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AGRONOMY

Effect of Cultivar, Growth Regulators and $CaCl_2$ on *In Vitro* Culture of Potato (*Solanum tuberosum* L.)

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ABSTRACT

The investigation was carried out aiming to develop a technique for rapid in vitro micropropagation of potato with three different popular cultivars in middle Gujarat. Different surface sterilization treatments were carried out and axenic cultures were established on agar solidified (0.8%) Murashige and Skoog's (1962) medium containing 2% sucrose and 10 mgl^{-1} GA_3. The axenic culture of different cultivar reported the difference among them and concluded that a higher concentration is good for vegetative growth whereas the lower concentration favors the root growth in the presence of 2.0 mgl^{-1} calcium pantothenate. Effect of $CaCl_2$ on the growth of *in vitro* cultures was evaluated and it was found that Ca nutrition is one of the most important factors for growth. The produced *in vitro* cultures were used for further production of microtuberisation study.

Highlights

- The axenic culture of different cultivar reported varietal differences.
- MS salt with full concentration is good for the vegetative growth whereas the reduced concentration favors the root growth.
- Calcium nutrition is one of the most important factors for growth and it reduces vitrification in a long storage.

Keywords: Micropropagation, *in vitro*, *Solanum tuberosum*, $CaCl_2$

Potato (*Solanum tuberosum* L.) is one of the most important food crops grown worldwide. It stands fourth in most valuable food crop for human consumption in the world after wheat, rice and maize (Levy *et al.* 2013). In order to support the importance of potato, especially within the developing countries, the year 2008 was highlighted by the U.N as the year of potato (Levy and Veilleux, 2009). Virus, fungus and other bacterial infections can drastically affect both the production and the quality of potato yield. *In vitro* micropropagation is an alternative to conventional (vegetative) propagation of potato whereas aseptically meristem culture were used which had given pathogen tree plants (Fawzia *et al.*, 2015).

In vitro propagation techniques for growing potato involves various steps i.e. selection of explants, its sterilization, establishment, shoot proliferation and production of in vitro tubers. The first condition for the success of axenic cultures is asepsis. The maintenance of aseptic, free from all microorganism or sterile condition is an essential procedure for a successful tissue culture. In vitro potato micropropagation helps to proliferate and multiply the shoots which in turn develops microtuber production. Microtuber is an important propagules

to the potato seed industry for qualitative and qualitative potato production. Philomina and Jerold (2013) indicated that for a plant tissue culture, an ecofriendly technology includes micropropagation which leads to mass propagation of true to type, high quality planting material within a limited period. They also stated that the primary application of micropropagation has been to produce elite planting material irrespective of the season or the crop, which in turn leads to increased productivity in agriculture as well as better economy to the developing nations like India. The availability of an efficient and reproducible protocol for the regeneration of plants through adventitious shoot formation from explants is essential for the application of micropropagation and genetic engineering which helps in the improvement of plants (Sharma and Srivastava, 2014). Rapid multiplication of plants and homogeneous materials, say a single explant can be reproduced into several thousands of plants in a minimum time with virus free stock of potato. Chanakya *et al.* (2015) reported that potato produces more protein and calories per unit area, per unit time and per unit of water than any other major plant food. Globally, the tubers are considered as vital dietary source of starch, protein, antioxidant and vitamins, helping the plant to remain both as a storage organ and work as a vegetative propagation system (Barrell *et al.* 2013). In vitro tuber can be produced throughout the year and thus holds benefit over conventional tubers (Hoque 2010). After a long storage of in *vitro* shoots turn brown and its tip dies i.e. the shoot tip necrosis.

The investigation was carried out with an objective to develop a technique for rapid in vitro micropropagation of potato and find out the effect of basal nutrients for*in vitro* plant growth with three different popular cultivar viz, Kufri Badshah, Kufri Pukhraj and Kufri Chipsona-lpopular among the farmersof middle Gujarat.

MATERIALS AND METHODS

The investigation was carried out at the Department of Agricultural Botany, B.A. College of Agriculture, AAU, Anand. Healthy harvested tubers of the above indicated three cultivars were treated with a fungicide (0.05% Bavistin) for 15 minute and dried. In case the tubers were not required for immediate use, it was stored at 4 ºC. For immediate use, dormancy breaking treatment (50 mgl^{-1}) GA_3 for half an hour were given by soaking the tubers for 30 minutes. The tuber cuttings were placed on moist sand and were allowed to sprout. Sprouted tuber tips carrying apical meristem (0.2 – 0.4cm) were dissected and used for the initiation of *in vitro* cultures.

Surface sterilization treatments were given in the laminar flow clean air working area. The shoot apices were dissected and inoculated to study the effect of surface sterilization treatment and the establishment of axenic cultures on to Murashige and Skoog, (1962) medium with the incorporation of 10.0 mgl^{-1} GA_3 and 2% sucrose for the initiation of axenic culture. The percentage of contamination, percentage of survival and the total number of shoot bud sprouted per explant were recorded and expressed against the total number of nodes inoculated. Further to find out the optimum levels of the nutrient for the shoot growth different basal nutrient media were studied.

The *in vitro* shoots (Fig. 2) were selected with 2 node explant for the effect of $CaCl_2$ on the growth of potato apical meristem. They were cultured on MS medium with different concentrations of $CaCl_2$ (0, 5, 10, 50, 100, 200, 300 mgl^{-1}) and subsequent observations were made for its growth.

RESULTS AND DISCUSSION

Effect of surface sterilization treatment and the establishment of axenic cultures

Initial response in cultured explants was observed after 3-4 days of culturing. Results indicated (Table 1) that the lowest percentage of contamination was observed in T_1 surface sterilization treatment when compared to the other treatments. Maximum number of sprouts and the highest percentage of survival rate were also reported in T_1 treatment followed by T_2 and T_3 surface sterilization treatment.

Varietal response for different varieties were also reported. In all the treatment, the higher survival rate was in Kufri Chipsona-1, and was followed by Kufri Pukhraj. Kufri Badshah had minimum survival rate when compared to the other varieties. Shoot growth in potato had also been observed by Dhingra *et al*. (1992); Randhawa and Chandra (1990), Jarret *et al*. (1980) suggested that GA_3 was essential for both shoot initiation and subsequent shoot

development. Ullah *et al.* (2012) also reported that GA_3 is involved in cell elongation and its addition in MS medium enhanced the shoot growth in *in vitro*propagated plants of potato variety "Desiree".

Dhingra *et al.* (1992) reported that explants surface carries a wide range of microbial contaminants, which are to be taken care of by surface sterilization before transferring it into the nutrient medium. In this study, besides $HgCl_2$, the fungicide Bavistin and the antibacterial antibiotic "Streptocycline" as well as Kanamycine were used for the bacterial infections. Randhawa and Chandra (1990) also used $HgCl_2$ for surface sterilization of six Indian potato cultivar at 0.1 % for 8 minutes.

Table 1: Effect of surface sterilization treatment and the establishment of axenic cultures in potato

Treatments	No. of nodes inoculated	No. of shoots sprouts	% contamination	% survival
T_1				
V_1	48	30	36.8 ± 2.36	63.20 ± 2.36
V_2	48	36	26.25 ± 1.53	73.75 ± 1.53
V_3	48	35	27.87 ± 2.28	72.1 ± 2.30
T_2				
V_1	48	29	40.73 ± 1.50	59.20 ± 1.48
V_2	48	31	32.37 ± 0.94	67.62 ± 0.94
V_3	48	29	37.81 ± 1.51	62.05 ± 1.49
T_3				
V_1	48	26	45.92 ± 2.51	54.07 ± 2.52
V_2	48	28	39.75 ± 0.67	60.23 ± 0.68
V_3	48	26	45.97 ± 2.63	54.03 ± 2.63

V_1 = Kufri Badshah; V_2 = Kufri Chipsona-1; V_3 = Kufri Pukhraj

T_1 = 1. Tetracycline 200 ppm 10 min.
2. Bavistin 1000 ppm 20 min.
3. Kanamycin 400 ppm + Streptocycline 200 ppm 10 min.
4. 0.1 % $HgCl_2$ 8 min.

T_2 = 1. Tetracycline 200 ppm 10 min.
2. Bavistin 1000 ppm 20 min.
3. Kanamycin 400 ppm + Streptocycline 200 ppm 5 min.
4. 0.1 % $HgCl_2$ 8 min.

T_3 = 1. Chlortetracyclin 200 ppm 10 min.
2. Bavistin 1000 ppm 20 min.
3. Kanamycin 400 ppm
4. 0.1 % $HgCl_2$ 8 min.

Establishment media: MS + 10 mg $^{-1}$ GA_3 + 2% sucrose

Effect of basal nutrients on *in vitro* growth

Effect of basal nutrients on *in vitro* shoot growth was evaluated and different observations like Sprout length, Number of nodes per shoot, Number of leaf, Number of internodes, Length of internodes, Number of roots and Length of roots were noted. The results represented (Table 2) the number of nodes per shootwhich indicates that the first week and the fourth weeks observation were found to be non-significant, however, with maximum number (8.73) in full MS basal nutrient treatment while lower numbers (7.53 and 8.13) in reduced concentration of the nutrients. Varietal response was found to be significant during both the time of observation. Final observation significantly recorded maximum number in Kufri Chipsona-1 which was at par with Kufri Pukhraj. The interaction between the treatment and the varietal effect were reported to be significant during the fourth week observations i.e. S_1V_2 is the best combination. Results narrated (Table 2) that the effect of the nutrients for the number of leaf has no significant effect during the first and the fourth week of observations however the full strength nutrients had given the maximum number of leaf. It was observed that the effect of nutrients were significantly effective for the Kufri Chipsona-1 and it was at par with Kufri Pukhraj for the shoot growth.

The results (Table 3) revealed that S_1 treatment shows the least number of roots and significantly maximum number of roots in S_3 during the first and the fourth week observations. The numbers of roots were increased during the last weeks observation i.e. the 4th week observations.

The varietal response was found to be significant during the first week observation and V_2 reported maximum (5.73) number of roots and the least number of roots in V_1 (4.00) Kufri Badshah. The fourth week observations were non-significant for the varietal response.

The effect of basal nutrients treatment for the length of roots were reported in (Table 3) which indicates that the effect was significant during the first week of observations in the S_1 treatment, with the full strength of the nutrients, but it was found non-significant at the end of 4th week observations.

The varietal response was also reported with the same results and was found that the maximum

Table 2: Effect of basal nutrient media on shoot growth

Treatment	Sprout length		No. of nodes/ shoot		No. of internodes		No. of leaf	
	1st week	4th week	1st week	4th week	1st week	4th week	1st week	4th week
S_1	3.13	6.6	3.93	8.73	3.73	8.00	5.33	8.7
S_2	2.67	5.4	3.93	7.53	3.40	6.86	5.06	7.5
S_3	2.50	5.8	3.46	8.13	3.26	8.13	4.53	8.13
S.Em.±	0.173	0.294	0.209	0.405	0.194	0.306	0.324	0.405
CD	0.496	0.843	NS	NS	NS	0.878	NS	NS
V_1	2.29	5.96	2.8	7.13	2.86	7.13	4.33	7.13
V_2	3.21	5.67	4.4	8.8	3.73	7.87	5.60	8.8
V_3	2.80	6.10	4.13	8.46	3.80	8.00	5.00	8.4
S.Em.±	0.173	0.294	0.209	0.405	0.194	0.306	0.324	0.405
CD	0.496	NS	0.600	1.162	0.558	NS	4.53	1.162
S x VS.Em.±	0.299	0.508	0.362	0.701	0.557	0.529	5.06	0.701
C.D.	NS	NS	NS	2.012	NS	NS	NS	2.012
C.V. %	24.18	19.22	21.43	19.27	21.71	15.43	5.33	19.27

Table 3: Effect of basal nutrient media on shoot growth

Treatment	Length of internodes		No. of roots		Length of roots	
	1st week	4th week	1st week	4th week	1st week	4th week
S_1	0.70	0.79	4.26	4.3	3.29	5.46
S_2	0.72	0.73	5.53	6.2	2.86	5.66
S_3	0.76	0.71	5.40	12.4	2.18	5.10
S.Em.±	0.095	0.058	0.355	0.531	0.188	0.736
CD	NS	NS	1.020	1.523	0.538	NS
V_1	0.73	0.840	4.00	6.8	1.73	5.10
V_2	0.67	0.620	5.73	8.2	3.11	5.5
V_3	0.75	0.766	5.46	7.9	3.50	5.63
S.Em.±	0.95	0.068	0.355	0.531	0.188	0336
CD	NS	0.167	1.020	NS	0.538	NS
S x VS.Em.±	0.095	0.101	0.615	0.919	0.325	0.592
C.D.	NS	NS	1.766	NS	NS	NS
C.V. %	50.7	30.36	27.13	26.88	26.11	24.04

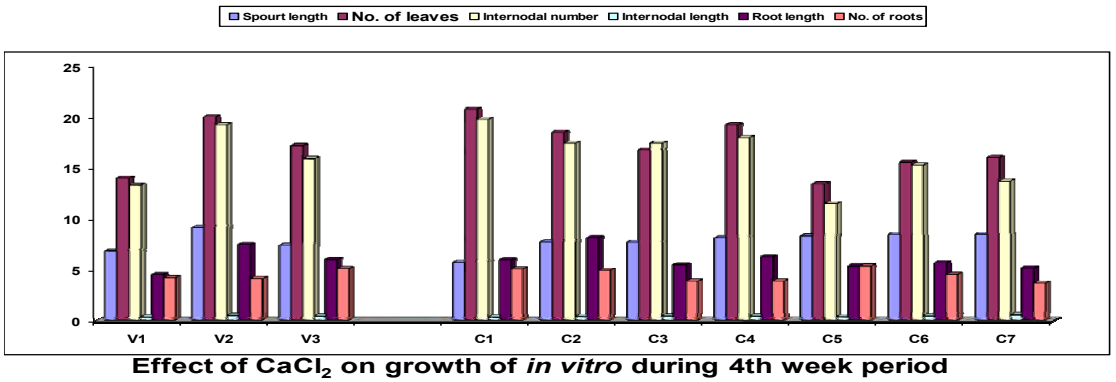
S_1 = MS + 100 mgl^{-1} inositol + 170 mgl^{-1} KH_2PO_4 + 0.4 mgl^{-1} thimine HCl + 0.005 NAA + 2.0 mgl^{-1} calcium pantothenate + 2% sucrose + 0.8% Agar

S_2 = ½ MS + 100 mgl^{-1} inositol + 170 mgl^{-1} KH_2PO_4 + 0.4 mgl^{-1} thimine HCl + 0.005 mgl^{-1} NAA + 2.0 mgl^{-1} calcium pantothenate + 2% sucrose + 0.8% Agar

S_3 = 1/4 MS + 100 mgl^{-1} inositol + 170 mgl^{-1} KH_2PO_4 + 0.4 mgl^{-1} thimine HCl + 0.005 mgl^{-1} NAA + 2.0 mgl^{-1} calcium pantothenate + 2% sucrose + 0.8% Agar

V_1 = Kufri Badshah; V_2 = Kufri Chipsona-1; V_3 = Kufri Pukhraj

length of roots in Kufri Pukhraj, was at par with Kufri Chipsona-1. The interaction effect (S × V) was found to be non-significant. The results concluded that the higher full concentration of MS media is good for the sprout length, and promotes the maximum number of nodes and the number of leaves. In case of the number of roots, the lower concentration of MS favors for the maximum number of roots. Length of roots were found to be a non-significant effect. The effect of the basal nutrients of MS media were significant for the plant growth and it exhibited good response in Kufri



Effect of $CaCl_2$ on growth of *in vitro* during 4th week period

C1=Control	C2=5 mgl^{-1} $CaCl_2$	C3=10 mgl^{-1} $CaCl_2$	C4=50 mgl^{-1} $CaCl_2$
C5=100 mgl^{-1} $CaCl_2$	C6=200 mgl^{-1} $CaCl_2$	C7=300 mgl^{-1} $CaCl_2$	

V_1 = Kufri Badshah; V_2 = Kufri Chipsona-1 V_3 = Kufri Pukhraj

Fig. 1: Effect of $CaCl_2$ on growth of in vitro shoots

Fig. 2: Axenic cultures of *Solanum tuberosum* L.

Chipsona-1. The results of Kanwal *et al*. (2006) also reported the maximum shoot formation in the full strength of MS medium.

Effect of $CaCl_2$ on the growth of *in vitro* cultures

The result indicates (Fig. 1) that the sprout length was maximum in C_7 treatment and V_2 variety while the other characters studied were very effective or gave higher response in C_1, C_2, C_4 treatment, and at the very same time C_5, C_6 and C_7 treatments gave poor responses. Kufri Chipsona-1 was found to be the most suitable followed by Kufri Pukhraj and Kufri Badshah. Patrick and Davinah (2014) evaluated potato varieties for the growth parameter like shoot, root, leaves and height that are indicated. The lower concentration of Ca promotes the better response to maximum number of nodes, maximum leaves and maximum internodal length while the higher concentration of $CaCl_2$ obtained maximum shoot length; (however, at the very same time control gave maximum sprouts). The increase in

the calcium concentration may favor the apical dominance resulting in long main shoot, while the decrease in the concentration resulting in auxiliary growth. However, it was observed that the results were somehow variable. This result is the conformity with the results of Ozgen and Palta (2005). Busse *et al.* (2008), narrated that the shoot tip necrosis has been attributed to calcium deficiency in *in vitro* cultures, resulting to the death of the shoot tip, loss of apical dominance and the axillary branch development. The results of Sarkar *et al*. (2005) documented that Ca nutrition is one of the most important factor determining the micro plant phenotype during the storage of plants. Calcium is an essential macronutrient for the plant growth derived from the growing medium. (Marschner, 1995)

CONCLUSION

The meristem culture was established in MS (1962) media which contains 10 mgl^{-1} GA_3 and 2% sucrose. The axenic culture was multiplied better in the lower level of (0.005mgl^{-1}) NAA in MS media containing 2.0 mgl^{-1} calcium pantothenate. The effect of calcium was reported as the better nutrient for the long storage of plants *in vitro*.

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