

10. Kamlekar, R. K. and Swamy, M. J., Molecular packing and inter-molecular interactions in two structural polymorphs of *N*-palmitoylethanolamine, a type-2 cannabinoid receptor agonist. *J. Lipid Res.*, 2006, **47**, 1424–1433.
11. Swamy, M. J., Ramakrishnan, M., Marsh, D. and Würz, U., Miscibility and phase behaviour of binary mixtures of *N*-palmitoylethanolamine and dipalmitoylphosphatidylcholine. *Biochim. Biophys. Acta*, 2003, **1616**, 174–183.
12. Kamlekar, R. K., Satyanarayana, S., Marsh, D. and Swamy, M. J., Miscibility and phase behaviour of *N*-acylethanolamine/diacylphosphatidylethanolamines binary mixtures of matched acyl chain lengths ($n = 14, 16$). *Biophys. J.*, 2007, **92**, 3968–3977.
13. Ramakrishnan, M., Kenoth, R., Kamlekar, R. K., Chandra, M. S., Radhakrishnan, T. P. and Swamy, M. J., *N*-Myristoylethanolamine-cholesterol (1 : 1) complex: First evidence from differential scanning calorimetry, fast-atom-bombardment mass spectrometry and computational modelling. *FEBS Lett.*, 2002, **531**, 343–347.
14. Feingold, L., *Cholesterol in Membrane Models*, CRC Press, Ann Arbor, MI, USA, 1993.
15. Ohvo-Rekilä, H., Ramstedt, B., Leppimäki, P. and Slotte, J. P., Cholesterol interactions with phospholipids in membranes. *Prog. Lipid Res.*, 2002, **41**, 66–97.
16. McMullen, T. P. W., Wong, B. C.-M., Tham, E. L., Lewis, R. N. A. H. and McElhaney, R. N., Differential scanning calorimetric study of the interaction of cholesterol with the major lipids of the *Acholeplasma laidlawii* B membrane. *Biochemistry*, 1996, **35**, 16789–16798.
17. Nakahara, H., Nakamura, S., Nakamura, K., Inagaki, M., Aso, M., Higuchi, R. and Shibata, O., Cerebroside Langmuir monolayers originated from the echinoderms: II. Binary systems of cerebroside and steroids. *Colloids Surf. B*, 2005, **42**, 175–185.
18. Zhao, X., Li, X.-M., Momsen, M. M., Brockman, H. and Brown, R. H., Lactosylceramide: Lateral interactions with cholesterol. *Biophys. J.*, 2006, **91**, 2490–2500.
19. Brown, D. A. and Rose, J. K., Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell*, 1992, **68**, 533–544.
20. Brown, D., Structure and function of membrane rafts. *Int. J. Med. Microbiol.*, 2002, **291**, 433–437.
21. Brown, D. A. and London, E., Functions of lipid rafts in biological membranes. *Annu. Rev. Cell Dev. Biol.*, 1998, **14**, 111–136.
22. Simons, K. and Ikonen, E., Functional rafts in cell membranes. *Nature*, 1997, **387**, 569–572.
23. Sheets, E. D., Holowka, D. and Baird, B., Membrane organization in immunoglobulin E receptor signalling. *Curr. Opin. Chem. Biol.*, 1999, **3**, 95–99.
24. Radhakrishnan, A. and McConnell, H. M., Cholesterol-phospholipid complexes in membranes. *J. Am. Chem. Soc.*, 1999, **121**, 486–487.
25. Radhakrishnan, A. and McConnell, H. M., Condensed complexes of cholesterol and phospholipids. *Biophys. J.*, 1999, **77**, 1507–1517; Erratum, *Biophys. J.*, 2001, **80**, 2037.
26. Radhakrishnan, A., Li, X.-M., Brown, R. E. and McConnell, H. M., Stoichiometry of cholesterol-sphingomyelin condensed complexes in monolayers. *Biochim. Biophys. Acta*, 2001, **1511**, 1–6.
27. Swamy, M. J., Ramakrishnan, M., Angerstein, B. and Marsh, D., Spin-label electron spin resonance studies on the mode of anchoring and vertical location of the *N*-acyl chain in *N*-acylphosphatidylethanolamines. *Biochemistry*, 2000, **39**, 12476–12484.
28. Li, X.-M., Ramakrishnan, M., Brockman, H. L., Brown, R. E. and Swamy, M. J., *N*-Myristoylated phosphatidylethanolamine: Interfacial behavior and interaction with cholesterol. *Langmuir*, 2002, **18**, 231–238.
29. Radhakrishnan, A. and McConnell, H. M., Chemical activity of cholesterol in membranes. *Biochemistry*, 2000, **39**, 8119–8124.
30. Radhakrishnan, A., Anderson, T. G. and McConnell, H. M., Condensed complexes, rafts, and the chemical activity of cholesterol

in membranes. *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 12422–12427.

ACKNOWLEDGEMENTS. This work was supported by a research grant from the Department of Science and Technology, New Delhi to M.J.S. M.R., P.K.T. and R.K.K. were supported by Senior Research Fellowships from CSIR, New Delhi. We thank the University Grants Commission, New Delhi for support through the UPE and CAS programmes, to the University of Hyderabad and School of Chemistry respectively.

Received 1 November 2006; revised accepted 13 March 2007

Studies on lower epidermal papillae, the site of storage of basmati rice aroma compounds in *Pandanus amaryllifolius* Roxb.

Kantilal V. Wakte¹, Altafhusain B. Nadaf^{1,*}, Sellappan Krishnan² and Ratnakar J. Thengane¹

¹Department of Botany, University of Pune, Pune 411 007, India

²Department of Botany, Goa University, Goa 403 206, India

***Pandanus amaryllifolius* Roxb. is the only species belonging to the family Pandanaceae that has fragrant leaves. In the higher plants, aroma compounds in leaves are stored in vacuoles and epidermal outgrowths like papillae, glandular hairs and trichomes. The lower epidermis of *P. amaryllifolius* has papillae as protrusions of lower epidermal cells. The papillae run parallel along the leaf length and are absent over the veins and midrib. The number of papillae varied from one to seven per cell. Papillae were also found surrounding the stomata forming a necklace-like structure. Quantitative analysis yielded 3.10 mg of 2-acetyl-1-pyrroline per kg of fresh leaves. Cell size, area and number of papillae were more in the clone of 'Sawantwadi' than in 'Pune'.**

Keywords: 2-Acetyl-1-pyrroline, basmati aroma, lower epidermal papillae, *Pandanus amaryllifolius* Roxb.

THE genus *Pandanus*, family Pandanaceae comprises approximately 600 species that are widely distributed in tropical and subtropical regions¹. Thirty-six species of *Pandanus* have been recorded in India, among which *P. odoratissimus* Linn. and *P. amaryllifolius* Roxb. are being exploited commercially by the flavour industry. In *P. odoratissimus* the flowers are scented, while in *P. amaryllifolius* the leaves are scented^{2,3}. *P. amaryllifolius* Roxb. is a native of the Philippines and Thailand⁴. It was introduced⁵ into India from Indonesia through the Botanical Garden at Kolkata in 1798. The principal aroma compound, 2-acetyl-1-pyrroline (2AP) is ten times higher in this

*For correspondence. (e-mail: abnadaf@unipune.ernet.in)

plant than in basmati rice⁶. Throughout Southeast Asia it is used in cooking to impart flavour and colour to rice, sweets, jellies, and in many other food products. It is widely used to flavour ordinary rice as a substitute for the expensive aromatic rice varieties^{7,8}. In non-tropical countries, it is difficult to get fresh *P. amaryllifolius* leaves. Hence the essence or paste of *P. amaryllifolius* is used as a substitute. In addition to aroma, *P. amaryllifolius* is also potentially valuable as a candidate for new medicinal principles^{9,10}.

Plants synthesize diverse forms of secondary products and store them in specialized organs such as vacuoles, glandular trichomes, non-glandular trichomes, hairs and papillae¹¹. Papillae are protrusions of epidermal cells, which give a velvety appearance to the plant's surface. Typical examples are the papillae of pansy flower (*Viola tricolor*) and the leaf surfaces of many species from rain-forests¹². *P. amaryllifolius* has epidermal papillae on the abaxial surface¹³. 2AP was reported as the principle aroma compound in *P. amaryllifolius*⁶. It was identified for the first time as the principle aroma compound in cooked rice by Buttery *et al.*¹⁴. Other than scented rices, 2AP has a wide range of occurrence, viz. in the leaves and flowers of *Vallis glabra*¹⁵, the crust of wheat and rye bread¹⁶, urine of tiger¹⁷, pearl millet¹⁸, popcorn¹⁹, *Bacillus cereus* strains isolated from cocoa fermentation boxes²⁰, *Bassia latifolia*^{21,22}, cooked meat²³, honey²⁴, etc. In an earlier study we have histochemically localized papillae as the site of storage for 2AP in *P. amaryllifolius*²⁵. In the present attempt, a detailed study of these papillae and quantitation of 2AP in *P. amaryllifolius* has been made. In addition, interpopulation anatomical variation among the clones grown at two different environmental conditions has also been studied.

Clones of *P. amaryllifolius* Roxb. were obtained from local nurseries at Pune and Sawantwadi, Maharashtra, India. Identity of the species was confirmed with the help of the literature, experts from botanical gardens and herbaria. The seedlings of this species were grown in pots in the botanical garden of the Department of Botany, University of Pune, Pune.

Leaf epidermal peels (adaxial and abaxial) of *P. amaryllifolius* were taken by scraping out the epidermis and observed under a compound microscope. The abaxial surface showed the presence of papillae. Measurements pertaining to cell size, number of papillae per cell and diameter of papillae were recorded using micrometre scale in the clones of Pune and Sawantwadi. The average values for these parameters were calculated by taking measurements of five random field views under compound microscope (42,500 sq. µm area of each field) and statistically analysed for standard error, standard error of mean and the test of significance using MSTAT-C software (version 1.42). The lower epidermal peels were taken and photographed under compound microscope (Olympus BX 40, Japan) at various magnifications using photographic attachments. In addition, transverse hand-cut sections of leaf were also taken and

photographed. The lower epidermal papillae observed in *P. amaryllifolius* were taken further for scanning electron microscopic studies. For this, clean leaf pieces of 1 sq. cm size were made and fixed in 2.5% glutaraldehyde fixative prepared in phosphate base saline (PBS). The leaf material was dehydrated by passing through grades of ethanol from 10 to 100% by leaving the material in each grade for 60 min. The material was further dried and mounted on stubs and coated with platinum in an auto fine coater (JEOL JFC-1600, Japan). The thickness of the coat was 30 µm. The leaf surface was scanned using analytical scanning electron microscope (JEOL JSM-6360A, Japan) at different magnifications ranging from 400 to 10,000×.

2AP from the Pune clone was extracted under reduced pressure using steam distillation unit. Five millilitres of 30 ppm collidine (2,4,6-trimethylpyridine, Fluka Chemicals; 98% pure) was used as an internal standard. The distillate was made alkaline (pH 8) by adding sodium bicarbonate (15 g) and the volatiles were taken in diethyl ether. Total volume of ether was dried over 40 g sodium sulphate and condensed using Rota vapour (Equitron roteva, India) by keeping 80 rotations per minute. Next, 1 µl of the concentrate was injected to a Gas Chromatography and Mass Spectrophotometer (GC-MS, QP 5050A, Shimadzu, Japan) in split mode having DB-5 column (length 30 m, diameter 0.25 µm) and QP detector. Oven temperature was kept at 50°C for 2 min and then increased from 50 to 170°C at 7°C per min and was held at 170°C for 5 min. The peak of 2AP was identified by matching its mass spectrum with that of standard 2AP from the published literature. Quantitation of 2AP was done using the following formula based on the ratio of peak areas of 2AP and collidine^{20,26}.

$$2AP \text{ amount } (\mu\text{g/kg}) = ((A/B)(111/121)C)/D,$$

where *A* and *B* represent the peak areas for 2AP and collidine respectively, 111 and 121 represent the molecular weights of 2AP and collidine respectively, *C* represents the amount of internal standard (in µg), and *D* is the sample weight (in kg). Since the standard 2AP was not available, the relative responses for 2AP and collidine were assumed to be equivalent to their molecular weight ratios.

P. amaryllifolius showed epidermal papillae on the lower epidermis (Figure 1 b–f). The presence of papillae makes the lower epidermal surface velvety in contrast to the upper epidermal surface. The papillae were found distributed parallel to the leaf length and were found missing over the veins and midrib. The number of papillae varied from one to seven per cell. SEM studies clearly revealed that the papillae were protrusions of the lower epidermis (Figure 1 d–f). Interestingly, papillae were also found surrounding guard cells of stomata in the lower epidermis, giving a necklace-like appearance (Figure 1 g). On an average four to eight papillae were found surrounding the guard cells and two longer papillae protruded over the

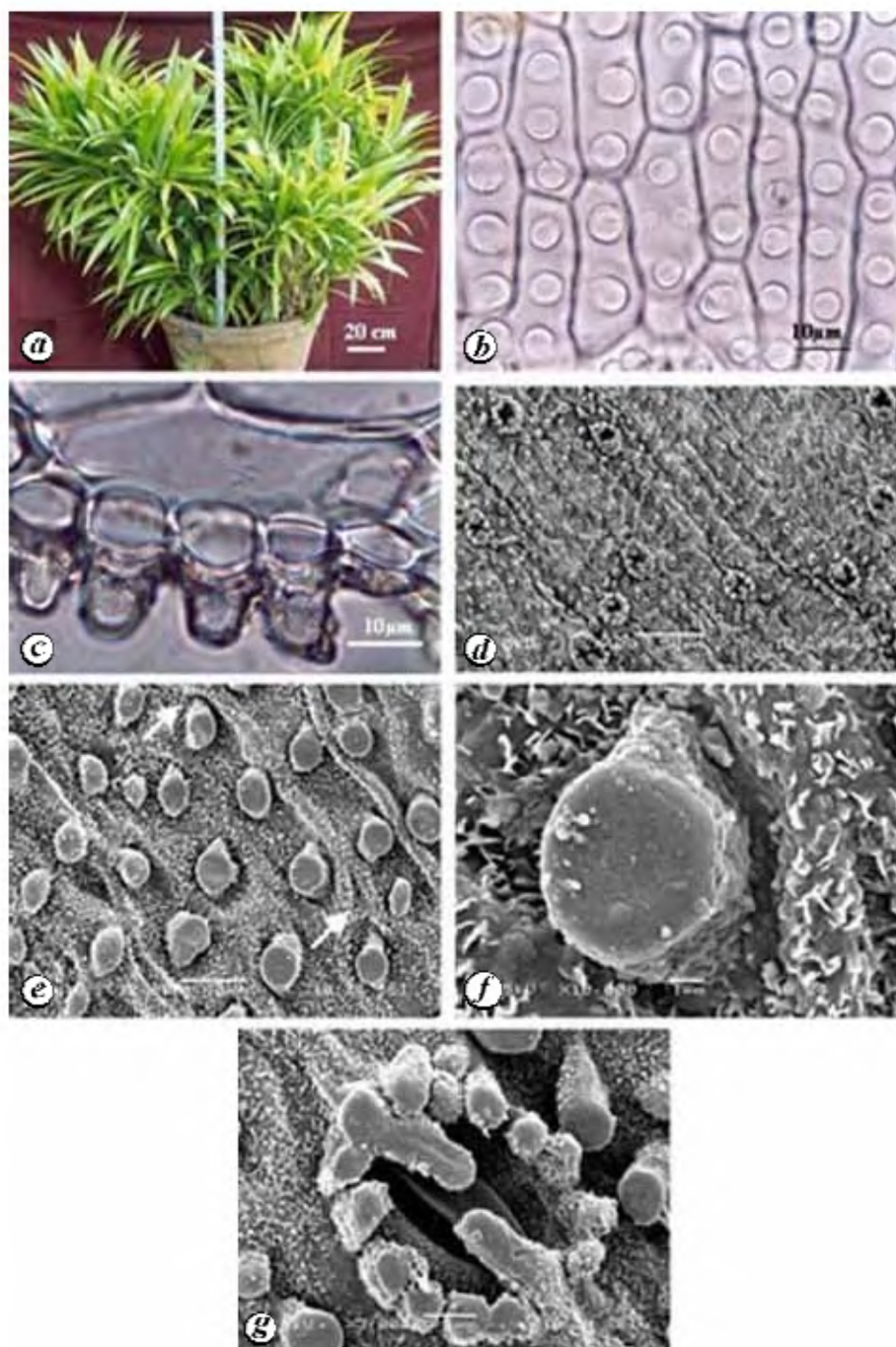


Figure 1. Epidermal papillae in *Pandanus amaryllifolius* Roxb. on the lower leaf epidermis. **a**, Seedlings of *P. amaryllifolius*. **b**, Lower epidermal peel showing papillae under compound microscope (330 \times). **c**, Papillae in transverse section (500 \times). **d**, SEM of lower epidermal papillae (400 \times). **e**, Magnified SEM view; single cell marked by arrows showing three papillae (2000 \times). **f, g**, SEM of individual papilla (10,000 \times) (**f**) and papillae around stoma (3000 \times) forming necklace-like structure (**g**).

stomatal pore. We observed that the development of these epidermal papillae in *P. amaryllifolius* begins at very early stages of leaf development; when leaves are tightly closed in the leaf apex and are shaded-off at the senescent stage.

GC-MS analysis of extracted volatiles by steam distillation recovered 3.10 mg/kg of 2AP (Figure 2).

A comparative study of papillae in the Pune and Sawantwadi clones of *P. amaryllifolius* revealed significant dif-

ferences in length, breadth and area of lower epidermal cells (Table 1). The cell length was found to be inversely proportional to cell breadth, while the number of papillae per cell was also inversely proportional to the diameter of the papillae. The number of papillae per cell was one to five in the Pune clone, as against one to six in the Sawantwadi clone. Overall number of papillae per cell was more in the Sawantwadi clone than in the Pune clone.

Table 1. Lower epidermal analysis of two *P. amaryllifolius* clones

Locality	No. of papilla(e) per cell	Cell length** (μm)	Cell breadth* (μm)	Cell area* (sq. μm)	Diameter of each papilla ^{ns} (μm)	Area of each papilla ^{ns} (sq. μm)	Area occupied by the papilla(e) per cell ^{ns} (sq. μm)
Pune	1	14.332 ± 1.65	12.166 ± 0.50	174.166 ± 31.21	7.732 ± 0.18	47.138 ± 2.29	47.138 ± 5.43
	2	23.252 ± 1.65	12.462 ± 0.50	289.922 ± 31.21	7.126 ± 0.18	38.562 ± 2.29	81.130 ± 5.43
	3	34.835 ± 1.65	11.674 ± 0.50	406.354 ± 31.21	7.044 ± 0.18	40.088 ± 2.29	120.274 ± 5.43
	4	42.664 ± 1.65	12.002 ± 0.50	502.410 ± 31.21	6.804 ± 0.18	37.400 ± 2.29	149.612 ± 5.43
	5	58.224 ± 1.65	10.854 ± 0.50	632.740 ± 31.21	6.860 ± 0.18	37.144 ± 2.29	186.340 ± 5.43
Average		34.665 ± 3.18	11.832 ± 0.23	401.118 ± 35.03	7.113 ± 0.09	40.066 ± 1.19	116.899 ± 10.26
Sawantwadi	1	19.000 ± 3.64	15.000 ± 0.59	285.000 ± 38.29	7.400 ± 0.52	45.410 ± 6.29	45.410 ± 13.97
	2	31.976 ± 3.64	14.230 ± 0.59	450.680 ± 38.29	7.492 ± 0.52	45.336 ± 6.29	90.923 ± 13.97
	3	42.324 ± 3.64	13.472 ± 0.59	558.922 ± 38.29	7.086 ± 0.52	40.108 ± 6.29	120.362 ± 13.97
	4	56.050 ± 3.64	13.120 ± 0.59	724.916 ± 38.29	6.940 ± 0.52	38.598 ± 6.29	154.400 ± 13.97
	5	71.872 ± 3.64	11.910 ± 0.59	832.606 ± 38.29	6.650 ± 0.52	35.391 ± 6.29	176.876 ± 13.97
Average		44.244 ± 4.04	13.546 ± 0.32	570.425 ± 42.61	7.114 ± 0.22	40.969 ± 2.69	117.594 ± 11.07

Data are shown as mean ± standard error; **P* < 0.01%; ***P* < 0.05%; ^{ns}Non-significant.

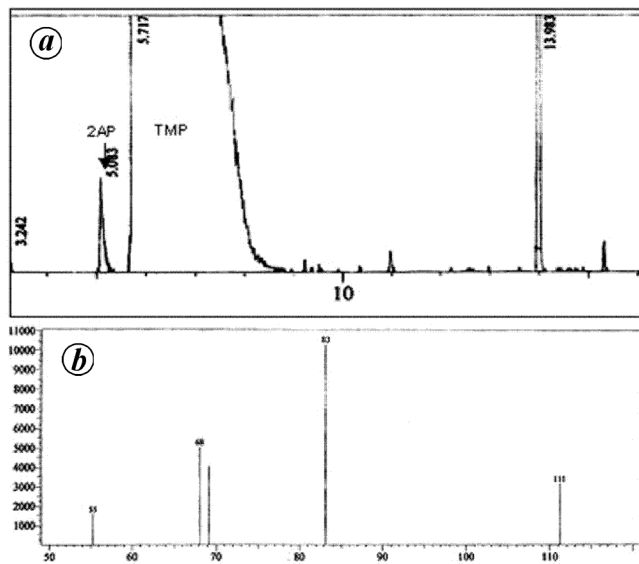


Figure 2. Gas chromatography and mass spectrophotometry analysis of volatiles in *P. amaryllifolius*. **a**, Gas chromatograph of extracted volatiles by steam distillation showing peaks of 2AP and TMP (2,4,6-trimethylpyridine; collidine). **b**, Mass spectrum of 2AP.

Papillae are small thickenings of the cuticle, which may be hollow or solid. They occur on normal epidermal cells as well as on cells encircling the stomatal complex. In such cases the papillae may partly cover the stomatal pores. In other cases they are restricted to the latter²⁷. On the basis of SEM studies of lower epidermal papillae in *P. amaryllifolius*, Stone²⁸ confirmed that 'large' and 'small' forms of this species grown in Malaysia and other countries are one and same. Our observations of epidermal papillae are in conformity with those of Stone. Kam¹³ has studied comparative systematic foliar anatomy of Malayan *Pandanus* species. Interestingly, he has reported the presence of abaxial papillae in several species of *Pandanus*. However, none of them has aroma in leaves. The presence of pleasant aroma in the leaf was confirmed in our labora-

tory through histochemical test²⁵. In this study, the histochemical test clearly revealed the presence of principle basmati aroma compound 2AP in the papillae. Pichersky *et al.*²⁹ reported that in vegetative organs, plant volatiles might be synthesized in surface glandular trichomes and then secreted by the cells and stored in a sac created by the extension of the cuticle. Thus the papillae of *P. amaryllifolius* are taken as the sites of storage for the aroma compounds. However, in basmati rice, no such extracellular structures are developed but are stored internally. The volatile aroma compounds serve multiple functions in both floral and vegetative organs, and these roles are not always related to their volatility³⁰. Some monoterpenes have been implicated as allelopathic agents, and they often directly or indirectly protect plants from herbivores and pathogens^{31–35}. Brahmachary²² reported the utility of 2AP as marking fluid (pheromone) of the tiger and also suggested its probable role in antifungal activity. However, Buttery *et al.*¹⁴ who have reported this compound for first time, correlate the presence of aroma in rice with pest insect attack. The presence of papillae can also be taken as a key character in distinguishing *P. amaryllifolius* from other *Pandanus* species.

Buttery *et al.*⁶ reported higher quantities of 2AP in *P. amaryllifolius*, of the order of 1 ppm; more than ten times that found in milled rice and 100 times that found in common rice. Our analysis yielded 3.10 mg/kg (3.10 ppm) 2AP, and thus is in agreement with this and other reports¹⁵.

Interpopulation studies clearly revealed that cell size and area and number of papillae vary among the clones of *P. amaryllifolius*. Pérez-Estrada *et al.*³⁶ observed that leaf trichomes of *Wigandia urens* show environmentally induced variation in terms of type and frequency. The clone collected from Sawantwadi is near the coastal region (Arabian Sea) with humid climate, whereas the clone from Pune is away from the coastal region with dry climate. *P. amaryllifolius* is a natural inhabitant of moist tropical islands². In India also, it is commonly found along coastal

regions⁵. Therefore, under similar conditions like Sawantwadi, it shows favourable growth in terms of the above-mentioned parameters. The quantity and quality of aroma compounds in general and 2AP in particular, may also vary among the populations as reported³⁷ in scented rice variety Khao Dawk Mali 105. This aspect is under study. Thus, the site of storage for 2AP and other aroma compounds is in the lower epidermal papillae. However, its site of synthesis is unknown. It may be either the epidermal cell or the papilla itself.

1. Takayama, H., Ichikawa, T., Kitajima, M., Nonato, M. G. and Aimi, N., Isolation and structure elucidation of two new alkaloids, pandamarilactonine-C and -D, from *Pandanus amaryllifolius* and revision of relative stereochemistry of pandamarilactonine-A and -B by total synthesis. *Chem. Pharm. Bull.*, 2002, **50**, 1303–1304.
2. Bhattacharjee, P., Kshirsagar, A. and Singhal, R. S., Supercritical carbon dioxide extraction of 2-acetyl-1-pyrroline from *Pandanus amaryllifolius* Roxb. *Food Chem.*, 2005, **91**, 255–259.
3. Zaheer, S. H., Prasad, B., Chopra, R. N., Krishnan, M. S., Santapau, R. H. and Deshaprabhu, S. B., *The Wealth of India: Raw Materials*, Publications and Information Directorate, CSIR, New Delhi, 1966, vol. 7, pp. 216–221.
4. Takayama, H., Ichikawa, T., Kitajima, M., Aimi, N., Lopez, D. and Nonato, M. G., A new alkaloid, pandanamine; finding of an anticipated biogenetic intermediate in *Pandanus amaryllifolius*. *Tetrahedron Lett.*, 2001, **42**, 2995–2996.
5. Vartak, V. D., Note on Ambemohar-pat (*Pandanus amaryllifolius* Roxb.) from western India. *J. Bombay Nat. Hist. Soc.*, 1981, **78**, 196–198.
6. Buttery, R. G., Juliano, B. O. and Ling, L. C., Identification of rice aroma compound 2-acetyl-1-pyrroline in pandan leaves. *Chem. Ind. (London)*, 1983, **23**, 478.
7. Ravindran, P. N. and Balachandran, I., Under utilized medicinal spices – III. *Spice India*, 2005, **18**, 16–24.
8. Samy, J., Sugumaran, M. and Lee, K. L. W., *Herbs of Malaysia: An Introduction to the Medicinal, Culinary, Aromatic and Cosmetic use of Herbs*, Federal Publication, Malaysia, Times edition, 2005, pp. 180–181.
9. Linda, O. S. M., Samuel, S. S. M. and Vincent, O. E. C., Purification and characterization of a new antiviral protein from the leaves of *Pandanus amaryllifolius* (Pandanaceae). *Int. J. Biochem. Cell Biol.*, 2004, **36**, 1440–1446.
10. Salim, A. A., Garson, M. J. and Craik, D. J., New alkaloids from *Pandanus amaryllifolius*. *J. Nat. Prod.*, 2004, **67**, 54–57.
11. Fahn, A., *Secretory Tissues in Plants*, Academic Press, New York, 1979, pp. 162–164.
12. Bergfeld, A., Bergmann, R. and Sengbusch, P. V., Botany online – The internet hypertextbook; Cell types of the epidermis, 2002 (<http://www.biologie.uni-hamburg.de/b-online/e05/05a.html/>).
13. Kam, Y. K., Morphological studies in Pandanaceae III. Comparative systematic foliar anatomy of Malayan *Pandanus*. *Bot. J. Linn. Soc.*, 1971, **64**, 315–351.
14. Buttery, R. G., Ling, L. C. and Juliano, B. O., 2-Acetyl-1-pyrroline: An important aroma component of cooked rice. *Chem. Ind. (London)*, 1982, **24**, 958–959.
15. Wongpornchai, S., Sriseadka, T. and Choonvisase, S., Identification and quantitation of the rice aroma compound, 2-acetyl-1-pyrroline, in bread flowers (*Vallis glabra* Ktze). *J. Agric. Food Chem.*, 2003, **51**, 457–462.
16. Schieberle, P. and Grosch, W., Identification of the volatile flavour compounds of wheat bread crust comparison of rye bread crust. *Z. Lebensm.-Unters.-Forsch.*, 1985, **180**, 474–478.
17. Brahmachary, R. L., Sarkar, M. P. and Dutta, J., The aroma of rice ... and tiger. *Nature*, 1990, **344**, 26.
18. Seitz, L. M., Wright, R. L., Waniska, R. D. and Rooney, L. W., Contribution of 2-acetyl-1-pyrroline to odors from wetted ground pearl millet. *J. Agric. Food Chem.*, 1993, **41**, 955–958.
19. Schieberle, P. and Grosch, W., Potent odorants of the wheat bread crumb; differences from the crust and the effect of a longer dough fermentation. *Z. Lebensm.-Unters.-Forsch.*, 1991, **192**, 130–135.
20. Romanczyk Jr, L. J., McClelland, C. A., Post, L. S. and Aitken, W. M., Formation of 2-acetyl-1-pyrroline by several *Bacillus cereus* strains isolated from cocoa fermentation boxes. *J. Agric. Food Chem.*, 1995, **43**, 469–475.
21. Midya, S. and Brahmachary, R. L., The aroma of *Bassia* flower. *Curr. Sci.*, 1996, **71**, 430.
22. Brahmachary, R. L., The expanding world of 2-acetyl-1-pyrroline. *Curr. Sci.*, 1996, **71**, 257–258.
23. Gasser, U. and Grosch, W., Identification of volatile flavour compounds with high aroma values from cooked beef. *Z. Lebensm.-Unters.-Forsch.*, 1988, **186**, 489–494.
24. Blank, I. and Fischer, K. H., Intensive neutral odorants of linden honey. Differences from honeys of other botanical origin. *Z. Lebensm.-Unters.-Forsch.*, 1989, **189**, 426–433.
25. Nadaf, A. B., Krishnan, S. and Wakte, K. V., Histochemical and biochemical analysis of major aroma compound (2-acetyl-1-pyrroline) in basmati and other scented rice (*Oryza sativa* L.). *Curr. Sci.*, 2006, **91**, 1533–1536.
26. Buttery, R. G., Ling, L. C., Juliano, B. O. and Mon, T. R., Quantitative analysis of 2-acetyl-1-pyrroline in rice. *J. Agric. Food Chem.*, 1986, **34**, 112–114.
27. Anon., Plant cuticles and some of their applications in paleobotany, 15 August 2006; <http://www.uni-muenster.de/Geo-Palaeontologie/Palaeo/Palbot/cuticles.html>
28. Stone, B. C., Studies in Malaesian Pandanaceae XVII. On the taxonomy of 'Pandan Wangi' – A *Pandanus* cultivar with scented leaves. *Econ. Bot.*, 1979, **32**, 285–293.
29. Pichersky, E., Joseph, P. N. and Dudareva, N., Biosynthesis of plant volatiles: Nature's diversity and ingenuity. *Science*, 2006, **311**, 808–811.
30. Pichersky, E. and Gershenzon, J., The formation and function of plant volatiles: Perfumes for pollinator attraction and defence. *Curr. Opin. Plant Biol.*, 2002, **5**, 237–243.
31. Pickett, J. A., Lower terpenoids as natural insect control agents. In *Ecology, Chemistry and Biochemistry of Plant Terpenoids* (eds Harborne, J. B. and Tomas-Barberan, F. A.), Clarendon Press, Oxford, 1991, pp. 297–313.
32. Harborne, J. B., Recent advances in the ecological chemistry of plant terpenoids. In *Ecology, Chemistry and Biochemistry of Plant Terpenoids* (eds Harborne, J. B. and Tomas-Barberan, F. A.), Clarendon Press, Oxford, 1991, pp. 399–426.
33. Langenheim, J. H., Higher plant terpenoids: A phyto-centric overview of their ecological roles. *J. Chem. Ecol.*, 1994, **20**, 1223–1280.
34. Wise, M. L. and Croteau, R., Biosynthesis of monoterpenes. In *Comprehensive Natural Products Chemistry: Isoprenoids including Carotenoids and Steroids* (ed. Cane, D. E.), Elsevier, Oxford, 1999, vol. 2, pp. 97–153.
35. Hallahan, D. L., Monoterpenoid biosynthesis in glandular trichomes of labiate plants. *Adv. Bot. Res.*, 2000, **31**, 77–120.
36. Pérez-Estrada, L. B., Canto-Santan, Z. and Oyama, K., Variation in leaf trichomes of *Wigandia urens*: Environmental factors and physiological consequence. *Tree Physiol.*, 2000, **20**, 629–632.
37. Yoshihashi, T., Nguyen, T. T. H. and Kabaki, N., Area dependency of 2-acetyl-1-pyrroline content in an aromatic rice variety, Khao Dawk Mali 105. *Jpn. Agric. Res. Quart.*, 2004, **38**, 105–109.

ACKNOWLEDGEMENT. We gratefully acknowledge the funds received from University of Pune, Pune for this work.

Received 19 September 2006; revised accepted 21 March 2007