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CHARACTERIZATION OF TARGET-SITE MUTATION IN 5-ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASE (EPSPS) GENE FROM GLYPHOSATE-RESISTANT BIOTYPE OF *Eleusine indica* (L.) Gaertn FROM MALAYSIA

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The population of goosegrass (*Eleusine indica*), resistant to glyphosate (an EPSPS–inhibiting herbicide) was found widespread in several states in Malaysia. However, the resistance levels in this plants which could lead to the understanding of its resistance mechanisms are not well studied. This study was therefore carried out to investigate different levels of resistance of goosegrass to glyphosate by the identification of mutations in the EPSPS gene. Whole plant bioassay confirmed that 9 out of the 20 populations collected were resistant, six populations were susceptible, while five populations were a mixture of resistant and susceptible biotypes. Allele-specific PCR (AS-PCR) was established based on nucleotide point 319 of the EPSPS gene, considering the amino acid substitutions (Pro-106-Ser) caused by mutation (**C**CA to **T**CA) at this point that adequately confers resistance to glyphosate. AS-PCR primers (EG-F, EC-F, EA-F, ET-F) had successfully revealed the allelic composition of EPSPS gene from different biotypes of goosegrass. Screening results through AS-PCR showed that individual plants from resistant populations consisted of either homozygous TT or heterozygous CT. Meanwhile, all susceptible populations showed the presence of only homozygous CC, except population E5 with homozygous GG. The partial EPSPS gene from one susceptible (PS) and five resistant populations (P2, P4, P6, P8 and P11) with different level of resistance were

cloned and sequenced. Besides the Pro-106-Ser substitution, the sequencing of these individuals also revealed a putative new amino acid substitution of Thr-102-Ile (A**C**T to A**T**T) in all resistant populations, while another substitution of Pro-381-Leu (C**C**G to C**T**G) occurred only in P4 (21-fold), P6 (28-fold) and P11 (41-fold). However, there was no specific pattern or combination of mutations at nt-308 (Thr-102-Ile), nt-319 (Pro-106-Ser) and nt-1145 (Pro-381-Leu) that contributed to different level of resistance in the resistant populations. Statistical analysis shows that the occurrences of T-allele in EPSPS gene of glyphosate-resistant goosegrass are weakly related to the different level of resistance in glyphosate-resistant goosegrass. Therefore, it is important to consider other factors such as the non-target-site resistance mechanism alongside with the target-site mechanism, in order to further understand the occurrence of different level of resistance in glyphosate-resistant goosegrass.

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PENCIRIAN MUTASI PADA TAPAK SASARAN DALAM GEN 5-ENOLPIRUVILSHIKIMAT-3-FOSFAT SYNTHASE (EPSPS) DARIPADA BIOTIP *Eleusine indica* (L.) Gaertn YANG RINTANG TERHADAP GLIFOSAT DI MALAYSIA

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Dalam kajian ini, beberapa populasi rumput sambari (*Eleusine indica*) yang rintang terhadap glifosat (herbisid perencat enzim 5-enolpiruvil-shikimat-3-fosfat) telah didapati tersebar luas di beberapa buah negeri di Malaysia. Walau bagaimanapun, kajian terhadap tahap kerintangan berbeza yang membawa kepada kefahaman tentang mekanisma kerintangan tidak begitu diketahui. Oleh yang demikian, kajian ini dijalankan bagi mengetahui dengan lebih mendalam tentang kejadian tahap kerintangan berbeza dengan memfokuskan kepada pencirian mutasi pada tapak sasaran dalam gen EPSPS. Bioasai keseluruhan tumbuhan menunjukkan 9 daripada 20 populasi rumput sambari adalah biotip rintang, manakala 6 populasi ialah biotip rentan dan selebihnya terdiri daripada biotip campuran. Tindakan polimerase berantai alel spesifik (AS-PCR) berdasarkan titik mutasi 319 pada gen EPSPS telah dijalankan bagi mengetahui komposisi alel dalam sesebuah populasi rumput sambari. Mutasi tersebut menyebabkan penggantian asid amino Pro-106 kepada Ser (kodon **C**CA-**T**CA) yang mengakibatkan kerintangan terhadap glifosat pada kadar yang disyorkan. Kajian penyaringan melalui AS-PCR menunjukkan bahawa individu tumbuhan daripada populasi rintang mengandungi alel homozigot TT atau heterozigot CT. Bagi populasi rentan, kesemua individu memiliki alel homozigot CC, kecuali populasi rentan E5 yang mengandungi alel homozigot GG. Di samping itu, sebahagian gen EPSPS bagi satu populasi rentan (PS) dan 5 populasi rintang (P2,

P4, P6, P8 and P11) yang mempunyai aras kerintangan yang berbeza telah diklon dan dijujuk. Keputusan menunjukkan selain daripada penggantian asid amino Pro-106-Ser, satu titik mutasi putatif baru yang melibatkan penggantian asid amino Thr-102-Ile (A**C**T kepada A**T**T) telah dikesan dalam semua populasi rintang. Manakala, penggantian asid amino Pro-381-Leu (C**C**G kepada C**T**G) turut berlaku hanya dalam populasi P4, P6 dan P11. Walaupun demikian, tiada corak spesifik atau kombinasi mutasi pada titik 308 (Thr-102-Ile), 319 (Pro-106-Ser) dan 1145 (Pro-381-Leu) yang menyumbang kepada aras kerintangan yang berbeza dalam populasi rintang. Analisis statistik telah menunjukkan perkaitan yang agak rendah di antara kewujudan alel T dalam gen EPSPS kepada aras kerintangan yang berbeza dalam populasi rintang. Oleh yang demikian, faktor selain daripada gen EPSPS perlu dititikberatkan bagi memahami fenomena berlakunya aras kerintangan berbeza dalam populasi rumput sambari.