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Factors affecting clonal multiplication in Kokam (*Garcinia indica* Choicy.)

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ABSTRACT

In vitro studies in Kokam (*Garcinia indica*) were carried to standardize protocol for aseptic culture shoot establishment of Kokam. During the investigation, treatments for surface sterilization, explant size, stage of explants and gelling agents were studied. Studies revealed that the $HgCl_2$ at 0.1% for 4 minutes and $HgCl_2$ at 0.1% concentration for 5 minutes were found to be at par as the best surface sterilization treatments to obtain maximum Kokam shoot establishment in vitro. The shoot tip explant of length 4 cm in length, dark green coloured were the ideal explants for *in vitro* establishment. Incorporation of Phytagel as a gelling agent in the Murashige and Skoog's medium exerted good conditions for *in vitro* shoot establishment in Kokam.

Key words: *Garcinia indica, Kokam, Clonal*

Introduction

Softwood grafting and side grafting results in slower propagation of Kokam (family Clausiaceae, a popular and economically important plant) and was unable to fulfill the increasing demand of Kokam seedlings from farmers. Dioceous nature of species creates a hurdle in selection of female trees at early seedling stage. To fulfill the increasing demand for Kokam seedlings from farmers and to overcome major bottlenecks existing in grafting techniques, *in vitro* study in Kokam was undertaken at Plant Biotechnology Unit, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri.

Materials and Methods

Mature field grown Kokam trees were used as Source of explant. Shoot tip explant was used as source of explant. MS (Murashige and Skoog's, 1962) medium was used for *in vitro* establishment of Kokam explants throughout the experiment. For aseptic culture establishment, sterilization procedure was standardized. In the primary sterilization, explants were sterilized with 0.1 % teepol solution for 15 minutes and then rinsed with running tap water. In the secondary sterilization treatment explants were treated with 01 % Bavistin, 0.1% Polyvenyl Pyrillidone and 0.1 % Citric acid. This solution was kept on shaker at 100-110 rpm for 30 minutes. After this explants were rinsed with Double Distilled Water and tertiary sterilization was carried out to in Laminar Air Flow with $HgCl_2$ 0.1% and 0.2% for different durations.

In case of explants, different stages of explants such as immature, semi mature and mature shoot tips of Kokam were obtained from mature Kokam trees. Shoot tip explant for varying length as 1 cm, 2 cm, 3 cm and 4 cm was studied in MS medium along

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Table 1. Surface sterilization treatment for shoot establishment in Kokam :

	Treatments	Duration	% aseptic culture	% proliferating cultures	Mean
T_1	$HgCl_2$ (0.1 %)	1	34.72	25.83	30.27
T_2	$HgCl_2$ (0.1 %)	2	40.23	31.94	36.08
T_3	$HgCl_2$ (0.1 %)	3	52.77	40.93	46.45
T_4	$HgCl_2$ (0.1 %)	4	81.94	81.52	81.73
T_5	$HgCl_2$ (0.1 %)	5	83.23	76.10	79.71
T_6	$HgCl_2$ (0.1 %)	1	40.27	52.08	46.17
T_7	$HgCl_2$ (0.1 %)	2	72.22	58.88	65.55
T_8	$HgCl_2$ (0.1 %)	3	76.38	53.88	65.13
T_9	$HgCl_2$ (0.1 %)	4	86.11	31.52	58.81
T_{10}	$HgCl_2$ (0.1 %)	5	93.05	17.49	55.27
T_{11}	$HgCl_2$ (0.1 %)	0	00	00	00

with two gelling agents viz: Phytagel and Agar.

Results and Discussion

It is evident from Table 1 that the maximum percent for *in vitro* aseptic culture was obtained from treatment T10 (93.05 %), $HgCl_2$ 0.2% for ten minutes followed by the treatment T9 (86.11 %) $HgCl_2$ 0.2 % for 5 minutes. Maximum proliferating cultures were recorded for the treatment T4 (81.52 %), $HgCl_2$ 0.1 % for 4 minutes. Although the $HgCl_2$ 0.2 % for 5 minutes duration showed maximum aseptic cultures (93.05 %), it showed that only 17.49 percent of proliferating cultures. There was no further proliferation. The maximum aseptic culture establishment (81.73 %) was exerted by the treatment T4 i.e. $HgCl_2$ 0.1 per cent for 4 minutes followed by $HgCl_2$ 0.1 per cent for 5 minutes with aseptic culture establishment as 79.71 per cent. Hence, the treatment $HgCl_2$ 0.1 percent for 4 minutes duration was found to be better treatment for surface sterilization in Kokam shoot establishment.

Stage of explant

Various explants stages viz; immature, semi mature and mature explants were investigated to standardize the protocol for *in vitro* shoot establishment in Kokam shoot tip explants. The mature explants were greenish in colour with well developed leaves from apical leaf primordia, while immature shoot tip explants were in between them. The study revealed that mature explants execute highest shoot establishment (81.94 %) than the semi mature shoots (52.77 %). The immature explants showed poorest performance for shoot establishment (34.71 %) as compared to both mature and semi mature explants.

Length of explant

Mature greenish coloured shoot tips of different sizes were undertaken to and their effect on shoot establishment per cent was studied. It is evident from the shoot tip explants of length 3 cm gave higher percent of shoot establishment i.e. 81.94 per cent than other, while shoot tips of 2 cm length execute 80.55 per cent shoot establishment. The minimum performance for shoot establishment was observed from shoot tip explants of 1 cm length as 70.83 % and 72.22 % on 4 cm length.

Gelling agents

The effect of gelling agent with growth medium on in vitro culture establishment of Kokam was studied using three growth regulator treatments and two gelling agents viz; Agar agar and Phytagel. Media were gelled by using agar agar 8 g/l and Phytagel as 2.25 g/l. Shoot tip cultures of Kokam were inoculated in three plant growth regulator combinations in Murashige and Skoog's medium. After three weeks interval they caused marked difference in shoot growth reaction in medium. Slower shoot growth with cracking in some cases was observed on culture medium gelled by Phytagel exhibit normal growth rate without any cracking in the medium. Hence it can be said that Phytagel was better as gelling agent for *in vitro* shoot establishment in Kokam than agar.

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