Abstract

Tuberose or *Polianthes tuberosa* L. (Calcutta single) is a horticultural crop of tropical origin, widely cultivated for its adorable and intense floral fragrance emitted at night. Scant information exists on chemical diversity of floral scent volatiles. Chemical diversity of floral volatilome in *P. tuberosa* has been elucidated in the present study leading to identification of around 57 emitted volatile organic compounds. Altogether 19 different adsorbent/solvent combinations were used for determination of total emitted volatilome from un-plucked as well as plucked flowers using headspace and gas chromatography–mass spectrometry platform. A mixture of porous polymers (such as, Porapak^{TM}Q or Tenax$^{®}$TA) with carbotrap could serve as an unsurpassed trapping matrix followed by solvent desorption (with either hexane or DCM) for volatiles collection. Distinct three major classes of volatile organic compounds were categorized viz. aromatics, terpenes, and fatty acid derivatives. Maximum diversity along with higher quantity was found with the aromatic group. A few of them could be used as floral biomarkers because of their exclusive presence in tuberose, amongst the previously studied Amaryllidaceae flowers. Total chemical divergence of volatilome from plucked and un-plucked flowers, when compared, were found almost same except a significant rise of a few volatiles and a handful of new minor volatile components detected in the volatilome of the plucked flowers.

Further investigations have been made on the physiological and cell biological aspects of floral scent biosynthesis, tissue localization and emission, which was not previously examined. Volatiles collected from floral headspace were analysed through GC-MS for identification of individual compounds and elucidation of emission patterns. Transcripts accumulation and the amount of active enzyme were measured to throw light on scent volatiles biosynthesis. Localization of scent volatiles was investigated by histochemical and ultrastructural studies. Scent emission was found rhythmic and nocturnal under normal day-night influence, peaking at night. Enhanced enzyme activities and transcripts accumulation were recorded just prior to maximum emission. Through SEM analysis, presence of large number of floral stomata in the adaxial surface of tepal was revealed which might have bearing on tissue-specific emission. Guard cells of stomata responded significantly to histochemical tests which also indicated that epidermal tissues are mostly involved in scent emission. High metabolic activity was found in epidermal layers during anthesis as shown from TEM analysis. Further, a new insight into the scent compounds localization, plausible tissue involved in their release along with the preceding ultrastructural changes at cellular levels have been presented. Finally, ultrastructural analysis of tepal surface has been able to fill up a major gap in knowledge of stomatal involvement during scent emission.

Keywords: Volatiles, Rhythmic emission, *Polianthes tuberosa*, Benzenoids, Metabolic pathway, Gene expression, Floral stomata.