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Some of Cheaper Alternatives to Ms Media for *in vitro* Culture of Potato

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Abstract: The most important attempts during the whole investigation were taken to make *in vitro* propagation protocol, cost effective by using economically cheaper alternatives to MS salts, agar and sucrose. MS (Murashige and Skoog) and low cost (LC) media with different hormonal combinations of Kn (0.04, 0.06 and 0.08 mg/l) and IAA (0.50 mg/l) were used for shoot and root proliferation. In low cost media, tapioca was used as substitute of agar and replacing sucrose with sugar cane, because of low cost and easy availability. Calcium ammonium nitrate (6.6 gm/l), Single super phosphate (1 gm/l), Muriate of potash (10.6 gm/l) and sugar cane (30 gm/l) were used as low cost media in place of MS salts. Amongst the two media used for proliferation, the shoot height (14.73 cm), number of nodes (23.7) and root length (14.3 cm) were significantly higher in LC media with 0.04 mg/l Kn and 0.50 mg/l IAA hormonal combination, as compared to MS media after 80 days of growth. The transplanted plantlets to the soil were survived well as 70-80%. The results obtained from the present investigation indicate that LC media was consistently better for shoot and proliferation in cultivar *Kufri Himalini*. From the present investigation, it is concluded that through reduction of the cost on the techniques, the cost of the product also be reduced and farmers get benefited using low cost, disease free and clonal planting material with high production and saving land resources.

Key words: MS media % Low cost media % Tapioca % Table sugar % Shoot and root proliferation

INTRODUCTION

Potato is an important human food with wider adaptability potential to fill the gap between food needs and cereals. The importance of vegetables in human nutrition is well known. In a country with limited resources, where the nutrition level of the population has to be maintained under inhospitable situations, the potato has a special value as food [1]. As major cereals are fast approaching the practical limits of their production, the yield potential of potato is still underutilized [2]. Conventional propagation of potato is done vegetatively using seed tubers which ensure uniformity of the crop in terms of growth and yield, but results in degeneration of the crop due to virus infection. The rates of degeneration vary from place to place and from one cropping season to other cropping season [3]. The viruses are transmitted through different ways as well as by planting infected tubers. If the seed stock is not maintained well or frequently replaced with fresh ones, the virus infiltration can reach up to 100% in 3 - 4 successive crop seasons resulting in almost half or one third yields [4]. This is the major problem faced by seed producers.

Potato poses various problems to the plant breeders, by being a tetraploid, vegetatively propagated crop. These problems include a high level of heterozygosity, the common occurrence of pollen sterility, difficulties in germplasm storage and transport and the build ups of viruses. The planting material is also quite bulky, as tubers are used for propagation and a seed rate of 2.0 to 2.5 tons/ha is required for it; therefore much amount of potential food is wasted [5]. The main problem of growing potato worldwide is huge economic losses due to late blight. *Phytophthora infestans* can destroy all parts of potato plant within two weeks in wet conditions [6-8]. *Phytophthora infestans* can survive under adverse conditions and winter over in the form of oospores. The pathogen however, invades and infects potato plants in the field via zoosporangia which disperse via soil water, rain splash and wind [8]. Generally tuber is used as a seed. Due to progressive accommodation of viral disease in potato seed stock, availability of good quality seed is a

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major constraint in potato production, which is approximately 50% of the total production cost. Besides high cost of seed potato, the productivity is also influenced by characterized by low multiplication rate of only 4-6 times. Large scale production of clonal material i.e., to produce uniform, identical seed material of potato, micropropagation can be the better alternative over conventional propagation of potato. By using the technique, which involves low cost components, large-scale clonal material can be produced in short time duration. Use of micropropagation for commercial seed production has moved potato from test tubes to field [9].

In Vietnam, a simple low - cost rapid multiplication system has been developed for farmers using *in vitro* plantlets. These are multiplied *in vitro*, producing single node cuttings that are transferred and rooted in sand beds at high density. Apical and axillary cuttings are taken and rooted in beds with subsoil-manure mixture. Then cuttings are taken for rooting in the small banana leaf pots. Three *in vitro* plantlets can provide sufficient material to plant 1 ha in 7 months [10]. Using the tissue culture technique of micropropagation, it is possible not only to reduce the number of field exposures but also to increase the rate of multiplication several times. Plant tissue culture comprises a set of *in vitro* techniques, methods and strategies that are part of the group of technologies called plant biotechnology. Hence to produce the disease free planting material and for decreasing the production cost new methods of propagation are to be derived and adopted. To large production of clonal material i.e. to produce the uniform, identical seed material of potato, micropropagation is the better alternative over to conventional propagation of potato

MATERIALS AND METHODS

The tubers of *Kufri Himalini* obtained from Central Potato Research Institute, Shimla, were grown as mother plant and nodal segments were selected for *in vitro* culture. Two types of media with different hormonal combinations were used for the study. The MS media was composed of micro and macronutrients containing 3% sucrose and 0.8% agar. In low cost media, tapioca was used as substitute of agar and sugar cane was used in the place of sucrose, because of low cost and easy availability. Calcium ammonium nitrate (CAN- 6.6 gm/l), Single super phosphate (SSP- 1 gm/l), Muriate of potash (MoP- 10.6 gm. /l) and sugar cane (30 gm. /l) were used as low cost media [11]. Four concentrations (9, 12, 15 and 18 g /l) of tapioca were used as gelling agent in LC media (Table 1). The rates were fixed based on prior information and pilot investigations.

Sterilized explants were cultured in MS and LC media supplemented with different hormonal combinations and variations of tapioca in laminar air flow cabinet. These cultures were incubated in culture growth room at 25°±1°C temperature with 16 hrs light and 8 hrs dark conditions. The observations were recorded for shoot height, node number and root length

Table 1: MS and LC media along with the hormonal combinations for shoot proliferation and multiplication

	Hormone mg /l			
Symbol used of media	Kn	IAA	Carbon source used (gm./l)	Gelling agent used (gm./l)
MSH1	0.04	0.05	Sucrose (30)	Agar (8)
MSH2	0.06			
MSH3	0.08			
LCT9H1	0.04	0.05	Sugar (30)	Tapioca (9)
LCT9H2	0.06			
LCT9H3	0.08			
LCT12H1	0.04	0.05	Sugar (30)	Tapioca (12)
LCT12H2	0.06			
LCT12H3	0.08			
LCT15H1	0.04	0.05	Sugar (30)	Tapioca (15)
LCT15H2	0.06			
LCT15H3	0.08			
LCT18H1		0.05	Sugar (30)	Tapioca (18)
LCT18H2				
LCT18H3				

after 40, 60 and 80 days of culture growth. The shoots were sub-cultured on its parent media after the interval of 25-30 days, by cutting it into small pieces of around 3 cm in a way that each subsection had at least 2-3 nods. The plantlets developed in the culture media were transplanted to soil for survival evaluation.

The data were analyzed according to the procedure of analysis of variance for Randomized Block Design, Factorial RBD (Single Factor and Double Factor) and Least Significant Difference *(P<0.05)* during the whole investigation [12].

RESULTS AND DISCUSSION

The potato (*Solanum tuberosum* L.) is the fourth ranked world crop which has a production of nearly 325 million tons annually. It is the most widely cultivated food crop after wheat, rice and maize, therefore, it is considered as the most important tuber crop [13]. Potato production is being seriously hampered due to certain viruses, fungus and bacterial diseases. The total loss caused by diseases is estimated between 30 - 100% during cultivation and in a period of 2 - 6 months of storage [14]. Potatoes are normally propagated by planting the buds, or eyes, present on the tubers. This method of propagation allows viruses to be transmitted to the new reduce yields [15-17]. During 1980s, with an advance in plant biotechnology new methods in plant pathology have been developed [17]. For example, micro-propagation of potatoes in laboratories has shown to eliminate virus diseases thus ensuring a virus-free material resulting in increased yields.

The present study was undertaken to develop the low cost component protocol for *in vitro* culture of potato cv. Kufri Himalini. Indian Council of Agriculture Research has identified *Kufri Himalini* for commercial cultivation in hilly regions. The new variety, with media maturity of 110-120 days has been recommended for cultivation in the north- western and eastern hills during summer. Kufri Himalini provides a yield advantage of over 10% over Kufri Jyoti and Kufri Giriraj, in the plains and its keeping quality is better than all the cultivars developed so far for hill regions [18].

The sterile nodal segments were propagated on MS and LC media to find the economically cheaper basal media, as the low cost media has an input of commonly used fertilizers. Although the MS media [19] contains most suitable combination of organic and inorganic compounds and has been overwhelmingly used for shoot proliferation by number of workers [20-32]

Table 2: Effect of MS and LC media on shoot height after 40, 60 and 80 days of culture

	Periods (day)		
Media Used	40	60	80
MSH_1	8.28±0.5	10.91±1.1	14.1±1.7
MSH_2	7.15±0.5	8.38±0.5	10.19±1.1
MSH_3	6.51±0.6	7.33±0.6	8.77±0.6
LC_9H_1	4.60±1.2	5.25±0.5	5.86±0.4
LC_9H_2	3.45±0.6	4.42±0.5	4.97±0.3
LC_9H_3	3.61±0.3	4.42±0.3	4.71±0.2
$LC_{12}H_1$	6.06±0.4	8.00±0.5	9.22±0.5
$LC_{12}H_2$	5.63±0.4	6.90±0.5	7.74±0.4
$LC_{12}H_3$	5.02±0.4	5.91±0.3	7.18±0.2
$LC_{15}H_1$	9.32±0.8	12.24±0.3	14.73±1.1
$LC_{15}H_2$	8.61±1.0	12.14±0.2	14.54±0.5
$LC_{15}H_3$	7.48±0.9	10.59±0.3	12.71±1.3
$LC_{18}H_1$	5.20±0.8	6.28±0.5	7.60±0.3
$LC_{18}H_2$	4.94±0.2	5.65±0.3	6.76±0.4
$LC_{18}H_3$	4.24±0.6	5.04±0.5	5.96±0.6
F value	65.00**	112.37**	175.77**
LSD (*P< 0.05*)	0.52	0.58	0.60

***Significant*

but in the present study the shoot height and node number was significantly better in LC media as compared to MS media. In MS media maximum shoot height reached 14.72±.7 cm and node number was counted 23.4±2.1 in H_1 combination. In LC media different tapioca concentrations showed much variation in shoot height and node number. With the increasing concentration of tapioca, shoot height and node number were increased but after 15 gm/l tapioca concentration both were decreased as observed in the media with 18 gm/l tapioca. Maximum shoot height (14.73±1.1 cm) and node number (23.7±1.8) in LC media have been observed with 15 gm/l tapioca and H_1 hormonal concentration. A perusal of values for shoot height (Table 2; Plate 1b) and node number (Table 3; Plate-1 b) indicates clearly that the Low Cost (LC) media responded better than MS media (Plate-1 a). The H_1 hormonal combination in both the media showed better growth than all other combinations. In LC media, between various tapioca concentrations, 15 gm/l tapioca reported better with all three hormonal combinations (H_1, H_2 and H_3), while 12 gm/l tapioca with H_1 hormonal combination also showed good result.

MS media contains macro and micronutrients, sucrose, vitamins and agar. Agar is the most commonly used gelling agent for routine propagation experiments, it contributes to 60% of the cost of media and makes the whole *in vitro* work expensive. India imports tones of agar

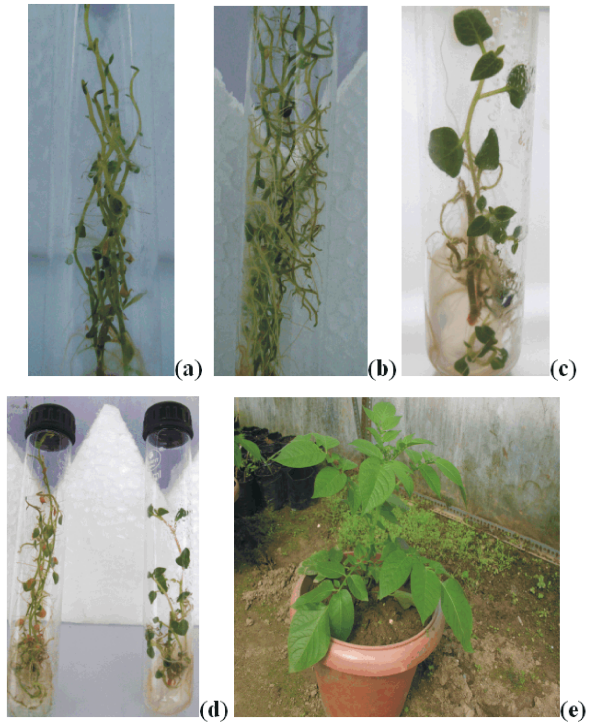

Plate 1(a-e): Plantlet Growth and Transplantation;
(a) Plantlets in MSH_1 media,
(b) Plantlets in $LC_{15}H_1$ media, (c-d) rooting on shoots and
(e) Transplantation of plantlets to soil

Table 3: Effect of MS and LC media on number of nodes after 40,60 and 80 days of culture

	Periods (day)		
Media Used	40	60	80
MSH_1	9.4±1.0	16.2±1.4	23.4±2.1
MSH_2	8.2±1.0	14.1±0.9	20.4±1.3
MSH_3	6.3±0.9	10.6±2.3	16.2±2.0
LC_9H_1	5.6±1.1	7.9±1.1	9.6±1.1
LC_9H_2	2.3±0.9	4.4±1.5	5.9±1.4
LC_9H_3	1.4±0.8	2.8±1.5	5.0±1.6
$LC_{12}H_1$	12.4±2.0	16.6±2.0	20.0±2.1
$LC_{12}H_2$	9.2±1.6	13.8±1.3	17.7±1.6
$LC_{12}H_3$	7.1±1.1	11.9±1.7	15.1±1.8
$LC_{15}H_1$	14.3±2.6	19.5±2.2	23.7±1.8
$LC_{15}H_2$	12.0±1.4	16.6±1.7	21.5±2.4
$LC_{15}H_3$	8.4±1.7	13.1±2.5	16.9±2.5
$LC_{18}H_1$	3.0±1.1	5.6±1.1	8.5±1.1
$LC_{18}H_2$	1.9±0.8	4.4±0.6	7.4±1.0
$LC_{18}H_3$	1.5±0.5	4.1±0.7	6.3±0.6
F value	92.68**	108.70**	145.60**
LSD (*P< 0.05*)	1.01	1.22	1.29

***Significant*

annually which costs more than US $ 20-500/ kg depending on its purity. Tapioca, a potential gelling agent and a good substitute to agar cost only $ 1/kg and hence were incorporated in the present study to see that could it serve as a viable alternative to agar. The other costly ingredient in MS media is sucrose that costs around US $ 03-10/ kg and that too depending upon its purity and hence was substituted with table sugar which only costs US $ 0.5-0.6/ kg [33].

During proliferation of shoots when MS, agar and sucrose were substituted with LC nutrients (fertilizers), tapioca and table sugar, the LC nutrients, tapioca and sugar were found statistically better for shoot height and node number, however to initial establishment LC media showed some ignorable difficulties. It is also reported that in-spite of the difficulty in initial establishment, further sub-culturing was easy on tapioca based media because the roots were retained in the media and it was easy to remove shoots for further sub-culturing [34].

Various combination of Kn (0.04, 0.06 and 0.08 mg/l) with IAA (0.50 mg/l) were used in the present investigation. The combination of Kn and IAA had consistently given good results for improving shoot height which was in confermation of the findings of several workers [35-39]. The H_1, H_2 and H_3 hormonal combination i.e. Kn (0.04, 0.06 and 0.08 mg/l) with IAA (0.50 mg/l) had significant difference between mean shoot height, node number, root length, fresh weight of root and shoot and root: shoot ratio, whereas the H_3 hormonal combination having higher concentration of Kn (0.08 mg/l) responded the least. In considering the individual values most suitable hormonal combination was 0.04 mg/l Kn with 0.50 mg/l IAA for both the MS and LC media.

In the findings of the present investigation, the shoot height in MS media reached 14.10±1.7, 10.17±1.1 and 8.77±0.6 cm. (Table 2) and node number was reported 23.4±2.1, 20.4±1.3 and 16.1±2.0 (Table 3) in H_1, H_2 and H_3 combination respectively. In LC media different tapioca concentrations showed much variation in shoot height and node number. It was reported that with the increasing concentration of tapioca, shoot height and node number were increased but after 15 gm/l tapioca concentration both were decreased in the media up to 18 gm/l tapioca. In LC_{15} media the shoot height reached 14.73±1.1, 14.54±0.5 and 12.71±1.3 cm. and node number reported 23.7±1.8, 21.5±2.4 and 16.9±2.5 in H_1, H_2 and H_3 combination respectively. The increase in plantlet growth in tapioca based media represents a substantial increase in propagation.

Table4 : Effect of MS and LC media on root length, fresh weight of root and shoot and root: shoot ratio after 80 days of culture

Media Used	Root length	Root fr.	Wt. Shoot fr. Wt.	Root:Shoot ratio[z1]
MSH_1	9.4±1.0	16.2±1.4	23.4±2.1	1.07±0.1
MSH_2	8.2±1.0	14.1±0.9	20.4±1.3	0.78±0.7
MSH_3	6.3±0.9	10.6±2.3	16.1±2.0	0.86±0.5
LC_9H_1	5.6±1.1	7.9±1.1	9.6±1.1	0.55±0.5
LC_9H_2	2.3±0.9	4.4±1.5	5.9±1.4	0.49±0.4
LC_9H_3	1.4±0.8	2.8±1.5	5.0±1.6	0.50±0.01
$LC_{12}H_1$	12.4±2.0	16.6±2.0	20.0±2.1	0.62±0.07
$LC_{12}H_2$	9.2±1.6	13.8±1.3	17.7±1.6	0.58±0.05
$LC_{12}H_3$	7.1±1.1	11.9±1.7	15.1±1.8	0.68±0.04
$LC_{15}H_1$	14.3±2.6	19.5±2.2	23.7±1.8	1.05±0.1
$LC_{15}H_2$	12.0±1.4	16.6±1.7	21.5±2.4	1.29±0.1
$LC_{15}H_3$	8.4±1.7	13.1±2.5	16.9±2.5	1.22±0.1
$LC_{18}H_1$	3.0±1.1	5.6±1.1	8.5±1.1	1.27±0.1
$LC_{18}H_2$	1.9±0.8	4.4±0.6	7.4±1.0	1.42±0.1
$LC_{18}H_3$	1.5±0.5	4.1±0.7	6.3±0.6	1.33±0.08
F value	111.92**	254.31**	322.34**	52.83**
LSD (*P< 0.05*)	0.72	0.01	0.03	0.10

***Significant*

Similar observations have been reported by, Gebre and Sathyanarayana, [31], in which they observed that tapioca at 11-15% strength, shows the values ranged between 8.9-9.8 cm shoot height and 11.3-12.1 node number, same as in MS media with agar (8.9 cm shoot height and 10.5 node number). These results are comparable or even better than the most rapid node production (8 to 10 per month) as has already been reported earlier using agar by Hussey and Stacey, [21].

A perusal of values for shoot length and node number indicates clearly that the Low Cost (LC) media responded better than MS media. The H_1 hormonal combination in both the media showed better growth than all other combinations. In LC media, between various tapioca concentrations, 15 gm/l tapioca reported better with all three hormonal combinations (H_1, H_2 and H_3), while 12 gm/l tapioca with H_1 hormonal combination also showed good result.

The root length, root and shoot fresh weight and root: shoot ratio was also recorded to evaluate the strength of the plantlet. The root length in MS media reached 9.4±1.0, 8.2±1.0, 8.2±1.0 and 6.3±0.9 cm. in H_1, H_2 and H_3 combination respectively. In LC media with the increasing concentration of tapioca, root length was also increased from 9 gm/l to 15 gm/l, but after that 18 gm/l tapioca concentration showed least mean of root length (3.0±1.1, 1.9±0.8 and 1.5±0.5 cm in H_1, H_2 and H_3 combination respectively). The root length in LC media with 15 gm/l tapioca concentration reported higher (14.3±2.6, 12.0±1.4 and 8.4±1.7 with H_1, H_2 and H_3 combination respectively) than all other concentrations (Table 4; Plate-1 c and d). Similar findings have been published by Gebre and Sathyanarayana, 2001 [31] in which they found that tapioca 9-14% strength supported higher root growth than agar. Root fresh weight in MS media, was observed 16.2±1.4, 14.1±0.9 and 10.6±2.3 gm and shoot fresh weight was 23.4±2.1, 20.4±1.3 and 16.1±2.0 gm, with H_1, H_2 and H_3 combination respectively. In LC media, as the concentration of tapioca was increased i.e. 9 gm/l to 15 gm/l the root and shoot fresh weight were also increased but with the higher concentration of tapioca (18 gm/l), root and shoot fresh weight was decreased. Highest root fresh weight (19.5±2.2, 16.6±1.7 and 13.1±2.5) and shoot fresh weight (23.7±1.8, 21.5±2.4 and 16.9±2.5) were reported in LC_{15} media in H_1, H_2 and H_3 combination respectively which is presented in Table 4. The root: shoot ratio in MS media was observed 1.07±0.1, 0.78±0.7 and 0.86±0.5 in H_1, H_2 and H_3 combination respectively. The ratio was increased with the increasement of tapioca concentration i.e. 9 gm/l to 18 gm/l. LC_{18} media with H_2 combination that showed higher ratio (1.42±0.1) in comparison to all other media.

The above data indicates that the plantlets of LC media showed better strength than MS media. The 15 gm/l tapioca concentration of LC media showed best result for root and shoots fresh weight and root: shoot ratio. The H_1 combination in both the media showed higher growth while root: shoot ratio was observed higher in LC_{18} media with H_2 combination. The above data indicates that LC media exhibited the better strength

than MS media. The 15 gm/l tapioca concentration of LC media showed best result for root and shoots fresh weight. and root: shoot ratio. The H_1 combination in both the media showed higher growth while root: shoot ratio was observed higher in LC_{18} media with H_2 combination. The transplanted plantlets to the soil were survived well as 70-80% (Palte-1 e).

In the last of the summarization of the present work and on the basis of observation and the results, it can be concluded that the LC nutrients having 30 gm/l table sugar and 15 gm/l tapioca with 0.04 mg/l Kn and 0.50 mg/l IAA hormonal combination has been proved better than MS nutrients having 30 gm/l sucrose and 8 gm/l agar with 0.04 mg/l Kn and 0.50 mg/l IAA hormonal combination, for shoot and root proliferation. The results of the present study showed that tapioca and table sugar are the best alternative of agar and sucrose respectively, to reduce the cost of media. In the place of MS nutrients, LC nutrients may be used and the cost of whole media may be reduced 100%, without any adverse effect, although further investigation is essential for establishment of this fact. From the present investigation, it is concluded that through reduction of the cost on the techniques, the cost of the product automatically also be reduced and farmers get benefited using low cost, disease free and clonal planting material with high production and saving land resources.

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