

## INDUCTION OF RESISTANCE TO BARLEY YELLOW DWARF VIRUS (PAV) IN BREAD WHEAT USING POST-TRANSCRIPTIONAL GENE SILENCING (PTGS) \*

M.YASSAIE<sup>1\*\*</sup>, A.R. AFSHARIFAR<sup>1</sup>, A. NIAZI<sup>2</sup>, S. SALEHZADEH<sup>1</sup> and K. IZADPANAHI<sup>1</sup>

(Received: 3.7.2010 ; Accepted: 6.12.2011)

### Abstract

Barley and cereal yellow dwarf are the most important cereal diseases causing serious damages worldwide. Limited number of resistance and tolerance genes, their inefficiency and their complex inheritance justifies the adoption of the pathogen derived resistance approaches. In the present study, three types of constructs including sense, antisense and intron hairpin-loop (ihp-loop) were prepared from a sequence of BYDV-PAV polymerase gene and cloned into pWBVec8 binary vector. Agrobacterium-mediated transformation of mature embryos of bread wheat Alvand was carried out using the three types of constructs in the optimized culture media supplemented with hygromycin as a selective marker. The transformed explants were evaluated by ELISA, polymerase chain reaction, and dot blot assay using a nonradioactive probe. Artificial inoculation of plants was carried out employing BYDV-PAV viruliferous aphids. The average rates of transformation were evaluated as 1.8, 1.3 and zero percent for ihp-loop, sense and antisense constructs, respectively, based on ELISA values. Transformed plants remained healthy while control plants developed yellow dwarf symptoms after artificial inoculation of BYDV-PAV.

**Keywords:** *Barley yellow dwarf virus-PAV*, Post transcriptional gene silencing, DNA construct, Resistance, Gene silencing constructs.

See Persian text for figures and tables (Pages ۱۷-۱۲).

---

\*: Part of PhD. Thesis of the First Author, Submitted to College of Agriculture, Shiraz University, Shiraz, Iran.

\*\* : Corresponding Author, Email: [yassaie@shirazu.ac.ir](mailto:yassaie@shirazu.ac.ir)

1. PhD. Student (Currently, Scientific Member of Fars Research Center for Agriculture and Natural Resources), Assoc. Prof., MSc. and Prof. of Plant Pathology. and Plant Virology Research Center (PVRC), Respectively, Shiraz University, Shiraz, Iran.

2. Assis. Prof. of Biotechnology, Institute of Biotechnol., Shiraz University, Shiraz, Iran.

## References

- ABBOT, D., WANG, M.-B. and WATERHOUSE, P. 2002. A single copy of virus-derived transgene-encoding hairpin RNA confers BYDV immunity. Pp. 22-26. *In*: M. Henry and A. McNab (Eds.), **Barley Yellow Dwarf Disease: Recent Advances and Future Strategies**. CIMMYT, Mexico.
- AFSHARIFAR, A., MASUMI, M., SADEGHI, M.S., YASSAIE, M., ESMAEELZADEH-HOSEINI, S.A. and IZADPANA, K. 2004. The status of Barley yellow dwarf and Cereal yellow dwarf viruses in Iran. **Proc. Iran. Plant Protec. Cong., Tabriz, Iran.** 76(Abstr.).
- BAULCOMBE, D.C. 1996. RNA as a target and an initiator of post-transcriptional gene silencing in transgenic plants. **Plant Mol. Biol.** 32: 79-88.
- BAULCOMBE, D. C. 2004. RNA silencing in plants. **Nature** 431: 356-363.
- BHALLA, P.L., OTTENHOF, H.H. and SINGH, M.B. 2006. Wheat transformation- an update of recent progress. **Euphytica** 149: 353-366.
- BUCHER, E., Lohuis, D., van POPPEL, M.J.A., GREEKS-DIMITRIADOU, C., GOLDBACH, R. and PRINS, M. 2006. Multiple virus resistance at a high frequency using a single transgene construct. **J. Gen.Virol.** 87: 3697-3701.
- BURNETT, P., COMEAU, A. and QUALSET, C.O. 1995. Host plant tolerance or resistance for control of barley yellow dwarf. Pp. 321-343 *In*: C.J. D'Arcy and P. Burnett (Eds.), **Barley Yellow Dwarf: 40 Years of Progress**. The American Phytopathological Society, St. Paul MN.
- CHUANG, C.F. and MEYEROWITZ, E.M. 2000. Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*. **Proc. Natl. Acad. Sci.** 97: 4985-4990.
- CONVERSE, R.H. and MARTIN, R.R. 1990. ELISA methods for plant viruses. Pp. 179-196. *In*: R. Hampton, E. Ball and S. De Boer (Eds.), **Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens. A Laboratory Manual**. APS Press, USA.
- DOUGHERTY, W.G., LINDBO, J.A., SMITH, H.A., PARKS, T.D., SWANEY, S. and PROEBSTING, W.M. 1994. RNA-mediated virus resistance in transgenic plants: Exploitation of a cellular pathway possibly involved in RNA degradation. **Mol. Plant-Microbe Interact.** 7: 544-552.
- DUPRE, P., HENRY, M., POSADAS, G., PELLEGRINESCHI, A., TROTTET, M., and JACQUOT, E. 2002. Genetically engineered wheat for *barley yellow dwarf virus* resistance. Pp.27-28. *In*: M. Henry and A. McNab (Eds.), **Barley Yellow Dwarf Disease: Recent Advances and Future Strategies**. CIMMYT, Mexico.
- FITCHEN, J.H. and BEACHY, R.N. 1993. Genetically engineered protection against viruses in transgenic plants. **Annu. Rev. Microbiol.** 47: 739-763.
- GAO, L., MA, Q., LIU, Y., XIN, Z. and ZHANG, Z. 2009. Molecular characterization of the genomic region harboring the BYDV-resistance gene *Bdv2* in wheat. **J. Appl. Genet.** 50: 89-98.
- HELLIWELL, C. and WATERHOUSE, P. 2003. Constructs and methods for high-throughput gene silencing in plants. **Methods** 30: 289-95.
- HELLIWELL, C. and WATERHOUSE, P. 2005. Constructs and methods for hairpin RNA-mediated gene silencing in plants. **Methods Enzymol.** 392: 24-35
- HEWINGS, A.D. and EASTMAN, C.E. 1995. Epidemiology of barley yellow dwarf in North America. Pp. 75-106. *In*: C.J. D'Arcy and P. Burnett (Eds.), **Barley Yellow Dwarf: 40 Years of Progress**. The American Phytopathological Society. St. Paul. MN.
- HIEI, Y., KOMARI, T. and KUMASHIRO, T. 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. **Plant J.** 6: 271-282.
- HUNTLEY, C.C. and HALL, T.C. 1996. Interference with *brome mosaic virus* replication in transgenic rice. **Mol. Plant-Microbe Interact.** 9: 164-170.

- JINEK, M. and DOUDNA, J.A. 2009. A three-dimensional view of the molecular machinery of RNA interference. **Nature** 457: 405-412.
- JONES, H.D., DOHERTY, A. and WU, H. 2005. Review of methodologies and a protocol for the *Agrobacterium*-mediated transformation of wheat. **Plant Methods** 1: 5. Doi: 10.1186/1746-4811-1-5
- KOEV, G., MOHAN, B.R., DINESH-KUMAR, S.P., TORBERT, K.A., SOMERS, D.A. and MILLER, W.A. 1998. Extreme reduction of disease in oat transformed with the 5' half of the *barley yellow dwarf virus*-PAV genome. **Phytopathology**. 88: 1013-1019.
- KOHLI, A., TWYMAN, R.M., ABRANCHES, R., WEGEL, E., STOGER, E. and CHRISTO, P. 2003. Transgene integration organization and interaction in plants. **Plant Mole. Biol.** 52: 247-258.
- LISTER, R.M. and RANIERI, R. 1995. Distribution and economic importance of barley yellow dwarf. pp. 29-53 *In*: C.J. D'Arcy and P. Burnett (Eds.), **Barley Yellow Dwarf: 40 Years of Progress**. The American Phytopathological Society. St. Paul. MN.
- McGrath, P.F., VINCENT, J.R., LEI, C.H., PAWLOWSKI, W.P., TORBERT, K.A. and GU, W. 1997. Coat protein-mediated resistance to isolates of barley yellow dwarf in oats and barley. **Eur. J. Plant Pathol.** 103: 695-710.
- MEISTER, G. and TUSCHL, T. 2004. Mechanisms of gene silencing by double-stranded RNA. **Nature** 431: 343-349.
- METTE, M.F., AUFSATZ, W., VAN DER WINDEN, J., MATZKE, M.A. and MATZKE, A.J. 2000. Transcriptional silencing and promoter methylation triggered by double-stranded RNA. **EMBO J.** 19: 5194-5201.
- MILLER, W.A. and YOUNG, M.J. 1995. Prospects for genetically engineered resistance to *barley yellow dwarf virus*. Pp: 345-369. *In*: M. Henry and A. McNab (Eds.), **Barley Yellow Dwarf Disease: Recent Advances and Future Strategies** CIMMYT, Mexico.
- MOZHAEVA, K.A. and KASTALYEVA, T.B. 2002. Barley yellow dwarf in Russia. Pp.120-122. *In*: M. Henry and A. McNab, (Eds.), **Barley Yellow Dwarf Diseases: Recent Advances and Future Strategies**. CIMMYT. Mexico.
- MURASIGHE, T. and SKOOG, F. 1962. A revised medium for rapid growth and bioassay of tobacco tissue culture. **Plant physiol.** 15: 473-497.
- OERKE, E.C., DEHNE, H.W., SCHONBECK, F. and WEBER, A. 1994. **Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops**. 808p. Amsterdam, Elsevier Science.
- ORTIZ, J.P.A., REGGIARDO, M.I., RAVIZZINI, R.A., ALTABE, S.G., CERVIGNI, G.D.L., SPITTELER, M.A., MORATA, M.M., ELIAS, F.E. and VALLEJOS, R.H. 1996. Hygromycin resistance as an efficient selectable marker for wheat stable transformation. **Plant Cell Rep.** 15: 877-881.
- PAKDEL, A., AFSHARIFAR, A., NIAZI, A., ALMASI, R. and IZADPANAH, K. 2010. Distribution of cereal luteoviruses and molecular diversity of BYDV-PAV isolates in central and southern Iran: Proposal of a new species in the genus Luteovirus. **J. Phytopathol.** 158: 357-364
- PALUKAITIS, P. and ZAITLIN, M. 1997. Replicase-mediated resistance to plant virus disease. **Adv. Virus Res.** 48: 349-377.
- PATNAIK, D. and KHURANA, P. 2001. Wheat biotechnology: A minireview. **Electr. J. Biotechnol. [online]** 4: 74-102.
- PATNAIK, D., VISHNUDASAN, D. and KHURANA, P. 2006. *Agrobacterium*-mediated transformation of mature embryos of *Triticum aestivum* and *Triticum durum*. **Curr. Sci.** 91: 307-317.
- PAWLOSKI, L.C., DEAL, R.B., MCKINEY, E., BURGOS-RIVERA, B. and MEAGHER, R.B. 2005. Inverted repeat PCR for rapid assembly of constructs to induced RNA interference. **Plant Cell Physiol.** 46: 1872-1878.

- PLUMB, R.T. and JOHNSTONE, G.R. 1995. Cultural chemical and biological methods for the control of barley yellow dwarf. Pp. 307-319. *In*: C.J. D'Arcy and P. Burnett (Eds.), **Barley Yellow Dwarf: 40 Years of Progress**, APS Press.
- ROCHOW, W.F. 1969. Biological properties of four isolates of *barley yellow dwarf virus*. **Phytopathology**. 59: 1580-1589.
- SAGHI-MAROOF, M.A., SOLIMAN, K.M., JORGENSEN, R.A. and ALLARD, R.W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance chromosomal location and population dynamics. **Proc. Natl. Acad. Sci.** 81: 8014-8018.
- SAHRAGARD, N. and MASUMI, M. 2004. Yield loss assessment of *barley yellow dwarf virus* on wheat in Shahrekord town. **Proc. 16<sup>th</sup> Iran. Plant Protec. Cong., Tabriz, Iran.** 59 (Abstr).
- SINGH, R.P., BURNETT, P.A., ALBARRAN, M. and RAJARAM, S. 1993. *Bdv1*: A gene for tolerance to *barley yellow dwarf virus* in bread wheat. **Crop Sci.** 33: 231-234.
- SIOMI, H. and SIOMI, M. 2009. On the road to reading the RNA-interference code. **Nature** 457: 396-404.
- SMITH, N.A., SINGH, S.P., WANG, M.-B., STOUTJESDIJK, P., GREEN, A. and WATERHOUSE, P.M. 2000. Total silencing by intron-spliced hairpin RNAs. **Nature** 407: 319-320.
- TOMARI, Y. and ZAMORE, P.D. 2005. Perspective: machines for RNAi. **Genes Dev.** 19: 517-529.
- TRAVELLA, S., KLIMM, T.E. and KELLER, B. 2006. RNA interference-based gene silencing as an efficient tool for functional genomics in hexaploid bread wheat. **Plant Physiol.** 142: 6-20.
- WANG, M.-B. and WATERHOUSE, P.M. 2001. Application of gene silencing in plants. **Curr. Opin. Plant Biol.** 5: 146-150.
- WANG, M.-B., ABBOTT, D.C. and WATERHOUSE, P.M. 2000. A single copy of a virus-derived transgene encoding hairpin RNA gives immunity to *barley yellow dwarf virus*. **Mol. Plant Pathol.** 1: 347-356.
- WANG, M.-B., Li, Z., MATTHEWS, P.R., UPADHYAYA, N.M. and WATERHOUSE, P.M. 1998. Improved vectors for *Agrobacterium tumefaciens* mediated transformation of monocot plants. **Acta Hort.** 461: 401-407.
- WATERHOUSE, P.M., WANG, M.-B. and LOUGH, T. 2001. Gene silencing as an adaptive defense against viruses. **Nature** 411: 834-842.
- WEIGEL, D. and GLAZEBROOK, J. 2006. Transformation of *Agrobacterium* using the freeze-thaw method. **Cold Spring Harb. Protoc.** Doi: 10-1101/pdb.prot4666.
- WESLEY, S.V., HELLIWELL, C.A., SMITH, N.A., WANG, M.-B., ROUSE, D.T., LIU, Q., GOODING, P.S., SINGH, S.P., ABBOTT, D., STOUTJESDIJK, P.A., ROBINSON, S.P., GLEAVE, A.P., GREEN, A.G. and WATERHOUSE, P.M. 2001. Construct design for efficient, effective and high-throughput gene silencing in plants. **Plant J.** 27: 581-590.
- WU, H., SPARKS, C., AMOAH, B. and JONES, H.D. 2003. Factors influencing successful *Agrobacterium*-mediated genetic transformation of wheat. **Plant Cell Rep.** 21: 659-668.
- YAN, F., ZHANG, W.W., XIAO, H., Li, S.F. and CHENG, Z.M. 2007. Transgenic wheat expressing virus-derived hairpin RNA is resistant to *barley yellow dwarf virus*. **Yi Chuan** 29: 97-102.
- YAN, P., SHEN, W., XINZHENG, G., DUAN, J. and ZHOU, P. 2009. Rapid one-step construction of hairpin RNA. **Biochem. Biophys. Res. Commun.** 383: 464-468