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In vitro propagation in Kokam (*Garcinia indica* Choisy.)

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ABSTRACT

Kokam (*Garcinia indica*) an important spice crop is under commercial cultivation in tropical rain forests of Western Maharashtra, face major bottleneck in identifying female plant at seedling stage. *In vitro* clonal multiplication in Kokam was standardized with the help of shoot tip explant in Murashige and Skoogs modified medium. The plant growth regulator combination as 10 ppm BA + 5 ppm NAA + 5 ppm GA_3, was found to be optimum level for establishment of Kokam shoot tip cultures *in vitro*. However, various concentrations of auxins in MS medium were found ineffective to induce root initiation in Kokam shoot cultures.

Key words: *Garainia indica, In vitro, Shoot tip.*

Introduction

Kokam, popularly known as 'Ratamba' is a slender and beautiful evergreen tree belongs to family Guttiferae. It grows widely in tropical rain forests of Western Ghat region. This tree is also called as Brindania fallow tree, 'Kokam butter tree' (Murashige and Skoog, 1962). It is one of the very important spice crops in Konkan region of Maharashtra State. Kokam fruit is edible part of the plant which, has agreeable flavor and is sweetish, acidic in taste. Kokam seed is good source of fat and Kokam butter can be replaced with buttermilk. Kokam butter is only solid fat at room temperature fetch tremendous potential in cosmetic and medicine sector. In addition to this, Kokam tree has great economical and ecological potential to make it ideally suited to the restoration of natural flora.

Softwood grafting and side grafting results in slower propagation of Kokam and was unable to fulfill the increasing demands of Kokam seedlings from farmers. Dioceous nature of Kokam species creates a great hurdle in selection of female seedlings produced through grafting techniques. To overcome these major bottlenecks, *in vitro* propagation of Kokam was undertaken to achieve true to type multiple seedlings at Plant Biotechnology unit. Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri, (M.S.)

Materials and Methods

Mature field grown Kokam trees were used as source of explant. Shoot tip explant and nodal explants of new season growth having dark green colour and with 4 cm length were used for shoot proliferation. Murashige and Skoog's medium (1962) and Lloyd and McCown's Woody Plant Medium (Lloyed and

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Mc Cown, 1981) were used for in vitro establishment of Kokam cultures and shoot regeneration throughout the experiment. Treatments for shoot regeneration were formulated with two media and various levels of plant growth regulators.

For aseptic culture establishment, sterilization procedure was standardized. In the primary sterilization, explants were sterilized first with 0.1% teepol solution for 15 minutes and then kept under running tap water for few minutes. In the secondary sterilization, explants were treated with 0.1% Bavistin, 0.1% Polyvenyl pyrillidone and 0.1% citric acid for the duration of 4 minutes in the Laminar air flow cabinet. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20-25 minutes. Cultures were maintained at 27 ± 2°C with 10 hours light under 3000 lux.

Other procedure for inoculation and incubation was followed as usual. The growth regulator medium with 0.1 percent charcoal proved very effective against leaching of phenols. Hence charcoal was added to the medium throughout the experiment. The experiment was carried out under factorial Randomised Block Design. The analysis was done according to Panse and Sukhatme (1985).

Results and Discussion

The effect of type of explant and media composition for in vitro establishment of Kokam was studied using two tissue culture media along with various growth regulator combinations and two types of explants. Media used were Murashige and Skoog's medium and Woody Plant Medium by Lloyd and McCown's. The explants used were shoot-tip explants and nodal explants. It is evident from the Table 1 that by using various culture media compositions and type of explant i.e. shoot-tip and nodal explants. The shoot- tip explant was found to be better for initial establishment of Kokam cultures. Shoot-tip explant showed maximum shoot establishment (81.94%) as compared to nodal shoot explant.

Various treatments of plant growth regulators showed significant difference for shoot establishment by using shoot-tip explant. The maximum percentage for shoot establishment was observed on the treatment MS ½ N+10 ppm BA+5 ppm NAA + 5 ppm GA_3 and shoot establishment was 81.94 percent. Minimum shoot establishment percentage was observed in treatment MS ½ N without growth regulators and shoot establishment observed was 30.55 percent.

The nodal explants gave highest shoot establishment (69.44 %) on the growth regulator combination MS ½ N+10 pm BA+5 ppm NAA+5 ppm GA_3 followed by the treatment WPM+10 ppm BA+5 ppm NAA+5 ppm GA_3 for (59.72%).

The highest shoot establishment percentage in Kokam was recorded by Murashige and Skoog's medium (81.94 %) and Woody Plant Medium (63.88 %) for the shoot tip explant and growth regulator combination as 10 ppm BA+5 ppm NAA+5 ppm GA_3. Thus, the shoot tip explant was found to be better for

Table 1. Effect of type of explants and media composition on *in vitro* shoot establishment of Kokam.

S. No.	Culture media composition	Shoot tip	Nodal	Mean
1.	MS ½ N (Without groeth regulators)	30.55 (33.58)	22.22 (28.11)	26.38 (30.92)
2.	MS ½ N + 10 ppm BA + 2.5 NAA + 2.5 GA_3	52.77 (46.61)	47.22 (43.39)	49.99 (45.00)
3.	WPM + 10 ppm BA + 2.5 NAA + 2.5 GA_3	34.72 (36.09)	30.55 (33.58)	32.63 (34.82)
4.	MS ½ N + 5 ppm BA + 1.5 NAA + 2.5 GA_3	69.11 (56.23)	52.77 (46.61)	60.94 (51.30)
5.	WPM + 5 ppm BA + 1.5 NAA + 2.5 GA_3	31.94 (34.39)	36.10 (36.93)	34.02 (35.67)
6.	MS ½ N + 10 ppm BA + 5 NAA + 5 GA_3	81.94 (64.82)	69.44 (56.42)	75.69 (60.47)
7.	WPM + 10 ppm BA + 5 NAA + 5 GA_3	63.88 (53.07)	59.72 (50.59)	61.80 (51.83)
8.	MS ½ N + 5 ppm BA + 2.5 NAA + 2.5 GA_3	43.77 (41.44)	36.10 (36.93)	39.93 (39.17)
9.	WPM + 5 ppm BA + 2.5 NAA + 2.5 GA_3	40.27 (39.41)	27.77 (31.88)	34.02 (35.67)
	Mean	49.88 (44.94)	42.43 (40.63)	46.15 (42.82)
	Range	30.55 (33.58) to 81.94 (64.82)	22.22 (28.11) to 69.44 (56.42)	26.38 (30.92) to 75.69 (60.47)
		Media	Ex plant	M X E
	SE±	0.7	1.49	2.11
	C.D.	2.02	4.29	6.06

Note : Figures in parenthesis are arc sin transformed values.

shoot establishment (81.94%) over Lloyd and McCown's Woody Plant Medium which gave lower shoot establishment (63.88 %). MS ½ N + 10 ppm BA + 5 ppm NAA and 5 ppm GA_3 was superior over other treatments for shoot establishment (81.94% for Kokam culture.

Efforts were made towards root induction in Kokam shoot cultures through various growth regulator combination of auxins with MS and modified MS media. *In vitro* established shoots with well developed leaves were used to induce root. Various auxin levels such as IBA , NAA and 2,4 –D were tried alone or in combination to induce roots. Among the various treatments of auxin and modified media, growth regulator combination as 2, 4 and 6 ppm IBA along with 2 ppm NAA and 2 ppm 2,4-D was found to be effective to induce swelling at the base of the Kokam shoots. However, IBA, NAA and 2,4–D at various concentrations in MS medium were unable to induce rooting or either swelling. Hence, it can be concluded that not a single treatment was found to be effective to induce roots in Kokam.

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