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Evaluation of Suitable Medium in Certain Mulberry Varieties of M5, V1, S36, Anantha, India

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KEYWORDS

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A B S T R A C T

A defined nutrient medium consists of inorganic salts, a carbon source, vitamins, and growth regulators. Other components may be added for specific purposes. They include organic nitrogen compounds, organic acids, and plants extracts. The Murashige and Skoog (MS) or Linsmaier and Skoog (LS) are the most widely used salt compositions, especially in procedures where plant regeneration is the objective. The B5, Nitsch and Nitsch (NN) media is also having importance in tissue culture. Between the two different media such as MS and B5 with 3mg/l BAP + 0.05 mg NAA tested for Morus Spp. varities M_5, S_{36}, V_I and Anantha, MS medium was found to be best basal medium for shoot induction when compared to the B5 media. Further, the auxiliary buds exhibited high percentage of callus formation and weight of callus in all the mulberry varieties. It concluded that MS media is suitable for mulberry invitro regeneration.

Introduction

One of the most important factors governing the growth and morphogenesis of plant tissues in culture is the composition of the culture medium. The basic nutrient requirements of cultured plant cells are very similar to those of whole plants. Plant tissue and cell culture media are generally made up of the following components like macronutrients, micronutrients, vitamins, amino acids or other nitrogen supplements, sugar(s), other undefined organic supplements, solidifying agents or support systems, and growth regulators.

The macronutrients provide the six major elements-nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S)-required for plant cell or tissue growth. The optimum concentration of each nutrient for achieving maximum growth rates varies considerably among species. Culture media should contain at least 25-60 mM of inorganic nitrogen for adequate plant cell growth. Plant cells may grow on nitrates alone, but considerably better results are obtained when the medium contains both a nitrate and

ammonium nitrogen source. Certain species require ammonium or another source of reduced nitrogen for cell growth to occur. Nitrates are usually supplied in the range of 25-20 mM; typical ammonium concentrations range between 2 and 20 mM. However, ammonium concentrations in excess of 8 mM may be deleterious to cell growth of certain species. Cells can grow on a culture medium containing ammonium as the sole nitrogen source if one or more of the TCA cycle acids (Gamborg *et al*., 1968) are also included in the culture medium at concentrations of approximately 10 mM. When nitrate and ammonium sources of nitrogen are utilized together in the culture medium, the ammonium ions will be utilized together in the culture medium, the ammonium ions will be utilized more rapidly and before the nitrate ions. Potassium is required for cell growth of most plant species.

Materials and Methods

Selection and collection of plant Material

Actively growing shoots from 75 - 90 days old (days after pruning) plants were selected. Top 15 - 25 cm long shoots were chosen and 4-5 nodes are rejected (from the top downwards). The selected shoots were free from any visible signs of diseases and pests. The shoots were cut with a scissor and kept in a conical flask containing water and brought to the laboratory. The petiole, shoot tips and auxiliary buds of surrounding portion (brown dead and dried) of the stem are gently scraped with a scalpel. The nodal explants were cut into small pieces of about 1 cm. consisting of a single auxiliary bud and placed in a conical flask. The explants were washed in running tap water for about 30 minutes. Then a drop of liquid soap labolene was put into the flask and the explants were agitated in the soap solution for a minute and poured off. The explants were again washed in running tap water for 30 minutes. Then 100 ml of water was added to the flask and 200 mg Bavistin, 50 mg streptomycin and 50 mg cephatoxin was added and the flask was vigorously agitated. The explants were kept in the disinfection solution for 3 - 3.5 hours. The disinfection solution was poured off and the explants were washed in running tap water for 30 minutes. The media to be used for the inoculation, autoclaved water in flasks, 1 liter capacity flask (to collect the washings), instruments used for aseptic transfer work such as inoculation forceps, scalpel, coupling jar, etc. were placed in laminar air flow chamber. The steel table of the chamber was swiped with rectified spirit and the ultra violet (UV) lamp was switched on to make the interior of the chamber free of contamination. After 20 minutes the airflow was switched on along with the UV lamp to blow away all the dust, spores, mycelia, etc. so as to make the inside of the chamber sterile as aseptic conditions were a pre-requisite for the culture work. After 20 minutes the UV lamp was switched off and the fluorescent lamp switched on. The material (nodal, petiole and shoot tips explants) to be inoculated were placed inside the chamber. The hands and fingers were swiped with rectified spirit. The explants in the flask were covered with 0.05% mercuric chloride (aqueous solution) and gently agitated from time to time for 7 to 9 minutes depending on the thickness of the explants and type of the explants. The solution was carefully decanted into the 1 liter flasks without disturbing the explants. Then the explants were washed with autoclaved water and slowly poured into the 1 liter flask. This process was repeated three times. The explants were covered with 70% ethanol and agitated for 30 seconds and the ethanol poured off into the 1 liter flask.

Results and Discussion

From the data presented in figure 1, it is observed that the callus formation and fresh weight of callus is significantly high in all explants i.e., petiole, auxiliary buds and shoot tips in M5 cultivar. However the order of callus formation and weight of callus was auxiliary buds>shoot tips>petiole. From the data presented in figure 2, it is observed that the callus formation and fresh weight of callus is significantly high in all explants i.e. petiole, auxiliary buds and shoot tips in S36 cultivar and the order of callus formation and weight of callus was auxiliary buds>shoot tips>petiole. From the data presented in figure 3, it is observed that the callus formation and fresh weight of callus is significantly high in all explants i.e., petiole, auxiliary buds and shoot tips in V1 cultivar. However the order of callus formation and weight of callus was auxiliary buds>shoot tips>petiole. From the data presented in figure 4, it is observed that the callus formation and fresh weight of callus is significantly high in all explants i.e., petiole, auxiliary buds and shoot tips in Anantha variety and the order of callus formation and weight of callus was auxiliary buds>shoot tips>petiole. On the whole, the auxiliary buds exhibited high percentage of callus formation and weight of callus in all the mulberry varieties.

Further, in the present study two individual media viz., MS (Murashige and Skoog, 1962), and B_5 (Gamborg *et al.,* 1968) medium supplemented with 6-BAP ($3mg^{-l}$) and NAA ($0.05\ mg^{-l}$) were tried to evaluate the most suitable medium for the *in vitro* propagation of different Mulberry varieties from petiole, auxiliary buds, shoot tips. Evaluation of the most suitable medium out of the two media was carried out based on the average number of shoots produced per explants and average shoot length after a stipulated period of incubation (4 weeks) and also callusing response under the conditions described in materials and methods. Explant proliferation, shoot bud initiation and sign of morphogenesis were observed after 8 days of incubation on all the media employed during the present investigation. The percentage of explants producing shoots was observed to be varying on different media. The degree of success in any technology employing plant cell, tissue or organ culture is related to relatively few factors. A significant factor is the choice of nutritional components and growth regulators. A defined nutrient medium consists of inorganic salts, a carbon source, vitamins and growth regulators. Other components may be added for specific purposes. They include organic nitrogen compounds, organic acids and plant extracts (Gamborg, 1996). The present study clearly indicate about the superiority of MS medium over other B5 media tried in promoting shoot/plantlet regeneration from petiole, auxiliary buds, shoot tips explants of different varieties of *Morus Spp*, Between two culture media Murashige and Skoog (MS) (1962) medium is the most widely used salt compositions, especially in procedures where plant regeneration is the objective. The B_5 medium (Gamborg *et al.,* 1968) originally designed for cell suspension or callus cultures and with modifications proved valuable for protoplast culture. It may also be useful in regeneration of protoplast-derived plants (Gamborg and Phillips, 1996).

Evaluation of the most suitable medium out of the two media was carried out based on the average number of shoots produced per explant and average shoot length after a stipulated period of incubation (4 weeks). In our study the average shoot length and number of shoots per explants were significantly higher in MS media in all the

mulberry varieties. This indicates that the superiority of MS media over B5 media. Since B5 media (Gamborg *et al.*, 1968) was originally prepared for protoplast culture and cell suspension or callus cultures, B5 media could not support high percentage of average shoot length or number of shoots per explants.

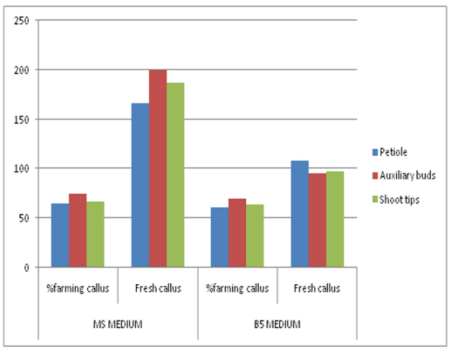

Fig. 3. 1: Evaluation of suitable medium for callus induction mulberry cultivar M5

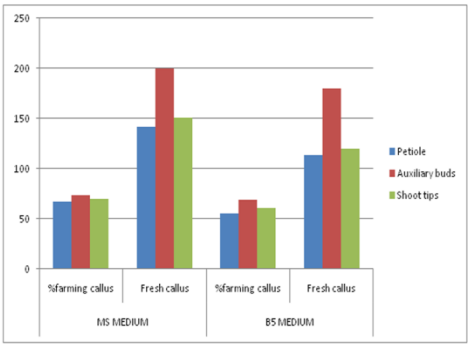

Fig. 3.2: Evaluation of suitable medium for callus induction mulberry cultivar V1

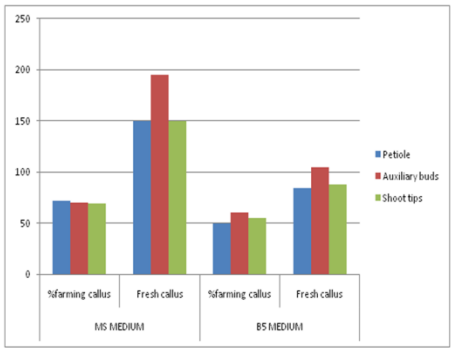

Fig. 3. 3: Evaluation of suitable medium for callus induction mulberry cultivar S36

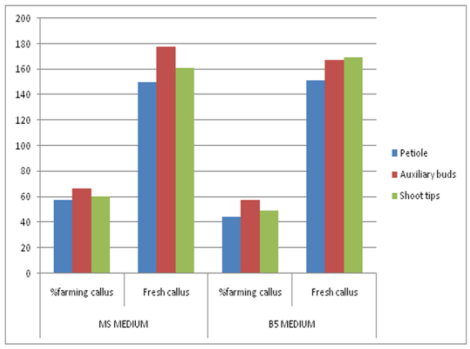

Fig. 3. 4: Evaluation of suitable medium for callus induction mulberry variety Anantha

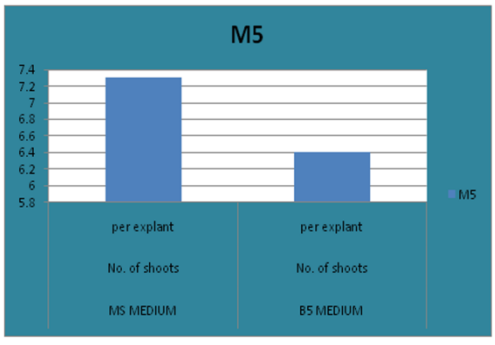

Fig. 3. 5: No. of shoots per explant in mulberry variety M5 with medium MS and B5 supplemented with BAP ($3mg^{-1}$) and NAA ($0.05mg^{-1}$)

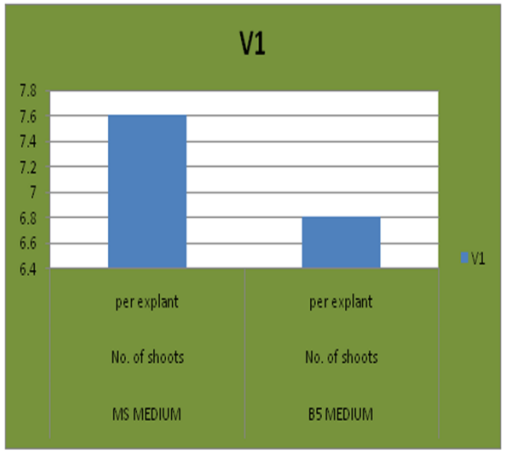

Fig. 3. 6: No. of shoots per explant in mulberry variety V1 with medium MS and B5 supplemented with BAP (3mg^{-1}) and NAA (0.05mg^{-1})

Fig. 3. 7: No. of shoots per explant in mulberry variety S36 with medium MS and B5 supplemented with BAP (3mg^{-1}) and NAA (0.05mg^{-1})

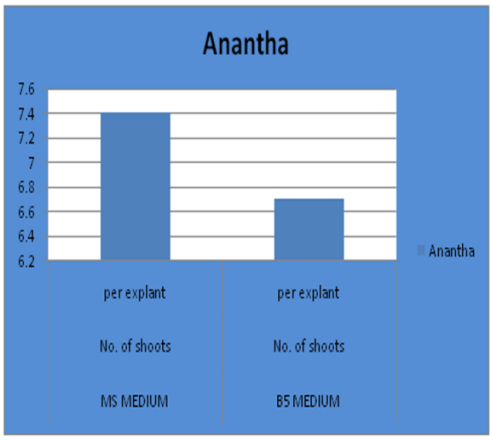

Fig 3.9 : Average Shoot length (cms) in mulberry variety S36 with medium MS and B5 supplemented with BAP (3mg^{-1}) and NAA (0.05mg^{-1})

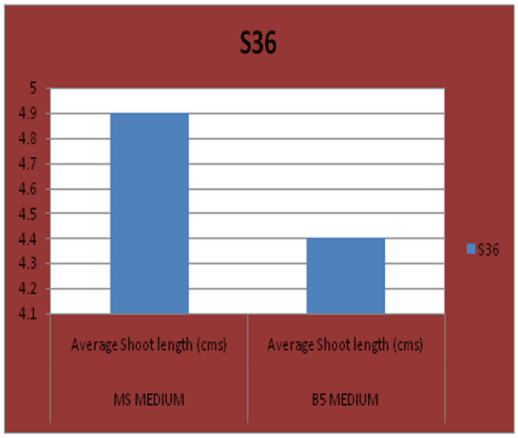

Fig 3.8 : No. of shoots per explant in mulberry variety Anantha with medium MS and B5 supplemented with BAP (3mg^{-1}) and NAA (0.05mg^{-1})

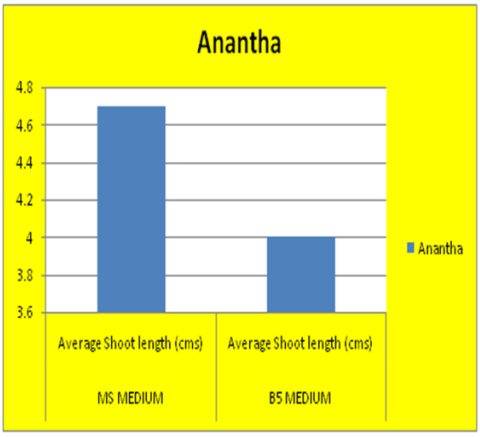

Fig 3.10 : Average Shoot length (cms) in mulberry variety Anantha with medium MS and B5 supplemented with BAP (3mg^{-1}) and NAA (0.05mg^{-1})

Success in employing these various media in all probability lies in the fact that the ratios as well as the concentrations of nutrients nearly match the optimum requirement with regard to the growth and differentiation of respective cell or tissue systems. In general, the choice of media is dictated by the purpose of the tissue culture technology which is to be employed and the plant species or a variety.

Hence, all the further studies were carried out by employing MS medium as the medium of choice. Since the auxiliary buds exhibited a significant response for MS medium, for further studies auxiliary buds were selected from all varieties (M_5, V_1, S_{36} and Anantha) of mulberry.

Conclusions

MS medium was found to be best basal medium for shoot induction when compared to the B5 media. Further, the auxiliary buds exhibited high percentage of callus formation and weight of callus in all the mulberry varieties. Hence, only MS medium was used to carry out all the *invitro* experiments for plantlet regenerations.

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