Evolution and Spread of Glyphosate Resistant Barnyard Grass (*Echinochloa colona* (L.) Link) from Australia

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Abbreviations

ACCase: Acetyl-CoA carboxylase

AFLP: Amplified fragment length polymorphism

AGRF: Australian Genome Research Facility

ALS: Acetolactate synthase

EPSP: 5-enolpyruvylshikimate-3-phosphate synthase

HAT: Hour after treatment

LD_{50}: Lethal dosage (dose required to control 50% of individuals in the population)

LSD: Least significant different

NSW: New South Wales

PCR: Polymerase chain reaction

QLD: Queensland

R/S: Resistance/susceptibility

RAPD: Randomly amplified polymorphic DNAs

RFLP: Restriction fragment length polymorphism

SA: South Australia

SE: Standard error

SSR: Simple sequence repeats

VIC: Victoria

WA: Western Australia

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Abstract

Echinochloa colona is an important summer-growing weed species in northern Australian cropping regions. The intensive use of glyphosate in summer fallow operations has led to the appearance of glyphosate resistant *E. colona* populations at a large number of sites. Studies of the genetic diversity, resistance mechanisms, inheritance and spread of resistance were undertaken to better understand the evolution of glyphosate resistance in this species. A survey of 65 barnyard grass populations collected from Queensland and New South Wales determined 34 populations were resistant to glyphosate with resistance levels ranging from 2 to 11-fold. High genetic diversity within three populations and between 62 populations was identified by the AFLP technique. A total of 99.2% of alleles identified within populations were polymorphic with a higher percentage of polymorphic alleles within the two resistant populations compared to the susceptible population. The level of glyphosate resistance in populations was dependent on the ambient temperature. Resistant populations showed a noticeably higher level of resistance at 30^{o}C compared to 20^{o}C whereas there was no effect of temperature on the response of the susceptible population. Experiments were carried out on glyphosate absorption and translocation in resistant and susceptible plants to identify the reason for these differences and the results showed a considerable decrease in glyphosate absorption into leaves at 30^{o}C. Differences were also identified in glyphosate translocation between the treated leaves and the other sections of plants at the different temperatures. There were no differences in glyphosate absorption or translocation between the susceptible population and the resistant populations suggesting that differences in absorption and translocation of the herbicide are not the mechanism of resistance in the studied populations. Studies of *EPSPS* gene copy number showed gene amplification was not the resistance mechanism either. A mutation was detected at codon 106 (proline substituted by serine) of the *EPSPS* gene of the most resistant population, A533.1, indicating the presence of target-site resistance in this population. Gene flow by pollen exchange between the glyphosate resistant

population A533.1 and the susceptible population Echi S occurred at a frequency of 1.38% when progeny from the susceptible parent was tested at 240 g a.e. ha^{-1} of glyphosate. The mutation in the *EPSPS* gene was detected in 24 F_1 progenies of this population pair. Segregation of resistance in the gene flow experiment between resistant and susceptible individuals occurred at a 3:1 resistance : susceptibility ratio in the F_2 generation indicating the trait of glyphosate resistance is a single dominant trait of *E. colona*. Sequencing the *EPSPS* cDNA of five parental and F_2 filial individuals revealed at least two *EPSPS* genes present in *E. colona*. Shikimate accumulation of the F_1 hybrid and the glyphosate response of F_2 progenies were intermediate between the two parental populations.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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