Abstract

Agave amica (Medik.) Theide and Govaerts (syn. *Polianthes tuberosa* L.) or commonly known as tuberose from the family Asparagaceae is a well-known plant for its fragrant flowers. Tuberose are of demand for their post-harvest utility as cut flowers. Two most common cultivars of tuberose, Calcutta single (CS) and Calcutta double (CD) have been used for the present study to explore the properties of the cuticular wax and its isolation for possible industrial uses. For a detailed analysis of tuberose cuticular wax, the flowers were subjected to basic analysis using gravimetric and colorimetric procedures to detect the presence of cuticular wax in the flowers. This was followed by qualitative analysis using gas chromatography-mass spectrometry (GC-MS) to confirm the presence of major classes of compounds. Based on this data, a detailed analysis of the cuticular material was designed incorporating the analyses of the total epicuticular, total intracuticular, total wax, and surface specific epicuticular wax. For understanding the hydrophobicity induced by the epicuticular wax, contact angle measurement was carried out. From the GC-MS analysis of the total epicuticular and intracuticular wax, it was found that epicuticular wax was more diverse in nature than intracuticular wax. In the total wax analysis of tuberose, it was found that CD contained a much higher wax load than CS. In surface specific analysis, it was observed most of the higher wax load of CD is actually contributed by the outer whorl. Using gum Arabic impression, a detailed analysis of the abaxial and adaxial surface from both cultivars were carried out, which showed that the adaxial side contains higher wax load than the abaxial side, which also explained the higher contact angle and hydrophobicity shown by the adaxial side than abaxial also. Microscopic examination of the tuberose cuticle was carried out using epifluorescence, bright field and scanning electron microscopy (SEM). In epifluorescence microscopy, lipid droplets have been observed in real time, forming a larger lipid droplet by fusion of small droplets and ultimately moving towards the plasma membrane in the epidermal cells of CD outer whorl. An efficient and cost-effective method for SEM sample preparation for observing cuticle has also been developed. SEM analysis revealed surface microstructural difference between the two whorls, CS was found to contain more crystalloid deposition of wax whereas, CD deposited the wax in the form of cuticular nanoridges. The stomatal nature also confirmed the pattern of wax deposition. In the bud stage, stomata from both CS and CD showed deposition of epicuticular wax materials in the stomatal aperture, in the mature stage stomatal behaviour changed to scent emitting stage in CS and CD inner whorl, but the out whorl stomata remained as sunken type loaded with waxy deposition. Based on the stomatal behaviour, GC-MS data, and SEM analysis an inter-relationship between scent emission and cuticle biosynthetic pathway has been proposed. Besides, floral concrete and absolute from CS and CD have been isolated, and their major chemical composition was revealed through GC-MS analysis. The floral concrete was tested for its capacities to inhibit several in vitro enzyme activities, and to scavenge free-radical including DNA nicking properties to check its potential to be used as a skin-care product. The photoperiod and temperature treatment of tuberose cut flowers showed increase in fatty acid esters upon exposure to continuous light and increase in wax load upon low-temperature exposure.

Keywords: Cuticular nanoridges; Fatty acids; Floral concrete; Floral cuticle; GC-MS; Histochemistry; *Polianthes tuberosa*; SEM