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Accelerating regeneration of threatened wild banana (*Musa paramjitiana* L.J. Singh) endemic to Andaman and Nicobar Islands, India

Andaman and Nicobar (A&N) Islands in the Bay of Bengal are known to harbour 2426 species of angiosperms, of which about 300 are endemic to these islands[1]. This diversity includes taxa of ecological and economic importance. The wealth of horticulturally useful species, including their wild relatives present in these Islands has been documented[2]. However, new species having potential agricultural importance are being discovered and reported regularly as large areas of these islands are yet to be explored systematically[3]. Recently, a new endemic species of seeded wild banana, viz. *Musa paramjitiana* L.J. Singh has been reported from the Andaman Islands[3], with observations on multiplication of species under field conditions. However, the threats posed by human interventions and damage by elephants have led to restricted distribution of the species in Andaman Islands and its categorization as 'Critically Endangered' species[3]. Under such conditions, assisted regeneration of species by raising nursery would be advantageous. Here, we studed the effect of seed treatments and substrates on germination and seedling growth parameters in the species for facilitating its conservation and further use in research.

Fully mature fruits of *M. paramjitiana* were collected from Dhanikhari Experimental Garden cum Arboretum of Botanical Survey of India, Andaman and Nicobar Regional Centre, Port Blair, and were allowed to ripe naturally. Seeds were extracted from fruits, pulp was washed and seeds were soaked overnight in fungicide (carbendazim, 0.1%) as prophylactic measure before undergoing any treatment. Seeds were divided into different groups and subjected to the following treatments. T_1: untreated control; T_2: water soaking (24 h); T_3: gibberellic acid (GA_3) (500 mg/l, 24 h); T_4: KNO_3 (0.1%, 24 h); T_5: mechanical scarification + water soaking (24 h); T_6: mechanical scarification + GA_3 (500 mg/l, 24 h) and T_7: mechanical scarification + KNO_3 (0.1%, 24 h). Scarification was done by carefully removing a small portion of seed coat using sharp scissors. Seeds were sown in pro-trays filled with coir pith. The experiment was laid in completely randomized design with three replications of 20 seeds each.

Table 1. Growth parameters in seedlings (48 days) of *Musa paramjitiana* as affected by various seed treatments

Treatment	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Number of leaves	Number of roots	Collar thickness (mm)
T_1	6.69 ± 0.384c	6.11 ± 0.291a	12.80 ± 0.583b	2.5 ± 0.167b	12.8 ± 0.490a	2.82 ± 0.089a
T_2	8.80 ± 0.242b	6.85 ± 0.576a	15.65 ± 0.665a	2.5 ± 0.167b	12.0 ± 0.494a	2.85 ± 0.076a
T_3	9.49 ± 0.441b	6.15 ± 0.411a	15.64 ± 0.651a	3.0 ± 0.000a	10.7 ± 0.367b	2.90 ± 0.067a
T_4	10.71 ± 0.418a	5.73 ± 0.561a	16.44 ± 0.795a	2.9 ± 0.100a	11.8 ± 0.359ab	2.85 ± 0.076a

Values are mean ± standard errors. Values followed by similar alphabets in a column do not vary significantly at 5% level of significance using least significant difference.

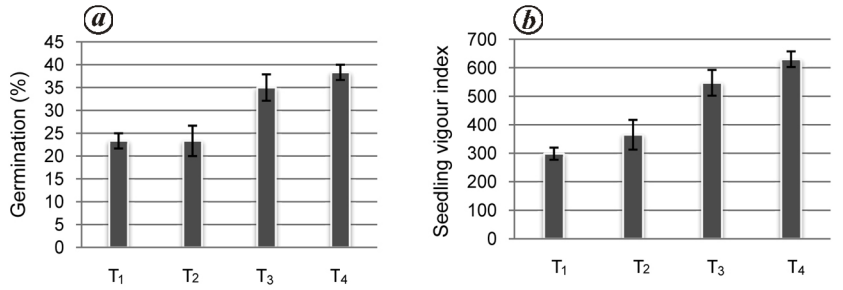

Figure 1. Germination percentage (***a***) and seedling vigour index (***b***) in *Musa paramjitiana* as influenced by different seed treatments. T_1, untreated control; T_2, water soaking (24 h); T_3, GA_3 (500 mg/l, 24 h); T_4, KNO_3 (0.1%, 24 h).

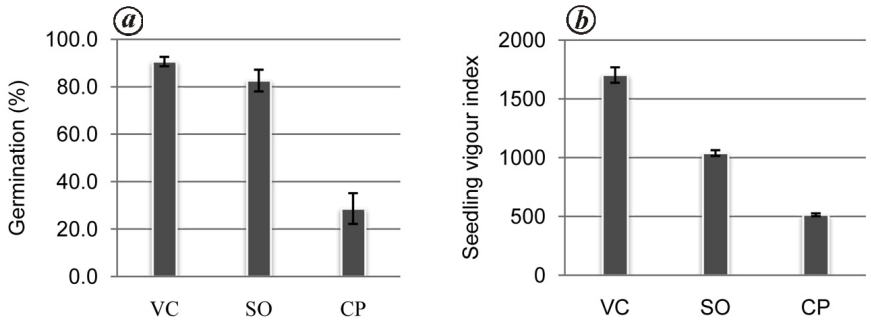

Figure 2. Germination percentage (***a***) and seedling vigour index (***b***) in *Musa paramjitiana* as influenced by different substrates, viz. VC, Vermicompost; SO, Soil; CP, Coir pith.

Considering the limited germination success in experiment 1, the effect of substrates (vermicompost, soil and coir pith) was studied with 0.1% KNO_3 as pretreatment in experiment 2. Six replications of 25 seeds each were used and the experiment was laid in completely randomized design. Seed germination was observed every day and seedling growth parameters were recorded for 10 randomly selected seedlings in each treatment after 48 days of sowing. Seedling vigour index, an expression of vigour of regenerated seedlings, was calculated by multiplying seedling length with germination percentage[4]. Data were subjected to analysis of variance using WASP 2.0 (ICAR-CCARI, Ela, India).

Seed germination in *M. paramjitiana* was significantly influenced by scarification and chemical treatments studied. A total of 23.33% germination was reported in untreated control, which remained statistically similar with water soaking treatment. Soaking with GA_3 (35.00%) and KNO_3 (38.33%) efficiently improved the germination percentage over control (Figure 1 *a*). Both chemicals might have assisted the germination process by mobilization of starch and activation of enzymes, which has been well documented in a number of other species including native species of these Islands[5]. Though Singh[3] reported 250–300 seeds/fruit, a mean of 137.1 seeds/fruit was observed in the present study. It was probably because of the fact that due to limited availability of fruits, first hand was used for analysis as against recommended (second hand) for *Musa* spp. descriptors. Even though considerably high numbers of seeds per fruit were noticed, germination of 23.33% in untreated control was noticeably low. In general, germination of intact seeds in *Musa* species is reported to be poor and erratic, and factors governing the germination process are not fully understood[6]. Relatively low germination efficiency of *Musa ornata* (30–40%) also suggests difficulties in the regeneration of wild bananas[7] as observed in the present study.

Interestingly, mechanically scarified seeds showed poor germination percentage, even though scarification was combined with chemical pre-treatments. Only 3.33% and 5.00% germination was observed in scarified seeds treated with water and GA_3 respectively; whereas no germination was possible with KNO_3 treatment. Previous reports on wild *Musa acuminata* ecotypes indicated the role of mechanical scarification in improving water uptake[8]; however, poor germination in the scarified seeds in T_1 could be attributed to the differences in the genetic make-up of both the species[6].

Seedling growth parameters recorded after 48 days of sowing were significantly influenced by the chemical pre-treatments studied (Table 1). None of the seedlings obtained from treatments involving scarification could survive, whereas those without scarification grew normally. Shoot length increased significantly from 6.69 cm in untreated control to 10.71 cm in KNO_3-treated seeds. Though length of the longest root did not increase with chemical treatments, seedling length was significantly better in all the treatments over untreated control. Seedlings from untreated seeds were 12.8 cm long (Table 1), whereas seedling length in different treatments improved up to 15.64–16.44 cm with chemical treatments.

For improving leaf production per seedling, seed treatments with GA_3 and KNO_3 were significantly superior (3.0 leaves) over untreated control and water soaking treatment. Root length and the number of roots did not show any significant differences among the treatments.

Collar thickness of 2.82–2.85 mm was noticed in the regenerated seedlings and was not affected by the pre-treatments studied. Seed treatment had a profound influence on seedling vigour index, as it improved from 298.67 in untreated control to almost double in seeds treated with KNO_3 (Figure 1 *b*).

Substrate is known to have profound influence on nursery success of a species; however, limited information is available in case of the genus *Musa*[9]. In experiment 2, significantly improved germination percentage (from 28.7% to 90.7%) and seedling vigour index (from 513.73 to 1703.3) were recorded when vermicompost was compared with coir pith as substrate (Figure 2).

Considering improved germination characteristics, seed treatment with 0.1% KNO_3 for 24 h and vermicompost as substrate could be recommended for raising vigorous seedlings. This could help in conservation of this threatened species and its subsequent utilization. Further, habitat enrichment would also be helpful for island wildlife, including bats, birds, rats, elephants and other unknown fauna, which are dependent on fruits of this species for food.

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Gelatin-coated magnetic nanoparticles for genomic DNA isolation from biological samples

The selection of an appropriate DNA isolation method for a specific biological sample (whole blood, saliva, stool, urine, fresh tissue or paraffin-embedded tissue) is a prerequisite for any molecular testing. This selection is dependent on many factors, including yield, purity, time, safety, requirement of specialized equipment, trained personnel, intended downstream applications, cost and sample source.

The use of magnetic nanoparticles (MNPs) provides a number of advantages in biotechnological applications, including nucleic acid isolation[1,2]. Even though uncoated MNPs can bind to DNA and can be used for its isolation, polymer-coated MNPs provide higher DNA recovery. MNPs coated with different polymers such as agarose and silica have been previously described and used in bacterial cells[3–5]. In this study, we synthesized gelatin-coated magnetic nanoparticles (GMNPs) for use in the isolation of genomic DNA from biological samples.

GMNPs were synthesized by chemical co-precipitation of Fe^{+3} and Fe^{+2} in an alkaline solution under hydrothermal conditions[6,7]. Briefly, GMNPs were prepared by mixing 1 M $FeSO_4 \cdot 7H_2O$ and 2 M $FeCl_3 \cdot 6H_2O$ with 1 ml of gelatin solution (0.15 g/ml, previously melted), followed by the addition of 8 M ammonium hydroxide. The Fe_3O_4 (magnetite) nanoparticles were formed at approximately pH 10. Co-precipitation was done at 60°C for 30 min. In the gelatin solution, iron nanoparticles interact electrostatically with amide bonds of the gelatin chain[8]. GMNPs were purified by washing three times with deionized water and using a high-gradient magnetic field to remove impurities. To estimate the total yield, GMNPs were first dried at 100°C for 2 h. Then they were resuspended in deionized water at a concentration of 50 mg/ml. The nanoparticles obtained exhibited a strong magnetic response.

The particles were characterized, and the mean particle size determined by transmission electron microscopy (TEM). Briefly, GMNPs were suspended in 500 µl distilled water. Next, 5 µl of the suspension was dropped into a Formvar/Carbon 200 mesh grids and left to dry at room temperature. The particles were examined using TEM (Hitachi 7500 Transmission Electron Microscope operating at 80 kV). Images were obtained using an AMT camera equipped with AMT image capture engine (V602). Images were analysed using FIJI. For analysis, each image was scaled, and each particle was measured by drawing a line across the diameter. One hundred particles per sample were measured and used for analysis. GMNPs were stored at 4°C.

The procedure for DNA isolation was standardized for whole blood, saliva and stool samples. The samples were collected from 30 healthy participants. Written informed consent was obtained from all of them. For DNA standardization, whole blood samples were collected in ethylenediaminetetra acetic acid (EDTA)