeds clearly proves the presence of hard impermeable seed coat in musk mallow. Hydration is an important factor for initiation of germination in seed. Before dispersal, the moisture content of the seeds is reduced to a great extent for the post-dispersal damage management due to extreme cold and infections by microorganisms. Reduced moisture content also attenuates the metabolic activity so as to prevent germination until the favourable environmental condition is reached. However, persistent impermeable hard seed coat prevents germination even after the favourable environmental condition is available. Pre-germination treatments are mainly targeted to enhance seed hydration and to initiate pre-germinative metabolic processes ahead of radical protrusion (Sanjay *et al.*, 2011).

Mechanical removal of the seed coat was found to be practically not possible due to firm attachment with the embryo. Chemical scarification, including HNO_3, EtOH and H_2SO_4, was effective in improving permeability of the seed coat to water. Seeds treated with concentrated H_2SO_4, followed by addition of equal volume of distilled water, showed significantly higher germinability, compared to the seed treated with the chemical alone. The cumulative effect of high temperature liberated by the

exothermic reaction and action of acid on the seed coat might have improved the water facilitation to the embryo.

Exogenously supplied GA_3 showed a pronounced effect on the germination percentage in the scarified seeds. Our study shows that gibberellic acid moist chilling has increased the germination percentage by 75% in the seeds scarified with H_2SO_4 + H_2O. It is noteworthy that this scarification treatment, followed by GA_3 preconditioning, is the only treatment where T_{50} can be recorded, which is 48 h after incubation. A considerable effect of GA_3 concentration and chilling time on germination percentage was recorded in our study.

Viability test through TTC solution showed the percentage of viable seeds present in our sample, which further help us to correlate with our germinative values. The high viability and no germination in the untreated seeds proved the presence of functional embryo and very less physiologically damaged seeds. Staining of the embryo through TTC test is based on the reduction of colourless 2, 3, 5-Triphenyltetrazolium chloride to coloured substance formazan through hydrogenation from dehydrogenase enzyme present in the viable seeds. Staining of both halves of sectioned seeds helps to evaluate more accurately by narrowing the observational error, but it is difficult to cut the embryo exactly into two equal halves in small seeds (Souza *et al*., 2010).

In conclusion, the present work can be utilized effectively for artificially breaking the dormancy status of *Abelmoschus moschatus* subsp. *moschatus* seeds. The plant can be made available round the year in *in vitro* condition through seedling development for various scientific researches by this method. Further studies will be conducted to understand more precisely the developmental mechanism of seed dormancy in this plant to cater information for future crop improvement and germplasm conservation strategies.

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