mechanical scarification (manual and with sandpaper), chemical scarification (with H₂SO₄), cold stratification, soaking in distilled water and soaking in a gibberelic acid solution, was studied. Seeds were incubated under a 16-h light photoperiod at 25 ± 2°C. For each treatment the final germination percentage, the mean germination time (MGT) and the time to get 50% of the final germination percentage (Tₜ₀) for up to 30 days were recorded. The seed morphological features water content, size (length and width) and weight were also investigated. The final germination percentage of untreated seeds was considerably high, around 60%, demonstrating that *P. algarbiensis* seeds have good germination potential. Maximum germination percentages (100%) were obtained with manual scarification, showing that the mechanism of dormancy lies in the seed coat. On the other hand, no significant differences were observed among the germination percentages reached by seeds submitted to the other presowing treatments, including the other scarification techniques applied. Moreover, the lower MGT (2.7 days) and Tₜ₀ (1.7 days) values were also obtained with manual scarification. In conclusion, *P. algarbiensis* seeds exhibited slight physical dormancy that can be completely broken by manual scarification.

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**Proteomics Analysis of Polygonum minus Leaves**

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**Keywords:** Polygonum minus; proteomics; environmental stress; secondary metabolite

The essential oil produced by *Polygonum minus* possesses high economic value with high demands in the food, flavor and fragrance industry. The leaves of the plants grown at high altitude have been found to be less aromatic compared to those grown on low ground. Changes observed in the secondary metabolites produced by plants at high ground would be expected to be accompanied by differential expression of proteins that are involved in the metabolic pathway of these secondary metabolites. In this study, analysis of altitude responsive proteins was conducted wherein *P. minus* were obtained from high (Genting) and low (Lenggeng) land sites. Using proteomic techniques, proteins, extracted from leaves, were analyzed using two-dimensional electrophoresis and MALDI-TOF MS. At least 93 protein spots were found to be unique in the protein profiles of Genting leaves and 104 protein spots in the Lenggeng leaves. A total of 25 protein spots were found to be up-regulated in the protein profiles of Genting leaves whilst 26 protein spots were down-regulated. Of these, 16 proteins were identified, including 12 that are involved in photosynthesis including RubisCO and ATP synthase, three proteins are associated with plant response towards environmental stress including Copper-zinc superoxide dismutase and one protein with unknown function. Furthermore, majority of the proteins involved in photosynthesis were found to be up-regulated in plants from the high land hence indicating an increase in photosynthetic activity in these plants compared to the low land ones. Hence these results provide new information into our understanding on the cellular response of this aromatic plant.

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**[P-P&F.35]**

**Transient expression of dengue virus envelope protein in a plant system**

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**Keywords:** Dengue Virus Envelope Protein; Recombinant protein; Plants; Transient expression

**Introduction:** The utilization of plants as bioreactors presents several advantages related with its simplicity, biosafety and low cost. It retains the advantages of eukaryotic expression systems, as post-translational modifications, and prokaryotic expression systems, as scalability and economical production. However, long time-scale and low yield of recombinant proteins have been the main bottlenecks in plant-made biopharmaceuticals. A developed technique known as Magnifection can offset these disadvantages. This transient expression technology is based on replication of viral vectors delivered to the plant by *Agrobacterium* and allows very fast production, high recombinant protein expression levels. Dengue virus (DV) serotype 2 envelope glycoprotein (E) is the main protein associated with viral entry and the induction of immunity. In order to produce a candidate for subunit vaccines and to provide an antigen for diagnostic kits, E protein was expressed using this technology.

**Methods:** An E truncated version was designed to be expressed alone and co-expressed with DV structural proteins. As well, the critical Domain III of E protein was fused to Hepatitis B core antigen. *Nicotiana benthamiana* plants were co-infiltrated with 5′ Module, Integrase Module and three different 3′ Module carrying coding sequences for Et, CMEt and HBcore-DV2d3 proteins respectively.

**Results:** The recombinant proteins were produced successfully in *N. benthamiana* plants and were reactive with anti-E polyclonal antibody and the fusion was reactive with anti-E polyclonal and anti-HBcore antibodies.

**Discussion:** We have demonstrated that Magnifection system is suitable for the production of the recombinant DV-2 protein in *N. benthamiana* plants. This is the first report of a plant system being able to successfully produce the flaviviral DV-2 E truncated version and its co-expression with C and prM structural DV-2 proteins. Moreover, this is the first work reported in plants in which the HBcore antigen is co-expressed with the critical Domain III of DV-2 Envelope glycoprotein.

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