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A comparative UHPLC-Q/TOF–MS-based eco-metabolomics approach reveals temperature adaptation of four *Nepenthes* species

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***Nepenthes*, as the largest family of carnivorous plants, is found with an extensive geographical distribution throughout the Malay Archipelago, specifically in Borneo, Philippines, and Sumatra. Highland species are able to tolerate cold stress and lowland species heat stress. Our current understanding on the adaptation or survival mechanisms acquired by the different *Nepenthes* species to their climatic conditions at the phytochemical level is, however, limited. In this study, we applied an eco-metabolomics approach to identify temperature stressed individual metabolic fingerprints of four *Nepenthes* species: the lowlanders *N. ampullaria*, *N. rafflesiana* and *N. northiana*, and the highlander *N. minima*. We hypothesized that distinct metabolite regulation patterns exist between the *Nepenthes* species due to their adaptation towards different geographical and altitudinal distribution. Our results revealed not only distinct temperature stress induced metabolite fingerprints for each *Nepenthes* species, but also shared metabolic response and adaptation strategies. The interspecific responses and adaptation of *N. rafflesiana* and *N. northiana* likely reflected their natural habitat niches. Moreover, our study also indicates the potential of lowlanders, especially *N. ampullaria* and *N. rafflesiana*, to produce metabolites needed to deal with increased temperatures, offering hope for the plant genus and future adaption in times of changing climate.**

Nepenthes (*N.*), the sole genus under the family *Nepenthaceae*, is one of the largest families of carnivorous plants, with an extensive geographical distribution across the Malay Archipelago, specifically in Borneo, Philippines, and Sumatra. To date, 151 species have been documented, with most species displaying high degrees of endemism and often restricted to single areas, i.e. *N. villosa*, *rajah* and *burbidgeae* which can only be found in Mount Kinabalu and the neighboring Mount Tambuyukon in Borneo[1–3]. The characteristic pitcher and their adaptation to nutrient poor soils has been well documented[4–7].

Nepenthes can be clustered into two groups: lowlanders (with altitudinal distributions below 1100 m above sea level (asl)—hot and humid jungles) which can tolerate heat stress and highlanders (with altitudinal distributions beyond 1100 masl such as highland montane forests with warm days and cool to cold, humid nights) which can tolerate cold stress[2,8,9]. There are some exemptions such as *Nepenthes ampullaria* and *N. rafflesiana*, even though categorized as lowland species, both were recorded in highland environments but only very rarely[2–5]. Besides that, *N. minima* was the only highlander species able to grow well at our greenhouse under lowland conditions. Our current understanding on the adaptation or survival mechanisms acquired by the different *Nepenthes* species to their climatic conditions at the phytochemical level is, however, limited.

Heat stress has been shown to increase respiration, reduce photosynthesis, disrupt plant cellular structures and defensive mechanisms, and elevate stress metabolites production in plants[10–12]. Low temperature stress, on the other hand, can affect the photosynthesis rate of the plant thus causes the imbalance of the energy metabolism. Besides that, cellular DNA damage, physiological functions and metabolic sink disruption of plant cell were also

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