MOLECULAR STUDIES OF FLUAZIFOP RESISTANCE MECHANISMS IN GOOSEGRASS (*Eleusine indica*)

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Molecular studies of fluazifop resistance mechanisms in goosegrass (Eleusine indica) / Najihah Mohamed @ Ghazani.

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Goosegrass (*Eleusine indica*) populations resistant to fluazifop, which is an acetyl coenzyme A carboxylase (ACCase: EC 6.4.1.2) –inhibiting herbicide was found in three areas out of nine areas investigated in Malaysia. These *E. indica* populations which designated as P2, P3 and P4 biotypes respectively were originated from Kulim (Kedah), Grik (Perak) and Renggam (Johor). Based on ED_{50} values (fluazifop rate required to inhibit shoot fresh weight by 50%), the P2, P3 and P4 biotypes were 87.5-fold, 62.5-fold and 150-fold resistant than of susceptible (P1 biotype) from Taiping, Perak. Molecular analyses of plastidic acetyl coenzyme A carboxylase (ACCase) gene were conducted to determine mutation conferring resistance to fluazifop. The carboxyl-transferase (CT) domain of the plastidic ACCase was amplified by PCR and sequenced. Comparison at the amplified CT domain between the plastidic ACCase sequences of resistant and susceptible *E. indica* biotypes has revealed a nucleotide changes from G to C at target point (TP-III) of Fragment B, which cause amino acid substitution from Trp (TGG

codon) to Cys (TGC codon), corresponded to amino acid residue 2027 in *Alopecurus myosureides* plastidic ACCase (AJ310767). This non-synonymous point mutation was found in the two resistant biotypes, P2 and P3, but not in P4 implying that alteration of target enzyme ACCase conferred resistance in the P2 and P3 biotypes. Allele-specific PCR developed based on the mutation at the TP-III in Fragment B successfully discriminated between the resistant and susceptible alleles in the *E. indica* and correlated with initial sequencing results. Due to the absence of the putative mutation in ACCase gene in P4 biotype, the quantitative real-time PCR (qPCR) was performed to examine the expression of target ACCase gene in the P4 biotype associated with the fluzifop resistance. The expression level of ACCase gene was similar in the resistant (P4) and susceptible (P1) biotypes before being treated with fluazifop. At 72 hour of treatment, the ACCase gene level in the P4 biotype was significantly higher compared to the P1 biotype, indicating that the ACCase gene was relatively overexpressed. This result suggests that the resistance mechanism in the P4 biotype is associated with overproduction of the target enzyme, ACCase.

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KAJIAN MOLEKUL MENGENAI MEKANISME KERINTANGAN RUMPUT SAMBARI (*Eleusine indica*) TERHADAP FLUAZIFOP

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Populasi rumput sambari (*Eleusine indica*) yang rintang terhadap fluazifop, iaitu sejenis racun rumpai yang merencat asetil-koA karboksilase (ACCase: EC 6.4.1.2) telah ditemui di tiga kawasan daripada keseluruhan sembilan kawasan yang dikaji di Malaysia. Populasi *Eleusine indica* ini yang berasal dari Kulim (Kedah), Grik (Perak) dan Renggam (Johor) ini dilabelkan masing- masing sebagai biotip P2, P3 dan P4. Berdasarkan nilai ED_{50} (kadar fluazifop yang diperlukan untuk merencat 50% berat basah dedaun), aras kerintangan biotip P2, P3 dan P4 ialah masing-masing 87.5, 62.5 dan 150 kali lebih rintang berbanding biotip rentan (P1) yang berasal dari Taiping, Perak. Analisis molekul terhadap plastidik asetil-koA karbosilase (ACCase) dilakukan untuk menentukan mutasi yang menyebabkan kerintangan rumpai ini terhadap fluazifop. Domain karboksil transferase (CT) pada plastidik ACCase diamplikasi menggunakan tindakbalas polimerase berantai (PCR) dan seterusnya melalui proses penjujukkan. Hasil perbandingan pada domain CT yang diamplifikasi antara jujukan plastidik ACCAse bagi

biotip rintang dan rentan *E. indica* mendedahkan bahawa asid amino Trp (kodon TGG) telah ditukar kepada asid amino Cys (TGC), sejajar denga kedudukan asid amino 2027 pada *Alopecurus myosuroides* (AJ310767). Mutasi titik yang tidak sinonim ini ditemui pada biotip rintang, P2 dan P3 tetapi tidak pada biotip P4. Ini mencadangkan bahawa pengubahan pada enzim ACCase telah menyebabkan kerintangan biotip P2 dan P3 terhadap fluazifop. Alel-spesifik PCR yang dibangunkan berdasarkan mutasi pada titik TP-III (Fragmen B) berjaya membezakan antara alel rintang dan alel rentan *E. indica* dan menyokong hasil penjujukan kajian ini. Kuantitatif masa-sebenar PCR (qPCR) seterusnya dilakukan untuk mengkaji pengekspresan gen sasaran, ACCase bagi biotip P4. Aras pengekspresan gen ACCase didapati sama antara biotip rintang (P4) dan biotip rentan (P1) sebelum disembur dengan fluazifop. Selepas 72 jam rawatan, aras gen ACCase pada biotip P4 adalah lebih tinggi berbanding biotip P1, yang menunjukkan berlaku pengekspresan gen ACCase. Hasil kajian ini mencadangkan bahawa mekanisme kerintangan biotip P4 berkaitan dengan pengeluaran enzim sasaran, ACCase.