Anatomical and Ultrastructural Changes in Lotus
(Nelumbo nucifera Gaertn.) Petal Browning

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Abstract
The anatomical structure of 'Sattabhud' lotus (Nelumbo nucifera Gaertn.) petal on the first day of soaking in distilled water consisted of one layer of epidermis, layers of mesophyll and vascular tissue. Structure and shape of cells were turgid cells with distinct cytoplasm. After 3 days of soaking, the petal tissues showed a tremendous change of cells and tissues particularly the epidermis and parenchyma. The parenchyma cells lost their round or polyhedral shape with observed shrinkage and separation of protoplasm from cell wall. The ultrastructure of petal cells indicated that browning caused from the deterioration of epidermis and mesophyll parenchyma cells particularly collapsed of cells, precipitation of substances in cytoplasm and shrinkage of protoplasm. This also resulted in synthesis of toxic metabolites and petal turning black.

Keywords: lotus, ultrastructure, browning

Introduction
The lotus flowers usually turn brown or black within 1-2 days after harvest resulting in short vase life. This is a very serious postharvest problem for cut lotus flower. Therefore, to learn about the anatomical and ultrastructural changes during the browning process is necessary for solving this problem.

Materials and Methods
The 'Sattabhud' lotus flowers were harvested from Chonburi province, Thailand on July, 2009. The flower peduncle(36 cm in length) was immediately soaked in distilled water. The petals of 'Sattabhud' lotus were studied on their anatomical and ultrastructural changes on the first day and after 3 days of harvest. The plant micro-technique process was employed. The samples cut with ultra microtome of Leica UCT were stained with toluidine blue, uranyl acetate and lead citrate. These samples were investigated by a compound microscope (Zeiss with Azio plus II) and a transmitting electron microscope(Joel;JEM 1220).

Results and Discussion
1. Anatomy of 'Sattabhud' lotus petals on the first day
The petals of 'Sattabhud' lotus were composed of one-cell layer of upper and lower epidermis. The cell had a nipple papilla shape, 3 sides rather thick of cell wall, and some cells contained phenolic compound in the cytoplasm. The mesophyll layer consisted of 1 - 3 layers of parenchyma cells, in compact arrangement beneath the epidermal layer. The parenchyma cells were round or polyhedral in shape, rather thick in cell walls and consisted of numerous phenolic compound in all of the cells and resin substance in some cells. In the middle region of petals, the parenchyma and aerenchyma cells were rather loose in arrangement forming a large air space. The air spaces were alternately arranged with vascular bundle. The dispersed resin cells were commonly found in the middle of petals and in the vascular tissues. The vascular tissue was composed of phloem and xylem in the collateral type arrangement (Fig. 1 - 2).

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2. Cells ultrastructures of ‘Sattabhud’ lotus petals on the first day

Upper and lower epidermis of the petals contained 3 sides rather thick of cell wall. The cytoplasm of some cells was composed of small amount of phenolic compound. The 1 - 3 layers of parenchyma cells in line arrangement and adjacent to the epidermal layer consisted of large amount of full or almost full in capacity of phenolic compound and the resin cells scattered around this area. The inner structure of cells in this stage was still in complete form. Almost all of the cells in the petals contained significant amount of protoplasm, cell membrane closed to cell wall or very turgid (Fig. 3 - 5).

3. Anatomy of ‘Sattabhud’ lotus petals after 3 days of harvest

Shape and cell wall of epidermal cells were unchanged, except that of the content in protoplasm. Because of the shrinkage of protoplasm this resulted to the separation of cell membrane from the cell wall. The shrinkage of protoplasm in the parenchyma cells adjacent to epidermis were more than the epidermis cells. This change in turgidity shrunk the cells due to cell collapse hence reducing the air space in the middle of petals (Fig. 6 – 7).

4. Cells ultrastructures of ‘Sattabhud’ lotus petals after 3 days of harvest

Change observed in cell wall of parenchymas after 3 days of harvest were the shrinkage of protoplasm from cell wall, collapse structure in the cells and the adjacent cells, separation of protoplasm from cell wall and precipitation of substances in the protoplasm. (Fig. 8 - 10).

The browning symptom in the ‘Sattabhud’ lotus petals indicated that the area of parenchyma cells in the mesophyll layers appeared earlier more than other regions. The occurrence of browning symptom resulted from the change of cells and contents in the cells. The collapse of cells also caused the collapse of the petals. The distinct precipitation appeared in the cells with large amount of phenolic compounds which reacted with enzyme polyphenol oxidase and oxygen. This resulted to the shrinkage and dark color of the petals. The browning symptom occurred at the margin of outer petal blade and spread into the middle area. However the browning also resulted from the external environment factors such as temperatures, relative humidity and amount of oxygen which could accelerate or delay the symptom (Fig. 11 - 12).

Conclusion

The occurrence of browning symptom resulted from the deterioration of petal cells. The rapid changes of parenchyma cells particularly in the mesophyll layer were separation of protoplasm from cell wall and precipitation of protoplasm which caused the collapse of petals, altering of the inner component of cytoplasm, synthesis of toxic metabolites and induction of browning of petals.

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References


Figure 1  Structure of the lotus petal at the margin on the first day.
Figure 2  Structure of the lotus petal at the midrib on the first day.
Figure 3  Sectional view and ultra-structure of lotus petal on the first day.
Figure 4  Resin cell on the first day.
Figure 5  Cell wall and cell membrane on the first day.
Figure 6  Structure of margin lotus petal after 3 days of harvest.
Figure 7  Structure of lotus petal at the midrib after 3 days of harvest.
Figure 8  Wrinkle protoplasm of parenchyma cell of lotus petal after 3 days of harvest.
Figure 9  Collapsed parenchyma cells of lotus petal after 3 days of harvest.
Figure 10 Parenchyma cell wall of lotus petal after 3 days of harvest.
Figure 11 Lotus flowers on the first day.
Figure 12 Lotus flowers after 3 days of harvest.