MOLECULAR TAXONOMY OF CONTAMINANT GALIUM OF CANADIAN SHIPMENTS CANARY GRASS SEED

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Introduction. *Galium spurium* is a major weed yield-reducing in cereals and oilseeds up to 18%. This weed has developed resistance to certain herbicides such as sulfonylurea and triasulfuron (1). *G. spurium* and *G. aparine* are among the ten most abundant weeds along the Prairie region of western Canada and are increasing in abundance on the fastest rate since the seventies (2). In resistant biotypes, one or more mutations have been found, causing decreased sensitivity to inhibiting acetolactate synthase (ALS). Identified mechanisms of resistance to ALS inhibitors in weed species include alterations in the target site. Based on the Arabidopsis *ALS* gene have been described six sites which are major substitutions: Ala122, Pro197, Ala205, Asp376, Trp574 and Ser653 (3). Also there is great morphological similarity between *G. spurium* and *G. aparine* so they are confused and although both species are considered weeds, the Work Plan for Import canary grass seed from Canada to Mexico only restricts the entry of *G. spurium*. Thus we decided to determine the species of *Galium* occurring in canary grass seed imported from Canada by amplification, sequencing and Blast of a gene fragment of ALS.

Methods. DNA extraction was performed from *Galium* embryos with GeneJet Extraction Kit (Fermentas). Primers were designed to amplify a 260 bp segment based on the sequence of *ALS* gene *G. spurium* isolation MIN (JN038053). The amplification products were cloned into pGEMT-Easy (Promega). Three clones were sequencing and compared with the GeneBank Blast.

Results. Amplification of a 260 bp segment was performed with DNA *Galium* at concentrations of 10, 25 and 50 ng. Amplification was observed at all concentrations tested (Fig 1).

Fig 1. Amplification of a 260 bp band in 1, 2 and 3 with 10, 25 and 50 ng of DNA of *Galium* respectively.

The PCR products were purified and cloned in pGEM-Teasy, subsequently clones 4, 7 and 9 were sequencing. With the sequences obtained was performed the Blast (Table 1).

Table 1. Identity of the clones 4, 7 and 9 with the sequences obtained from Blast.

Clone	Blast	Identity (%)
4	*G. spurium* isolate MIN *ALS* gene	98
	G. aparine strain S ALS mRNA	96
7	*G. spurium* isolate MIN *ALS* gene	96
	G. aparine strain S ALS mRNA	94
9	*G. spurium* isolate MIN *ALS* gene	96
	G. aparine strain S ALS mRNA	94

It was also determined that these species have different degrees of resistance herbicides. Alignment was made of the amino acid sequences of the clones 4, 7 and 9 with *G. spurium* isolate MIN. We found one of the mutations reported by (1) which changes to Asp376Glu (Figure 2). This indicated that this specie is suspected of having resistance to florasulam, but is susceptible to fluroxypyr.

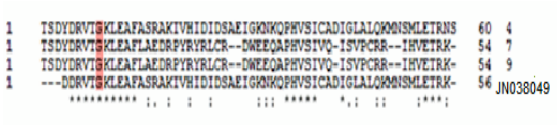

Fig 2. Alignment of clones 4, 7 and 9 against *G. spurium* isolation MIN (JN038049). Note the presence of the mutation Asp376Glu.

Conclusions. The specie of *Galium* ocurring in shipments of canary grass seed imported from Canada is *G. spurium.* This specie may also have a resistance level less than 10% to florasulam.

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