LOW COST SUPPORT MATRIX FOR POTATO MICRO-PROPAGATION

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ABSTRACT: Potato quality seed is supplied through minitubers produced by micropropagation technique. Lowering cost of production without loosing the quality is the major bottleneck in potato micropropagation. Agar is most widely used gelling agent in plant tissue culture. Agar as gelling agent not only limits the growth and cause vitrification of cultures but also adds to the cost of production. Various alternative gelling agents were tried earlier for lowering the production cost in potato micropropagation but hardly any of them was used in large scale micropropagation. In the present study, a successful attempt has been made to find out cotton as low cost plant support matrix in plant tissue culture. In comparison with agar as support matrix, there was an early shoot initiation followed by vigorous growth when cotton is used as a support matrix. Shoot height, number of nodes and biomass accumulation increased significantly in cotton incorporated cultures. The explant establishment was 97%, with 55% increase in number of internodes followed by 30% increase in shoot height and two-fold increase in dry matter content; increases production efficiency considerably in terms of producing significantly more number of nodes per shoot (4.6 ± 0.23) which results in more number of explants for further subcultures. This ultimately results in increased subculture efficiency. Cotton is very cheap than agar and there is a saving of twenty rupees per litre of media, which had high impact on economics in the cost of production in plant micropropagation. The results put together indicate that cotton can be used as low cost support matrix in potato micropropagation.

INTRODUCTION

Potato micropropagation is a common practice all over the world for mass production of quality seed tubers. Potato minitubers are produced through micropropagation of disease-indexed mother cultures. The cost of seed tubers raised through micropropagation is higher than normal tubers. For plant micropropagation use of liquid media for shoot multiplication has advantages in terms of better nutrition, easier dispensing, uniform nutrient and temperature dispersion (1). However liquid media requires illuminated shakers, which needs high initial investment. Different support matrices/gelling agents are used to support explants in liquid media. Support matrix tried so far lack inertness, large-scale availability for commercial use which adds to the cost of production. Agar is considered to be non-toxic and biologically inert gelling agent in plant tissue culture and hence most widely used. However, it accounts for 80% of media cost (5, 8). Study has revealed that impurities and variability in composition of agar cause complex interactions in plant growth, alters the availability of mineral components and results in variation of gas exchange, reduces heat dissipation and enhances microbial contamination and functional abnormalities in root development (5, 10). Identification of suitable gelling agent/support matrix for micropropagation is an essential need in commercial potato micropropagation. Identification of low cost matrices to replace agar use in micropropagation was attempted by many workers (8, 7). Although starch from different sources was used as cheap alternative, it gets metabolised resulting in decreased media consistency and reduced growth rate of cultures (10). In the present study, cotton has been tested as a supporting matrix alternative to agar because it is cheaper than agar, easily available, easy to handle, requires no pre-melting like other gelling agents and media clarity can be used as a measure to check microbial contamination. In present work

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absorbent cotton as a support matrix instead of agar in in-vitro culture studies and effect on potato shoot multiplication and its impact economics has been discussed.

Five SNCs were placed in each bottle. Each replication contained 50 bottles and experiment was repeated thrice with three replications. The data recorded was statistically analysed for significance using standard statistical methods.

RESULTS AND DISCUSSION

When starch from various sources was used as alternative gelling agent in plant micropropagation, it suffered drawbacks like weak solidification and significant ionic variation in batch to batch. Elemental and organic impurities affect morphological and molecular responses of seedlings to deficiencies of nutrients (5, 2). Starch upon autoclaving yields sugars which caused the enhancement of medium osmotic potential that resulted in growth reduction. Starch is not soluble in water at ambient temperature and at high temperature its granules gelatinize to form an opalescent dispersion, which acts as potential substrate for enzymes such as amylase and amyloglucosidase. During sterilization, using such gelling agents, decrease in pH of the medium alters the availability of nutrients for explants to grow (10). Aseptic conditions need to be maintained with high vigil to dispense the hot media, a step that can result in microbial contamination. Use of cotton for replacing agar has no such limitation as it provides support without any detrimental effects like heat and nutrient diffusion gradient, drop of pH or change in EC, etc. It is easily available and can be easily incorporated in/removed from media bottles and does not pose any threat to the environment. Cotton (cellulose) is a highly crystalline polymer completely insoluble in water, most of organic solvents and is inert during the micropropagation process.

Morphological parameters like per cent explant establishment, height of the plant, number of internodes and number of leaves were found better in cotton-supported medium.

MATERIALS AND METHODS

The mother cultures were established according to Naik and Karihaloo (9). About 40 ml growth medium with no gelling agent/support matrix, or with 7.5% agar (Hi Media) as gelling agent or ~1 g of absorbent cotton was dispensed per bottle. Cultures were grown in standard growth chamber maintained at 22 ± 2° C temperature under 16 h photoperiod. Single Node Cuttings (SNCs) of 1 to 1.5 cm from three-week old potato shoots were used as explants for culture multiplication.

The growth parameters like percentage of explant establishment, period required for sprouting of axillary bud, height of microplant, number of nodes/leaves/multiple shoots per plant, were recorded on 4th and 20th day after culture initiation. The dry matter accumulation was recorded on 20th day. The plants were visually evaluated for symptoms of hyperhydricity (gross morphological change and/or translucent appearance). pH and EC of the medium was recorded before and after autoclaving of the medium, at inoculation and subsequently on 20th day after inoculation as described by Cassells and Collins (4).
(Fig. 1) at significance at p<0.1. The plant height increased significantly with cotton as support matrix (3.9 ± 0.27 cm) as compared to agar (3.0 ± 0.17 cm) i.e. 30% increase in plant height. Number of nodes increased significantly with cotton as support matrix (4.6 ± 0.23) as compared to agar (3.0 ± 0.67), i.e. 53% per cent increase in number of internodes and 40% increase in number of leaves was observed (Fig. 2). Plants in liquid media showed poor growth. There was no difference in per cent explants establishment with agar incorporated and cotton supported media but initiation of the axillary bud development was noticed within two days of inoculation with cotton as support matrix while those in agar and liquid cultures required four and eight days respectively. The plants grown on cotton matrix showed significantly higher fresh (0.393) and dry (0.035) biomass at p≤0.05) (Fig. 2).

that the cotton matrix used, remained inert during the growth period of culture. Callus like growth at base of explants was observed in plants grown in liquid and agar medium. Use of cotton as support matrix probably helps in better acquisition of nutrients and aeration of plants, reduction in diffusion gradient in nutrient supply. Liquid medium favours higher nutrient assimilation and dry matter accumulation, however in the present study cultures grown on cotton support matrix showed higher biomass accumulation.

ECONOMICS

To calculate the economics, current market price for agar Rs.1408/500g and Rs.50/500g for cotton has been used. The approximate cost of matrices per bottle works out to be Rs. 0.10 per bottle (Rs. 2.5/ l) in case of cotton and Rs. 0.85 per bottle (Rs.13.25/ l) for agar. This results in direct saving of Rs.0.75 per bottle in the media cost, a significant saving (Rs. 20.00/ l) on cost in commercial micropropagation. When cotton was used as support matrix, pre-heating of media to melt agar/ starch is not required and media dispensing becomes easy and saved energy usage. It was observed that there was increase in production efficiency in terms of getting higher number nodes resulting in higher number of shoots with same media.
amount with cotton, instead of agar as support matrix. The work presented here has detailed the benefits for lowering not only the cost of media ingredient but it has also shown the betterment for improving the quality of product and production process. Hence, cotton, as support matrix, has tremendous potential in commercial micropropagation potato and also in other plants. The investigations suggest that cotton is best and cheap supporting matrix for potato micropropagation. It also avoids overexploitation of natural resources.

LITERATURE CITED

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