Glyphosate Resistance in Annual Ryegrass (*Lolium rigidum* Gaud.) with Multiple Resistance Mechanisms

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ABSTRACT

Glyphosate (*N*-(phosphonomethyl)glycine) is a post-emergent, systemic and non-selective herbicide for the control of annual and perennial weeds. This herbicide has very low toxicity to the mammals. The target enzyme for glyphosate in plants is 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Glyphosate inhibits the biosynthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan in the plant. The first case of glyphosate resistance was reported in *Lolium rigidum* in Australia after 15 years of persistence use of this herbicide and the number weeds reported resistant to glyphosate has increased around the world. So far, two mechanisms known to be involved in resistance to glyphosate are target-site mutation and reduced herbicide translocation. Recently, two populations of *L. rigidum* from Australia have been discovered with very high levels of resistance to glyphosate. This project aims to determine the levels of glyphosate resistance in these populations, investigate glyphosate resistance mechanisms in the populations and finally assess the mode of inheritance of resistance.

In this project, four resistant (NLR70, SLR77, SLR80 and SLR88) and one susceptible (VLR1) *L. rigidum* populations were evaluated for their response to glyphosate. From the dose response experiments, the susceptible population of VLR1 was completely controlled with the recommended rate of glyphosate (450 g a.e ha^{-1}). In contrast, the resistant populations were not fully controlled by this herbicide rate. There was considerable variation between the populations in their resistance to glyphosate. In comparison to the susceptible population VLR1, SLR77 was 2.2 to 3.5 fold resistant to glyphosate, NLR70 was 3.7 to 8.4 fold resistant to glyphosate, SLR88 was 5.6 to 11.4 fold resistant to glyphosate and SLR80 was 8.2 to 76.7 fold resistant to glyphosate.

The mechanism of glyphosate resistance in the populations was investigated. ^{14}C-glyphosate was used to determine the absorption and translocation of glyphosate among the populations. There was no significant difference on the absorption of ^{14}C-glyphosate 48 hours after treatment in the population. However, the accumulation of ^{14}C-glyphosate in the stem region was higher in the susceptible VLR1 population (25.9%) and in resistant SLR77 (25%) than the other three populations. The resistant populations NLR70, SLR88 and SLR80 had about half the amount of glyphosate

accumulating in the stem region. These three resistant populations appear to be resistant to glyphosate as a result of reduced translocation of glyphosate to the shoot meristem.

Part of the EPSP synthase gene of the susceptible and four resistant populations was amplified and sequenced to identify any changes in the nucleotide sequence. The predicted amino acid sequence from the susceptible population VLR1 was the same as the consensus sequence from other plant species in the conserved region sequenced. However, the resistant populations of NLR70, SLR77, SLR80 and SLR88 showed polymorphisms within the nucleotide sequence in this region. Single nucleotide substitutions of A for C at codon 106 were observed in the resistant populations SLR77 and SLR80. This nucleotide change is predicted to substitute threonine for proline at position 106. In the resistant population SLR88, a nucleotide substitution of T for C was observed at the same codon. This nucleotide substitution is predicted to change the amino acid from proline 106 to serine. Therefore, these three populations appear to be resistant to glyphosate as a result of a target-site mutation.

An inheritance study was conducted by cross pollinating the susceptible VLR1 and resistant SLR88 population. From the dose response, the parent susceptible was completely killed with the recommended rate of glyphosate and higher rates of glyphosate were required to control parental resistant and both F_1 progenies (maternal susceptible and resistant). Both F_1 progenies showed an intermediate response to glyphosate compared with the parental populations. This indicated that the resistance to glyphosate in population SLR88 is inherited by nuclear gene(s) through the transfer of pollen during the cross pollination.

It is suggested that SLR88 and SLR80 population contain both glyphosate resistant mechanisms due to the cross pollination between individuals with different resistant mechanisms. Having two resistant mechanisms results in populations being highly resistant to glyphosate compared to those with one resistance mechanism. The higher level of glyphosate resistance in these multiple glyphosate resistance populations will likely make them harder to manage.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree in any university or other tertiary institution and to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference is made in the text.

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Yazid Bostamam
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Pisang emas dibawa belayar,
Masak sebiji di atas peti,
Hutang emas boleh ku bayar,
Hutang budi ku bawa mati.

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