Disease-free Pre-Basic Seed Potato Production through Tissue Culture in Nepal

Binesh M. Sakha*, Gyan P. Rai, Shambhu P. Dhital and Ram B. Nepal

National Potato Research Programme-Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal

ABSTRACT

Pre-basic seed potatoes are disease free potato minitubers produced by transplanting pathogen free *in vitro* potato plantlets under protected condition in aphid-proof glasshouse and/or screen house. Double antibody sand witched - enzyme linked immuno-sorbant assay is used to test six major potato viruses, namely PLRV, PVS, PVX, PVY, PVA and PVM. Thermotherapy cum meristem tip excision techniques are used to eliminate these viruses. Virus free *in vitro* potato plantlets are rapidly propagated by single nodal cuttings on modified MS media. For pre-basic seed production disease free *in vitro* potato plantlets are transplanted in the sterile sand soil substrate under glasshouse and screen house, once in autumn season and next in spring season. Since 1990, National Potato Research Program has been producing about 200,000 pre-basic seeds annually. So far, PBS of 19 different recommended and released potato cultivars has been produced. Till date 3,465,799 PBS had been produced and 3,217,666 pre-basic seeds distributed to the different seed potato growers groups, District Agriculture Development Offices, government farms/research stations, and NGOs/INGOs for subsequent basic seed potato production. After establishment of tissue culture facilities in National Potato Research Program, the productivity of potato has been increased by 71% due to utilization of pre-basic seed potatoes.

Key words: DAS-ELISA, meristem excision, potato, pre-basic seed, thermotherapy

INTRODUCTION

Potato, being vegetatively propagated crop, is very prune to seed degeneration as several potato viruses accumulate to the seed tubers overtimes resulting into its reduced yield potential. So far, six major potato viruses, namely PLRV, PVS, PVX, PVY, PVA and PVM had been reported to infect potato crops in Nepal (Akius and Kloos 1990, Ranjit et al 1994). To overcome seed tuber degeneration, seed potatoes should be replaced by high quality seed potatoes at regular intervals (Sakha and Rai 2004). A continuous source of high quality seed potatoes is, therefore, necessary in the country. For this purpose, the National Potato Research Program (NPRP) has been established well equipped tissue culture facilities to produce a limited quantity of pre-basic seed (PBS) potatoes each year. These pre-basic seed potatoes are used as source seeds for the production of high quality seed potatoes for several generations. The adopted use of high quality seed potatoes on a large scale will, therefore, help significantly increase the productivity of the potato in Nepal.

PBS potatoes are virus tested, disease free minitubers produced under aphid proof glasshouse and/or screenhouse. When multiplied using improved and recommended potato production techniques, these seeds produce large quantities of high quality seed potatoes for several generations. Cultivation with well-managed agronomic practices, these quality seeds will give a higher potato yield. The objective of PBS production is to increase productivity by replacing old degenerated seed potatoes from the major seed potato production pocket areas of the country.

MATERIALS AND METHODS

Virus elimination technique

Double Antibody Sandwitched - Enzyme Linked Immunosorbant Assay, DAS-ELISA, (Clark and Adams 1977) technology was used to assess the presence of six major potato viruses in potato

cultivars. Then, thermotherapy cum meristem tip excision technique was used to eliminate potato viruses. For virus elimination, tubers were allowed to sprout at 37^{o}C for 2-3 weeks as a thermal treatment and shoot tips were excised from the sprouts and washed in detergent water. Under laminar airflow chamber, their surface were treated with 70% Ethanol for 30 seconds, washed with sterile distilled water and then sterilized with 2% sodium hypochlorite solution for five minutes and again washed at least three times with sterile distilled water. By using a stereoscopic microscope, the apical meristem (with one or two leaf premordia, about 0.2 – 0.3 mm in diameter) was excised from the shoot tip and placed on top of a filter paper bridge on a liquid MS medium (Murashige and Skoog 1962) supplemented with 0.5 mgl^{-1} IAA, 0.4 mgl^{-1} Kinetin and 0.1 mgl^{-1} GA_3 (Mellor and Stace-Smith 1977). The meristem was then cultured in an incubation room under 20 ± 2^{o}C with proper illumination (2000 lux) and 16 h photoperiod. After few weeks, it became green and gave rise to stem and leaves. It was then transferred to a solid MS medium for proper rooting and shooting. This plantlet was multiplied into several clones by nodal cuttings.

Virus testing

Clones regenerated from meristem culture were utilized for virus testing by DAS-ELISA. In NPRP, there were testing facilities for major six potato viruses namely, PLRV, PVS, PVX, PVY, PVA and PVM. Once the virus-free clones were obtained, they were propagated by nodal cuttings for further multiplication and maintained *in vitro* as clean germplasm. Virus testing of the standing crops in the glasshouse/screenhouse was further conducted randomly after tuberization stage.

Germplasm maintenance

Virus eliminated clones were maintained with subsequent subcultures as germplasm in the tissue culture laboratory of NPRP. Majority of the potato germplasm were imported from the International Potato Centre (CIP), Lima, Peru as disease-free *in vitro* plantlets, some introduced from India and the rest were of Nepalese origin.

Rapid propagation

Virus-free *in vitro* plantlets, maintained as mother plantlets, were rapidly propagated by single nodal cuttings on modified MS solid media supplemented with 2 mgl^{-1} calcium pantothenate. With subsequent subcultures, desired numbers of *in vitro* plantlets of required cultivars were obtained for transplantation in each season. The cultures were incubated in growth chambers with culture conditions of 16 h photoperiod, 2000 lux light intensity and 20 ± 2^{o}C temperature.

PBS production under controlled condition

Four to six weeks old *in vitro* plantlets were transplanted into sterile mixture of 2:1 sand and soil substrate under aphid-proof glasshouse and screenhouse twice a year for pre-basic seed production. Special cultivation techniques such as planting at 20- × 10-cm spacing, irrigation with UV sterilized water, fertilizer application, earthing up, plant protection and haulm pulling were used between transplantation and harvest. Chemical fertilizers were applied at the rate of 200:200:120 kg N:P:K per hectare. Irrigation was stopped at least two weeks before harvest and haulms pulled a week ahead harvesting. Depending up on cultivars, minitubers were harvested three to four months after transplantation.

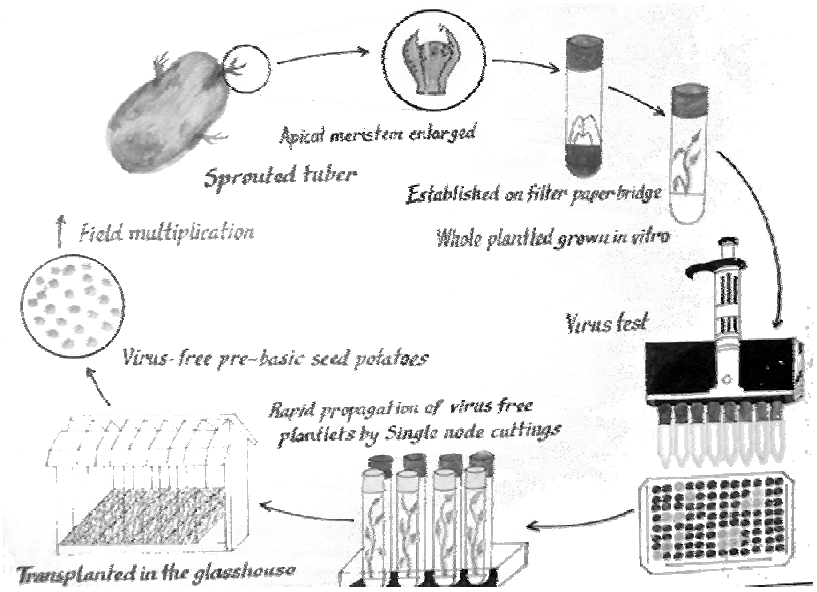

Figure 1. PBS production scheme adopted at NPRP, NARC.

PBS packaging and storage

After harvesting, PBS were graded into four different weight size categories, as >5 g, 1-5 g, 0.5-1 g and <0.5 g, and packed into screen bags with proper tagging. PBS produced during autumn season were harvested in November – December and stored in the cold store till August to October for about eight to ten months before being distributed for succeeding Tarai season field plantation. In case of spring season production, PBS were harvested in April - May and stored in the cold store till November - January for about six to eight months before being distributed for succeeding hill season field plantation.

RESULTS AND DISCUSSION

Virus elimination and germplasm maintenance

So far 12 potato cultivars had been cleaned from the major potato viruses in NPRP (Table 1). Before virus cleaning, cultivars were found to be infected with single to multiple viruses and percentage of virus elimination was higher in case of single infestation as compared to multiple infestations. Out of six major potato viruses tested through DAS-ELISA technique, PVS infestation was found to be the highest for eight clones followed by PVM infecting three clones. More than hundred potato germplasms were maintained under *in vitro* condition in the NPRP's tissue culture laboratory (NPRP 2006).

Table 1. Potato viruses eliminated cultivars in NPRP, Khumaltar

Cultivar	Eliminated viruses	Reference	Remark
Sarkari Seto	NA[†]	NA	Cleaned in 1989
Syang Dorje	NA	NA	Cleaned in 1989
Kufri Sindhuri	PVS and PLRV	Akius and Kloos 1990	
Cardinal	PVX, PVY and PVS	Shakya et al 1992	
Kathmandu Local	PVS and PVM	Ranjit et al 1994	
Tharu Local	PVS and PVM	Ranjit et al 1994	
NPI-106	PVS	Ranjit et al 1994	
Kufri Badshah	PVX	Sakha and Dhital 2006	
Jumli Local	PVX, PVS, PLRV & PVM	Sakha and Dhital 2006	
CIP 388572.1	PVS	NPRP 2006	
CIP 388572.4	PVS	NPRP 2006	
Gui Valley	PVY	Dhital et al 2006	

[†] *NA, Not available*

Rapid propagation

During rapid propagation, the explants developed into fully grown plantlets within four to six weeks depending up on cultivars. It was found that multiple explants grow rapidly as compared to single explant per culture tube. About ten to twenty thousands *in vitro* plantlets are being propagated and supplied to the glasshouse/screenhouse each season for PBS production purpose (Table 2).

Table 2. *In vitro* plantlets supplied to the glasshouse and screen house for PBS production

Year	Autumn season, n	Spring season, n	Total, n
1990/91	12,400	14,625	27,025
1991/92	10,976	13,280	24,256
1992/93	11,409	13,550	24,959
1993/94	12,546	17,599	30,145
1994/95	16,775	17,720	34,495
1995/96	16,058	8,720	24,778
1996/97	14,593	7,666	22,259
1997/98	14,094	22,264	36,358
1998/99	21,330	18,402	39,732
1999/00	21,578	18,823	40,401
2000/01	20,193	21,150	41,343
2001/02	22,600	15,170	37,770
2002/03	17,844	21,830	39,674
2003/04	22,570	15,606	38,176
2004/05	26,345	21,479	47,824
2005/06	24,894	17,655	42,549
Total	286,205	265,539	551,744

PBS production and distribution

Pre-basic seed potatoes were produced for the first time in Nepal during 1989/90 (Akius et al 1990). Since then PBS has been produced twice a year, once during autumn season and next during spring season. So far PBS of 19 cultivars has been produced in NPRP (Table 3). Out of them Cardinal, Desiree, Kufri Jyoti, Khumal Seto-1 and NPI-106 found suitable for both seasons. Depending up on the demand, PBS of five to ten cultivars are produced each season. There are altogether 20 benches of 12.88 m^2 area each in the glasshouse and eight benches of 14.28 m^2 area each in the screenhouse for PBS production in NPRP. So far about 200,000 PBS are being produced each year.

Table 3. Potato cultivars suitable for autumn and spring season plantation

SN	For Tarai season/autumn plantation	For hill season/spring plantation
1	Cardinal†	Cardinal†
2	Desiree†	Desiree†
3	Kufri Sindhuri†	Kufri Jyoti
4	Kufri Jyoti	NPI-106
5	NPI-106	Khumal Seto-1
6	Khumal Seto-1	I-1124
7	Janakdev†	CFM 69-1
8	Khumal Rato-2†	CFJ 69-1
9	BR 63-65†	Syang Dorje
10	Tharu Local	Sarkari Seto
11	Kufri Badshah	Kathmandu Local†
12	Perricholi†	Jumli Local†

† *Red skinned cultivars, rest are white skinned.*

Study of PBS production during last five years revealed that total number of PBS production during spring season is about 24% higher than that produced during autumn season (Table 4). However, production of larger sized (>1 g) PBS was much more (58.3%) during autumn season as compared to 37.6% only during spring season. Thus, production of higher number of potato tubers per unit area results in the decrease in average tuber size, and vice versa. It was found that *in vitro* plantlets produced about five minitubers per plantlet with the mean tuber size of 2.8 g (Sakha and Rai 2004).

Table 4. Size distribution of PBS produced during 2001/02 to 2005/06 in NPRP, Khumaltar

Year	Autumn PBS					Spring PBS				
	Production, n	Size distribution, %				Production, n	Size distribution, %			
		>5 g	1-5 g	0.5-1 g	<0.5 g		>5 g	1-5 g	0.5-1 g	<0.5 g
2001/02	110,331	17.9	48.6	23.0	10.5	131,003	8.5	37.4	33.0	16.4
2002/03	67,945	22.3	42.2	25.2	10.4	192,113	3.2	33.3	46.6	16.9
2003/04	104,289	7.1	40.3	44.0	8.5	103,186	7.7	42.4	48.2	11.7
2004/05	100,170	6.8	47. 7	37.6	7.8	90,760	7.8	32.1	40.3	19.7
2005/06	84,718	12.1	49.1	24.3	14.5	62,445	2.0	23.9	48.6	25.6
Mean	93,491	13.2	45.1	30.8	10.3	115,901	5.8	31.8	43.3	18.1

Initially PBS was distributed mainly to seed potato growers and seed potato grower groups (SPGs) through Field Coordinators in each Developmental Regions. After the establishment of Memorandum of Understanding (MoU) between Nepal Agriculture Research Council (NARC) and Department of Agriculture (DoA) in 1997, PBS are being distributed to the seed potato producers through District Agriculture Development Offices in collaboration with Potato Development Program, Department of Agriculture (Sakha and Dhakal 2006). Accordingly, 80% PBS has been distributed to the seed potato producers groups and the rest 20% to the Government farms, research stations, NGOs and INGOs within the country. Till date 3,365,799 PBS had been produced and 3,217,666 PBS had been distributed so far (Table 5).

Table 5. Production and distribution of pre-basic seed potatoes after the inception of tissue culture facilities in NPRP, NARC

Year	Production target, n	Production, n	Distribution†, n
1990/91	100,000	135,860	149,550
1991/92	100,000	108,722	123,696
1992/93	100,000	84,991	103,207
1993/94	200,000	222,987	86,000
1994/95	200,000	440,533	197,095
1995/96	200,000	297,268	400,802
1996/97	200,000	283,240	237,342
1997/98	200,000	162,843	203,525
1998/99	200,000	142,780	254,985
1999/00	200,000	208,496	140,057
2000/01	200,000	231,119	195,393
2001/02	200,000	241,334	226,624
2002/03	200,000	260,058	238,201
2003/04	200,000	207,475	239,167
2004/05	200,000	190,930	233,568
2005/06	150,000	147,163	188,454
Total		3,365,799	3,217,666

† *PBS, produced in the preceding year distributed during succeeding year.*

Utilization of pre-basic seed potatoes

PBS has been utilized for the production of high quality basic seed potatoes. Depending up on the environmental condition of the plantation areas and cultivation practices, PBS could be used to produce quality basic seeds for several generations: at least 3-4 years in plain areas, 5-6 years in mid-hills and 8-10 years in high hills. Depending up on the size of PBS, field plantation should be done at 60- × 10-cm, 60- × 15-cm and 60- × 20-cm spacing for PBS of <1 g, 1-5 g and >5 g sizes, respectively (Sakha and Dhakal 2006). Size of PBS, environment and cultivar were the three main factors affecting both multiplication rate and productivity. Average multiplication rates of basic seed-1 tubers produced per unit PBS were found to be 4.9 for the hills and 5.2 for the Tarai with average production of 116 g and 171 g of basic seed-1 per tuberlet in the hills and Tarai, respectively (Schulz et al 1998).

After the establishment of tissue culture facilities in NPRP in 1990, the continuous use of PBS potatoes as source seeds resulted in the significant increase in productivity of potato (Figure 2).

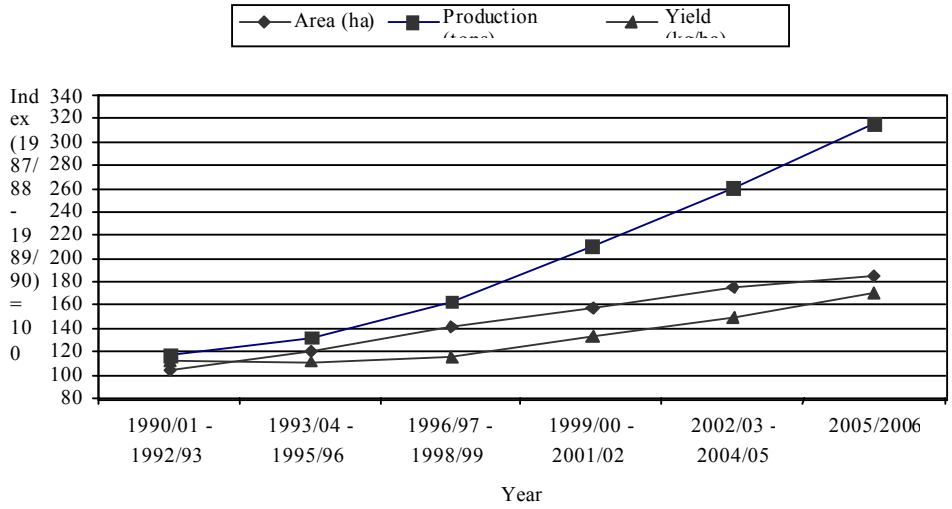

Figure 2. Index of potato area, production and yield in Nepal.
Base Year (3 Years Avg.) 1987/88 - 1989/90 = 100

Source: ABPSD, 2006.

CONCLUSION

PLRV, PVS, PVX, PVY, PVA and PVM are known to be the major potato viruses of economic importance in Nepal. National Potato Research Program under Nepal Agricultural Research Council has facilities to test these viruses. Since 1990, NPRP has been producing about 200,000 PBS annually. So far, PBS of 19 different recommended and released potato cultivars has been produced. Till date 3,465,799 PBS had been produced and 3,217,666 PBS distributed to the different seed potato growers groups, District Agriculture Development Offices, Government Farms/Research stations, NGOs/INGOs and others for subsequent basic seed potato production within the country.

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