World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:11, No:08, 2017

Isoflavonoid Dynamic Variation in Red Clover Genotypes

Authors : Andrés Quiroz, Emilio Hormazábal, Ana Mutis, Fernando Ortega, Loreto Méndez, Leonardo Parra

Abstract : Red clover root borer, Hylastinus obscurus Marsham (Coleoptera: Curculionidae), is the main insect pest associated to red clover, Trifolium pratense L. An average of 1.5 H. obscurus per plant can cause 5.5% reduction in forage yield in pastures of two to three years old. Moreover, insect attack can reach 70% to 100% of the plants. To our knowledge, there is no a chemical strategy for controlling this pest. Therefore alternative strategies for controlling H. obscurus are a high priority for red clover producers. One of this alternative is related to the study of secondary metabolites involved in intrinsic chemical defenses developed by plants, such as isoflavonoids. The isoflavonoids formononetin and daidzein have elicited an antifeedant and phagostimult effect on H. obscurus respectively. However, we do not know how is the dynamic variation of these isoflavonoids under field conditions. The main objective of this work was to evaluate the variation of the antifeedant isoflavonoids formononetin, the phagostimulant isoflavonoids daidzein, and their respective glycosides over time in different ecotypes of red clover. Fourteen red clover ecotypes (8 cultivars and 6 experimental lines), were collected at INIA-Carillanca (La Araucanía, Chile). These plants were established in October 2015 under irrigated conditions. The cultivars were distributed in a randomized complete block with three replicates. The whole plants were sampled in four times: 15th October 2016, 12th December 2016, 27th January 2017 and 16th March 2017 with sufficient amount of soil to avoid root damage. A polar fraction of isoflavonoid was obtained from 20 mg of lyophilized root tissue extracted with 2 mL of 80% MeOH for 16 h using an orbital shaker in the dark at room temperature. After, an aliquot of 1.4 mL of the supernatant was evaporated, and the residue was resuspended in 300 µL of 45% MeOH. The identification and quantification of isoflavonoid root extracts were performed by the injection of 20 µL into a Shimadzu HPLC equipped with a C-18 column. The sample was eluted with a mobile phase composed of AcOH: H_2O (1:9 v/v) as solvent A and CH_3CN as solvent B. The detection was performed at 260 nm. The results showed that the amount of aglycones was higher than the respective glycosides. This result is according to the biosynthetic pathway of flavonoids, where the formation of glycoside is further to the glycosides biosynthesis. The amount of formononetin was higher than daidzein. In roots, where H. obscurus spent the most part of its live cycle, the highest content of formononetin was found in G 27, Pawera, Sabtoron High, Redqueli-INIA and Superqueli-INIA cvs. (2.1, 1.8, 1.8, 1.6 and 1.0 mg g^{-1} respectively); and the lowest amount of daidzein were found Superqueli-INIA (0.32 mg g^{-1}) and in the experimental line Sel Syn Int4 (0.24 mg g^{-1}). This ecotype showed a high content of formononetin (0.9 mg g^{-1}). This information, associated with cultural practices, could help farmers and breeders to reduce H. obscurus in grassland, selecting ecotypes with high content of formononetin and low amount of daidzein in the roots of red clover plants. Acknowledgements: FONDECYT 1141245 and 11130715.
Keywords : daidzein, formononetin, isoflavonoid glycosides, trifolium pratense
Conference Title : ICASTE 2017 : International Conference on Agricultural Science, Technology and Engineering
Conference Location : Venice, Italy
Conference Dates : August 14-15, 2017