

## Comparison of crops raised with tissue culture *vis-a-vis* conventional seed tubers of potato

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**ABSTRACT** An attempt was made to compare stage-III and stage-IV seed crops (conventional tubers) with their respective counterpart's generation-1 and generation-2 seed crops (tissue culture tubers) in Kufri Sindhuri, Kufri Lauvkar and Kufri Chandramukhi in factorial RBD replicated three times during 2010-11 and 2011-12. For morphological parameters, there were no significant differences among respective counterparts respectively. In case of yield parameters, generation-1 (8, 19,000) and generation-2 (7, 42,000) recorded significantly higher tuber number over their respective counterpart stage-III (5, 96,000) and stage-IV (6, 53,000) respectively. For tuber weight (t/ha) there was no significant difference among the respective counterparts. ELISA testing results revealed that generation-1 (1.11%) and generation-2 (1.83%) recorded significantly lower disease percent over their respective counterpart's stage-III (3.33%) and stage-IV (4.33%). The hi-tech seed tubers were better than conventional seed tubers health wise and can be integrated with conventional system for breeder seed production.

**Keywords:** Health standard, hi-tech tubers, conventional tubers, seed potato, comparison

Presently, Central Potato Research Institute, Shimla is producing about 2600 t breeder seed of potato annually, which is sufficient to meet the country's demand of certified seed if properly multiplied by the state and other agencies to which the breeder seed is being supplied. Central Potato Research Institute produces breeder seed using both tubers produced from Micro-propagation technology and conventional system. Stage-III and stage IV of conventional system are considered as equivalent to generation-1 and generation-2 of Micropropagation system during breeder seed production. However, extra field exposure for one year in conventional system of seed production may favour contamination with viruses, which causes severe reduction in yield.

All conventional potato seed production systems are characterized by low multiplication rate and progressive accumulation of degenerative viral diseases during clonal propagation. About 30 viruses

and virus like agents infect potato. These being systemic pathogens, are perpetuated through seed tubers and pose a major threat to potato seed production [1-2]. Some of these viruses, notably potato leaf roll (PLRV), potato virus A (PVA), potato virus Y (PVY), potato virus V (PVV), potato virus M (PVM), potato virus X (PVX), potato virus S (PVS), potato mop top virus (PMTV) and potato aucuba mosaic virus (PAMV) occur worldwide in potato crops; others are important only in some geographical areas [3]. In this event, biotechnological approach through Micropropagation offers a great potential to complement conventional seed production [4].

Minitubers are the intermediate stage of potato seed production between laboratory and field multiplication [1]. It is known as G-0, multiplied under insect proof net house conditions, and further multiplied under field conditions as Generation-1 and Generation-2, which is supposed to be equivalent to Stage-III and Stage-IV of conventional system.

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Serological procedures form the most reliable method for identification and quantitative assay of viruses. In this context, the direct double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) is used widely for virus detection [5]. The advantage of this assay is that the virus particles are concentrated from extracts by the coating antibody, and inhibitory components of extracts are removed by rinsing before addition of detecting antibody and enzyme substrate.

### MATERIALS AND METHODS

Under conventional system the nucleus seed developed by clonal selection and tuber indexing. For clonal selection, apparently, healthy plants from the field are marked and four uniform tubers are selected from each plant and serially numbered. The health status of each tuber is checked by tuber indexing by growing their eye plugs and testing the resulting plants for freedom from viruses through ELISA test.

The disease free indexed tubers are multiplied in field in Stage-I at wider spacing of 1.2x1.2 m and tested individually by ELISA for viruses also inspected visually for mosaic and other abnormalities/ varietal mixture. The healthy produce of single hill in stage-I is then planted in row as a clone at wider inter row spacing of 1m and 20cm intra row spacing as per size of tubers. The crop in stage-II is tested against viruses by serological methods and inspected visually for mosaics. Variety wise bulked produce of stage-II is planted in stage-III for bulk multiplication at normal spacing 60x20cm. Whole crop was visually inspected for mosaics. The healthy produce is bulked for multiplication in Stage-IV.

Micropropagation is a tissue culture (*in vitro*) method used for rapid and true to type multiplication of plants on artificial nutrient media under controlled environment. The controlled and aseptic environment of the tissue culture laboratory provides optimum

conditions for multiplication of plant cultures. Nodal segment culture in which the auxiliary and apical buds grow into new plants is predominantly used for initial shoot multiplication of mericlones. The auxiliary/apical buds of these nodal segments grow into full plants (microplants) within a period of about 3 weeks. These plants can be further sub-cultured on fresh medium. Assuming effective generation of minimum 3 nodal cuttings by an *in vitro* plant and sub-culturing interval of 25 days, theoretically  $3^{15}$  (14.3 million) microplants can be obtained from a single virus-free microplant in a year. In this method, 15-20 day old cultures are kept in glasshouse or polyhouse for 8-10 days for hardening without removing culture tube plugs or lids of magenta boxes. The hardened *in vitro* plants are removed from culture vessels with the help of forceps, washed to remove adhering medium and cut into two pieces after cutting out the root zone. Microtubers are miniature tubers developed *in vitro* under tuber induction conditions. They are very small (100-150mg) and convenient for handling, storage and transportation. Multiplication of these microplants and microtubers under aphid proof net house conditions is known as generation-0, which is further multiplied under field conditions as generation-1 and generation-2. The seed tubers of stage-III and stage-IV (conventional tubers), generation-1, and generation-2 (hi-tech tubers) are supposed to be equivalent and are compared in the present study for yield potential and health status.

A field experiment was conducted at the Central Potato Research Station, Gwalior (MP) during 2010-11 and 2011-12 for comparison of stage-III and stage IV seed crops (conventional seed tubers) with their respective counterparts generation-1 and generation-2 seed crops (hi-tech seed tubers) in three popular varieties namely Kufri Lauvkar (early) Kufri Chandramukhi (early medium) and Kufri Sindhuri (late).

The experiment was conducted in factorial randomized block design and treatments were replicated three times. Planting was done in the second week of November each year. A uniform inter and intra row spacing of 60cm × 20cm was kept in both the years. Nitrogen (N), phosphorus (P) and potassium (K) were applied @ 150 kg, 80 kg and 100 kg/ha, respectively. Full doses of P through single super phosphate, K through muriate of potash and half dose of N (90 kg/ha) through ammonium sulphate were applied at the time of planting. Remaining half dose of N (90 kg/ha) was applied through urea at the time of earthing up. Hoeing and earthing up were done in the second week of December during both years. Overall, 5 irrigations were applied during both the years. Growth parameters like, germination percentage was recorded after 30 days and plant height, Number of stem and number of compound leaves was recorded after 45 days after planting. Crop was harvested after 85 days of planting during both the years and yield parameters were recorded. Health data through visual observations in field and through enzyme-linked immunosorbent assay (ELISA) testing was also recorded where testing was done for six viruses namely, potato leaf roll (PLRV), potato virus A (PVA), potato virus Y (PVY), potato virus M (PVM), potato virus X (PVX) and potato virus S (PVS). The experiment was conducted twice, data were pooled over the years and analyzed statistically and means were separated according to the least significant differences (LSD) at 0.05 level of probability.

## RESULTS AND DISCUSSION

### Growth attributes

For growth attributes *viz.*, germination percentage, number of stem/plant, plant height, number of leaves/plant and canopy cover/plant at 25, 50 and 75 days, there were no significant differences among stage-III and generation-1 as well as stage-IV and

generation-2 respectively (Table 1). No significant differences were reported in emergence in microtubers and conventional seed tubers and among cultivars in different planting dates [6] and leaf area increased with the age of plants in both the methods and was maximum at 75 days of plant growth [4]. Similar trend was recorded in case of canopy cover of the plants in both the system of propagation in the present experiment. Among varieties, morphological parameters *viz.*, number of stems/plant, number of leaves/plant and canopy cover/plant at 50 and 75 days was significantly higher in Kufri Sindhuri over Kufri Lauvkar and Kufri Chandramukhi but for other parameters, there was no significant difference.

### Health standard

Enzyme-linked immunosorbent assay (ELISA) testing results revealed that generation-1 (1.11%) and generation-2 (1.83%) recorded significantly lower disease percent over their respective counterpart's stage-III (3.33%) and stage-IV (4.33%) respectively (Fig.1). Among varieties, Kufri Chandramukhi had highest disease incidence (3.42%) followed by Kufri Lauvkar (2.75%) and Kufri Sindhuri (1.79%) (Table 1). Non-significant differences were recorded in case of foliar diseases like stem necrosis, leaf spot, and early blight among respective counterparts. Among varieties, only Kufri Lauvkar recorded the significantly higher incidence of foliar diseases like leaf spot, and early blight over other two varieties, but in case of stem necrosis, it was non-significantly higher over other two varieties (Table1).

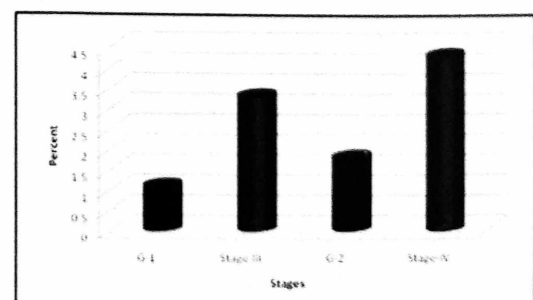


Fig. 1. Comparison of percent disease incidence through ELISA test

**Table 1. Comparison of growth attributes and percent foliar diseases in conventional and hi-tech tubers**

Tuber type/ Varieties	Growth attributes							Foliar diseases %			
	Germination (%)	No. of stem/plant	Plant height (cm)	No. of leaves/plant	Canopy cover at 25 DAP (M <sup>2</sup> )	Canopy cover at 50 DAP (M <sup>2</sup> )	Canopy cover at 75 DAP (M <sup>2</sup> )	(%) diseased plant in ELISA	Stem necrosis (%)	Leaf spot (%)	Early blight (%)
Generation-1	87.17	3.6	43.6	39.7	0.06	0.09	0.16	1.11	0.08	0.16	0.16
Stage-III	88.17	3.2	41.9	39.3	0.07	0.10	0.16	3.33	0.00	0.24	0.00
Generation-2	86.37	3.9	44.3	46.6	0.06	0.10	0.16	1.83	0.00	0.32	0.16
Stage-IV	86.18	3.4	42.5	38.6	0.06	0.10	0.17	4.33	0.00	0.40	0.32
CD (0.05) T	NS	0.53	2.38	7.42	0.01	NS	NS	1.07	NS	NS	0.29
K. Sindhuri	85.75	4.2	42.8	47.3	0.06	0.12	0.18	1.79	0.00	0.00	0.00
K. Lauvkar	88.35	3.2	44.8	39.0	0.06	0.08	0.15	2.75	0.06	0.83	0.48
K. Chandramukhi	86.81	3.2	41.7	36.9	0.07	0.08	0.15	3.42	0.00	0.00	0.00
CD (p=0.05) V	NS	0.46	2.06	6.43	0.01	0.01	0.02	0.92	NS	0.32	0.25
CD (p=0.05) TxV 7.74		0.92	4.12	12.86	0.02	0.03	0.03	1.84	0.20	0.65	0.50

In case of field observations, severe mosaic was recorded in all the three rouging and in totality observed; non-significant differences were among respective counterparts (Table 3). In case of mild mosaic, stage-III recorded significantly higher total mild mosaic (0.63%) than its counterpart's generation-1 (0.00%). Among stage-IV and generation-1, there was no significant difference. In case of off type plants, no significant difference was recorded among respective counterparts (Table 3).

#### Yield parameters

In case of <25g tubers grade category per ha, generation-1 tubers recorded significantly higher tubers numbers (4,56,000) than its counterpart Stage-III (2,85,000) and among generation-2 and stage-IV there was no significant difference. Similar trend was recorded in yield (t/ha) in this grade. In case of 25-125g grade (seed size) tubers/ha, no significant difference was recorded among respective counterparts both in number of tubers per hectare and yield per hectare (Table 2). In case of >125g grade tuber category, stage-IV had significantly higher tuber number (47,000) than its counterparts generation-2 (35,000), but tuber weight was significantly higher in stage-III (7.7 t/ha) than generation-1 (5.7 t/ha), In case of total tuber

yield parameters/ha, generation-1 (8,19,000) and generation-2 (7,42,000) recorded significantly higher tuber number over their respective counterpart stage-III (5,96,000) and stage-IV (6,53,000) respectively. It indicated that hi-tech system produces higher number of tubers than conventional system; hence, the planting area can be increased in the further multiplication if the tubers are produced from hi-tech system [7]. For total yield t/ha, there was no significant difference among the respective counterparts (Table 2). Higher number and weight of tubers per plant in <25 g and total tuber category in micropropagated tubers than conventional tubers and vice versa in >125 g category of tubers were recorded [4].

Among varieties, in case of tuber numbers/ha, Kufri Sindhuri recorded significantly higher tubers (6,09,000 in <25g grade tuber category, 4,18,000 in 25-125g grade tuber category and 10,58,000 in total tubers) than Kufri Lauvkar and Kufri Chandramukhi. However, in >125g grade tuber category tubers, Kufri Lauvkar and Kufri Chandramukhi recorded significantly higher tubers over Kufri Sindhuri. Similar trend was recorded in case of weight of tubers (Table 2). The seed size of tubers and total yield in Kufri Sindhuri was very high in number as well as in weight in comparison to other two varieties

**Table 2. Comparison of yield parameters in conventional and hi-tech tubers**

Tuber type/ Varieties	No. of tubers (000) /ha				Weight of tubers (t/ha)			
	< 25g	25-125g	>125g	Total	< 25g	25-125g	>125g	Total
Generation-1	456	325	38	819	5.2	18.0	5.7	29.1
Stage-III	285	268	44	596	3.4	16.5	7.7	27.9
Generation-2	372	335	35	742	5.1	17.5	6.3	29.3
Stage-IV	326	280	47	653	4.2	18.2	8.2	30.9
CD (0.05) T	76.7	NS	11.3	68.4	1.18	NS	1.64	NS
K. Sindhuri	609	418	31	1058	7.0	19.2	5.0	31.6
K. Lauvkar	204	236	47	487	2.5	17.0	8.1	27.8
K. Chandramukhi	266	252	45	563	3.9	16.5	7.8	28.5
CD (p=0.05) V	66.4	54.0	9.8	59.2	1.02	2.10	1.42	2.85
CD (p=0.05) TxV	132.8	108.0	19.6	118.4	2.04	4.20	2.85	5.70

**Table 3. Comparison of health parameters in conventional and hi-tech tubers in field through visual observations**

Tuber type/ Varieties	Percent field health data of conventional and hi-tech tubers											
	Severe Mosaic				Mild Mosaic				Off type			
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	Total	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	Total	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	Total
Generation-1	0.63	0.08	0.12	0.83	0.00	0.00	0.00	0.00	0.12	0.28	0.12	0.52
Stage-III	0.71	0.32	0.12	1.15	0.32	0.20	0.12	0.63	0.12	0.00	0.00	0.12
Generation-2	0.60	0.28	0.52	1.39	0.24	0.24	0.04	0.52	0.28	0.20	0.12	0.60
Stage-IV	0.67	0.32	0.32	1.31	0.28	0.44	0.24	0.95	0.36	0.40	0.16	0.91
CD (0.05) T	NS	NS	0.40	NS	0.31	0.22	0.23	0.51	0.22	0.28	NS	0.46
K. Sindhuri	0.71	0.06	0.60	1.37	0.24	0.09	0.03	0.36	0.15	0.18	0.15	0.48
K. Lauvkar	0.65	0.24	0.03	0.92	0.18	0.09	0.03	0.30	0.03	0.18	0.00	0.21
K. Chandra mukhi	0.60	0.45	0.18	1.22	0.21	0.48	0.24	0.92	0.48	0.30	0.15	0.92
CD (p=0.05) V	NS	0.23	0.35	NS	NS	0.19	0.20	0.44	0.19	NS	0.15	0.52
CD (p=0.05) TxV	0.74	0.47	0.69	1.39	0.54	0.39	0.41	0.88	0.39	0.49	0.31	0.60

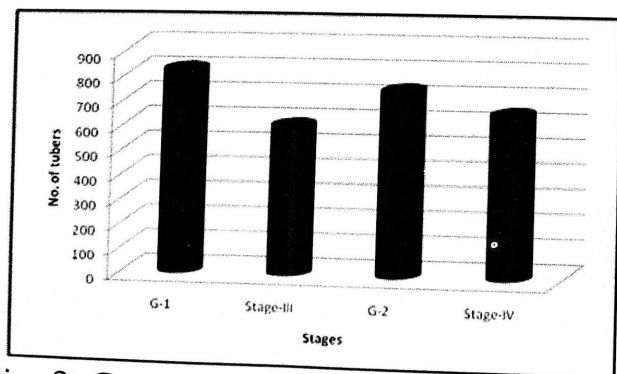


Fig. 2. Comparison of total number of tubers (thousand per hectare)

**CONCLUSIONS**

Health wise hi-tech tubers were better than conventional tubers. The planting area can be increased in further multiplications as number of tubers produced are higher in hi-tech system of seed production, hence can be integrated with conventional system for breeder seed production. In the present study, overall comparative performance in terms of germination, number of stems/ plant,

plant height, canopy cover, foliar diseases, severe mosaic and total tuber yield weight is more or less similar in hi-tech and conventional seed production.

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