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# A review on Comparison of Tissue Culture v/s Conventional System of Seed Potato Production

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ABSTRACT

Seed production in potato is largely being done by conventional seed production system which involves clonal multiplication by the use of potatoes. This is the transition phase of shifting whole seed production system from conventional system to hi-tech seed production system which involves micro-propagation techniques. The study of comparison of these two production system is important for researchers and seed producers. The main drawback of conventional seed production system is rapid degeneration of seed tubers by the viruses and other pathogens, low seed multiplication rate varying from 1:4 to 1:15, larger size of tubers hence high cost of seed production and time duration is more to get breeder seed. *In-vitro* multiplication of virus-free microplants and microtuber followed by minituber production in net house and aeroponic techniques is most important components of high-tech seed production which overcomes drawbacks of conventional seed production system. Generation-1 and Generation-2 which is supposed to be equivalent to Stage-III and Stage IV of conventional system are multiplied under field conditions as pre-breeder seed (Generation-1 and Stage-III) and breeder seed (Generation-2 and Stage IV). Comparatively hi-tech system tubers were at par with conventional system tubers with respect to growth parameters. 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 during Enzyme-linked immunosorbent assay (ELISA) testing of virus detection. Hi-tech system produces higher number of tubers than conventional system and at par total yield t/ha. The large scale multiplication with its vertical increase in half of the required time (2 years) and space, desired seed size for producing nucleus planting material along with round the year production, free from all diseases, very good quality, healthy tubers and easy to transport with very low cost confirms high-tech seed production system has an edge over conventional seed production system.

## 1. Introduction

There are a number of potato propagation techniques that are currently used worldwide to multiply seed potato and among them are: (1) conventional seed potato production, (2) Tissue culture techniques of seed production (micropropagation), (3) hydroponics, (4) aeroponics, (5) Bioreactor and (6) NFT. However, most farmers of the developing countries are engaged in potato seed multiplication using the conventional method of producing seed tubers. While the remaining other methods have got limitations and challenges, the conventional technique has the highest limitations in reducing high quality seed tubers under the poor resource farmer's conditions.

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## 2. Conventional system of potato seed production

Potato seed production is normally through vegetatively propagated. Conventional techniques of seed potato production involve clonal multiplication by the use of potatoes that are propagated by harvesting and replanting the tubers in the field. The tubers used for planting are known as "seed potatoes". Seed potato growers select better quality tubers for seed and discard those of poor quality. The diseased and healthy plants are identified and separated, and the healthy tubers are used for the next season's production (Chiipanthenga 2012). Conventional potato seed production systems involves successive multiplication of nucleus seed tubers through super elite, elite and certified seed stage (Ahloowalia 1999). However, this method of seed production has proved to be laborious (labour intensive), prone to pest and disease infestation and time consuming (Chiipanthenga 2012).

### *2.1 Problems in Conventional Seed Potato Cultivation*

Successful cultivation of seed potato primarily depends upon the availability of disease free seed, plant protection measures, low temperature and short day conditions during tuberization phase. Potato plant is very sensitive to ecological factors such as temperature, rainfall and photoperiod (Singh 2002). Seed tuber quality is an extremely important factor for potato yield. Since it is a vegetatively propagated plant, fungal, bacterial and, particularly viral disease, agents are easily transmitted through the tubers. Viral diseases are, for the most part, responsible for degeneration, characterized by a decrease in vigor, productivity and resistance to diseases of potato cultivars after successive cultivation from the same lot of tubers (Silberschmidt 1937; Sangar et al. 1988). In India, farmers use the traditional methods for potato cultivation and face many problems. The most severe problem faced by the farmers, regarding is the non-availability of foundation and certified seed. The farmers use pieces of potato or whole potato tuber, as a seed and therefore a large quantity of food material is also lost. Some major problems in conventional potato cultivation are as follows:

### *2.2 Disease and Insect problems*

If the mother potato plant becomes infected with a disease during the growing season, each of the new daughter tubers is likely to be infected as well (Chiipanthenga 2012). The infection of a single seed tuber produces a tenfold increase in diseased tubers in the subsequent propagation (Ahloowalia 1999). Considering that vegetatively propagated crops especially potatoes are prone to both viral and bacterial diseases, the conventional production of seed potatoes favors disease build-up, which drastically reduces crop yield (Badoni and Chauhan 2010; El-Komy 2010). 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 (Nyende 2005 and Naik and Karihaloo 2007). Some of these viruses, notably potato leaf roll virus (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 (Brunt 2001)

### *2.3 Quantity and quality of the product may be decreased*

Rapid degeneration of seed tubers by the viruses and other pathogens is the most common problem in seed potato production. If the seed stock is ill- maintained 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. This is the major problem faced by seed producers (Badoni and. Chauhan 2010).

### *2.4 Availability of good quality seed is a major constraint*

Area under potato during 2011-12 was 1.90 mha with the production of 46.2 million tons in India (Economic survey, 2012). To cover this area it requires about 4.50 to 5.43 million tonnes quality seed. To fulfill this requirement area to be planted under seed crop is 0.36 mha. To cover this area our country require 3491 tonnes of breeder seed after four multiplication at state/ farmers level for 1.54 mha area under ware Potato. Central Potato Research Institute produces about 2600 tons of breeder seed per annum. Having huge gap between supply and demand, of late private sector has entered in a big way (Singh et al 2011; Venkatasalam et al 2011 and Economic survey 2012). But as private companies and seed growers have entered in seed production, quality of seed production is a matter of concern.

### *2.5 High cost of potato seed*

Usually tubers produced in conventional system are of large size and the whole seed tubers have to be planted for good quality crop. Therefore, the seed cost become very high under conventional system. Seed cost in potato accounts for 40-50 percent of total production cost (Badoni and Chauhan 2010).

### *2.6 Low multiplication rate*

The conventional method of propagation is one of the slowest methods of seed multiplication. Compared with other seed propagation techniques like tissue culture and aeroponics, this traditional method would create approximately 8 daughter tubers only in the course of a year (Hussey and Stacey 1981 and Otazu 2008). The multiplication rate in potato is low varying from 1:4 to 1:15 (one tuber yields 4 to 15 tubers) depending upon variety, agro- climatic conditions and crop management practices (Badoni and Chauhan 2010). This method has also shown to be time specific particularly in tropical and sub-tropical regions where potato is a winter crop (Burton, 1989). In addition, the method requires a seed producer to have enough land if he is to enter into commercial seed production. This however, is associated with high labour cost in managing big fields. All conventional potato seed production systems are characterized by low multiplication rate and progressive accumulation of degenerative viral diseases during clonal propagations. That is why, despite tremendous efforts little success had been achieved in conventional seed plant potato production scheme (Singh et al 2008).

## 3. Tissue culture techniques of potato seed production

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. The *in vitro* propagation method is most suitable alternative to produce seed material of potato. By using the technique, which involves low cost components, the large scale clonal material can be achieved in short time duration. Use of micro propagation for commercial seed production has moved potato from test tubes to field (Wang and Hu 1982). Micropropagation is a sophisticated technique of regenerating plants using small pieces of plants (so called explants) that is proliferated on an artificial medium under sterile conditions. Importance of micropropagation lies in very fast clonal multiplication of vegetable crops. Micropropagation is used mainly for getting disease- free plants of superior vigour and productivity (Singh, 1997). Plant tissue culture is the science of growing plant cells, tissues or organs isolated from the mother plant, on artificial media. This is facilitated through the use of a liquid, semi-solid or solid growth media in sterilized tubes or containers. Tissue culture is one of the important new methods of plant propagation available to growers. The use of tissue culture technique in seed production has resulted into mass production of potato plants in a very short period of time. The system is characterized by very flexible rapid multiplication giving a high rate of multiplication (Beukema and Van de Zaag 1990 and Pruski, 2001). *In vitro* propagation by nodal cutting has become an established method of rapid multiplication in potatoes (Ranalli et al 1994) Meristem culture is one of the important plant tissue culture applications for elimination of viruses from planting materials (Naik and Karihaloo 2007; Badoni and Chauhan 2010). It is a procedure in which apical/axillary growing tip (0.1 to 0.3 mm) are dissected and allowed to grow into plantlets on artificial nutrient media under controlled conditions. This technique for virus elimination is based on the principle that, many viruses are unable to infect the apical/axillary meristems of a growing plant and that a virus free plant can be produced if a small piece of meristematic is propagated (Wang and Hu 1982 and Kassanis 2008).

Apical meristem has a number of unique characteristics that has made elimination of virus possible and some of the features include:

**1.** Vascular system through which viruses are spread is not developed in the meristematic region.

**2.** Chromosome multiplication during mitosis and high auxin content in the meristem may inhibit virus multiplication through interference with viral nucleic acid metabolism

**3.** Existence of virus inactivating system with greater activity in the apical region than elsewhere (Naik and Karihaloo 2007).

**4**. Maintenance of genotype identity, since meristem cells preserve their genetic stability more uniformly (Grout, 1990). When materials have been cleaned of the pathogens, they can be mass multiplied for use as planting materials.

Tissue culture is not limited by the time of the year or weather. Healthy plants can be grown in a laboratory at any time of the year. In addition, conditions in the laboratory are ideal and therefore, conducive to all year round production scheduling. It also saves an enormous amount of daily care required by conventional cuttings and seedlings (Tudge 1988 and Mahmond 2006). Application of tissue culture alone has been practiced in different countries such as India as a revolutionized seed potato production. This technology is now well mastered and has been used in the successful micropropagation of several plant species in several countries (Bachraz 1995). However, in the case of potato, seed tubers are the best planting materials hence, application/ adoption of plant tissue culture alone in seed potato multiplication has been low. Most developing countries fail to maximize tissue culture technology due to high operational costs involved as it requires specialized equipment which is very expensive to acquire.

In addition, different nutrients, energy sources, vitamins and growth regulators used for media formulation are also very expensive (Badoni and Chauhan 2010). The techniques of tissue culture require specialized skills and knowledge which can only be acquired after going through formal training. Inadequate sterilization can result in 100% contamination, particularly when using field grown material. The success of any tissue culture propagation depends on the ability to transfer plants from a sterile environment to a non-sterile environment. For tissue culture to be adopted commercially, this stage must be done with high survival rates at low cost. Such ways of reducing contamination in tissue culture have been proved to be time consuming, labour intensive and therefore, very costly (Shahsavari 2010 and ISAAA 2010). Plants in tissue culture grow under very humid conditions in the culture tubes and have very little layer of wax on their surface. The wax is important in preventing excess water loss and to some extent, protecting against disease attack (Hamilton 2004). As a result, plants obtained from tissue culture are more susceptible to transporting shock and prone to wilting, pests and diseases attack once transported to the field. The micropropagated plantlets therefore, require a hardening off period every time planting materials are produced prior to planting them in the field (Dhawan and Bhojwani 1987). The use of plant tissue culture as a routine method of potato seed production would be costly but these techniques can be used to eliminate the pathogens, produce required initial material and then, use another efficient and cheaper system to rapidly produce high quality seed tubers for commercial production.

### *3.1 Process*

Micropropogation has been successfully used in almost all potato producing countries to speed up initial stages of seed production. The process typically consist of

**1.** Development of virus free potato plants using meristem culture and micropropogation of virus free plants.
**2.** Production of micro-and or / minitubers from micropropogated plants in net/poly house.
**3.** Growing healthy seed crop in breed using minitubers as a planting material.

### *3.2 Minituber production and its use in seed production*

Seed potato production is mostly based on in-vitro plantlets or micro-tubers, and on the subsequent production of mini-tubers as first ex vitro generation (Ranalli 1997). Minitubers can be produced from plantlets which are planted in beds (Wiersema 1987; Hassanpanah and Azimi, 2011). Minitubers are the intermediate stage of potato seed production between laboratory and field multiplication (Naik and Karihaloo, 2007). Minitubers are small seed potato tubers produced after acclimatization from plants propogated in-vitro and planted at high density in glass house seed beds or in containers using different substrate mixtures (Lommen, 1994). By using minitubers in seed minitubers in seed programme, the number of field multiplication can be reduced. This may increase the flexibility of seed production, improve health status of ultimate commercial seed produced (Özkaynak and Samanci 2006). Rapid multiplication of disease free clones using micropropogation coupled with conventional multiplication methods has now become the integral part of seed production in many countries (Donnally et al 2003). It is known as G-0 and 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. One year extra field exposure in conventional system of seed production are likely to be more contaminate with viruses which causes severe reduction in yield.

## 4. Comparison of field performance of tissue culture v/s conventional seed tubers of potato

In vitro production of microplants and microtubers in high-tech is corresponding to stage-1 in conventional system while G-O in nethouse *i.e.* corresponding to stage-2 which considered as nucleus seed. Generation-1 and Generation-2 which is supposed to be equivalent to Stage-III and Stage IV of conventional system are multiplied under field conditions as pre-breeder seed (Generation-1 and Stage-III) and breeder seed (Generation-2 and Stage IV).

### *4.1 Growth attributes*

For growth attributes parameters *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 (Sadawarti et al 2013). No significant differences were reported in emergence in microtubers and conventional seed tubers and among cultivars in different planting dates (Kawakami et al 2005) and leaf area increased with the age of plants in both the methods and was maximum at 75 days of plant growth (Singh et al 2008). Similar trend was recorded in case of canopy cover of the plants in both the system of propagation (Sadawarti et al 2013). The microtuber plants of Kitaakari had a lower initial increase in leaf area index than conventional seed tuber

Plants, but at the maximum shoot growth had the same leaf area index. This pattern was also observed in the other cultivars. Tuber initiation and tuber bulking occurred on average five days later in microtuber plants than in conventional seed tuber plants of cultivar Kitaakari. At maximum shoot growth, microtuber plants had on average 65% of tuber dry weight of conventional seed tuber plants, with small variation among cultivars (Kawakami et al 2005).

### *4.2 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. Non-significant differences were recorded in case of foliar diseases like stem necrosis, leaf spot, and early blight among respective counterparts (Sadawarti et al 2013). Singh et al 1997 reported that in generation-2, the virus level was much lower (0-0.0035%) as compared to stage-IV (0.09%-0.034%). In all the three roguings and in totality, non significant differences were among respective counterparts for severe mosaic, stage-III recorded significantly higher total mild mosaic (0.63 %) than its counterpart's generation-1 (0.00%) for mild mosaic. Among stage-IV and genration-1, there was no significant difference for mild mosaic. No significant difference was recorded among respective counterparts for off type plants (Sadawarti et al 2013).

### *4.3 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. It indicated that hi-tech system produces higher number of tubers than conventional system (Sadawarti et al 2013), hence the planting area can be increased in the further multiplication if the tubers are produced from hi-tech system. Seed size tubers were higher in generation-2 than Stage- IV conventional tubers (Singh et al 2008). No significant difference among the respective counterparts for total yield t/ha. Higher number and weight of tubers per plant in <25 g and total tuber category in micropropogated tubers than conventional tubers and vice versa in >125 g category of tubers were recorded (Singh et al 2008). Conventional tubers appeared superior to minitubers in all characteristics except radiation conversion coefficient, which was similar. Differences in performance between minitubers and conventional tubers were attributed to weight and age of seed tubers, pre-sprouting method and crop husbandry (Lommen and Struik 1994). Irrespective of maturity period, microtuber plants showed a higher tuber increase after maximum shoot growth, achieving around 86% of tuber dry weight of conventional seed tuber plants at harvest. Microtuber plants of early and late cultivars have a similar yield potential relative to conventional seed tuber plants, and microtubers of both early and late cultivars might be used as an alternative seed tuber source for potato production, if necessary (Kawakami et al 2005). Health wise tissue culture tubers are better than conventional tubers also the planting area can be increased in further multiplications as numbers of tubers produced are higher in tissue culture system of seed production, hence can be integrated with conventional system for breeder seed production.

During 2011-12 cropping season at CPRS, Gwalior the average germination, number of leaves and stems per plant was almost same in both the system total number of seed tubers were higher in Genartion 1 and 2 than their counterpart Stage-III and IV in all the three varieties (Fig.1). Howerver, in Kufri Sindhuri and Kufri Chandramukhi seed yield was higher in Genaration-1 than its counter part stage III. But among Generation-2 and stage IV, it was at par in Kufri Sindhuri. Figure-2 clearly indicates that the number of rejected diseased plants through ELISA test were highest in stage-III (3.2%) and stage IV (4.4%) as compared to their counterparts G-1(1.0) and G-2 (1.9). Comparative study during 2012-13 revealed that the average germination, number of leaves and stems per plant was almost same in both the system of seed production but plant height was more in conventional system than hi-tech system at Modipuram. The average yield of test varieties in terms of no. of tubers per hectare was maximum in conventional system than tissue culture raised seed crop and record of maximum 616.3 q/ha yield was recorded in Kufri Pukhraj in stage-3 at Jalandhar. Virus incidence in visual rouging as well as ELISA was comparatively less in hi-tech seed production. At Gwalior, higher number of stem and leaves were recorded in Stage-IV (5.12 and 47) and Stage-F1 (4.7 and 47) than their respective counterparts G-2 (4.0 and 42) and G-3 (4.6 and 40). In G-2 and G-3 total number of seed tubers were higher than their counterpart Stage-III and IV (Fig-3).

## Conclusion and Future Research

Seed potato production is mainly based on clonal selection of indexed tubers and its multiplication in subsequent stages in field which is generally called as conventional system. It is vegetatively propagated by progeny tubers in open field hence infection, multiplication and accumulation of virus is a common problem in potato seed production.

**Figure 1**. Comparison in yield

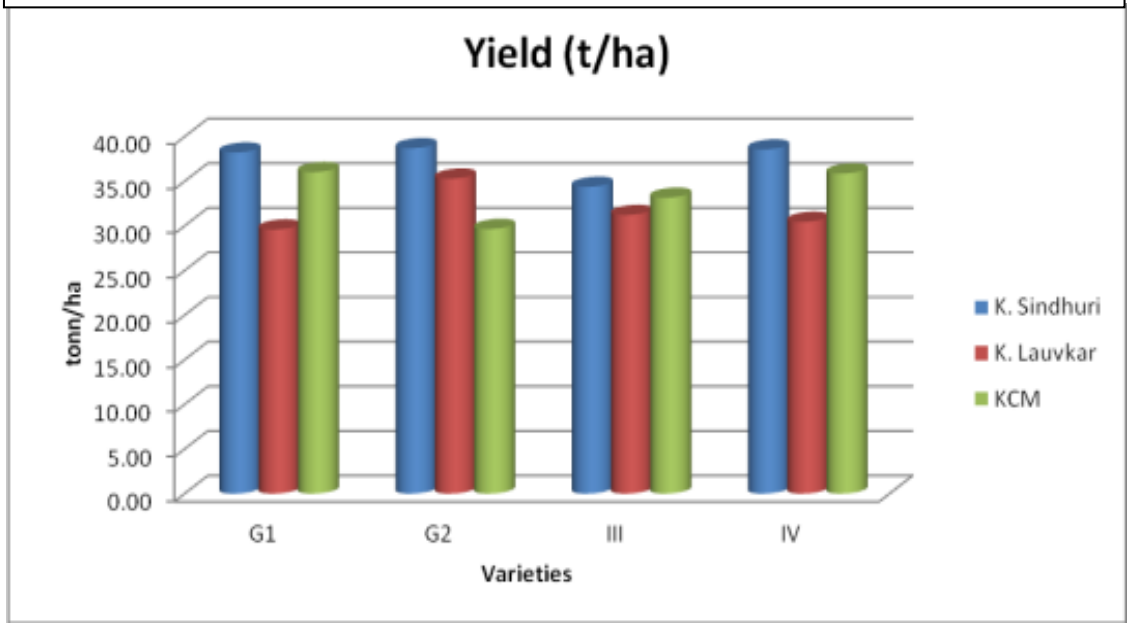



**Figure 2.** Comparison in virus status

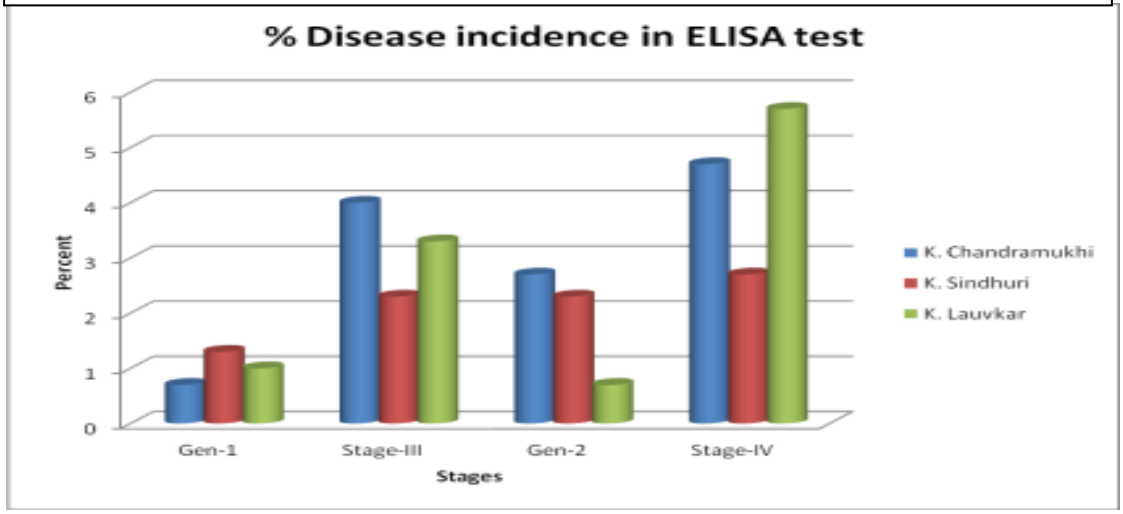
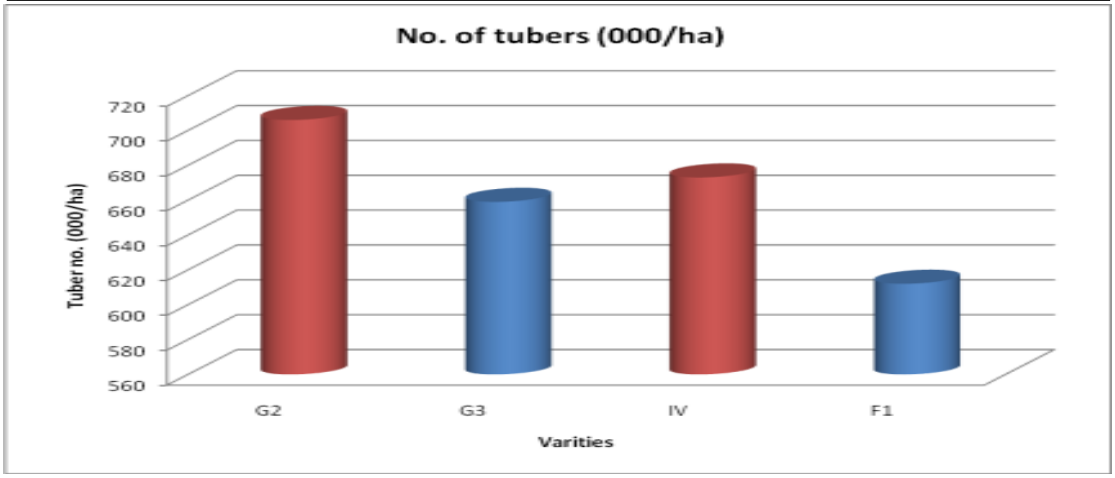



**Figure 3**. Comparison in yield by number



Secondly, the seed multiplication rate is low and time duration is more to get breeder seed from conventional system. To overcome these two major problems, tissue culture based seed potato production was started which is called high-tech system. *In-vitro* multiplication of virus-free microplants and microtuber followed by minituber production in net house and aeroponic techniques is most important components of high-tech seed production. The large scale multiplication with its vertical increase in half of the required time (2 years) and space, desired seed size and round the year production is very essential to get millions of nucleus planting material. Moreover, the uniform seed tuber of comparatively small size, free from all diseases, very good quality, healthy tubers and easy to transport with very low cost are other benefits of high-tech seed production. However, we need to caution on use of growth hormones and no. of multiplication cycle to avoid any change including epigenic changes in the progeny tubers.

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