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In vitro regeneration of potato (Kufri Pukhraj)

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Abstract
Potato (*Solanum tuberosum*) is the most important non-cereal food crop of the world. Kufri Pukhraj is a variety of potato grows in many parts of Maharashtra. For rapid sprouting of potato tubers were *in vivo* treated with different concentration of GA_3 to between the explants 200ppm GA_3 showed greater ability for *in vitro* sprouting of potatoes. The 200ppm GA3 showed ability of number of sprout to tuber and their shoot length are large. The mean of no. of sprout formed was 2.66, and sprout length was 2.06. In which also study the callus induction on MS-media with 2,4-D (3.0mg/l) and kin (1.0mg/l) the callus induction was 2.01cm after 30 days and shoot regeneration of potato plant from callus and meristem by using the growth hormones such as, BAP and NAA.(3.0 mg/L and 2.0 mg/L resp.) Shoot regeneration mean was 0.92 after 30 days of regenerate.

Keywords: Potato, regeneration, tissue culture

Introduction
Potato (*Solanum tuberosum*) is the most important non-cereal food crop of the world. It belongs to family solanaceae, chromosome no. 2n= 48. It ranks fourth in the world after wheat, rice and maize. It produces the largest quantity of carbohydrates per day per unit area among the food crops. Potato in which consists of 80% water, 2-3% protein and 18% carbohydrate. Central Potato Research Institute, Shimla, India over the past 55 years has resulted in the development of 35 high yielding potato varieties for diverse agro climatic conditions and innovation of seed plot technique for augmenting the seed production (Kumar *et. al*.). According to Aykroyd (1941), it contains 74.7 per cent water, 22.9 per cent sugar, 0.6 per cent protein, 0.1 per cent fat, 0.6 per cent mineral matters, 0.01 per cent calcium, 0.03 per cent phosphorus and 0.0007 per cent iron. For best yields, a 120 to 150 day crop requires from 500 to 700 mm (20 to 27.5 inches) of water, before coming to the planting operation. It should be kept in mind that the sufficient soil moisture is available for satisfactory sprouting. The rate of water use is low till 30-35 days after planting; it means that the first irrigation is essentially done within 30-35 days after planting. Kufri Pukhraj is a variety of potato grow in Maharashtra, This variety is suitable in Bihar, Gujarat, Haryana, Himachal Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Punjab, Uttar Pradesh and West Bengal. it is released in 1998 from CPRI, Shimla. Early maturing varieties grow in 70-90 days. It is resistant to early and late blight diseases. The average yield potential is 350-400 q/ha. The availability of tissue culture technology for rapid multiplication of disease free planting material has facilitated potato seed production to a great extent. meristem culture is being successfully employed to obtain virus-free potato clones.
Cultivation of axillary or apical shoot meristems, particularly of shoot apical meristem, is known as meristem culture. Rapid multiplication of these disease-free clone using micropropagation coupled with conventional multiplication methods. The conventional planting material for potato is the vegetative tuber. In tissue culture various types of method are used, micropropagation for rapid and true type multiplication of plants on artificial nutrient media. The most significant advantages offered by micropropagation are large numbers of disease-free propagules can be obtained from a single plant in a short period, propagation can be carried out throughout the year, the propagating material can be accommodated in a small space, production of virus-free plants using meristem culture. (Naik & Karihaloo; 2007) [31]. Tissue culture techniques are used worldwide to produce pre-basic, virus-free seed potatoes known as microtubers. The microtubers are sown in a protected environment to produce minitubers (basic seed).

Materials and Methods
Plant materials: The samples of potatoes were collected from National Agriculture Research Project Marathwada Agriculture University, Aurangabad. Krushi Vigyan Kentra near railway

station, Aurangabad. Kufri pukhraj varieties tubers are also collected from nearby jadhavwadi, bhajimandi, Aurangabad.

Instrument and sterilization: The glassware properly cleaned with tween 20 detergent, washed under running tap water. The glassware was then steam sterilized in an autoclave at pressure of 15 lb/in2 at 121 °C for 20 minutes and hot air oven. Ethanol, MS Media stock solution, $HgCl_2$, Plant growth hormons viz, 2,4-D, BAP, NAA, kinetin, Stress inducing reagent sorbitol.

Treatment: Tubers of variety kufri pukhraj, taken as explants for planting in pots. They washed under tap water for 30 minutes. The tubers were treated with different concentration solution of GA_3. For treatment to tuber to break dormancy used at rate of control, 100ppm, 200ppm, 300ppm, 400ppm and 500ppm, soaked for 16 hours.

Preparation of MS-media: Contamination of media could be controlled by adding antimicrobial agents, acidification or by filtration through microporous filters. Sterilization of media is routinely achieved by autoclaving at the temperature ranging from 115 ℃.

Selection of explants and sterilization: Collection of shoot tips for explants. washed under tap water for 30 minutes. Few drops of Tween 20 for 20 minutes. again washed with sterile double distilled water for 10 minutes. Sterilization with ethanol (70%) and 0.1% mercuric chloride for 1-2 minutes each is carried out. Finally rinsing is done thrice with sterile double distilled water to completely remove any mercuric chloride.

Meristem culture: The sterilized shoot tips were inoculated in MS-media giving various treatment of growth hormones in concentration of 2,4-D and kinetin such as 1-1, 1-2, 2-1, 2-2, 3-1, 1-3mg/L. Cultures were maintained at 25±2 °C in light and dark 16/8 hrs. conditions. Callus sub culturing was done regularly after 25 days using visual observations to select morphogenic tissues prior to transfer. Media used for sub cultured callus was same as inoculation medium for number of callus induction.

Shoot regeneration from callus: Callus induced on MS media containing combination of 2,4-D and kinetin were sub cultured on MS media incorporated with concentration of BAP and NAA 1-2, 2-1, 2-2, 3-2, 2-3mg/l for shoot regeneration.

Shoot multiplication: Baksha *et al*, 2002, reported Multiple shoots from shoot tip explants of potatoes by culturing on MS medium supplemented with BAP and Kinetin 0.5-1, 1-0.5, 1-1, 1-2mg/L were used for shoot multiplication.

Results

The following results were obtained from the above experiment these are as follows.

The complete procedure and stepby step results asr shown in figure no 1. The potato tuber treated with different concentration of GA_3 viz, control, 100ppm, 200ppm, 300ppm,400ppm, 500ppm. Treatment with 100 ppm and 200 ppm of GA_3 was efficient for the variety (kufri pukhraj). After the 10 days form the sprout. For treatment to tuber to break dormancy used at rate of 200ppm to 500ppm. Seed soaked for 16 hours in GA_3 solution at 200 ppm gives the highest number of sprout with mean 4.33 where as the GA3 AT 500ppm gives the lowest number of sprout with mean 1.33.

Table 1: The number of sprout formation

Replication/ GA_3 Treatment	R_1	R_2	R_3	Mean
T_0 (control)	3	3	4	3.33
T_1 (100ppm)	2	6	2	3.33
T_2 (200ppm)	4	3	6	4.33
T_3 (300ppm)	1	2	2	1.66
T_4 (400ppm)	3	2	1	2.00
T_5 (500ppm)	1	2	1	1.33
Mean	2.33	3.00	2.66	2.66
Std. error	1.73			
Std. deviation	1.53			
Geometric mean	2.28			

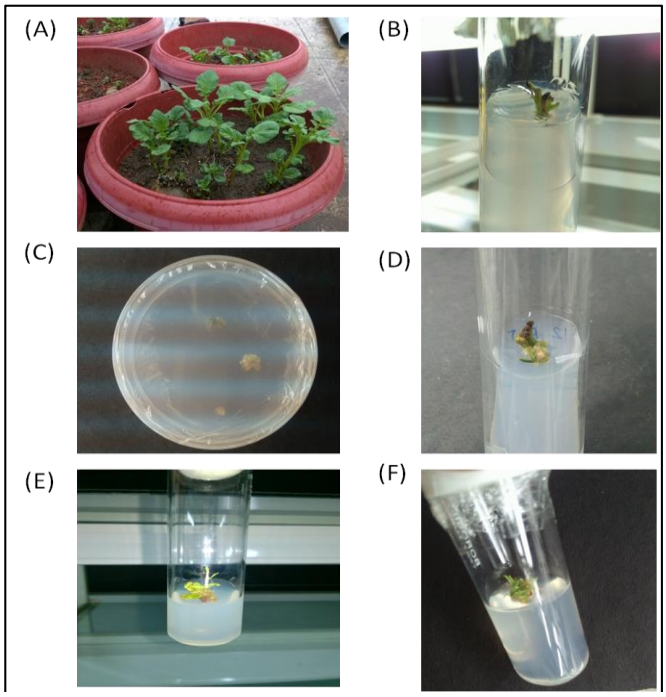

Fig 1: A) The explants grown in the pot. B) Shoot tip transfer on the callus induction medium. C) The induction of callus. D) Callus multiplication E) Shoot regeneration F) Shoot multiplication.

The sprout length

There is significant increase in the sprout length at 200 ppm with mean sprout length 4.33 observed, whereas at 100ppm the mean sprout length was 1.35.

Table 2: The sprout length

Replication/ GA_3Treatment	R_1	R_2	R_3	Mean
T_0 (control)	1.50	2.16	1.65	1.77
T_1 (100ppm)	1.50	1.56	1.00	1.35
T_2 (200ppm)	3.75	2.50	3.37	**3.20**
T_3 (300ppm)	1.00	2.50	2.00	1.83
T_4 (400ppm)	2.50	2.75	2.00	2.41
T_5 (500ppm)	2.00	1.00	2.50	1.83
Mean	2.04	2.07	2.08	2.06
Std. error	1.00			
Std. deviation	0.77			
Geometric mean	1.92			

In this study complete regeneration was done from shoot tips of *solanum tuberosum* cultivars (kufri pukhraj) through meristem culture. The sterilized shoot tips were inoculated in MS-media containing 2,4-D (3.0mg/l) and kinetin (1.0 mg/l).

Callus induction from shoot tips on media was observed after 13 days. After callus induction from shoot tips and their growth increases were observed and recorded periodically at 10 days intervals.

Table 3: Callus induction after 10 days intervals.

Induction of callus (Diameter of callus in cm)				
2,4-D+ KIN(mg/L)	10 days	20 days	30 days	Colour
3.0+1.0	0.91	1.21	2.01	Yellowish green
1.0+3.0	0.12	0.73	0.89	Light yellowish

Shoot regeneration from Callus induced on MS media containing combination of 2,4-D and kinetin were sub cultured on MS media incorporated with concentration of BAP (3.0mg/l and 2.0mg/l) and NAA (2.0mg/l and 3.0mg/l). The shoot was regenerated after 20 days Observed by visually. The shoot multiplication used the growth hormone with MS-media, BAP (0.5-1.0 mg/l) and Kin (1.0- 0.5 mg/l). Final observation was taken at 3rd subculture stage in multiplication as same inoculation medium. Observations were taken for evaluating the growth of shoots by taking parameters like average shoot length and number of shoots at 10, 20, 30 days after inoculation.

Table 4: Shoot regeneration from callus after 10 days intervals.

Mean of Shoot regeneration from callus clump (length in cm)			
BAP + NAA	10 Days	20 days	30 days
3.0+2.0	0.34	0.61	0.92
2.0+3.0	0.23	0.56	0.79

Discussion

Treatment with 200ppm of GA_3 was efficient for the variety (kufri pukhraj). Treated tubers result shows effect of 100% sprout formation after the 10 days form the sprout. For treatment to tuber to break dormancy used at rate of 200ppm GA_3 solution in tuber soaked for 16 hours. The high concentration GA3 solution not effective for breaking dormancy they will be die.

In the present study regeneration was achieved from *in vivo* shoot tips explants of *s. tuberosum* cultivars (Kufri pukhraj) through meristem culture. The sterilized shoot tips were inoculated on MS media containing of 2,4-D (3mg/l) and kinetin (1mg/l). *Vijay kumar et.al* (2014) [41] reported best callus growth from the cultivars (kufri pukhraj) was observed on Murashige and Skoog (MS 1962) [24] media containing 3.0mg/l 2,4-D and 1.0mg/l kin. Callus formatiom from shoot tips of *s. tuberosum* on media were observed and recorded after some days. The combination and concentration of auxin and cytokinin are effectives for callus formation. Equal amount of auxin and cytokinin produces callusing. MS medium supplemented with combinations of BAP and NAA were employed for shoot regeneration. Best shoot regeneration from callus was observed on MS media containing 3.0mg/l BAP and 2.0mg/l NAA. Koleva Gudeva Liljana *et al.* (2012) [19] reported the shoot from callus of potatoes by culturing on MS medium supplemented with BAP and NAA (3.0mg/l and 2.0mg/l, 2.0mg/l and 3.0mg/l respectively) as a control were effectively used for shoot regeneration.

Shoot multiplication after two cycles of sub culturing in the solid media, regenerated shoot were transferred to combinations of liquid multiplication media and compared the multiplication rates. Baksha *et al*, 2002, reported Multiple shoots from shoot tip explants of potatoes by culturing on MS medium supplemented with BAP (0.5-1.0 mg/l), Kin (0.1-0.5 mg/l) as control were used for shoot multiplication. Final observation was taken at 3rd subculture stage in multiplication. Observations were taken for evaluating the growth of shoots by taking parameters like average shoot length and number of shoots at 10, 20, 30 days after inoculation. Axillary shoots initiated from the base progressively increases in size in each subculture and number of shoots multiplied vary from medium to medium.

Conclusion

Potato (*Solanum tuberosum*) is the most important food crop of the world. It produces the largest quantity of carbohydrates per day per unit area among the food crops. We choose Kufri Pukhraj is a variety of potato grow in Maharashtra. It is resistant to late and early blight disease. For rapid sprouting of potato tubers were *in vivo* treated with 200ppm concentration of GA3 showed greater ability. The 200ppm GA_3 showed ability of number of sprout to tuber and their shoot length are large. The mean of no. of sprout formed was 2.66, and sprout length was 2.06. In which also study the callus induction on MS-media with 2,4-D (3.0mg/l) and kin (1.0mg/l) the callus induction was 2.01cm after 30 days and shoot regeneration of potato plant from callus and meristem by using the growth hormones such as, BAP and NAA.(3.0 mg/L and 2.0 mg/L resp.). shoot regeneration mean was 0.92 after 30 days after transfer. The shoot multiplication by culturing MS-media with BAP (0.5-1.0 mg/l), Kin (0.1-0.5 mg/l) as control were used for shoot multiplication. Shoot multiplication rate will be slow.

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