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DEVELOPMENT OF LOW COST PROTOCOL FOR MICROPROPAGATION OF *SOLANUM TUBEROSUM* L.

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Abstract

A cost-effective protocol for micropropagation of potato was established. Balanga (*Lallemantia royleana)* seeds were used as gelling agent in semi-solid/Murashiage and Skoog medium in order to find cheaper substitute of agar. After a pilot study, 45 g/l of balanga seeds were selected as suitable concentrations to be used for micropropagation of potato. Axillary shoots of potatoes plants were used as explants and data on various parameters *viz.* number of roots, root length, shoots length and number of nodes etc. was recorded. Results of the experiment indicated that balanga seeds show parallel even better briefed than agar to some extent and it is about 32% cheaper than agar. Based on these results it may be concluded that balanga seeds could be used as gelling agent for micropropagation of potato throughout the world as cost effective source to save hard earned money and disease free potato seeds simultaneously.

Introduction

Potato (*Solanum tuberosum* L.) is fourth major food crop after wheat, rice and maize. Annually more than a billion people consume potato throughout the world (Venkatasalam *et al*. 2013). It is rich in carbohydrates and approximately 90 kilocalories of energy can be obtained from 100 grams of potato (Szalay 2014). In Asian-Pacific countries about 121.7 million tons of potatos are prduced on area of approximately 7.3 million hectares with an average production of about 16.49 tons per hectare (Naik 2007). While in Pakistan, during 2013, potato was cultivated on an area of about 161.9 hectares with a production of 3507.5 tons (Arain 2014).

Seed is considered as the most expensive output in potato cultivation. About half of the total expenses are consumed for seeds (Venkatasalam *et al*. 2013). However, despite spending a huge amount of money, most of the seeds obtained are non-certified, impure and contaminated by various pathogens (Shah *et al.* 2003). Plant biotechnology is offering great deal in this regard by producing disease free and healthy potato seeds via clonal propagation for large scale or commercial production because it has an advantage of immediate 'age transfer' that saves time, reduce cost and the only way to conserve true to type plant (Anon. 2006, Pattnaik *et al.* 1996, Konstas *et al*. 2003).

But lack of budget, limited resource allocation and relatively high recurrent cost (chemical expenses) of this technology has been envisaged as a major obstacle in benefiting from this technology especially for developing countries like Pakistan. The only alternative is to develop cost-effective technology for *in vitro* clonal propagation of disease free potato that requires controlled environment and defined medium. The principal components of most plant tissue culture media are inorganic nutrients (macro- and micronutrients) carbon source (sucrose) organic supplements (vitamins), growth regulators and agar. Agar is widely used as gelling agent in tissue culture due to its high clarity, stability and non-toxic nature (Henderson *et al.* 1988). However, pure culture grade agar is found to be the most expensive component of this technology (Sahu *et al.* 2013).

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Therefore, in the present research work an attempt has been made to substitute agar by balanga seeds as gelling agent to decrease the cost of micro propagation without compromising on quality of production.

Materials and Methods

This experiment was performed at Plant Tissue Culture Laboratory, Department of Botany, Hazara University, Mansehra.

Red skinned potato cultivar "desiree" was used as explant. *In vitro* propagated virus free plantlets were kindly provided by Hazara Research Centre Abbottabad. Three apical nodes were used in order to maintain physiological uniformity throughout the experiment.

Basal Murashige and Skoog (1962) medium containing gibberellic acid (0.50 mg/l), sucrose (30 g/l) and meso-inositol (100 mg/l) was used as nutrient media solidified with 6 g/l of agar for control group. Agar was replaced by *Lallemantia royleana* seeds for experimental group. Nodal sections from virus free plantlets were aseptically cultured on 15 ml media in 15 × 30 mm test tubes. Cultures were incubated at almost 26^{o}C temperature and 16/8 hrs (day/night) photoperiod.

Three different concentrations i.e., 40, 45 and 50 g/l of balanga seeds were used as a gelling agent in pilot experiment.

Growth of the shoots and roots was measured in centimeters (cm) by the methods of Martinez *et al.* (1996) after 7, 14, 21 and 28 days of culturing.

Results and Discussion

Although agar has been found as best gelling agent for tissue culture due to its clarity, non-toxic nature and stability (Henderson and Kinnersely 1988) however, its high cost always compelled the scientists of developing countries to find out a cheaper alternative without compromising on quality of production.

Seeds of *Lallemantia royleana* (balanga seeds) were used as gelling agent in this study and almost similar results were found as in media supplemented with agar. Data regarding number of roots/plantlet, number of shoots/plantlet and shoot length/plantlet showed parallel results in both treatments. Even it was observed that plantlets supplemented with balanga seeds were healthier and morphologically better than plantlets supplemented with agar. Especially roots were stronger and well developed in media supplemented with balanga seeds. Gelling agent greatly influence the plant growth as the growth of cultured plantlet is the result of interaction of gelling agent and nutritive media. Semisolid media allows better absorption of required nutrients from media for plantlet (Venkatasalam *et al.* 2013, Shah *et al*. 2003). Mucilage of *L. royleana* has gelling properties as reported by Abdulrasool *et al.* (2011) and can be used as gelling agent in tissue culture. It is non-toxic organic material and it is not metabolized throughout the experiment. Moreover, balanga seeds might reduce the chances of contamination as reported by Mahmood *et al.* (2013) because balanga seeds possess antibacterial activities against four bacterial strains *E. coli*, *E. cloaceae*, *P. aeruginosa* and *S. aureus* (Figs 1-4 and Table 1).

Despite of tissue culture a best way to produce virus free plants, it is limited to developed countries only and developing countries like Pakistan are unable to afford its expenses and they are compelled to rely on non-certified, impure and contaminated seeds. However our experiment suggested balanga seeds to be used as gelling agent instead of agar as there is no significant difference between them. Even, throughout the experiment balanga seeds were more efficient to support overall growth of potato than agar solidified medium. The study reveals that (i) balanga seeds are suitable substitute of agar, (ii) balanga seeds did not exhibit any disorder or adverse

effect on the overall development and growth of potato plant rather it showed better performance on overall physiological traits of potato plants, (iii) bud proliferation completed in same number of days in both the medium, (iv) the plants showed better survival in the medium solidified with balanga seeds and (v) the cost of production/plant of potato for balanga seeds is lower than agar. These results are in conformity with the findings of Gasper *et al.* (1996) who reported that synthetic growth regulator work much in the same way as natural growth regulators present in plants while Anon. (2003) stated that large amount of plant growth regulators are hazardous for environment and human health and should be replaced by less hazardous compounds having natural source.

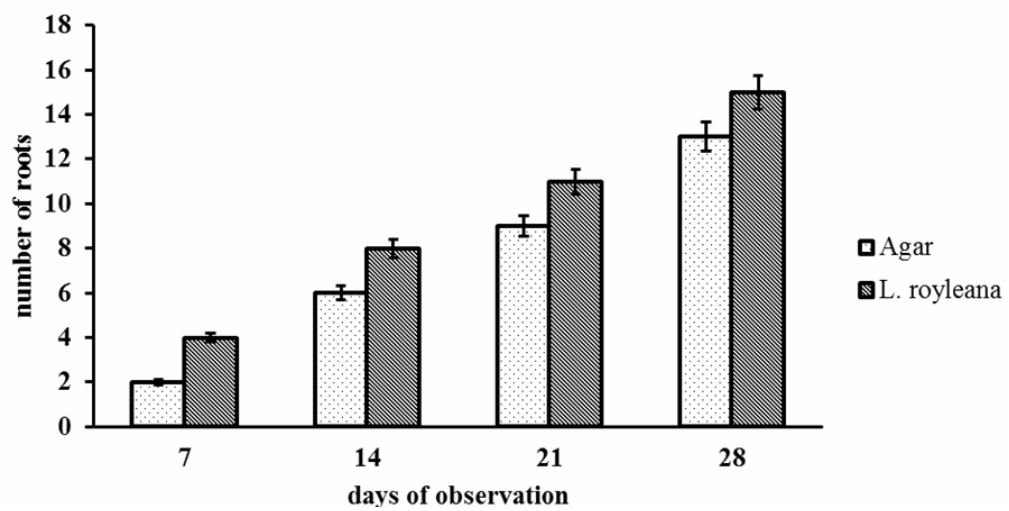

Fig. 1. Showing average number of roots in two treatments.

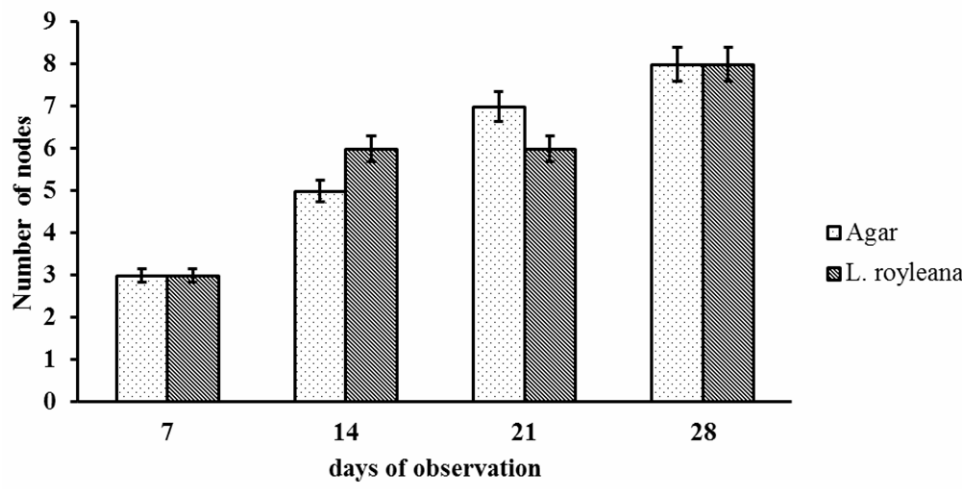

Fig. 2. Showing average number of nodes in two treatments.

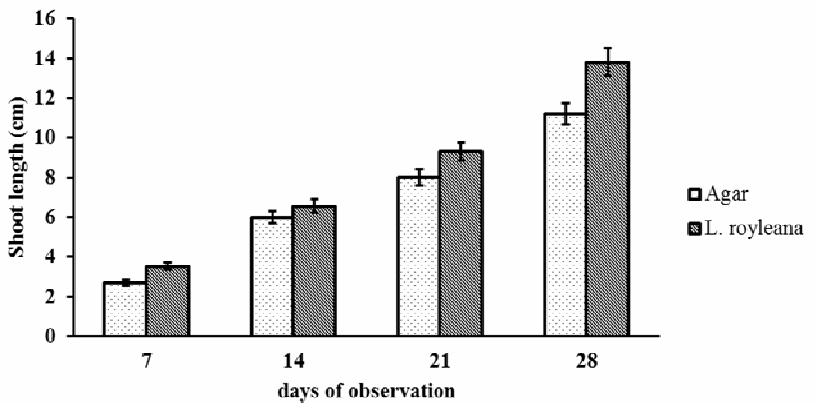

Fig. 3. Showing average shoot length in two treatments.

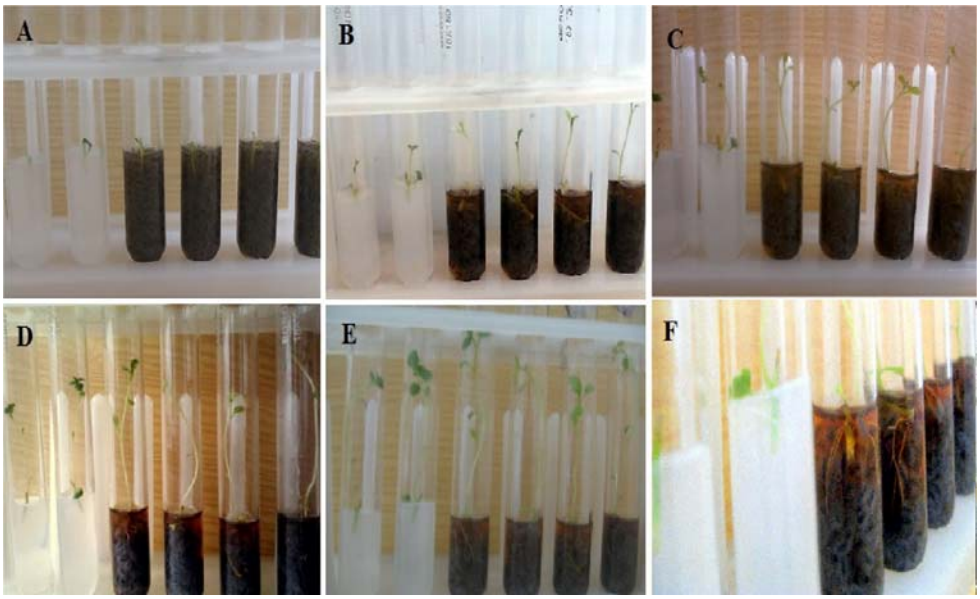

Fig. 4. Axial shoots of red skinned potato variety "desiree" were cultured on two treatments i.e. agar and *L. royleana*. Bud proliferation and emergence of first roots observed after two days of experiment (A), while highest number of roots and shoots were observed after 28 days of experiment (E), highest shoot length and number of nodes were also observed after 28 days of experiment (E), while roots in two treatments are shown in (F).

Table 1. Showing effect of gelling agents on number of roots/plantlet and number of nodes/plantlet of potato cv. "Desiree".

Gelling agent	Number of roots/plantlet (mean)	Number of nodes/plantlet (mean)	Shoot length in cm (mean)
Agar	7.5	3.75	6.97
L. royleana	9.5	5.75	8.32

However, result of present study revealed that balanga seeds showed parallel performance with agar as gelling agent and it has no adverse effect on quality of plantlets. Hence seeds of balanga seeds can be used as the most suitable alternative of agar for micro propagation of potato. By using balanga seeds as gelling agent for micropropagation of potato Rs. 2.27 per plantlet can be saved as it is about 32% cheaper than agar. There is every possibility of balanga seeds becoming a universal gelling agent.

It is strongly recommended that this protocol for adaptation in all tissue culture labs in the whole country, where for the basic seed production commercial *in vitro* micropropagation is used. Hence, it would be possible to reduce the drain off hard earned foreign exchange.

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