IN VITRO PROPAGATION OF POTATO (*SOLANUM TUBEROSUM* L.) FOR VIRUS TESTED PATHOGEN-FREE PRE-BASIC SEED PRODUCTION IN NEPAL

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INTRODUCTION

Pre-basic seed potatoes are tuberlets of minitubers produced in an aphid-proof glasshouse or screen house from micro propagation of virus-tested plantlets of selected cultivars. These potatoes are different from other tissue cultured potatoes in that they are derived from the plants which have been positively eliminated from six most important potato viruses, rapidly multiplied by nodal cuttings in *in vitro* conditions and transplanted in sterile soil mix under glasshouse or screenhouse conditions. These disease free seed potatoes have been produced for the first time in Nepal since 1990 (Akius *et al.*, 1990) and utilized gradually by the seed potato growers of the country. The objectives of pre-basic seed production and utilization scheme are to: rapidly propagate virus-free seed potatoes through tissue culture, and replace old and infected seed stocks with clean and virus free stocks derived from pre-basic seed potatoes in the main seed pockets of Nepal.

When virus infected tubers are planted, more virus will be accumulated on the potato plant giving rise to so called degenerated tubers with drastically reduced yield potentials. Since no chemical remedy is available against virus, propagation of virus free seed potatoes is a must. So, it is very essential to eliminate the source seed potatoes from the virus before they are used for further multiplication.

Virus testing

The first step in viral elimination is virus testing. Recommended cultivars and old stocks of potatoes are tested for the presence of six viruses namely, PVA, PVM, PVS, PVX, PVY and PLRV by Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (Clark and Adams, 1977). Each virus is detected with the help of their corresponding antibodies and conjugated antibodies. Virus samples are taken either from leaf sap or sap from tuber sprouts. First, polystyrene plates are coated with antibody in which is added crude leaf or sprout extract. Then, the conjugated antibody or the antibody linked with an enzyme is added. If the sample contains a virus, it will hold on to both the antibody attached on the plate and the conjugated antibody on the top. Upon addition of a substrate to the attached enzyme, a colour reaction will occur confirming the detection or presence of the virus in the sample. If there is no colour reaction, it means that the sample is free from virus.

Virus can be eliminated by thermo therapy (Kassanis, 1954) or meristem culture (Morel and Martin, 1952). Infected plants are raised at high temperature in big incubators

(often at > 30°C for few days) until viruses are inactivated. An alternate method is meristem excision in which apical meristem of the shoot apex is excised together with 1 or 2 leaf primordia and cultured in liquid medium. Few weeks after incubation, the meristem will become green and start differentiating into shoot and leaves. The shoot is then transferred to agar medium for rooting. The rooted plantlet is grown to 6-8 nodes and is then used for virus testing.

After viruses have been eliminated, the in-vitro plantlets are rapidly propagated by nodal cuttings and cultured in a Modified Murashige/Skoog medium (Murashige and Skoog, 1962) in the incubation room at 20°C ± 2°C under a light intensity of 2000-4000 Lux and 16 hrs day length for four to six weeks. Then, each nodal cutting will grow into a full plantlet with roots and shoots with 6-10 nodes. They can be either used for further multiplication through nodal cutting or transplanted in an aphid-proof glass or screen house for the production of pre-basic seed potatoes.

PRODUCTION OF PRE-BASIC SEED POTATOES

The virus free *in vitro* plantlets are directly transplanted on sterile mixture of soil, sand and compost (1:1:1 by volume) in concrete benched inside an aphid proof glasshouse or screenhouse at a spacing of 10 x 10 cm. The plantlets are irrigated with water sterilized by ultra violet radiation. As these plantlets grow and produce lateral shoots, stem cuttings are harvested from them and induced to root in jiffy cups or decomposed saw dust. Rooted cuttings are handled in the same manner as the *in vitro* plantlets. Sterile soil mix is added as the plants grow taller. The plants are staked as necessary and dehaulmed a week prior to the harvest.

The pre-basic seeds, also harvest, are graded into 3 categories namely, > 1 g, 1-5 g and >5 g and stored in the cold store at 4°C. After 5-9 months of cold storage, the dormancy will be broken and the seeds should be pre-sprouted under diffused light before planting directly in the field. Seeds less than 1 g are recycled under controlled conditions of the glasshouse for the production of pre-basic seeds. Larger seeds are distributed to the Government Horticulture Farms and affiliated farmers for the production of basic seeds.

UTILIZATION OF PRE-BASIC SEEDS

The pre-basic seeds are so clean that they may be readily multiplied for 8-10 generations for seed production depending upon the field condition and precautionary measures taken during cultural operations. However, there are certain points that the growers should take note while handling the pre-basic seeds. Since, they are generally smaller than the conventional seeds (@ 35 g/tuber), they have to be planted more closely than 25 x 70 cm. A spacing of 50 x 15 cm was found adequate for all minituber sizes (Ojha, 1993). After plowing and preparing the ridge and furrow, the top soil should be pulverized well. Smaller

tubers less than 5 g should be planted in a ridge. The depth of planting should be about 3-4 cm. The soil should contain plenty of compost (> 25 t/ha). Chemical fertilizers and chicken manure should not be applied into the planting holes to avoid rotting of tubers. Water logging should be avoided at all costs.

Monitoring of aphid population is a must for the production of seed potato. As soon as the aphid population reaches the threshold, systematic insecticide such as Metasystox or Dimecron should be sprayed together with a fungicide Dithane-M 45 against the late blight (*Phytophthora infestans*). Roguing (though not expected) and haulmpulling should also be followed while producing basic seeds from the pre-basic seeds.

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