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Identification of Stress-related Proteins during the Growth and Development of *Piper nigrum* L.

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Abstract *Piper nigrum* L., also known as the pepper plant, produces pepper berries, which is a spice used worldwide. Changes in the natural environment have forced the plant to undergo acclimatization for plant growth. Consequently, un-adapted plants developed unhealthy, stunted growth, affecting the production of pepper berries, and thus, causing loss to agriculture. Proteomic analysis using two-dimensional polyacrylamide gel electrophoresis (PAGE) coupled with mass spectrometry was used to compare the differentially expressed proteins between healthy and unhealthy pepper plants. The proteins were successfully extracted from the stems and roots of *P. nigrum* and the two-dimensional protein profiles were compared. Two-dimensional analysis has indicated significant differences between the healthy and unhealthy parts with 41 and 25 differentially expressed proteins in the stems and roots respectively. A total of 10 differentially expressed proteins, characterized as unique (2) and over-expressed (8) proteins, from both plant parts were selected and sent for identification through matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry. The unique and over-expressed proteins in the unhealthy stems were identified as stress-related small heat shock proteins (sHSPs). These proteins act as a chaperone to protect other proteins against a stressful environment for plant defence and adaptation. Meanwhile, over-expressed proteins from the roots were found to be peroxidase 5, malate dehydrogenase, mitochondrial and gamma-glutamyl phosphate reductase which are involved in oxidative stress, photosynthetic activity, and plant development, respectively. The findings of the differentially expressed proteins in this study, therefore, may create opportunities for further investigations of stress management control and help to improve plantation practices in the future.

1. Introduction

Piper nigrum L., which is also known as pepper, is a woody perennial climbers native to South-Western India [1,2] and is commercially planted in Sarawak, Malaysia [3]. The berries or peppercorn of the plant are widely used as spice for food flavouring and often regarded as the King of Spice. Aside from that, peppercorn and the secondary metabolites of the plant are also used for medicinal benefits, preservatives and also act as bio-control agents [4]. Globally, South-East Asia has contributed to 60% of the black pepper production where the value of the trade reached 1.5 billion US dollars from the production of 4.6×10^5 tons in 2017 [5]. However, in Malaysia, the production of pepper has slightly decreased in recent years. Since 2010, about 24, 227 tons of pepper were produced

