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RESEARCH ARTICLE

EFFECT OF IBA PRE-SOAKING IN THE REGAIN OF LOST VIABILITY IN THE SEEDS OF *MYRISTICA MALABARICA*, LAM.

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ABSTRACT

Seed recalcitrance is a major problem with tropical tree species limiting their natural regeneration and a constraint for their long term storage. The seeds are sensitive to desiccation and lose their viability very quickly. They do not have any molecular mechanism to tolerate dehydration such as metabolic 'switch off' and intracellular differentiation as orthodox seeds do (Berjak and Pammenter, 2013). *Myristica malabarica* is an indigenous arborescent species occurring in Western Ghats and has been red listed by IUCN. The major threats to this species are over exploitation of seeds for medicinal uses, conversion of its native swamp forests to agricultural land and the recalcitrance nature of seeds. The seeds are extremely sensitive to dehydration and lose viability in one month. In the present study, we report for the first time that the exogenous application of IBA for a period 24hrs. to the *Myristica malabarica* seeds desiccated for one month showed a reversal of their lost viability and cent percent germination could be achieved after eighteenth day of sowing. The results indicate that ABA/GA hormonal balance plays an important role in the germination of recalcitrant seeds and the exogenous application of IBA may disrupt this hormonal balance and regain the lost viability (Pieruzzi *et al.*, 2011).

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INTRODUCTION

Myristica malabarica (Family: Myristicaceae) is an indigenous arborescent species occurring in the swamp forests and other lowland localities in the Western Ghats. It has been rated as the highly traded top twenty medicinal plants in India (Ved *et al.*, 1998) and has been enlisted in the Red list of threatened species by IUCN (2013). The species is threatened because of the large scale of conversion of swamp forests to agricultural land and also the recalcitrant nature of seeds. Like many other tropical tree species, the seeds of *Myristica malabarica* are extremely sensitive to desiccation and loss viability in one month's time (Anilkumar *et al.*, 2002). Seed recalcitrance is a major problem with many tropical tree species including *Myristica malabarica* that limit their natural regeneration. Seeds of such species are extremely sensitive to dehydration and undergo little or no maturation drying and remain desiccation sensitive during development and after they shed. The seeds are shed in hydrated state and lose their viability very quickly. Such seeds do not have any mechanisms/processes that facilitate the acquisition and maintenance of desiccation tolerance as shown by orthodox seeds (Berjak and Pammenter, 2013). The tolerance mechanisms shown by the orthodox seeds have been extensively studied by many (Berjak and Pammenter, 2008;

Vertucci and Ferrant, 1995; Pammenter and Berjak, 1999; Berjak *et al.*, 2007; Kermode and Finch-Savage, 2002). Metabolic 'switch off' and intracellular differentiation have been highlighted by many authors as the possible mechanism of tolerance in orthodox seeds. However, recalcitrant seeds either lack, or do not express these mechanisms. The work carried out by Delahaie *et al.* (2013) showed that in the recalcitrant seeds of *Castanospermum australe*, there was no expression of LEAs which have shown to be crucial for desiccation tolerance in orthodox seeds. Three types of damages have been known during the dehydration of recalcitrant seeds such as mechanical damage as a result of reduction in volume, metabolism-induced ROS production as a result of loss of water and desiccation damage as a result of removal of water that maintains the structural integrity of macromolecules (Berjak and Pammenter, 2013). As a consequence of recalcitrance, the seeds of many species cannot be stored for a longer period of time limiting the scope for the species conservation especially endangered species. The recalcitrant seeds can no longer be cryopreserved as dehydration, a pre-requisite for cryopreservation, results in metabolism linked injury mediated by uncontrolled reactive oxygen species generation and failing anti-oxidant system (Berjak and Pammenter, 2013). In fact, sensitive to dehydration in recalcitrant seeds is still a puzzling problem and more research need to be carried out to understand this problem. Seed germination is a highly complex plant developmental process and many plant growth regulators are known to influence this process (Chen *et al.*, 2004). The prominent

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growth regulators are auxins which influence every aspect of plant growth and development, regulating transcription by rapidly modulating levels of Aux/IAA proteins throughout development (Mockaitis and Estelle, 2008). Plant developmental processes like gametogenesis, embryogenesis, seedling growth, vascular differentiation and floral development are influenced by auxins (Zhao, 2010). Cellular responses of auxins include cell division, elongation, differentiation as well as plant cell polarity (Tromas and Perrot-Rechenmann, 2010). Many literatures are available explaining the influence of auxins in the germination of orthodox seeds. However, information pertaining to the effect of auxins in the germination of recalcitrant seeds is scanty. A few works have shown that the natural auxins, IAA can increase the level of polyamines by activating the enzymes involved in its biosynthesis (Rastogi, Davies., 1991) and the increased polyamines can enhance the germination of recalcitrant seeds (Pieruzzi *et al.*, 2011). In this context, the present work was carried out to understand whether there is any effect of IBA pre-soaking on the germination of desiccated seeds of *Myristica malabarica*.

MATERIALS AND METHODS

Mature fruits of *Myristica malabarica* were harvested from the trees located at Ponmudi forests in Thiruvananthapuram district during the month of February 2014. The seeds were removed from the fruits and de-arillated. They were surface sterilized with 2.5% sodium hypochlorite for 30 minutes and washed extensively with distilled water. One set of fresh seeds were randomly selected from the seed lot and were scarified by cutting a small portion of the testa just near to the point of attachment of embryo in order to facilitate the entry of IBA into the seed as testa was hard. The seeds were pre-soaked in different concentrations of IBA (Sigma) for 24 hours just before sowing. There were four treatments; T1 (10mg IBA/L), T2 (50mg IBA/L), T3 (100mg IBA/L), T4 (200mg IBA/L) and a control (distilled water) with fifty seeds in five replicates (10x5) per treatment (ISTA, 1985). The seeds were put in the growth chamber; 80% relative humidity and 30-35°C temperature was maintained in the growth chamber during the period of study. Germination was assessed for a period of one month based on the occurrence of radicle protrusion. The other set of seeds were dehydrated under room temperature for one month in order to make the seeds non-viable. After this, the seeds were scarified and pre-soaked in different concentrations of IBA for 24 hours as described above.

Statistical Analysis

The data were subjected to Analysis of variance and the Fischer's least significant difference test at 5% level using the statistical package for social science (version 12.0 for Windows, SPSS Inc.).

RESULTS AND DISCUSSION

Analysis of variance showed significant differences in the germination of freshly harvested and desiccated seeds of *Myristica malabarica* (Table 1 & Fig.1). The IBA pre-soaking was found to be effective in smaller concentrations in the

germination of fresh seeds. After thirty days, optimum percent of seed germination (90%) was observed when the seeds were pre-soaked in 10mg IBA/L solution (T1) and at the same time, the control recorded only 82% germination. T2 (50mg IBA/L) and T3 (100mg IBA/L) did not exhibit and significant response as that of T1 and recorded only 78% germination. However, these two treatments had a significant effect in the early induction of germination process and achieved more than 70% germination in twenty days time. T4 (200mg IBA/L) showed only 42% germination after thirty days. This indicates that the germination process can be enhanced by IBA in lower concentration but inhibitory at higher concentrations. A significant finding was the reversal of lost viability in the seeds of *Myristica malabarica*. The thirty days desiccated seeds lost viability after thirty days of desiccation and showed only 9% germination (Fig.2). However, the desiccated seeds after pre-soaked in 10mg IBA/L (T1) showed cent percent germination after twentieth day of sowing and the process of germination had initiated after sixteenth day. Seeds pre-soaked in 50mg IBA/L (T2) did not show any significant effect on the germination. However, seeds pre-soaked in 100mg IBA/L (T3) and 200mg IBA/L (T4) had not germinated even after keeping thirty days in the growth chamber and this reflects the inhibitory effect of IBA at higher concentrations. The results indicate that pre-soaking of seeds at lower concentrations of IBA can bring back the lost viability. The results throw some light on the involvement of phytohormones in the regulation of seed recalcitrance.

Table 1. The percentage of seed germination of freshly harvested and desiccated seeds (thirty days after harvesting) of *Myristica malabarica* after thirty days of sowing. All the values are averages with \pm standard deviation (n=5). * indicates that the values are significantly different from the control at $P = 0.05$

Control/Treatments	Germination (%) after thirty days of sowing	
	Freshly harvested seeds	Desiccated seeds
Control	82 \pm 4.5	9 \pm 4.5
T1 (10mg IBA/L)	90 \pm 7.1*	100 \pm 4.5*
T2 (50mg IBA/L)	78 \pm 4.5*	12 \pm 4.5*
T3 (100mg IBA/L)	78 \pm 4.5*	0
T4 (200mg IBA/L)	46 \pm 5.5*	0

There are no literatures available explaining the role of IBA in the reversal of lost seed viability and the exact role of this growth regulator is still unknown. However, a close association between IAA and polyamines during the germination of recalcitrant seeds has been reported by some authors. The development of somatic embryos, and their conversion to plants, is closely related variations in endogenous phytohormone levels (Gemperlova *et al.*, 2009). Rastogi and Davies (1991) showed that IAA increased the level of polyamines and activities of enzymes involved in its biosynthesis. The interaction between IAA and polyamines during germination has been detailed by Hausman *et al.*, (1995). Pieruzzi *et al.* (2011) reported that there was an increase in the level of IAA and polyamines in the embryos during the germination of the recalcitrant seeds of *Araucaria augustifolia* and *Ocotea odorifera*, and it enhanced the synthesis of polyamines. They also observed a decrease in the level of ABA in association with the increase in IAA in the embryos of the recalcitrant seeds of *Ocotea odorifera* during germination. Gutierrez *et al.* (2007) reported that IAA activated

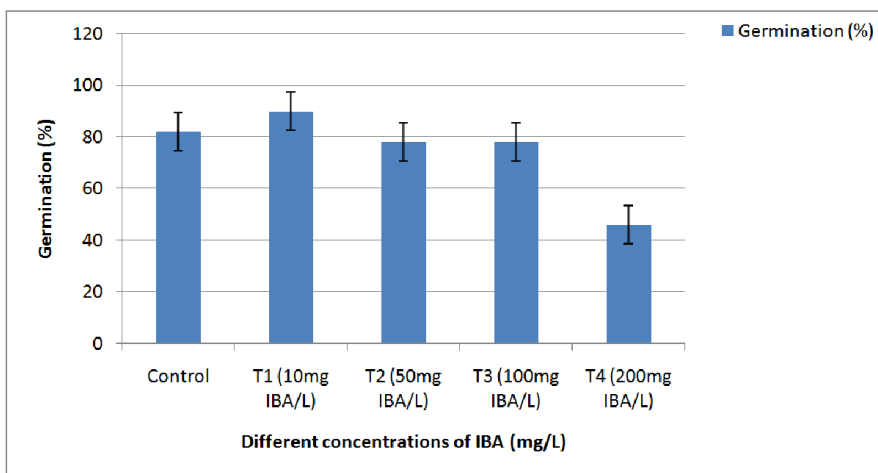


Figure 1. The percentage of germination of freshly harvested seeds of *Myristica malabarica* after thirty days of germination. The data are averages and are expressed as means of \pm standard error (n=5)

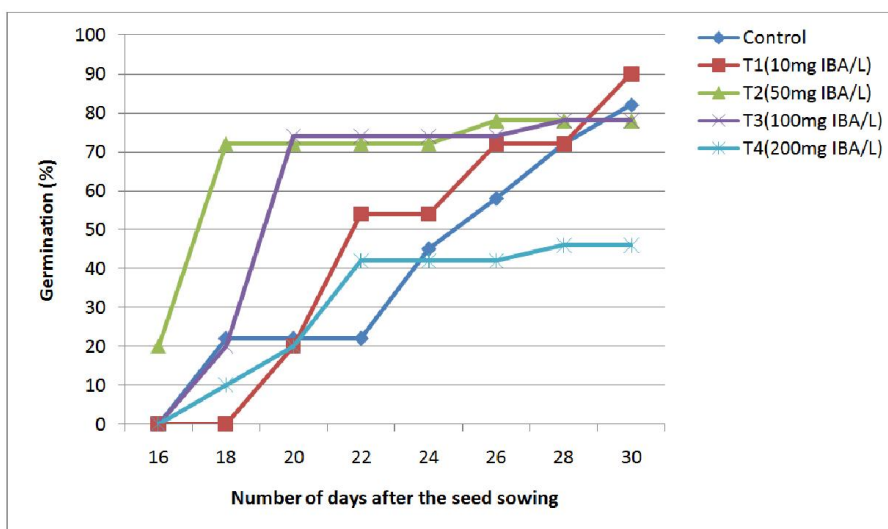


Figure 2. The percentage of germination of freshly harvested seeds of *Myristica malabarica* for a period of thirty days

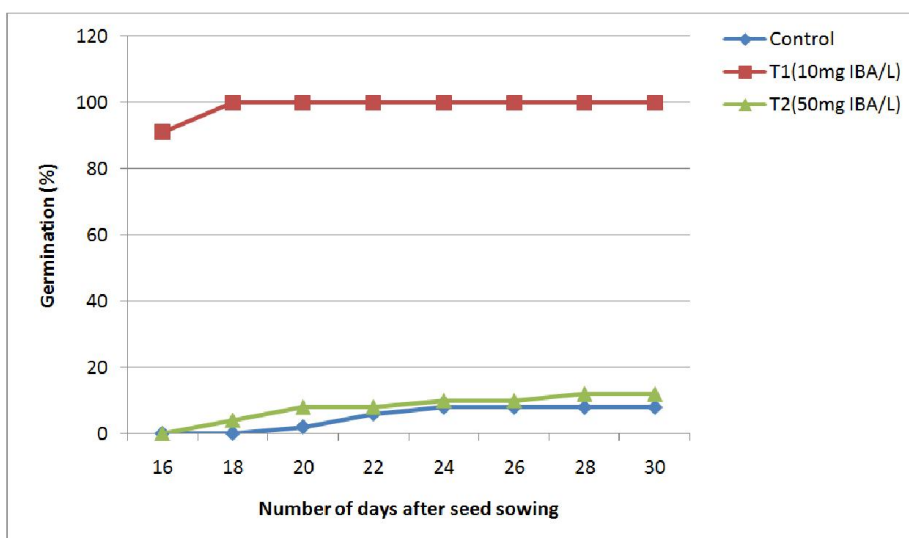


Figure 3. The percentage of germination of desiccated seeds (thirty days after harvesting) of *Myristica malabarica* for a period of thirty days (T3 and T4 have not shown due to zero germination)

the transcription factor (FUS3), one of the factors responsible for the modulation of ABA biosynthesis at the beginning of seed maturation. According to Curaba *et al.* (2003) and Gazzarini *et al.* (2004), ABA/GA hormonal balance is crucial to the maturation and germination process, and ABA is responsible for promoting maturation and inhibiting cell cycle progress, growth and germination. Finch-Savage *et al.* (2006) also reported the role of ABA in keeping the imbibed seeds in dormant stage. At the beginning of embryogenesis, ABA is produced by maternal tissue, suppressing vivipary and activating embryo growth and development (Chen *et al.*, 2002; Frey *et al.*, 2004) but during maturation, it is produced by embryonic tissue allowing the synthesis of storage proteins and lipids (Kermode, 1990; Guitierrez *et al.*, 2007). In many plant species, endogenous ABA is involved in the induction and maintenance of the dormant state and germination delay (Kucera *et al.*, 2005). A decrease in ABA levels is detected at the start of the germination process, based on synthesis, suppression as well as catabolism (Feurtado *et al.*, 2004; Kushihiro *et al.*, 2004). ABA is known as an antagonist of gibberellins that induce seed germination (Kermode, 2005). Based on research findings, it is reasonable to assume that the exogenous application of IBA in lower concentrations to the desiccated seeds of *Myristica malabarica* might disrupt the ABA/GA hormonal balance by activating the transcription factors responsible for the modulation of ABA biosynthesis and a low ABA/GA enhances germination. This crosstalk between IBA and ABA could be the reason for the reversal of lost viability in the seeds of *Myristica malabarica*. However, more work is required to understand the exact mechanism involved in this reversal process.

Conclusions

The present results showed that exogenous application of IBA has a significant effect in the process of reversal of lost viability in the seeds of *Myristica malabarica* and cent percent germination could be achieved when IBA was applied in lower concentrations (10mg/L) but it was not so effective at higher concentrations. This indicates that the recalcitrance in this species is linked to the ABA/GA hormonal balance and IBA application somehow disrupts this balance leading to the regaining of lost viability. Further studies are needed to understand the exact molecular mechanism behind this reversal.

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