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# Callus Formation in Certain Mulberry Varieties of $M_5$, $V_1$, $S_{36}$ and Anantha

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**Abstract:** ***Callus formation is central to many investigation and applied tissue culture procedures. Callus can be multiplied and later used to clone numerous whole plants. Since extensive callus formation can be induced by elevated hormone levels, tissue culture media designed to produce callus containg pharmacological additions of cytokinins and auxins. Callus tissue was green and friable on 2, 4-D medium, whereas on NAA and IAA medium brownish, compact callus was reported. Though the nature of callus in our present study was smooth and yellow in M5, V1, S36, Anantha, nodular and yellow colour was observed in M5 in the beginning, it has become compact and greenish patches appeared when sub-cultured on to the medium fortified with kinetin and IAA.***

**Keywords:** NAA, IAA, Mulberry Varities, MS Medium

## 1. Introduction

Callus is defined as an unorganized tissue mass growing on solid substrate. Callus forms naturally on plants in response to wounding, infestations, or at graft unions (Bottino, 1981). During callus formation, there is some degree of dedifferentiation (i.e. the changes that occur during development and specialization are, to some extent, reversed), both in morphology (a callus is usually composed of unspecialized parenchyma cells) and metabolism. One major consequence of this dedifferentiation is that most plant cultures lose the ability to photosynthesize. This has important consequences for the culture of callus tissue, as the metabolic profile will probably not match that of the donor plant. This necessitates the addition of other components such as vitamins and, most importantly, a carbon source to the culture medium, in addition to the usual mineral nutrients. Callus culture is often performed in the dark (the lack of photosynthetic capability being no drawback) as light can encourage differentiation of the callus. During long term culture, the culture may lose the requirement for auxin and/or cytokinin. This process, known as habituation, is common in callus cultures from some plant species (such as sugar beet).Callus cultures are extremely important in plant biotechnology.

Plant growth regulators are the critical media components in determining the developmental pathway of the plant cells. The plant growth regulators used most commonly are plant hormones or their synthetic analogues. There are five main classes of plant growth regulator used in plant cell culture, Namely:(1) auxins; (2) cytokinins; (3) gibberellins; (4) abscisic acid; (5) ethylene. Auxins promote both cell division and cell growth. The most important naturally occurring auxin is indole-3-acetic acid (IAA), but its use in plant cell culture media is limited because it is unstable to both heat and light. Occasionally, amino acid conjugates of IAA (such as indole-acetyl-l-alanine and indole-acetyl-l-glycine), which are more stable, are used to partially alleviate the problems associated with the use of IAA. It is more common, though, to use stable chemical analogues of IAA as a source of auxin in plant cell culture media. 2,4-Dichlorophenoxyacetic acid (2,4-D) is the most commonly used auxin and is extremely effective in most circumstances. Other auxins are available (Wagley et al.,1987), and some may be more effective or 'potent' than 2, 4-D in some instances. Cytokinins promote cell division. Naturally occurring cytokinins are a large group of structurally related purine derivatives. Of the naturally occurring cytokinins, two have some use in plant tissue culture media zeatin and 2 Plant tissue culture. Auxins and cytokinins are the most important hormones for transgenic work. Commonly used auxins, their abbreviations and chemical names

## 2. Materials and Methods

Actively growing plant tissues requires a continuous supply of inorganic chemicals, which constitute the macronutrients and micronutrients. Potassium nitrate was used in combination with ammonium nitrate in MS medium whereas potassium nitrate was used as a single nitrogen source in $B_5$. Phosphate was supplied as sodium dihydrogen phosphate in $B_5$ and potassium dihydrogen phosphate in MS. Calcium chloride was added for the calcium requirement in both media. Concentrated stock solutions of micronutrients listed in the tables were prepared. Iron stock was prepared separately to avoid problem with iron solubility, and it was prepared in a chelated form as the sodium salt of ferric ethylene di-amine tetra acetate. Different concentrations of 2, 4-D, IAA, NAA and Kinetin were used to study the callus initiating abilities and regenerating abilities of explants of mulberry varieties. These growth regulators were dissolved in suitable solvent before preparing stock solution. Thiamine- HCI, Nicotinic acid, pyridoxine HCI were added in both the media. The amount of thiamine was relatively more in $B_5$ medium than in MS medium. Glycine was added to MS medium. 0.1% activated charcoal was supplemented to the nutrient media as it adsorbs secondary products secreted by cultured tissues. 20,000 mg/l sucrose was added for both MS and $B_5$ media. Sterilized double distilled water was employed in all tissue culture media, including the water used during the culture procedure. For semi solid media, add agar at a final concentration of 6-10 g/litre prior to autoclaving. It is important to use a good quality,

bacteriological grade agar for plant cell culture work. Approximately 50 ml of double distilled water was taken in a 100 ml beaker. Salts were weighed according to the first column of the table I. weighed salts were dissolved separately. Solution was transferred to the 100 ml of volumetric flask and made up to the mark. This micronutrient stock was stored under refrigeration. To 50 ml of double distilled water, weighed $Na_2EDTA$ (according to column 1 in Table I) was added and boiled to dissolve. Weighed $FeSO_4.7H_2O$ was added to the boiling solution. After 5 minutes the solution was transferred to the volumetric flask of 100 ml capacity. $DDH_2O$ was added to make the solution to final volume. Iron stock was stored at room temperature. Vitamins are weighed according to the column 1 of table I and dissolved in 50 ml of $DDH_2O$. This vitamin mixture was transferred to the 100 ml volumetric flask and double distilled water was added to the final volume. Vitamin stock was stored under refrigeration.

## 3. Results and Discussion

Time Taken for callus initiation (days) from the auxiliary buds at different concentrations of 2, 4 D were significantly variable. In $M_5$ variety (Fig 1) time taken for callus initiation significantly decreased with the concentrations from 0.5 to 2.0. The order of decrease at different concentrations was 0.5 $mg^{-1}$ 2, 4 D > 1.0 $mg^{-}$ 2, 4 D, > 1.5 $mg^{-1}$ 2, 4 D > 2.0 $mg^{-1}$. A similar trend was also observed in $V_1$ (Figure 2), $S_{36}$ (Figure 3) and Anantha (Figure .4).

From the data presented table 4.5 and figure 4.5, it is observed that the percent frequency of callus initiation after 20 days at different concentrations of 2, 4-D (MS medium) in $M_5$. Percent frequency of callus initiation (days) from the auxiliary buds at different concentrations of 2,4 D were significantly increased with increased concentrations (0.5$mg^{-1}$ to 2.0 $mg^{-1}$). The order of increase Percent frequency of callus initiation (days) at different concentrations was 0.5 $mg^{-1}$ 2, 4 D < 1.0 $mg^{-}$ 2, 4 D, < 1.5 $mg^{-1}$ 2, 4 D < 2.0 $mg^{-1}$. A similar trend was also observed in $V_1$ (Figure 6), $S_{36}$ (7) and Anantha (8). In the present investigation auxiliary buds were used as the source material. Shoot culture (shoot tips or buds) is often sufficiently reliable with herbaceous plants to be used commercially (Holdgate, 1977). Oka and Ohyama (1975) performed material for *invitro* propagation of mulberry. They used three kinds of explants and various aged auxiliary buds for their study. Young greenish buds with long or short stem grew into leafy shoots on MS + NAA or MS + NAA + BA medium respectively. Excised greenish buds did not grow in var. Ichinose, but they grow in var. Kenmochi when supplemented with BAP. Excised brownish buds that were more aged than greenish buds did not show any growth in both varieties.

The physiological status of the explant has been of prime importance with adult trees. Extensive studies at the Association Forest - Cellulose (AFOCEL) research institute in France indicated that explants from adult forest trees were frequently slow to commence growth *invitro*, or failed completely unless selected from rejuvenated tissues. When natural rejuvenation is absent, it has been induced by various treatments such as grafting, shoot pruning, maintenance of high fertilizer levels, vegetative propagation or spraying with cytokinines (Franclet, 1979). Hence Two months after pruning, young rejuvenated twigs up to 5th node were collected in the present study. The response of explants varied depending upon the variety.

## 4. Conclusion

MS medium containing 2 $mg/1^{-1}$ 2,4-D produced maximum fresh and dry weight of callus in 20 days. Highest callus initiation was observed at the concentration of 2 $mg^{-1}$ of 2, 4. D. Thus the present study provides a scope of rapid callusing of $M_5$, $V_1$, $S_{36}$ and Anantha on MS + 2, 4. D (2,4-Dichloro phenoxy acetic acid).

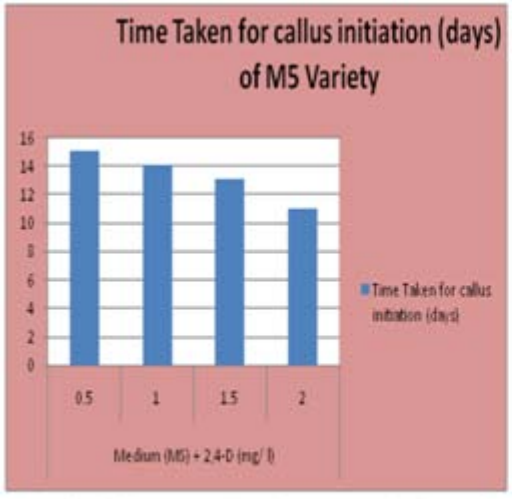



**Fig 4.1:** Effect of different concentrations of 2, 4-D 1 (MS medium) time taken for callus initiation in $M_5$

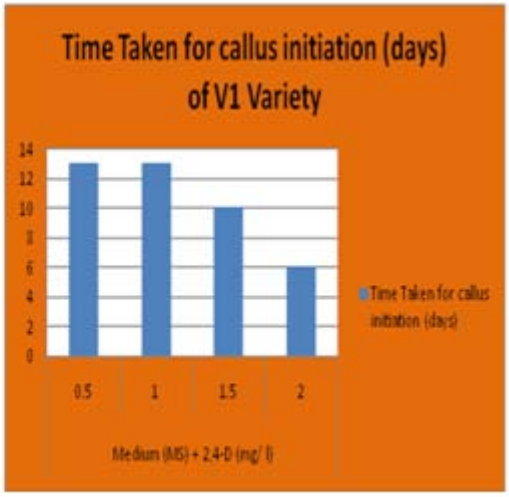



**Fig 4.2:** Effect of different concentrations of 2, 4-D 1 (MS medium) time taken for callus initiation in V1

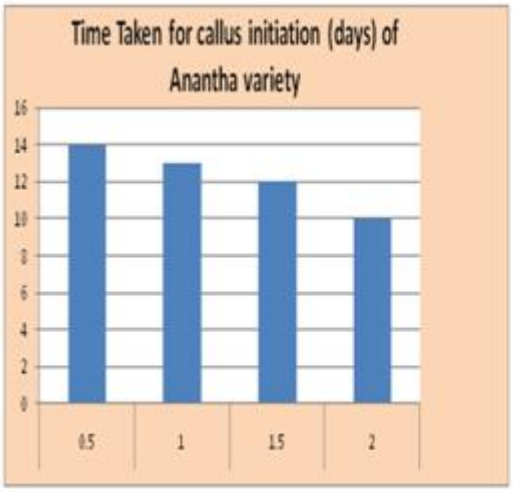



**Fig 4.3:** Effect of different concentrations of 2, 4-D 1 (MS medium) time taken for callus initiation in S36

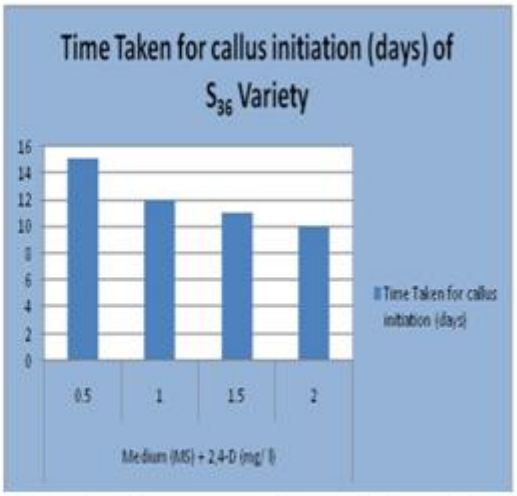
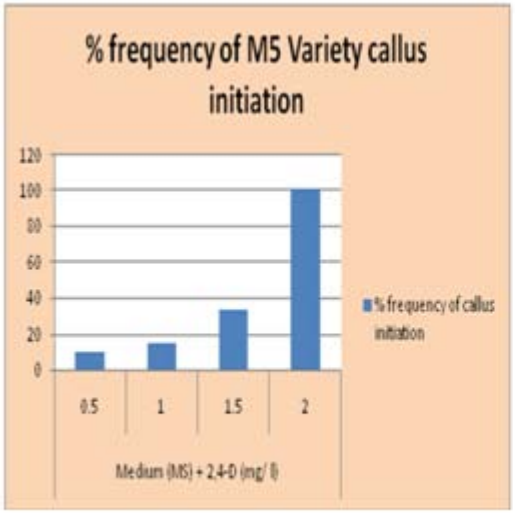



**Fig 4.4:** Effect of different concentrations of 2, 4-D 1 (MS medium) time taken for callus initiation in Anantha



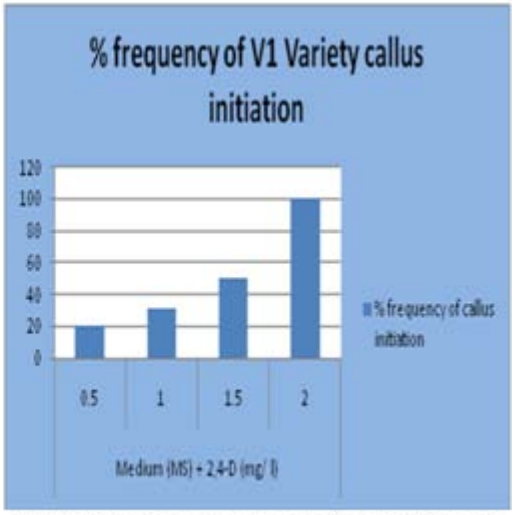




**Fig 4.6:** Effect of different concentrations of 2, 4-D 1 (MS medium) % frequency of callus initiation, after 20 days in V1

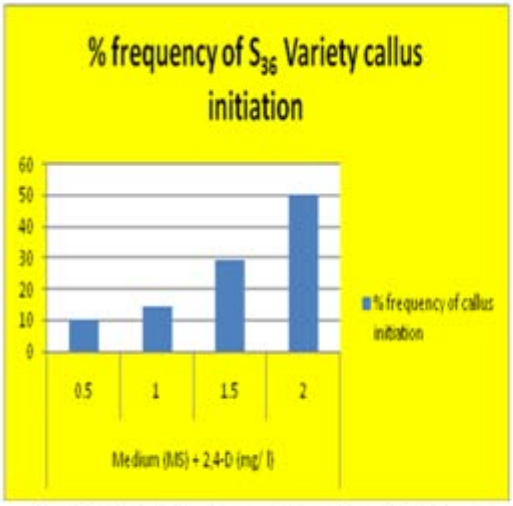



**Fig 4.7:** Effect of different concentrations of 2, 4-D 1 (MS medium) % frequency of callus initiation, after 20 days in S36

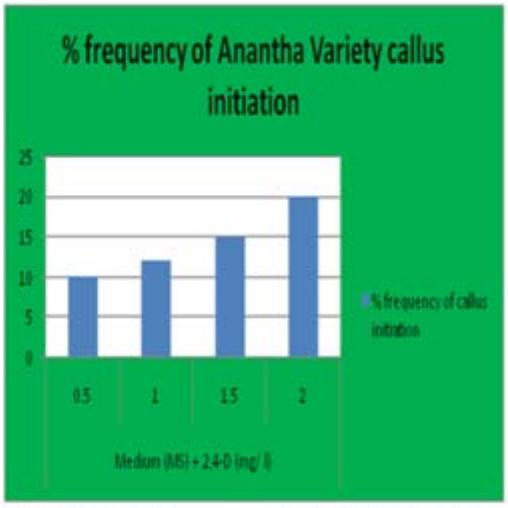



**Fig 4.8:** Effect of different concentrations of 2, 4-D 1 (MS medium) % frequency of callus initiation after 20 days in Anantha

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