Biological Forum – An International Journal **13(3): 375-380(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

In Vitro Propagation Studies for Late Blight Resistant Potato Variety Kufri Girdhari

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(Received 27 May 2021, Accepted 07 August, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Production of quality tubers, free from diseases and pests in potato is one of the foremost pre-conditions for obtaining good crop yields in the field. However, field multiplied tubers have low multiplication rate and prevalence of diseases and virus has been recognized as one of the major bottlenecks in potato cultivation in developing countries. An alternative method of production i.e., micropropagation can be effectively utilized for mass production of true-to-type, disease free quality planting material of potato. For the experiments on micropropagation, two explants viz., sprouts and nodal segments were cultured on MS basal medium supplemented with different hormonal combinations (BAP and kinetin). The best shoot initiation response was recorded on MS medium supplemented with BAP 0.75 mg/l in both the explants. For shoot multiplication the best response was obtained on MS medium supplemented with 0.75 mg/l BAP + 0.25 mg/l kinetin. For rooting, superior percentage rooting response was observed on 1.0 mg/l IBA. The *in vitro* raised plantlets were then transferred in pots filled with sterilized potting mixture, for plant acclimatization and establishment. Thus, a reliable and reproducible micropropagation protocol for potato variety 'Kufri Girdhari', an important high yielding, late blight resistant variety was developed in this study.

Keywords: Potato; Micropropagation; *in vitro*; sprouts; nodal segments; Kufri Girdhari.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a self-pollinating, important economic tuberous crop belonging to the family Solanaceae and cultivated worldwide in the temperate, tropical, and subtropical zones. It is the fourth most important food security crop in the world after rice, wheat, and maize (Haverkort *et al*., 2009). Potato is eulogized as 'King of Vegetables or Poor man's friend or Food for Future'. Potato produces more edible energy, greatest dry matter and protein per unit area and time than many other crops with exceptionally high yield. It is a nutrient dense food; thus, it has a key role in preventing malnutrition in impoverished areas and contributing to health where food is ample.

Potato can be propagated vegetatively, mainly through tubers and sexually through botanical seeds (true potato seed). In potato cultivation, seed tubers alone account for 40 to 50% of production costs (Kumar *et al*., 2007). The availability of disease-free quality seed/planting material in sufficient quantity is considered the most important factor for obtaining good yields. Despite spending a huge amount of money, most of the seeds obtained are non-certified, impure and is vulnerable to several biotic and abiotic stresses which limit its production, particularly amongst small and marginal farmers with limited resources. At present, the central seed production agencies of India and states can meet only 20-25% requirement of quality seed potatoes. ICAR-CPRI produces about 3,187 metric tonnes of nucleus and breeder seed of 25 popular potato varieties; out of which 70% is through conventional system whereas, 30% is through high-tech systems (Singh *et al.,* 2019). The shortage of good quality planting material has been recognized as one of the major bottlenecks in potato cultivation in developing countries. Due to low multiplication rate and high susceptibility to various diseases, there is a need to develop an alternate method of propagation. For bridging this wide gap, large scale integration of conventional and innovative methods like micropropagation at commercial level are needed for producing enough quantity of healthy seed tubers in minimum duration (Pandey, 2006). Micro propagation is the alternative to conventional propagation of potatoes (Chandra and Birhman 1994). *In vitro* propagation methods using sprouts and nodal segments

are more reliable for maintaining genetic integrity of the multiplied clones (Liljana *et al*., 2012).

In vitro technology facilitates production, mass multiplication and season independent production of disease-free planting material and conservation of potato in controlled and disease-free conditions. Micropropagation coupled with conventional propagation has now become an integral part of seed production in many countries. In this regard, the present study was undertaken at the Plant Tissue Culture Laboratory of the Division of Vegetable Science and Floriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, with the objective to find outoptimum concentration of suitable growth regulators for development of micropropagation protocol of important late blight resistant potato variety, 'Kufri Girdhari'.

MATERIALS AND METHODS

A. Planting material

Tubers from a popular high yielding and late blight resistant potato variety Kufri Girdhari tubers were used as a source for explants throughout the experiment. Sprouts and nodal segments were used as explants for the study. Healthy sprouted tubers were planted in pots in the green house. When the plants were 25 to 35 cm tall with 4 to 6 nodes, they were cut into single node and large leaves were removed to obtain nodal segments.

B. Sterilization of explants

The explants, viz., sprouts and nodal segments were first washed in running water to remove the debris. The explants were then cleaned with few drops of Tween-20 under running water for 15-20 minutes. They were then soaked in 0.2 percent Bavistin for 25-30 minutes followed bywashing with double distilled water for 3-4 times to avoid the scorching effect of fungicides. The explants were then dipped in 70 percent ethanol for 30 seconds followed by washing with sterile water. In the laminar air flow chamber the explants were surface sterilized with 2 % Sodium hypochlorite for 5 minutes followed by washing with sterilized double distilled water 5-6 times to remove the traces of Sodium hypochlorite.

C. Preparation of media

The basal medium as given by Murashige and Skoog (1962) with different modifications depending upon the experiment was used. The medium was prepared by mixing the stock solutions. Plant bio-regulators (BAP, kinetin and IBA) were added, depending upon the experiment. The solidifying agent viz., Agar and carbon source viz., sucrose was added. The pH of the medium was adjusted to 5.7±1 prior to adding gelling agents and carbon sources, with the help of Systronic digital pH meter (Saveer India Limited), using 0.1 N HCl or 0.1 N NaOH. Approximately, 10-15 ml and 25-30 ml of boiled medium was poured into the culture tubes and culture flasks, respectively, which were then plugged with cotton plugs made from non-absorbent cotton wrapped in muslin cloth.The culture vessels were then autoclaved at 15 lbs/sq. inch at 121ºC or 1.05 kg/cm^2 pressure for 15-20 minutes (culture tubes) and 20-25 minutes (culture flasks). The culture tubes/ flasks were allowed to solidify at room temperature overnight before use.

D. Establishment of explants and shoot induction

Maintaining the correct polarity of the sprouts and single nodal segments, these were individually inoculated in test tubes (Borosil, India) with Murashige and Skoog (MS) basal medium containing 3% sucrose and supplemented with different concentrations of BAP (0.0, 0.25, 0.5, 0.75, 1.0 and 2.0 mg/l) and kinetin (0.0, 0.25, 0.5, 0.75 and 1.0 mg/l) alone. The cultures were incubated in the culture room at 25±2^{o}C and uniform light from 3000-3500 lux with cool florescent tubes over a photoperiod of 16 and 8 hours of light and dark period, respectively.

E. Multiplication of shoots and root regeneration

All the regenerated shoots were inoculated on shoot multiplication medium supplemented with different concentrations of BAP (0.0, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 mg/l) alone and in combination with kinetin (0.25 mg/l). After multiplication of shoots, the regenerated shoot clumps were excised and transferred to medium supplemented with different concentrations of IBA (0.0, 0.25, 0.5, 1.0, 1.5 and 2.0 mg/l) for root induction.

RESULTS AND DISCUSSION

A. Effect of different concentrations of BAP and kinetin on shoot induction from sprout and nodal segment explants

For shoot induction using both sprouts and nodal segments as explant, MS medium supplemented with different concentrations of BAP and kinetin were used. The results obtained are in consonance with the previous works reported by various workers who have used different explants for establishment of fresh cultures with varying degree of success like nodal segments (Rai *et al*., 2012; Sharma *et al*., 2011), leaf explant (Al-Sulaiman, 2011) and shoot/meristems tip culture (Mohapatra *et al.*, 2018; Marcela *et al*., 2011; Yasmin *et al*., 2011).

Amongst all the treatments mentioned in Table 1, the significantly higher percentage response was recorded in T_4 - MS medium + 0.75 mg/l BAP (87.08). The lowest percentage response (60.44) was recorded in T_1 - MS basal medium. Significantly minimum number of days to shoot induction was observed in T_4 - MS medium + 0.75 mg/l BAP (9.58) whereas maximum number of days to shoot induction (15.03) was recorded in T_1 - MS basal medium.

For shoot induction using nodal segments as explant, the significantly higher percentage response (85.76) for shoot induction was recorded in T_4 - MS medium + 0.75 mg/l BAP. The lowest percentage response for shoot induction (55.43) was recorded in T_1-MS basal medium. As far as earliness of response is concerned, best response was observed in T_4 - MS medium + 0.75 mg/l BAP in terms of minimum days to shoot induction (7.43) whereas T_1 - MS Basal medium recorded maximum number of days to shoot induction (12.13).

Table 1: Effect of different concentrations of BAP and kinetin on shoot induction using different explants.

Treatment	Hormonal concentrations (mg/l) (BAP + kinetin)	Days to shoot induction		Percentage response (%)	
		Sprouts	Nodal Segments	Sprouts	Nodal Segments
T_1	MS Basal	15.03	12.13	60.44	55.43
T_2	0.25 + 00	13.31	10.32	64.96	68.63
T_3	0.50 + 00	12.48	8.80	73.97	77.10
T_4	0.75 + 00	9.58	7.43	87.08	85.76
T_5	1.0 + 00	11.33	7.88	83.83	82.10
T_6	2.0 + 00	11.41	8.02	85.53	79.66
T_7	0.0 + 0.25	12.80	10.42	68.83	79.00
T_8	0.0 + 0.50	13.54	10.10	71.43	72.30
T_9	0.0 + 0.75	14.25	9.58	72.57	68.76
T_{10}	0.0 + 1.0	13.77	9.31	72.40	67.06
C.D. (5%)		**0.97**	**0.50**	**1.54**	**1.98**

The results revealed better percentage response in sprout explants whereas nodal segment explants exhibited comparatively early response. The results were in conformity with the work reported by Gami *et al*., (2013); Hoque, (2010); Parvizi *et al*., (2008). Amongst the array of hormonal treatments used, the best results were obtained on T_4 treatment comprising of MS medium supplemented with 0.75 mg/l BAP in both sprouts and nodal segment explants. Similar results have been reported by Mohapatra *et al*., (2018) using sprout explants on MS medium supplemented with BAP 0.25 mg/l whereas Sharma *et al*., (2015) reported highest shoot regeneration response by using nodal segment explants on MS medium supplemented with 1.0 mg/l BAP.

It has been well established that shoot regeneration is dependent on optimum auxin: cytokinin ratio (De Ropp, 1955; Skoog and Miller, 1957) and solanaceous crops are known to have high levels of endogenous auxins which could be the probable reason for high regeneration response on BAP or kinetin. However, superiority of BAP over kinetin for shoot bud induction has also been demonstrated by various workers like Shahriyar *et al*., (2015); Bhuiyan (2013); Hussain *et al*., (2006); Sarker and Mustafa (2002). The effectiveness of BAP on shoot induction can be due to the abilities of plant tissue to metabolize the natural hormones more readily than artificial growth regulators or due to the abilities of BAP to induce natural hormones such as Zeatin within tissue and thus works through natural hormones (Zaerr and Mapes, 1982).

B. Effect of different concentrations of BAP and Kinetin on shoot multiplication

Effect of different concentrations of BAP (0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 mg/l) in combination with kinetin (0.25 mg/l) on shoot multiplication was recorded after four weeks from culture of *in vitro* single shoots. From the data as mentioned in Table 2, it was revealed that amongst all the treatments, superior response in terms of number of shoots per explant (15.50) was observed in T_{10} - MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin whereas minimum number of shoots per explant (4.50) was observed in T_1-MS basal medium. The maximum shoot length (8.13 cm) was observed in T_{10} - MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin.

The minimum shoot length (4.11 cm) was recorded in T_1 - MS Basal medium. The results further revealed that the maximum number of nodes per plantlet (8.54) was observed in T_7 -MS medium + 2.0 mg/l BAP. The minimum number of nodes per plantlet (4.18) was observed in T_2 - MS medium + 0.25 mg/l BAP.

The longest internodal length (1.12 cm) was observed in T_{10} - MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin whereas, shortest internodal length (0.84 cm) was observed in T_7 - MS medium + 2.0 mg/l BAP. Amongst all the treatments, best response was obtained in T_4 - MS medium + 0.75 mg/l BAP + 0.25 mg/l kinetin with sturdy and dark green multiple shoots besides better shoot length and number of internodes.

The synergistic effect of BAP and kinetin for increased shoot multiplication rate and proliferation was also reported on *B. tulda and M. baccifera* (Waikhom and Louis, 2014). The results reveal that a proper balance between two cytokinins may trigger more efficient shoot multiplication response. This may be due to variation in specific level of endogenous hormones as influenced by genotypes and environmental factors. Similar results were obtained by Genene *et al.,* (2018) in potato variety Gudenie, on MS medium supplemented with 0.5 mg/l BAP + 2 mg/l kinetin. Ahmed *et al.,* (2014) used MS medium supplemented with BAP 2.0 mg/l in combination with 0.1 mg/l kinetin to obtain maximum mean number of shoots. On the contrary, few workers like Mohapatra *et al.* (2018); Liljana *et al*., (2012); Rashid *et al.,* (2005) have also used combinations of auxins and cytokinins to obtain maximum shoot multiplication response. During experimentation for multiple shoot formation, a few microtubers have also been formed especially at higher concentrations of BAP (2.0 mg/l) alone or in combination with kinetin (0.25 mg/l). Donnelly *et al.,* (2003) have reported that growth regulators (BAP, kinetin and NAA) can induce microtubers in potato *in vitro*, but in some cases the effect of growth regulators was found to be genotype dependent (Hussain *et al*., 2006). Similar response was obtained by Meenakshi, (2020) using two potato varieties Kufri Bahar and Kufri Surya for testing *in vitro* microtuberization response in MS medium supplemented with three different concentrations of BAP (0.75, 1.5 and 2.25 mg/l).

Table 2: Effect of different concentrations of BAP and kinetin on shoot multiplication.

Treatment	Hormonal concentrations (mg/l) (BAP + kinetin)	Number of shoots per explant	Average shoot length (cm)	Number of nodes per plantlet	Internodal length (cm)
T_1	MS Basal	4.50	4.11	4.33	0.97
T_2	0.25 + 00	6.40	4.20	4.18	0.98
T_3	0.5 + 00	5.87	5.21	5.39	0.98
T_4	0.75 + 00	5.67	4.20	4.50	1.04
T_5	1.0 + 00	5.83	4.13	4.23	0.96
T_6	1.5 + 00	7.43	5.53	5.33	0.96
T_7	2.0 + 00	11.83	7.33	8.54	0.84
T_8	0.25 + 0.25	6.50	7.32	7.83	1.11
T_9	0.5 + 0.25	8.96	7.90	8.04	1.02
T_{10}	0.75 + 0.25	15.50	8.13	8.37	1.12
T_{11}	1.0 + 0.25	14.66	7.83	7.80	1.07
T_{12}	1.5 + 0.25	10.70	7.37	7.77	0.92
T_{13}	2.0 + 0.25	5.42	7.07	7.88	0.95
C.D. (5%)		**1.06**	**0.70**	**0.72**	**0.15**

C. Effect of different concentrations of IBA for root induction

For induction of rooting, healthy *in vitro* multiplied shoots (3-4 cm) obtained after shoot multiplication, were cultured on MS medium supplemented with different concentrations of IBA (0.0, 0.25, 0.5, 1.0, 1.5 and 2.0 mg/l). From the Table 3, it can be observed that the overall percentage rooting response in all treatments ranged between 83.13 to 97.50 per cent. Significantly superior percentage rooting response (97.50) was observed inT_4 - MS medium + 1.0 mg/l IBA whereas treatment T_6 –MS + 2.0 mg/l IBA recorded minimum percentage rooting response (83.13). The maximum number of roots per shoot (8.50) was recorded in T_4 - MS medium + 1.0 mg/l IBA whereas, T_6 - MS + 2.0 mg/l IBA recorded minimum of roots per shoot (5.84) as compared to all other treatments.

As far as root length is concerned, the significantly longer root length (6.97 cm) was observed in T_4 - MS medium + 1.0 mg/l IBA whereas, T_6 –MS medium + 0.50 mg/l IBA recorded shortest average root length (5.06 cm). The significantly higher number of leaves per plantlet was recorded in T_4 - MS medium + 1.0 mg/l IBA (15.79). Treatment T_1 -MS basal media recorded minimum number of leaves per plantlet (6.13) as compared to all other treatments. Data on increase in plant height was also recorded along with the rooting parameters which revealed maximum plant height in T_4 - MS medium + 1.0 mg/l IBA (14.83 cm) which was significantly superior to the other treatments. However, T_6 - MS medium + 2.0 mg/l IBA recorded minimum plant height (8.53 cm).

Table 3: Effect of different concentrations of IBA on rooting.

Treatments	Hormonal concentrations IBA (mg/l)	Percentage response (%)	Number of roots per shoot	Average root length (cm)	Number of leaves per plantlet	Plant height (cm)
T_1	MS Basal	84.57	6.82	6.12	6.13	8.89
T_2	0.25	88.65	7.83	6.10	10.50	11.47
T_3	0.50	95.13	8.03	6.18	13.50	13.03
T_4	1.00	97.50	8.50	6.97	15.79	14.83
T_5	1.50	87.10	7.50	5.59	7.10	10.42
T_6	2.00	83.13	5.83	5.06	6.76	8.53
C.D. (5%)		**1.59**	**1.04**	**0.67**	**0.97**	**0.92**

Among all the treatments the best results were obtained in treatment T_4 - MS medium supplemented with 1.0 mg/l IBA with significantly superior rooting response (97.50 %) and maximum number of roots per shoot (8.50). The results revealed relatively better rooting response in lower concentrations as compared to higher concentrations of IBA. This may be due to the higher level of endogenous auxins present in solanaceous vegetables as reported by Bhushan and Gupta (2017). Similar results have been reported by Mohapatra *et al.*, (2018); Khalafalla *et al.*, (2009); Khalafalla *et al.*, (2010); Uddin (2010). However, few researchers also reported good results by using combination of IBA and NAA (Genene *et al*., 2018), IAA (Sarkar and Mustafa, 2002) and MS Basal medium (Shahriyar *et al.*, 2015; Khalafalla *et al*., 2009) for root induction.

After *in vitro* root regeneration the rooted plantlets were removed from rooting medium and washed to remove adhering gel and kept on moist cotton for 3 days in the incubation room for initial hardening. These plantlets were then transferred in pots containing sterilized potting mixture soil, vermicompost and cocopeat (1:1:1). Perforated plastic bags were taken to cover the potted plantlets. Humidity was maintained inside the bag through regular spraying of water to protect the hardening plants from moisture stress and for the quick acclimatization.

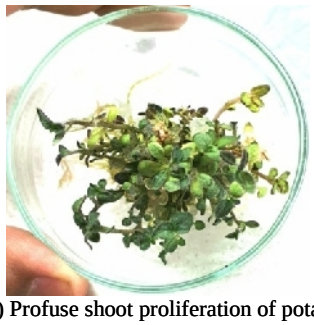
(A) Shoot induction in sprout explant

(B) Shoot induction in nodal segment explant

(C) Shoot growth and multiple shoot formation in sprout explant

(D) Shoot growth & multiple shoot formation in nodal segment explant

(E) Multiple shoot formation at 0.75 mg/l BAP + 0.25 mg/l kinetin



(F) Profuse shoot proliferation of potato at 0.75 mg/l BAP + 0.25 mg/l kinetin

(G) Rooting at IBA 1.0 mg/l

(H) Development of microtubers

(I) *In vitro* raised rooted plantlets

CONCLUSION

A micropropagation protocol for potato variety Kufri Girdhari was optimized from the study. This protocol will provide the base for the disease free and high-quality mass production of studied variety through *in vitro* technique.

FUTURE SCOPE

A Substantial number of micro propagated plantlets are not able to survive when transferred from *in vitro* conditions to field or greenhouse conditions. Thus, further experimental trials on a feasible hardening protocol for the *in vitro* generated plantlets are required for improving the survival percentage during acclimatization and field transplanting.

Acknowledgement. The authors are highly grateful to Late Prof. (Dr.) R. K. Gupta, Head, Division of Vegetable Science and Floriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, for planning, guiding and technically supporting the first author during her M.Sc. research programme. The financial support and laboratory facilities received from Division of Vegetable Science and Floriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, is also duly acknowledged.

Conflict of Interest. The authors report no conflicts of interest in this research work or in manuscript preparation.

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How to cite this article: Walia, A., Bhushan, A., Samnotra, R.K., Gaur, A. and Pandotra, P. (2021). *In Vitro* Propagation Studies for Late Blight Resistant Potato Variety Kufri Girdhari. *Biological Forum – An International Journal*, *13*(3): 375-380.