

TILLING: Examples of utilization in plant breeding

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Targeting Induced Local Lesions IN Genomes (TILLING), first published from McCallum *et al.* (2000a), is a reverse genetics tool that combines an ethyl methanesulfonate (EMS)-induced mutagenesis with the identification of novel genetic variation (reviewed in McCallum *et al.*, 2000b; Colbert *et al.*, 2001). This method generates a wide range of mutant alleles, is automatable and is applicable to any organism that can be chemically mutagenised. This technique was developed in *Arabidopsis thaliana* (McCallum *et al.*, 2000a) and also works in other plant species, including crops (reviewed in McCallum *et al.*, 2000b; Colbert *et al.*, 2001). TILLING is a non-transgenic tool to obtain crop species with advantageous characteristics as demonstrated in wheat (Slade *et al.*, 2005). Using the same detectable technology, naturally evolved polymorphisms can be identified between ecotypes (Eco-TILLING) (Comai *et al.*, 2004; Gilchrist *et al.*, 2006; Nieto *et al.*, 2007). Therefore, genes related to biotic or abiotic responses are potential targets for Eco-TILLING. A TILLING platform has been established for the model legume *Lotus japonicus* (<http://www.lotusjaponicus.org/tillingpages/Homepage.htm>) (Perry *et al.*, 2003). This facility is being used in the framework of the LOTASSA project with the objective of obtaining *Lotus* plants more tolerant to environmental constraints. In conclusion, TILLING and/or Eco-TILLING can be applied in plant breeding for detection of alleles associated with agronomically important traits.

References

- COLBERT T., TILL B.J., TOMPA R., REYNOLDS S., STEINE M.N., YEUNG A.T., MCCALLUM C.M., COMAI L. and HENIKOFF S. 2001. High-throughput screening for induced point mutations. *Plant Physiology*, **126**, 480-484.
- COMAI L., YOUNG K., TILL B.J., REYNOLDS S.H., GREENE E.A., CODOMO C.A., ENNS L.C., JOHNSON J.E., BURTNER C., ODDEN A.R. and HENIKOFF S. 2004. Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. *Plant Journal*, **37**, 778-786.
- GILCHRIST E.J., HAUGHN G.W., YING C.C., OTTO S.P., ZHUANG J., CHEUNG D., HAMBERGER B., ABOUTORABI F., KALYNYAK T., JOHNSON L., BOHLMANN J., ELLIS B.E., DOUGLAS C.J. and CRONK Q.C. 2006. Use of Ecotilling as an efficient

SNP discovery tool to survey genetic variation in wild populations of *Populus trichocarpa*. *Molecular Ecology*, **15**, 1367-1378.

MCCALLUM C.M., COMAI L., GREENE E.A., and HENIKOFF S. 2000a. Targeted screening for induced mutations. *Nature Biotechnology*, **18**, 455-457.

MCCALLUM C.M., COMAI L., GREENE E.A. and HENIKOFF S. 2000b. Targeting induced local lesions IN genomes (TILLING) for plant functional genomics. *Plant Physiology*, **123**, 439-442.

NIETO C., PIRON F., DALMAIS M., MARCO C.F., MORIONES E., GOMEZ-GUILLAMON M.L., TRUNIGER V., GOMEZ P., GARCIA-MAS J., ARANDA M.A. and BENDAHDANE A. 2007. EcoTILLING for the identification of allelic variants of melon eIF4E, a factor that controls virus susceptibility. *BMC Plant Biology*, **7**, 34.

PERRY J.A., WANG T.L., WELHAM T.J., GARDNER S., PIKE J.M., YOSHIDA S. and PARNISKE M. 2003. A TILLING reverse genetics tool and a web-accessible collection of mutants of the legume *Lotus japonicus*. *Plant Physiology*, **131**, 866-871.

SLADE A.J. and KNAUF V.C. 2005. TILLING moves beyond functional genomics into crop improvement. *Transgenic Research*, **14**, 109-115.

SLADE A.J., FUERSTENBERG S.I., LOEFFLER D., STEINE M.N. and FACCIOTTI D. 2005. A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING. *Nature Biotechnology*, **23**, 75-81. Epub 2004 Dec 2005.

TILL B.J., COOPER J., TAI T.H., COLOWIT P., GREENE E.A., HENIKOFF S. and COMAI L. 2007. Discovery of chemically induced mutations in rice by TILLING. *BMC Plant Biology*, **7**, 19.