# *ALS* GENE MUTATIONS IN *APERA SPICA-VENTI* CONFER BROAD-RANGE RESISTANCE TO HERBICIDES

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**Abstract:** Several biotypes of wind bentgrass in Poland have been identified as being resistant to acetolactate synthase (ALS) inhibitors. We screened these weeds with chlorsulfuron and performed a whole-plant bioassay with a range of doses based on these four herbicides: chlorsulfuron, sulfosulfuron, propoxycarbazone-sodium and mesosulfuron-methyl + iodosulfuron-methyl-sodium mixture. Ten biotypes, diverse in their levels of resistance, were submitted for molecular tests. PCR amplification and sequencing of *al*s domains demonstrated numerous single nucleotide polymorphisms. Nine biotypes showed non-synonymous substitutions in codon $Pro_{197}$, changing it to Ser or Thr. Mutation in $Pro_{197}$ conferred a high level of resistance to the tested herbicides. Analysis of four biotypes also revealed a substitution in the $Ala_{122}$ codon, changing it to Val. In one biotype this substitution was not accompanied by $Pro_{197}$ mutation and this biotype was resistant to chlorsulfuron and mesosulfuron + iodosulfuron, but not to sulfosulfuron or propoxycarbazone-sodium. Correspondence between mutations and levels of resistan ce to ALS inhibitors may support management of resistant weeds with the existing palette of herbicides.

**Key words:** grass weeds, wind bentgrass, acetolactate synthase, sulfonylurea, propoxycarbazone-sodium, proline 197

## INTRODUCTION

The phenomenon of weed resistance results from the long-term use of herbicides, where the herbicides were used in the same mode of action on one field. The resistant weed species rate-of-spread depends on the biology and genetics of each species. *Apera spica-venti* L. (Beauv.), also known as wind bentgrass, is a winter-annual, wind-pollinated, and primarily self-incompatible grass (Warwick *et al*. 1987). It is a common and noxious weed in central and eastern Europe, but it also grows in Asia, Great Britain, and North America. The resistance to sulfonylurea herbicides by wind bentgrass can be transferred by pollen from resistant to susceptible plants (Adamczewski and Matysiak 2009).

In Poland, wind bentgrass is one of the most noxious grass weeds in arable fields. It affects winter cereals, which are the most important cereal crops in Poland, as well as winter oilseed rape. About 60% of winter cereal fields in Poland, an area of about four million hectares, are infested with wind bentgrass. Heavy infestations are particularly prevalent in moist years. Wind bentgrass plants grow higher than winter wheat and can produces from 5 to 30 or sometimes more tillers. Wind bentgrass is a very competitive grass, particularly the short-straw varieties. This grass also shows high tolerance to temperature and light intentsity during germination.

Acetolactate synthase (ALS) inhibitors are widely used in Poland to control *A. spica-venti*. Sulfonylurea herbicides currently play a crucial role in the chemical weed control of winter cereals owing to their low cost and high biological activity. Biotypes that have developed resistance to ALS inhibitors represent 101 weed species and outnumber all other herbicide groups (Heap 2010). Wind bentgrass biotypes that are resistant to ALS inhibitors have been recorded in three countries to date, all in winter wheat fields. In 2001 in Poland, biotypes resistant to chlorsulfuron were found (Rola and Marczewska 2002); in 2005 in the Czech Republic, biotypes resistant to chlorsulfuron were found (Novakova *et al*. 2006); in 2005 in Germany, biotypes resistant to sulfosulfuron were found (Balgheim and Wagner 2006). In Poland, resistant *A. spica-venti* biotypes have been found in several localities on arable lands with common crop rotation and herbicide use (Adamczewski and Kierzek 2007) where sulfonylurea (*i.e.* chlorsulfuron) had failed to efficiently control the weed.

Resistance to ALS inhibitors occurs because mutations in *als* cause an altered target site, or due to enhanced metabolism of herbicide molecules (Corbett and Tardiff 2006). Most cases of resistance to ALS inhibitors appear to be caused by point mutations in *als* (Corbett and Tardiff 2006) in one or more of the following codons (Codon numbers refer to the *Arabidopsis thaliana* L. (Heyn.)

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sequence (Tranel and Wright 2002)): $Ala_{122}$, $Pro_{197}$, $Ala_{205}$, $Trp_{574}$, and $Ser_{653}$ (Tranel *et al.* 2010). Whaley *et al.* (2006) reported a new substitution in $Asp_{376}$ in *Amaranthus hybridus* L., which changes the amino acid residue to Glu. It was only in 2009 that a new mutation was discovered: substitution of $Gly_{654}$ conferred a high level of resistance to imidazolinones (Laplante *et al*. 2009). Although *als* shows variability among and within species, mutations conferring resistance are localised in highly conserved domains (Tranel and Wright 2002). According to Gressel (2002), domain A near the amino terminus of ALS includes codons from 124 to 205, and domain B near the carboxy terminus contains codons from 574 to 653. Boutsalis *et al.* (1999) discerned five domains, each encompassing one specific mutation site: Dom C ($Ala_{122}$), Dom A ($Pro_{197}$), Dom D ($Ala_{205}$), Dom B ($Trp_{574}$), and Dom E ($Ser_{653}$). For the experiments described in this paper, we used Gressel's (2002) domain A (broadened by codons 122 and 123) and B because they were spanned by the designed primers.

The objective of our study was (i) to evaluate the level of resistance of *A. spica-venti* to four ALS-inhibiting herbicides, and (ii) to check the existence of mutations in the *als* gene, which may underlie the resistance.

## MATERIALS AND METHODS

**Plant material**

Resistant biotypes of *A. spica-venti* were collected in 2005, 2006, and 2007 from farms where cereals were grown for several years with chemical weed management, and where herbicide control of the weed had become ineffective (Adamczewski and Kierzek 2007). One or more samples were collected separately from each field. In the laboratory, seeds were removed from the tillers, cleaned, and stored at –5°C for 1 week to break the dormancy. The seeds were used for screening and dose-response tests. In this paper, we describe 10 resistant biotypes, denoted '7', '11/2' (collected in 2005), '12/1', '13/6', '15/1', '17/2', '17/6', '20/2', '28/1' (2006), and '3' (2007). One susceptible biotype, WG, was harvested from a field in which no herbicides were used and was used as a standard for greenhouse screening and for dose-response experiments.

For whole-plant bioassays and dose-response experiments, 20–25 seeds were sown into 0.5 L, 9-cm diameter plastic pots. These pots contained a 1:1 (v/v) mixture of sandy loam soil and commercial peat-based potting material. Pots were placed in a greenhouse at 18–24°C with an 18 h photoperiod per day. After germination, plants were thinned to 10 plants per pot for dose-response experiments and to 15 plants for the screening test.

As reference for molecular analysis, an additional 10 susceptible biotypes (Bi, Bo, Gu, Mi, Pr, Sa, So, Soe, Wh, and Ze) were collected in July 2007 in central and southern Poland. Seeds were germinated on wet sand, and then planted in 10-cm diameter pots with commercial medium. At the three-to-four leaf-stage, plants were subjected to a screening test. Other plants from these biotypes were grown and then individually harvested, at the six-leaf stage, for DNA isolation.

**Whole-plant bioassay**

Greenhouse experiments were performed in three steps. In the first step (screening), plants were sprayed with chlorsulfuron at the two- to four-leaf stage (Glean 75 WG, 750 g a.s./kg, WG, DuPont de Nemours, France) in a dose of 18.75 g x ha. A greenhouse cabin sprayer with compressed air bottles, TeeJet 11002 nozzles, and a pressure of 300 kPa were used for the spraying. The spray volume used was 250 l x ha. Response to herbicide treatment was evaluated 2 and 4 weeks after application. For the next step, biotypes that showed only a minor reaction or no reaction to spraying were selected. In the second experiment, selected biotypes were exposed to various doses of chlorsulfuron. These were doses at the: recommended, double, triple, or four-fold the recommended dose (18.75, 37.50, 56.25, and 75.00 g x ha, respectively). For the last step, biotypes were individually sprayed with one of four possible doses (1x, 2x, 3x, or 4x the recommended dose) of three herbicides: sulfosulfuron (Apyros 75 WG, 750 g a.s./kg, WG, Monsanto Europe S.A/N.V., Belgium), propoxycarbazone-sodium (Attribut 70 WG 700 g a.s./kg, WG, Bayer CropScience AG, Germany), or mesosulfuron-methyl + iodosulfuron-methyl-sodium mixture (Atlantis 04 WG, 36 g a.s./kg, WG, Bayer CropScience S.A., France). The effect of the herbicide treatment was evaluated 4 weeks after treatment by weighing the fresh tissue of the above-ground portion of the plants. The effect of the herbicide treatment was expressed as the percent of fresh tissue weight of the unsprayed control (data not shown). In each experiment, the susceptible biotype was used as a standard. Biotypes 17/2 and 28/1 were subjected to only the screening test. Plants that survived the herbicide treatment were collected separately for DNA extraction.

Susceptible biotypes were sprayed only with chlorosulfuron and mesosulfuron+iodosulfuron at the recommended field dose, in order to verify their susceptibility to these sulfonylureas. The effect of the herbicide application was evaluated after 15 and 21 days.

**Dose-response experiments**

Three herbicide-resistant biotypes (3, 7, and 12/1) and one standard susceptible biotype (WG) were used in this study. At the two- to three-leaf stage, plants were sprayed with chlorsulfuron. To generate individual dose-response curves, each biotype was exposed to 11 doses: 0, 4.69, 9.38, 18.75, 37.5, 75, 112.5, 150, 187.5, 250, and 500 g x ha (the technical conditions were the same as in the screening test). In each experiment, the susceptible biotype was used as a standard. Four replicates of each treatment were performed. Plants were visually evaluated 2 and 4 weeks after treatment, and then the plants were cut at the soil surface, and their fresh weight was determined. Results were expressed as the percent of untreated sample biomass. Statistical analysis was performed using the Polo-Plus statistic program logic model (Robertson *et al.* 2003).

**Molecular analysis**

*DNA extraction*

Leaf tissue from each plant (200 to 500 mg) was placed in a 2 ml plastic Eppendorf tube and freeze-dried over-

night. Dried tissue was subjected to DNA extraction using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987) with minor modifications. Isolated DNA was diluted in water to a 100 ng/μl concentration, and was used for PCR. For each biotype, two or three selected plants were used for the molecular assay.

*Primer design*

Primers were designed in eprimer3 program, using the sequence of *Lolium multiflorum* Lam. ALS mRNA and *Bromus tectorum* L. ALS mRNA (GenBank accession numbers: AF310684, AF488771, respectively). Primers A3-F and A3-R were designed to anneal in the regions flanking domain A, so that codons from 120 to 209 would be clearly legible in sequencing. Primers B4-F and B4-R were designed similarly to hybridize in regions preceding and following domain B, so that the sequence from codon 570 to 660 would be legible. The amplified fragments spanned grand domains A and B distinguished by Gressel (2002). The primer sequences are shown in table 1.

Table 1. Primers used for domain A and B amplification

| Name | 5′→3′ sequence | Tm |
|---|---|---|
| A3-F | AAG GGC GCC GAC ATC CTC | 54.9°C |
| A3-R | CGA GGT AGT TGG CTT GG TGA | 54.4°C |
| B4-F | CAG GTG TCA CGG TTG TTG AC | 53.8°C |
| B4-R | GCA AAA CAC ATG CTT TAT TAG TTG A | 51.1°C |

Tm – melting temperature

*PCR reactions*

PCR of domain A was carried out using High Fidelity PCR Enzyme Mix (Fermentas) with a primer concentration of 0.5 μM. Domain A is rich in GC pairs, and therefore, 6 μl of Combinatorial Enhancer Solution (Ralser *et al.* 2006) was used per 30 μl of reaction mix to obtain a higher yield of amplicon. Thermocycling was performed in a Mastercycler (Eppendorf) at 94°C for 5 min, followed by 40 cycles of 95°C for 1.5 min, 63°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 7 min.

Domain B was amplified using High Fidelity PCR Enzyme Mix (Fermentas), 3 μl of Combinatorial Enhancer Solution per 30 μl of reaction mix, a primer concentration of 1 μM, and thermocycling conditions of 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 63°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 10 min.

*DNA sequencing and sequence analysis*

PCR products were purified and used directly for sequencing using **primers** A3-F and B4-F because they gave clearer results (data not shown). Purifying of the PCR product and sequencing was commissioned to Genomed Sp z.o.o. (Warsaw, Poland). Chromatograms were analyzed in *Chromas* and *FinchTV* programs. The comparisons of the sequences were prepared in the *ClustalW*, online-accessed program.

## RESULTS

### Whole-plant assay

Biotypes that showed no reaction to the dose they received were considered as resistant (R). Biotypes affected by the dose and showing slowed growth, reduction in weight, and chlorosis were marked as medium-resistant (MR). Results of the assay are compiled in table 2. All plants from biotypes Bi, Bo, Gu, Mi, Pr, Sa, So, Soe, Wh, and Ze were severely damaged on the 15th day after spraying, either with chlorosulfuron or with mesosulfuron+iodosulfuron at the recommended dose. Twenty-one days after the herbicide application, all the plants were dead.

### Dose-response experiment

The data presented refer to three *A. spica-venti* biotypes (3, 7, 12/1) that showed different level of resistance to four herbicides in whole-plant bioassay, and to the one biotype that was sensitive to all four herbicides. We generated a dose-response curve only for chlorsulfuron. The results, which were expressed in fresh weight as a percent of the unsprayed control, indicated differences between the four tested biotypes (Fig. 1). Biotype 3 was the most resistant, with a resistance index of 20.6 (based on $ED_{50}$) and 9.4 (based on $ED_{90}$). Biotype 7 was less resistant, with a resistance index for chlorsulfuron of 13.3 (based on $ED_{50}$) and 7.0 (based on $ED_{90}$). The different resistance indexes for chlorsulfuron are shown in table 3.

### Molecular characterisation

For domains A and B, the PCR products were approximately 380 and 520 bp, respectively. Codons 122–205 and 574–653 were clearly legible. As a first step, sequences obtained from all resistant biotypes and susceptible WG were aligned to find substitutions in codons 122, 197, 205, 574, 653 and 654.

In domain A mutation of $Ala_{122}$ to Val occurred in biotypes 12/1, 13/6, 17/6 and 28/1, as a result of a single nucleotide polymorphism (SNP) in the second position of the Ala codon. Biotypes 7, 15/1, 17/2 and 28/1 showed Pro to Ser substitution at position 197, with a SNP in the first position of the Pro codon. Codon of proline 197 was also substituted for codon of threonine, due to SNP in the first position. This was observed in five biotypes: 7, 15/1, 17/6, 20/2 and 3. Mutations in $Ala_{205}$ were not present. It is important to stress, that in several biotypes (7, 15/1, 28/1) not

Table 2. Results of whole plants assay. Biotypes were described as R, MR or S depending on their reaction to herbicide treatment

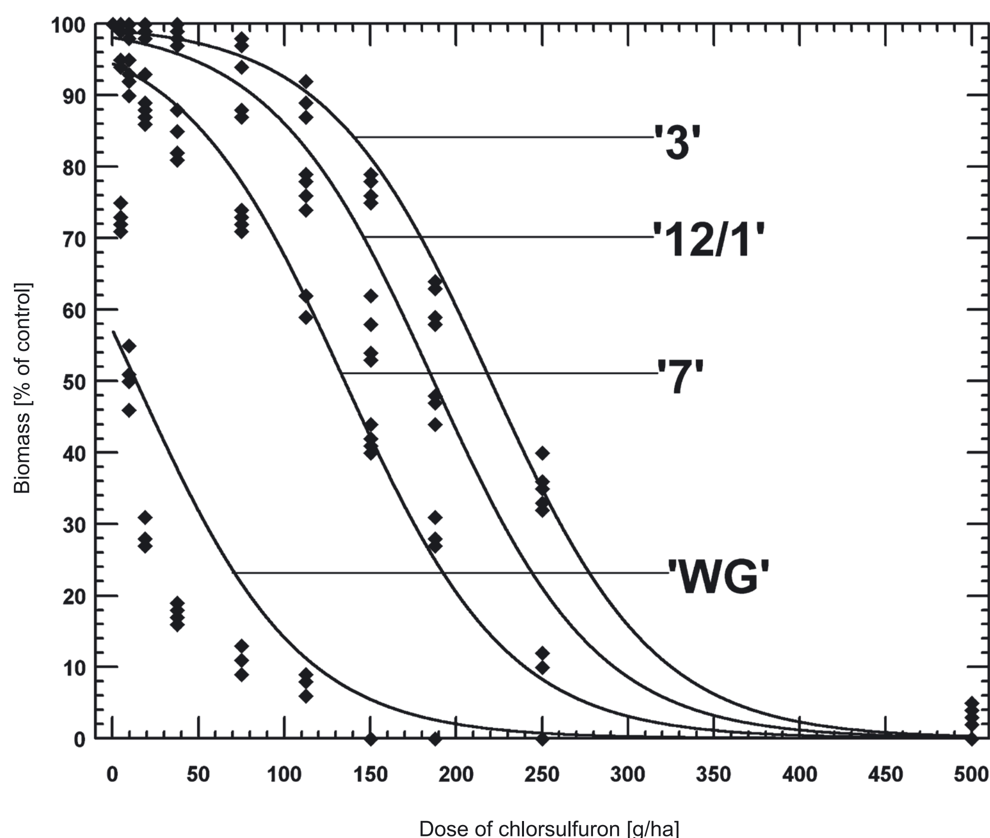
| Biotype | Year | Level of resistance for each herbicide and dose | | | |
|---|---|---|---|---|---|
| | | chlorsulfuron | sulfosulfuron | propoxycar bazone-Na | mesosulfuron + iodosulfuron |
| 7 | 2005 | 18.75 g R;<br>37.50 g; R<br>56.25 g; MR | 18.75 g; R<br>37.50 g; MR | 42.00 g; R<br>84.00 g; MR | 7.20 g; MR |
| 11/2 | 2005 | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 42.00 g; R<br>84.00 g; R<br>126.00 g; R<br>168.00 g; R | 7.20 g; R<br>10.80 g; R<br>14.40 g; R<br>18.00 g; R |
| 12/1 | 2006 | 18.75 g; R<br>37.50 g; R<br>56.25 g; MR | 18.75 g; S | 42.00 g; S | 7.20 g; R<br>10.80 g; MR |
| 13/6 | 2006 | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 42.00 g; R<br>84.00 g; R<br>126.00 g; R<br>168.00 g; R | 7.20 g; R<br>10.80 g; R<br>14.40 g; R<br>18.00 g; R |
| 15/1 | 2006 | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 42.00 g; R<br>84.00 g; R<br>126.00 g; R<br>168.00 g; R | 7.20 g; R<br>10.80 g; R<br>14.40 g; R<br>18.00 g; R |
| 17/2 | 2006 | 18.75 g; R<br>37.50 g; R<br>56.25 g; R | not tested | not tested | not tested |
| 17/6 | 2006 | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 42.00 g; R<br>84.00 g; R<br>126.00 g; R<br>168.00 g; R | 7.20 g; R<br>10.80 g; R<br>14.40 g; R<br>18.00 g; R |
| 20/2 | 2006 | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 42.00 g; R<br>84.00 g; R<br>126.00 g; R<br>168.00 g; R | 7.20 g; R<br>10.80 g; R<br>14.40 g; R<br>18.00 g; R |
| 28/1 | | 18.75 g MR | screening test only | | |
| 3 | 2007 | 18.75 g; R<br>37.50 g; R<br>56.25 g; R<br>75.00 g; R | 18.75 g; R<br>37.50 g; R<br>56.25 g; MR<br>75.00 g; MR | 42.00 g; R<br>84.00 g; R<br>126.00 g; MR<br>168.00 g; MR | 7.20 g; R<br>10.80 g; R<br>14.40 g; MR<br>18.00 g; MR |
| WG | 2006 | 18.75 g; S | 18.75 g; S | 42.00 g; S | 7.20 g; S |

R – resistant: no effect of herbicide application in indicated dose was observed; MR – medium resistant: distinct herbicide spraying effects were observed: slowed growth, yellowish tint of leaves, up to 50% reduction of biomass; S – susceptible: over 90% reduction of biomass

Table 3. Detection parameters of resistance, for resistant and susceptible to chlorsulfuron biotypes of *A. spica-venti*

| Biotypes | Detection parameters | | | | | |
|---|---|---|---|---|---|---|
| | slope of the curve | standard error | $ED_{50}$ | $RI_{50}$ | $ED_{90}$ | $RI_{90}$ |
| 3 | 0.232 | 0.525 | 208.2 | 20.6 | 489.3 | 9.4 |
| 7 | 0.183 | 0.404 | 169.4 | 16.8 | 473.8 | 9.1 |
| 12/1 | 0.179 | 0.384 | 133.9 | 13.3 | 367.4 | 7.0 |
| WG | 0.108 | 0.144 | 10.1 | – | 52.2 | – |

$ED_{50}$ – dose of chlorsulfuron causing 50% decrease in biomass; $ED_{90}$ – dose of chlorsulfuron causing 90% decrease in biomass
$RI_{50}$ and $RI_{90}$ – resistance index, calculated as the ratio between; ED parameters of resistant and susceptible biotypes




WG – susceptible standard; 7, 12/1, 3 – biotypes resistant to acetolactate synthose (ALS) inhibitors

Fig. 1. The effect of chlorsulfuron on fresh-weight reduction of resistant and susceptible biotypes of *A. spica-venti*

Table 4. Mutations observed in *als* gene in *A. spica-venti*

| Biotype | Year | Plant | Substituted aminoacids | |
|---|---|---|---|---|
| | | | Ala122 | Pro197 |
| 7 | 2005 | 1 | | Ser homozyg. |
| | | 4 | | Ser |
| | | 5 | | |
| 11/2 | 2005 | E1 | | Thr homozyg. |
| | | E3 | | Thr homozyg. |
| | | X3 | | Thr homozyg. |
| 12/1 | 2006 | 411 | | |
| | | 412 | Val | |
| 13/6 | 2006 | 451 | | Thr |
| | | 452 | Val | Thr |
| | | 456 | Val | Thr |
| 15/1 | 2006 | 473 | | |
| | | 476 | | Ser |
| | | 495 | | |
| 17/2 | 2006 | 504 | | Ser |
| | | 506 | | Ser homozyg. |
| 17/6 | 2006 | 522 | Val | Thr homozyg. |
| | | 526 | | Thr |
| 20/2 | 2006 | O1 | | Thr |
| | | X7 | | Thr |
| 28/1 | 2006 | 201 | | Ser |
| | | 202 | | Ser |
| | | 203 | Val | |
| 3 | 2007 | 686 | | Thr |
| | | 687 | | |
| 5 | 2007 | 686 | | Glu |
| | | 687 | | Glu |
| WG: susceptible control | 2006 | 54 | | |
| | | 55 | | |
| | | 56 | | |

Ala – alanine; Glu – glutamic acid; Pro – proline; Ser – serine; Thr – threonine; Val – valine; WG – susceptible standard

all plants showed mutations, although they had survived herbicide treatment. Also plants were homo- or heterozygotes concerning specific mutations (Table 4).

In domain B, no substitutions in $Trp_{574}$, $Ser_{653}$ or $Gly_{654}$ codons were observed.

Mutations in $Ala_{157}$ to Ser (biotypes 28/1, 18/1, and 15/1) were found. As it did not occurr in biotype WG, in the next step, sequences from susceptible biotypes from 2007, Bi, Bo, Gu, Mi, Pr, Sa, So, Soe, Wh, and Ze, were obtained to examine the role of $Ala_{157}$Ser substitutions in conferring resistance. Substitution $Ala_{157}$Ser was found in biotypes Bi, Mi, Pr, Soe, Wh, and Ze.

## DISCUSSION

Chlorsulfuron was the first sulfonylurea herbicide used in Poland and it is still used to control wind bentgrass. In 2002, Rola and Marczewska presented the first report of *A. spica-venti* resistance to chlorsulfuron in Poland. Since then, the Institute of Plant Protection in Poland has received many reports of poor management of *A. spica-venti* L. in cereal crops, mainly in north-western Poland where large farm areas are prevalent. The aim of this study was to identify the molecular background of *A. spica-venti* L. resistance to ALS inhibitors in Poland. Our secondary aim was to assign each mutation a separate category, as to whether it conferred a low or high level of resistance to specific herbicides that are currently widely used for grass weed management.

In greenhouse tests, a high level of resistance to doses of sulfonylurea herbicides which were 3- and 4-fold the recommended doses was observed. These tests suggest the presence of point mutations rather than enhanced metabolism of herbicide or enzyme overproduction. Some resistant biotypes appeared to have been selected by chlorsulfuron, but most of them also showed cross-resistance to other sulfonylureas and to propoxycarbazone-sodium. ALS-inhibiting herbicides registered in Poland represent only two groups: sulfonylureas and sulfonylaminocarbonyltriazolinones.

The substitution of $Ala_{122}$ codon to Val codon ($Ala_{122}$Val) occurred in three biotypes, 12/1, 13/6 and 17/6. In biotype 12/1 it was the only mutation in domain A and B that could have conferred resistance. The biotype was medium-resistant to chlorsulfuron in triple doses and medium resistant to mesosulfuron + iodosulfuron in double doses, but not to sulfosulfuron or propoxycarbazone-Na. Moreover, the $Ala_{122}$Val substitution was the first to be reported. As in other resistant species, a mutation in position 122, changing Ala to Thr, was observed: in *Xanthium strumarium* L. (Bernasconi *et al.* 1995), *Amaranthus hybridus* L. (Trucco *et al.* 2006), *Solanum ptycanthum* Dun. (Milliman *et al.* 2003), *Amaranthus retroflexus* L., and *Amaranthus powellii* S. Wats (McNaughton *et al.* 2005). In these cases, resistance to sulfonylurea was not observed. Therefore, mutation $Ala_{122}$Val seems to be responsible for a greater level of sulfonylurea resistance than mutation $Ala_{122}$Thr.

The mutation in $Ala_{157}$ to Ser occurred both in resistant and susceptible biotypes and is therefore unlikely to confer resistance to herbicides in wind bentgrass.

The biotypes with a substitution of $Pro_{197}$, either to Ser or to Thr, showed strong resistance to chlorsulfuron, sulfosulfuron, and mesosulfuron + iodosulfuron, as well as propoxycarbazone-Na. An additional mutation in $Ala_{122}$ to Val in two biotypes (13/6 and 17/6) did not seem to change the level or spectrum of resistance. These findings are consistent with other studies concerning substitution in position 197. This was the most commonly observed change in weeds with target site resistance to ALS inhibitors: 42 of 74 records in the list by Tranel *et al.* (2010). As the list shows, Pro197 substitution always conferred high (> 10-fold) resistance to sulfonylureas. Resistance to other ALS inhibitors depended either on the particular weed species, or was not determined. Resistance to sulfonylaminocarbonyltriazolinones was found in *B. tectorum* L. (for flucarbazone-Na, Park and Mallory-Smith 2004) and in *Chrysanthemum coronarium* L. (for flucarbazone-Na, Tal and Rubin 2004).

In biotypes 7, 12/1, 15/1 and 3, not all tested individual plants showed mutations in domains A or B, although they had survived herbicide treatment. The probable cause for this result might be the existence of another mechanism of resistance, *e.g.* enhanced metabolism.

The dose-response test resistance index ($RI_{50}$) for biotypes 3 ($Pro_{197}$ to Thr) and 7 ($Pro_{197}$ to Ser) was 20.6 and 16.4, respectively, showing that mutations in position 197 confer a high level of resistance. The difference between $RI_{50}$ for biotypes 3 and 7 is not high, but may correlate with a change to either Thr or Ser, or to other existing mechanisms of resistance.

The growing use of ALS inhibitors implies a serious risk of selecting new resistant biotypes and species. Molecular assays of resistant cases therefore, support weed management, *e.g.*, by indicating which group of ALS inhibitors can be successfully used for weed control of resistant biotypes.

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## POLISH SUMMARY

## MUTACJE GENU *ALS* W *APERA SPICA-VENTI* POWODUJĄCE ODPORNOŚĆ NA WIELE HERBICYDÓW

W Polsce odnaleziono biotypy miotły zbożowej odporne na inhbitory syntazy acetylomleczanowej ALS. Poddano je testowi przesiewowemu przy użyciu chlorosulfuronu, a następnie sprawdzono ich reakcję na wzrastające dawki 4 herbicydów: chlorosulfuronu, sulfosulfuronu, propoksykarbazonu sodu oraz mieszaniny mezosulfuronu i jodosulfuronu. Testom molekularnym poddano 10 biotypów o zróżnicowanym poziomie odporności. Namnożenie fragmentów genu *als* przy użyciu PCR, a następnie ich zsekwencjonowanie ujawniło obecność licznych mutacji punktowych. W dziewięciu biotypach odnaleziono niesynonimiczne substytucje, które zmieniały kodon $Pro_{197}$ na Ser lub Thr. Mutacja ta powodowała wysoki poziom odporności na wszystkie testowane herbicydy. W czterech biotypach wykryto także zamianę $Ala_{122}$ na Val. W jednym z biotypów mutacja ta występowała bez mutacji $Pro_{197}$, a biotyp posiadał odporność na chlorosulfuron i mezosulfuron + jodosulfuron, ale nie na propoksykarbazon lub sulfosulfuron. Zdefiniowanie relacji pomiędzy występującymi mutacjami a poziomem odporności na poszczególne herbicydy, może wspomóc ograniczenie zjawiska odporności chwastów, przy wykorzystaniu obecnie dostępnych środków ochrony roślin.