Transcriptome screening-based identification of candidate genes endowing non-target-site-based resistance to herbicides inhibiting ALS in Lolium sp

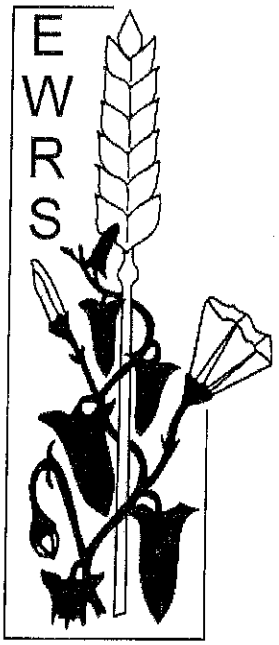
Arnaud Duhoux, Jerome Gouzy, Sebastien Carrere, Christophe C. Delye

2013-99

16th SYMPOSIUM

Samsun 2013

24-27 June, 2013



EWRS

European Weed Research Society

PROCEEDINGS

ISBN: 978-90-809789-12

EWRS European Weed Research Society
Europäische Gesellschaft für Herbologie
Sicété Européenne de Malherbologie

EWRS 16th SYMPOSIUM, Samsun 2013

Copies of the Proceeding may be obtained from:

EWRS Bookstore
P.O. Box 28
NL-6865 ZG Doorwerth
The Netherlands

Printing: Erol Ofset, Samsun

ISBN: 978-90-809789-12

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Rapid bioassay method for herbicide dose-response study and resistance diagnosis

C. J. Zhang, S. H. Lim, J. W. Kim, D. S. Kim
Department of Plant Science, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-742, Korea
dosoonkim@snu.ac.kr

This study was conducted to develop a rapid bioassay method for herbicide dose-response of *Echinochloa* species and herbicide resistance diagnosis in *Echinochloa* species. Germinated seeds of *Echinochloa* spp. were placed on the paper wick of 18 cm x 16.5 cm growth pouch containing herbicide solution at a range of concentrations. The herbicides tested in this study include bentazone (PSII inhibitor), cyhalofop-butyl (ACCase inhibitor), penoxsulam (ALS inhibitor), glufosinate (GS inhibitor) and glyphosat (EPSPS inhibitor). Shoot and root lengths of *Echinochloa crus-galli* were then measured after incubation for 6 days. Dose-responses in root length by the growth pouch method were well described by the logistic function and confirmed to be similar to those of whole plant assay, which was conducted by foliar spray of herbicides to *E. crus-galli* at the 5th to 6th leaf stage using a co mpressor pressurized belt-driven sprayer equipped with an 8002E flat-fan nozzle, regardless of herbicide modes of action. Thus, this result suggests that the growth pouch method can be used for herbicide bioassay.

The growth pouch method was also applied to rapid diagnosis of ACCase or ALS inhibitor resistance in *Echinochloa* spp.. Resistant and susceptible biotypes were discriminated by observing root length at 6 days after treatment. In cyhalofop-butyl dose-response study, the R/S ratios, which were obtained by dividing the GR_{50} value of tested biotype by that of reference susceptible biotype of *E. crus-galli*, were 4.3 and 1.6 by whole plant assay and 5.4 and 1.4 by the growth pouch method for Seosan-5 and Seosan-46, respectively. In penoxsulam dose-response study, the R/S ratios were 7.8 and 2.0 by whole plant assay and 7.4 and 1.7 by the growth pouch method for Seosan-5 and Seosan-46, respectively. The dose range which can discriminate betwen resistant and susceptible biotypes was 180 to 300 mg and 80 to 120 mg ai L^{-1} of cyhalofop-butyl for *E. crus-galli* and *E. oryzicola*, respectively, and 350 to 500 mg and 650 to 1000 mg ai L^{-1} of penoxsulam for *E. crus-galli* and *E. oryzicola*, respectively. This method was further applied to discriminate between resistant and susceptible *E. oryzicola* biotypes to other ALS inhibitors, azimsulfuron and bispyribac-sodium.

Therefore, it can be concluded that the growth pouch method can be used for herbicide dose-response study and diagnosis of herbicide resistance in *Echinochloa* spp. with significant time and cost-savings as compared to the conventional whole plant assay. As the growth pouch method requires very small amount of herbicide and small space, it can also be used in an early screening stage of herbicide discovery.

Transcriptome screening-based identification of candidate genes endowing non-target-site-based resistance to herbicides inhibiting ALS in *Lolium* spp.

A. Duhoux[1], J. Gouzy[2], S. Carrère[2], C. Délye[1]
[1]*INRA, UMR1347 Agroécologie, Dijon-France*
[2]*INRA, UMR441/2594 Laboratoire Interactions Plantes Micro-organismes, Castanet Tolosan-France*
aduhoux@dijon.inra.fr

Arable weeds have evolved herbicide resistance as an adaptation to herbicide applications. Non-target-site based resistance (NTSR) to herbicides is the major cause for grass weed resistance to herbicides inhibiting acetolactate synthase (ALS), but no diagnosis tools currently exist. NTSR is part of the weed response pathways to herbicide stress. Differential regulation of herbicide-stress-responsive genes between resistant and sensitive plants is a key in NTSR. Due to inherent intraspecific genetic variation, NTSR pathways can differ among individual resistant plants, which makes NTSR complex to study.

We implemented a deep-sequencing approach (RNA-Seq) to identify genes differentially expressed in the transcriptomes of two pools of *Lolium* spp. plants (3 plants resistant *versus* 3 plants sensitive to one herbicide inhibiting ALS) in a time-course experiment. We identified a set of 50 genes over-expressed in the resistant bulk compared to the sensitive bulk.

Candidate genes could show either a constitutive over-expression or an herbicide-induced up-regulation in resistant plants. Depending on the genes, the over-expression observed in resistant plants could be further increased after herbicide application. Genes over-expressed in resistant weed plants, and/or up-regulated by herbicide application encoded cytochromes P450, glutathione S-transferases, glycosyltransferases, as well as genes with other functions such as putative signalling proteins that could be involved in herbicide sensing and upstream regulation of NTSR. To confirm the role played by the NTSR candidate genes identified, the differences in expression observed were checked by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) in the plants used for RNA-Seq and in a range of additional resistant or sensitive plants from various populations from the field. A dozen genes specifically over-expressed in many resistant plants were identified. After further characterisation (i.e., characterisation of the associated cross-resistance patterns), these genes are interesting potential targets to develop molecular NTSR diagnosis.