Molecular Characterization of Glyphosate- and Acetolactate Synthase Inhibitor-Resistant Kochia from Montana

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Background and Problem

• Herbicide-resistant kochia is an increasing concern in the Northern Great Plains (NGP) (1)
• Glyphosate-resistant (Gly-R) kochia biotypes confirmed in seven NGP states including Montana (1,2,3)
• Based on dose-response assays, putative Gly-R biotypes (GIL01, CHES01, and JOP01) from MT had 4.6- to 11-fold levels of resistance relative to susceptible (Gly-S) biotype (2)
• Also, Gly-R kochia biotypes were found resistant to acetolactate synthase (ALS) inhibitors
• Evidence of multiple herbicide-resistant (MHR) kochia in MT (1)

Objective

• To investigate mechanism(s) conferring resistance to glyphosate and ALS-inhibitor herbicides in MHR kochia biotypes from MT

Materials and Methods

✓ Plant material:
  3 to 5 plants selected for each kochia biotype (GIL01, JOP01, CHES01, Gly-S)
  gDNA extraction using DNeasy Plant Mini Kit from Qiagen

✓ Mechanism of glyphosate resistance:
  1. Target site (EPSPS gene) mutations:
     • PCR amplification of the conserved region of EPSPS gene
     • Sequence alignment with CLC sequence viewer software
  2. EPSPS gene copy number:
     • qPCR performed to determine EPSPS gene copy number relative to ALS gene
     • EPSPS gene copy number calculated using delta CT method (2^ΔCT) (4)

Conclusions

• EPSPS gene sequencing showed no target-site mutation at Pro197 position in MHR kochia biotypes from MT

Results and Discussion

3. EPSPS protein expression (western blot)
   • Isolation and separation of protein on 10% SDS-PAGE
   • Incubation with EPSPS (primary) & Goat : rabbit (secondary) antibodies
   • Visualize protein bands in BIO-RAD ChemiDoc™ XRS

✓ Target site (ALS gene) mutations:
   • PCR amplification of 2 kb fragment of ALS gene for detecting known mutations
   • Alignment of nucleotide sequences using Multalin software

Figure 2. Sequence alignment of EPSPS gene showing no mutation at Pro197 position in MHR kochia biotypes from MT

Figure 3. Increased EPSPS gene copy number relative to ALS gene in MHR kochia biotypes from MT

Figure 4. Increased EPSPS protein in MHR kochia biotypes (JOP01, GIL01, & CHES01) compared with susceptible (Gly-S) kochia biotype in western blot

References


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