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Effect of sucrose concentration of MS nutrient media on *in-vitro* seed germination of sandalwood (*Santalum album*)

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Abstract
Present investigation aimed, to produce disease and virus free plantlets, by using sandalwood seeds on different media combinations amenable for tissue culture, to *in vitro* micro propagation of *Santalum album* was conducted in Completely Randomized Design at Centre of excellence in Plant Biotechnology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Dist. Akola (M.S.) during the academic year 2015-2017.

Seed germination when MS incorporated with sucrose 3%without GA_3 and MS 3%, 4%, and 6% of sucrose with GA_3 1mg/l was tried the highest seed germination was observed (86.66) when MS 3%+ GA_3 1 mg/l was used. The explants derived from *in vitro* grown seedling explants were (shoot, epicotyl, hypocotyl) inoculated on 8 different media combination. The highest survival percentage was recorded in treatment MS + BAP 2 mg/L + NAA 0.4 + IBA 0.4% in case of shoot (91.66%), epicotyls (50%), and hypocotyl (33.33%).

Keywords: Micropropagation, explant, *Santalum album*, survival, nodal explant, seed

1. Introduction
A priceless tree known as sandalwood (*Santalum album* L.) is connected to Indian culture. The world's second-most expensive wood is this one. One of the best natural carving materials is the tree's heartwood, which is prized for its scent. Pharmaceuticals, cosmetics, aromatherapy, and fragrances all use sandalwood oil. (Viswanath *et al*., 2008) [2]. Numerous studies have revealed that sandalwood has a significant genetic diversity for various features.

Santalum collection L., a medium-sized evergreen fractional root parasitic tree that's exceedingly prized for its fragrant heartwood, could be a part of the Santalaceous family. The Santalum collection has the foremost oil of any part of the genus. Across tropical and subtropical India, Sri Lanka, and Indonesia, the more well-known Indian sandal, Santalum album, develops over a wide extend of temperatures and soil sorts Western Australia is home to four *Santalum* species: *S. spicatum*, *S. accuminatum*, *S. murrayanum*, and *S. ianceolatum* (Sawyer and Jones, 2000) [4]. The oil content of *Santalum* spp. is highest in *S. album*, while *S. spicatum* and *S. ianceolatum* produce low-quality oil and wood, respectively (McKinnell, 1990) [8]. About 4000 tonnes of sandalwood are produced worldwide. India produces 400 tonnes or such. 1800 tons of Australian variety is produced in Australia; 350 tons are imported, primarily from Timor, Malaysia, Cambodia, Vietnam, Thailand, and Myanmar. The biggest consumers of sandalwood are China and Taiwan, with India and Indonesia as key suppliers. From Kerala within the south to Uttar Pradesh within the north, S. album is naturally spread all through India (Srinivasan *et al*., 1992) [16] in a variety of eco-climatic and edaphic conditions. Kanataka and Tamil Nadu states take up over 90% of the total area. It is primarily found in Kerala's Marayoor, Kasargod, Wayanad, and Thenmalai forest areas (Srimathi *et al*., 1995 [14] According to Jeeva *et al*. (1998) [10], sandal is also worn in Andhra Pradesh, Maharashtra, Madhya Pradesh, and Orissa.

To deal with the growing call for reforestation efforts, tissue culture or micropropagation technique in wooded area trees is said to be one of the best micropropagation techniques. It also has the advantage of producing several plantlets in a short amount of time Sandalwood in vitro propagation become tried as early as 1963. On a modified White's medium, the induction of callus from mature endosperm was described but the callus did now not maintain growing (Rangaswamy and Rao, 1963) [3].

Lakshmi Sita *et al*. (1980) [12] stated on the induction of callus, differentiation of embryoids, and subsequent production of plantlets from endosperm (immature seeds) which can be used to make sandal plants that are more effective commercially. As but various explants together with embryo (Rangaswamy and Rao, 1963 [3] hypocotyls (Bapat and Rao, 1979) [17], shoot tip (Lakshmi Sita and *et al*. 1995) [18], nodal segment.
(Bapat and Rao, 1979) [17], leaf disc (Mujib, 2005) [19], endosperm (Lakshmi Sita *et al*., 1979) [20] and cell suspension cultures (Dey, 2001) with various level of achievement have been used for speedy multiplication of sandal tree. only one study at the direct technology of shoot buds from in vitro grown leaves for sandal has been published (Mujib, 2005) [19]. When it comes to shoots that develop without a callus phase directly on leaves, relatively little information is available for woody species (Preece *et al*., 1993) [9].

2. Materials and Methods

2.1 Preparation of seed for culture inoculation

The Sandalwood seeds collected from well grown mother tree in Washim district of Vidarbha region were used for present study the mother tree identified to be 10-15 years age. The fallen seed were collected from the ground from the month of September. The collected seeds of sandalwood were cleansed thoroughly under running tap water for 20 min and washed with a solution of Bavistin 0.1% with few drops of Twin 20 for 25 min and again will be 4 times wash with sterile distilled water. And also wash Streptomycin 0.1% for 20 min. and again will be 4 times wash with sterile distilled water. The cleaned seeds finally treated with 0.1% (w/v) $HgCl_2$ for 5 min under aseptic conditions and washed 4 times with sterile double distilled water to remove traces of $HgCl_2$. After surface sterilization of seeds were transfer it individually on MS (Murashige and Skoog, 1962) [13] with different media combinations and plant growth regulators.

3. Results and Discussion

To determine the effect of sucrose concentration on sandalwood seed germination different concentrations of sucrose with MS medium were tested as, 3, 4, and 6%. The percentage of seed germination was counted.

3.1 Effect of sucrose level on seed germination

After surface sterilization of sandalwood seeds transfer it individually for inoculation with different media combination after blot drying.
Ten seeds per bottles were inoculated in three replications on MS containing 3 percent sucrose without GA_3 and MS supplemented with 3,4,6 percent sucrose with $GA_3$1mg/l. The blot dried seeds inoculated on media and placed in dark room for germination. The observation was recorded 30 days after inoculation.
62.66 percent seed germination was recorded when MS supplemented with GA_3 at 1 mg/l but in order to enhance seed germination further sucrose varying levels such as 3%, 4%, 6% supplemented with 1 mg/l GA_3 was used the observation were recorded after 15 days after inoculation MS with 3% sucrose and without GA_3 was treated as control.

Table 1: Effect of sucrose levels on seed germination of *Santalum album*

Treatments	Treatments details	% seed germination
T_1	MS 3% Control	46.66
T_2	MS 3% + GA_3 1mg/L	86.66
T_3	MS 4% + GA_3 1mg/L	73.33
T_4	MS 6% + GA_3 1mg/L	80.00
CV%		6.97
CD 1%		1.36

Data from Table 1, Fig. 1 and Plate 1 depicted that when seed inoculated on MS containing 3% sucrose and GA_3 at 1 mg/l, showed highest percent seed germination i.e. 86.66, followed by MS containing 6% sucrose 1 mg/l GA_3 (80%) and 73.33% seed germination was recorded when seed inoculation was done on MS containing 4% sucrose and GA_3 at 1 mg/l. Plate no.1 showed the seeds germination on liquid MS media as compared to solid MS media and also germination percent after 15 DAI, 30 DAI and 45 DAI.
Janarthanam *et al*. (2011) [21] reported in their experiments that when in vitro seedling was developed from seed explants inoculated in MS medium containing BA (0.5-2.0 mg L-1) with GA3 (0.5 mg L -1). Seed germination in most treatments was recorded within 3 weeks of cultivation. On MS base medium supplemented with 1.0 mg L-1 BA and 0.5 mg L-1 GA3, the developed healthy seedling showed a remarkable growth response, germination of 71.6±2.8%, average shoot length of 6.43±0.05 cm and root mean square. length 3.76±0.05 cm. 0.05 cm. 0.25 cm and healthy seedlings developed after 40 days of cultivation. Nikam and Barmukh (2009) [22] observed in their experiments when Santalum album endocarps were removed under aseptic conditions and seeds were treated with 2-, 4-, 6-, and 8-mM gibberellic acid (GA3) for 12 h and then inoculated. Murashige and Skoog (MS) on medium supplemented with or without 2.0 or 4.0 µM benzylaminopurine (BAP). Soaking seeds in 4 mM GA3 improved final germination to 80.6% in 30 days.

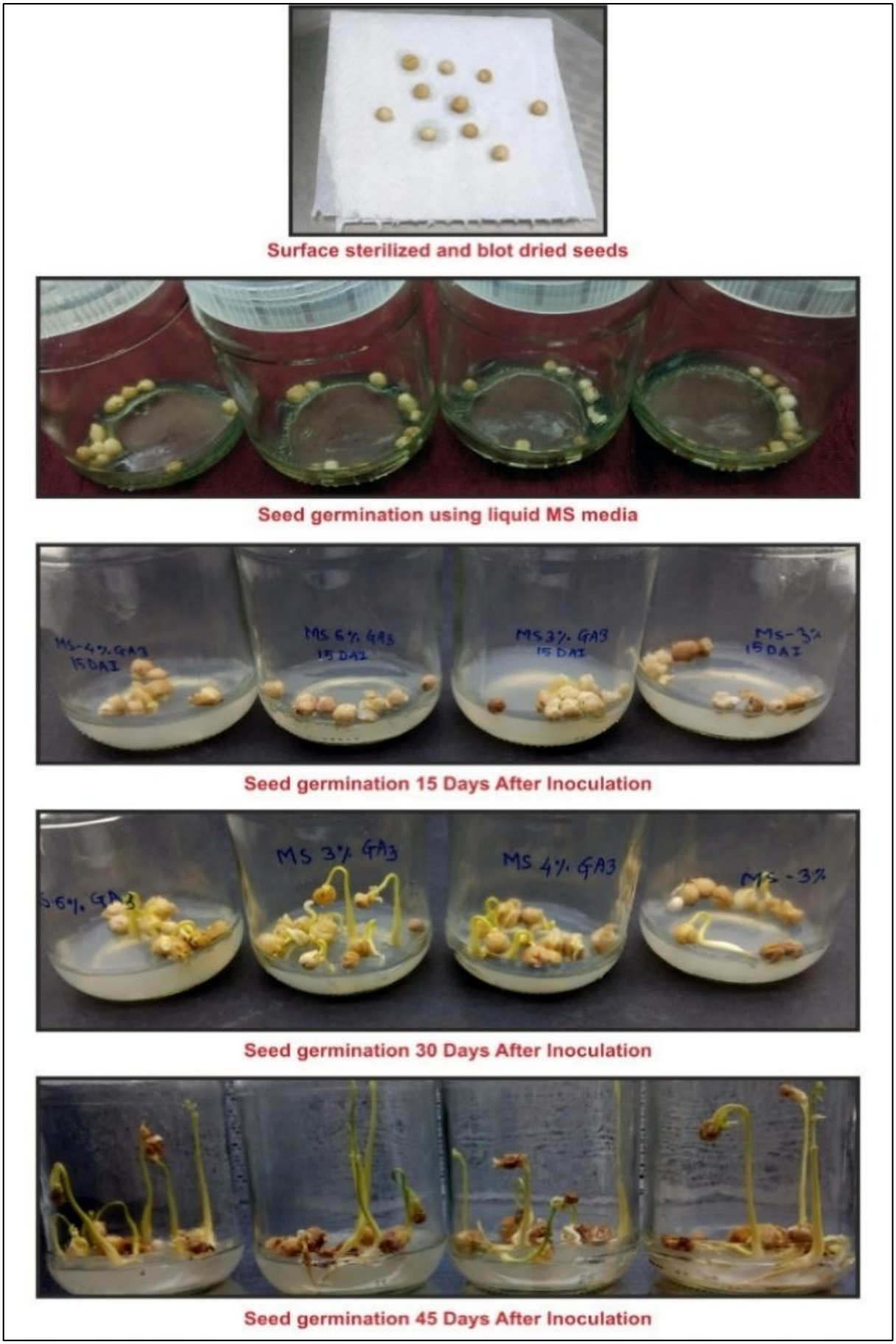

Plate 1: Effect of sucrose and GA, influencing seed germination

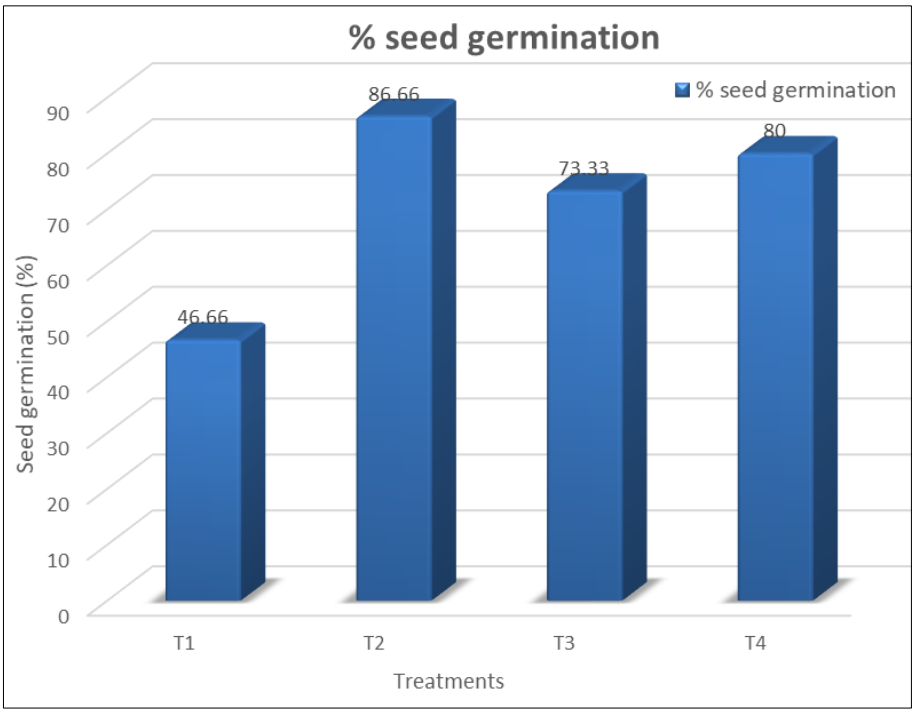

Fig 1: Effect of sucrose levels on seed germination of *Santalum album*

4. Conclusion

In vitro seed germination at 1 mg/L of GA3 showed the highest seed germination percentage i.e., 86.66% The results obtained from these studies clearly demonstrate that the standardized micropropagation protocol provides a successful and rapid technique that can be successfully used for *in vitro* mass propagation of elite species such as sandalwood.

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