

## Efficient way of potato tuber indexing by sprout ELISA

Y. P. SINGH, A. K. SOMANI, R. K. SAMADHIYA, A.N. MAURY<sup>1</sup> AND R. R. SINGH<sup>2</sup>  
Central Potato Research Station, Maharajpura, Bhind Road, Gwalior-474006, Madhya Pradesh  
E-mail : [ypsingh11@gmail.com](mailto:ypsingh11@gmail.com)

### Abstract

For the production of nucleus seed of potato, tuber indexing was done in glass house or polyhouse for 5 degenerative viruses - X, S, Y, M and A, by leaf ELISA (Enzyme-Linked Immunosorbent Assay) of plantlets raised from dormancy breaking triple treatment given scooped eyes one from each tuber in February-March during 2001-02 to 2009-10 seasons. After cold storage, another set from the same lot was indexed by substituting the sprouts for leaves (i.e. sprout ELISA). Comparison of the two methods of potato tuber indexing revealed that sprout ELISA is more sensitive and efficient in detecting the infection of the above 5 degenerative viruses. Sprout ELISA could detect a total of 6.3% virus infection resulting in 4.5% rejection of clones as compared to total 4.3% incidence of viruses resulting in 3.1% rejection of infected clones in Leaf ELISA during the 9 years of study.

Key words: Potato, tuber indexing, leaf ELISA, sprout ELISA, virus detection

### Introduction

Enzyme – Linked Immunosorbent Assay (ELISA) is one of the most suitable and modern methods for detecting virus and other pathogens. In ELISA method, virus or pathogen in the samples is trapped and immobilized by a specific antibody absorbed on a solid support - the polystyrene microtitre plates on which the testing is done. Trapped virus or pathogen is sandwiched with a specific antibody linked enzyme (i.e. enzyme- antibody conjugate). On adding substrate the positive sample gives yellow colour (Clark and Adams, 1977). This method of ELISA is called Double Antibody Sandwich ELISA (DAS-ELISA). Yellow colouration can be seen with naked eyes. However, the intensity of the colour (accordingly the concentration of the virus or pathogen) can be read with colorimeter (here, ELISA reader).

In potato (*Solanum tuberosum* L.), tuber indexing is done in glasshouse or polyhouse by compact pot method (Somani, 1982) by raising the plantlets from scooped eyes from the freshly harvested tubers after giving dormancy breaking triple treatment and testing their leaves for different viruses. Earlier the detection was done by chloroplast agglutination test and since 1984 by ELISA method (Khurana, 1999). It is the most

important step of nucleus seed production of potato. It requires a lot of glasshouse or polyhouse space, time, labour and off-course wastage of one eye from each tuber indexed. Alternatively, the tubers are sprouted just before planting and a sprout is substituted for leaf and tested by ELISA method for the viruses. The efficacy of sprout ELISA method was compared with Leaf ELISA method of tuber indexing and the results are presented in this paper.

### Materials and Methods

Scooped eyes (one from each tuber) from 400 to 700 four tuber clones of freshly harvested potato tubers of 4 to 7 varieties namely Kufri Chandramukhi, Kufri Jyoti, Kufri Sindhuri, Kufri Lauvkar, Kufri Chipsona-1, Kufri Chipsona-2 and Kufri Arun were given dormancy breaking triple treatment and indexed by compact pot method (Somani, *et al* 1982) during February – March from 2001-02 to 2009-10 in poly house at Central Potato Research Station, Gwalior. One compound leaf from each of the 4 plantlets of a clone were macerated and the extract was tested by leaf ELISA method for 5 potato viruses viz. PVX, S, Y, M and A. After cold storage, the remaining clones of the same lot (819 to 2772) of the above varieties of potato were sprouted and one sprout from each of the 4 tubers of a clone were macerated and the extract was ELISA tested (i.e. sprout ELISA) during

<sup>1</sup>Deptt. of Botany, D.S.(P.G.) College, Aligarh-202001 (UP)

<sup>2</sup>R. B. S. Collage, Bichpuri, Agra

September - October each year from 2001-02 to 2009-10 for the same 5 potato viruses - PVX, S, Y, M and A. In both the methods, clones infected with even one virus were rejected and the healthy clones were planted in the field the same season during the month of October. Indexing results i.e. the incidence of the 5 viruses tested and infected clones rejected by the two methods were compared.

### Results and Discussion

Many a times a clone was found to be infected with more than one virus. Comparison of the indexing results obtained by leaf ELISA (Table 1) and sprout ELISA (Table 2) reveals that all the 5 viruses were detected in both the methods. Year-wise concentration trend of individual virus is also similar in both the indexing methods though it was higher in sprout ELISA. Based on total 4.3% incidence of 5 viruses in leaf

ELISA, 3.1% diseased clones were rejected while in case of sprout ELISA total incidence of viruses was 6.3% and diseased clone rejection 4.5%. It might be due to the fact that in leaf ELISA indexing in poly house, the temperature increases at the time of ELISA which adversely affects the virus multiplication and thus their detection. Also, the concentration of different potato viruses might be more in sprouts as compared to leaves as Syller (1988) have reported higher concentration of potato leaf roll virus from sprouts than leaves and other plant tissues. Therefore, potato tuber indexing by sprout ELISA was found to be more efficient. Also, it is convenient, quick, cheaper and does not require costly infrastructure of glasshouse or poly house. Of course one eye from each tuber is also not lost in sprout ELISA thus yield potential is not reduced. In fact, only because of sprout ELISA, it become

Table 1: Leaf ELISA results of potato tuber indexing during 2001-02 to 2009-10.

Years	No. of clones	Average incidence (%) of potato virus					Total incidence of viruses	*Rejection percentage	Health standard
		X	S	Y	M	A			
2001-02	472	1.1	2.8	4.7	2.5	0.0	11.1	7.1	92.9
2002-03	460	2.0	1.1	2.4	0.2	0.2	5.9	5.7	94.3
2003-04	400	0.5	0.5	0.3	0.5	0.0	1.8	1.5	98.5
2004-05	400	0.3	0.5	0.8	0.0	1.5	3.1	2.5	97.5
2005-06	500	1.6	1.6	1.6	1.6	0.4	6.8	5.4	94.6
2006-07	480	0.8	0.6	0.0	0.5	0.0	1.9	1.9	98.1
2007-08	600	0.5	0.3	0.2	0.2	0.5	1.7	1.3	98.7
2008-09	700	0.3	0.1	0.3	0.6	0.7	2.0	1.1	99.0
2009-10	700	0.6	0.9	1.1	1.3	0.1	4.0	1.4	98.6
Average	524	0.9	0.9	1.3	0.8	0.4	4.3	3.1	96.9

\*Many of the clones rejected had more than one virus infection

Table 2: Sprout ELISA results of potato tuber indexing during 2001-02 to 2009-10.

Years	No. of clones	Average incidence (%) of potato virus					Total incidence of viruses	*Rejection percentage	Health standard
		X	S	Y	M	A			
2001-02	910	2.2	1.8	1.5	0.4	1.1	7.0	6.3	93.7
2002-03	1053	0.9	1.1	0.7	0.4	0.6	3.7	3.3	96.7
2003-04	1729	2.2	2.5	1.7	0.0	2.0	8.4	6.9	93.1
2004-05	1437	2.8	2.3	2.6	1.3	1.9	10.9	6.2	93.8
2005-06	1508	1.7	1.9	1.9	2.1	1.7	9.3	6.2	93.8
2006-07	1390	1.6	1.3	1.7	1.0	1.8	7.4	4.2	95.8
2007-08	2248	2.2	0.4	0.8	0.8	0.7	4.9	3.7	96.3
2008-09	2772	0.6	0.4	1.2	0.4	0.5	3.1	2.5	97.5
2009-10	819	0.0	0.1	0.1	0.8	0.2	1.2	1.1	98.9
Average	1541	1.6	1.3	1.4	0.8	1.2	6.3	4.5	95.5

\*Many of the clones rejected had more than one virus infection

possible to index all the clones before planting in field under stage-I and continuously following this method resulted in reducing the virus incidence in subsequent years. There is almost clear trend of reduction in virus incidence and diseased clone rejection percentage since the start of sprout ELISA ultimately resulting in improving the health standard of the nucleus seed. Therefore, in sub tropical region where the late February - March temperature is higher, sprout ELISA is a better option of tuber indexing for the production of nucleus seed of potato. However, it is efficient, convenient and cheaper method which does not reduce the yield potential not only in sub-tropical region but all the regions.

### Conclusion

Sprout ELISA method of potato tuber indexing for detecting degenerative potato viruses (PVX, S, Y, M and A) for the production of nucleus seed was more efficient as it is more sensitive, quick and cheaper as it does not require costly infra structure of glasshouse or polyhouse in comparison to leaf ELISA.

Also in sprout ELISA, one eye from each tuber is not lost thus does not reduce the yield potential. Sprout ELISA could detect a total 6.3% virus infection as compared to 4.3% in leaf ELISA. Therefore, sprout ELISA method of tuber indexing can be followed successfully replacing the leaf ELISA method.

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