To Committee members of James Rennie Bequest

Thanks to finacial support from the James Rennie Bequest, I was able to undertake the visit, as a part of my PhD study, to the Boyce Thompson Institute for Plant Resarch, Cornell University, USA from 23 April to 29 September 1998. This visit was successfully completed. I have finished the experimental work there as originally planned. The following is the report of my work I did in the Boyce Thompson Institute.

Expression of HIV gp 160 proteins in transgenic plants

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HIV is causative agent of AIDS. Development of a vaccine against HIV has been proved to be more difficult than first anticipated (Paul 1995). There is evidence that immunity to infection is possible. This protection may be mediated either by a individual component of the immune system or a combination of humoral, mucosal and cellular immunity (Haynes et al 1996). One approach to the development of HIV vaccine is the utilization of expression vectors for *in vivo* expression of HIV genes. Recently, viral proteins has been successfully expressed in tansgenic plants (reviewed in Mason et al. 1998). The transgenic plants have been shown capable of expessing antigenic proteins whose gene has been incorporated into their host genome. In most case, the proteins expressed in transgenic plants were fully functional as antigens and can elicit specific mucosal and systemic immune responses (Mason et al 1996). Thus the utilization of plants as expression vector for the production of immunogens might be an economic alternative to produce the subunit vaccine at lower cost, delivered orally, and distributed without refrigeration. This study was carried out to produce transgenic plants expressing HIV-1 gp160 and their use as an immunogen in animal model.

The construction of the transformation vector was based on the binary plasmid pGPTV-KAN. The HIV gp160 gene was amplified by PCR from plasmid HXB2-MCS containing full-length HIV-1 gp160 sequence. The pair of primers used introduced NcoI and SacI sites at 5' and 3' ends, respectively, of amplified products. The complete gp160 gene was then cloned into NcoI/SacI digested both pIBT240.1 containing tuber specific promoter patatin and pIBT210.1 containing leaf specific promoter 35S. The binary expression vector was constructed by inserting the EcoRI/Hind III fragment of both pIBT240.1-env and pIBT210.1-env containing HIV gp160 gene into EcoRI/Hind III digested pGPTV-KAN (designated as pGPTV-KAN-240.1-env and pGPTV-KAN-210.1-env).

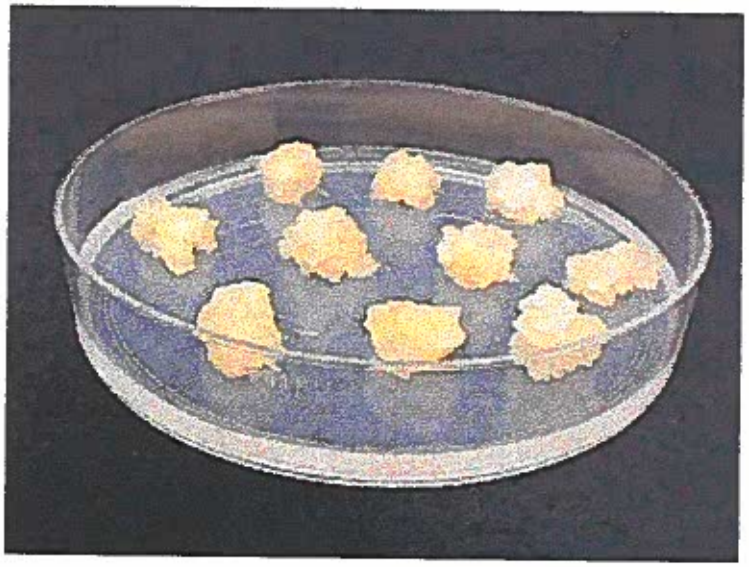
Plant transformation was performed as described by Mason et al (1996) with slight modifications. The plasmid pGPTV-KAN-210.1-env was used for

agrobacterium-mediated transformation of tobacco and NT-1 cells. 89 of 170 tranformed tobacco shoots had regenerated and rooted on the selective medium. 30 NT-1 tansformants were obtained. The plasmid pGPTV-KAN-240.1-env was used for *agrobacterium*-mediated transformation of potato. The transformed potato shoots are currently growing in selective rooting medium. Rooting percent is 69 -75%.

The presence of the gp160 gene in the transformed tobacco plantlets was detected by PCR. The result of PCR showed the presence of the expected size (2.7 kb) in 7 out of 11 extracts from transformed tobacco plantlet leaves growing on selective rooting medium.This product was absent in non-transformed plants.The results suggest that HIV gp160 gene has successfully been incorporated into their host genomes. The transcription of HIV gp160 in the tansgenic plant is being tested by using RT-PCR and the presence of HIV gp160 protein expressed in transgenic plants is currently being tested by ELISA.

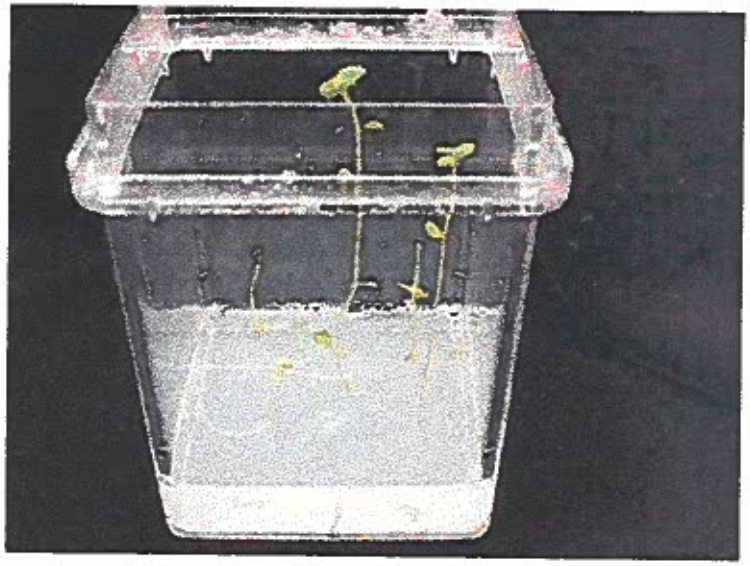
Haynes BF, Pantaleo G and Fauci AS. 1996. Towards an understanding of the correlates of protective immnuity to HIV infection. Science, 217:324-328.
Mason, HS, Ball JM, Shi JJ, Jiang X, Estes and Arntzen. 1996. Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. Proc. Natl. Acad.Sci USA., 93:5335-5340.

Picture1. Transgenic potato microtubers



Picture2. NT-1 transformants

Picture3. Transgenic tobacco *Samsun*



Picture4. Transgenic potato *FL1607*