

incubated in the presence of 90 mmol/L arsenate, the highest arsenic-tolerant ability was observed in the 120 Gy-treated group in which ferrous irons were completely oxidized within 5 d. While the oxidation capacities of other strains were inhibited severely, including original strain. The arsenite resistance of sample treated by 120 Gy carbon ion beam was higher than those reported previously. For example, Barrett<sup>[3]</sup> suggested that arsenite with a concentration of 30 mmol/L was a major toxin to *At.ferrooxidans*, and it can hardly grow anymore. The result showed that the carbon ion beam irradiation and domestication can improve the arsenic-tolerant ability of strain YS-1.

## References

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## 4 - 42 Study on the Mechanism of Flower Pigment Mutation Induced by Carbon Ions in Geranium (*Pelargonium × hortorum*)

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Flower color as the important economic indicator for ornamental plants has become the primary target for ornamental improving and breeding. Heavy ion mutation technology as a unique and efficient mutagen has been widely used in germplasm innovation and plant breeding. In our study, 150 young shoots of *Pelargonium* were exposed to 80 MeV/u carbon ions and finally one stable genetic flower color mutant was obtained at dosage of 30 Gy. Comparing with wild type, the physiological indexes of mutant displayed significant differences. The color of petal in mutant was changed from red to light pink, and the color of peduncle and torus was changed from green to red, as well as the pistils and stamens' color were changed from yellow green to purplish red. In order to elucidate underlying mutation mechanism of coloration change in mutant induced by carbon ions, the anatomical structures, pigment components and quantities and expression profiles of key genes involved in anthocyanin biosynthesis were investigated.

Anatomical structure observations suggested that the morphology of epidermal cells and the distribution of anthocyanins had no significantly differ between the mutants and the wild type, but the quantities and types of anthocyanidins were not same. As shown in Fig. 1, abundant purple-red and salmon pigments fully filled with the upper and lower epidermal cells of wild type, while only a small number of pale pink pigments in mutant. HPLC(High performance liquid chromatography) analyses were performed to identify the pigments in petal of wild type and mutant. The results indicated that the main pigments determined the flower color of wild type were abundant pelargonidin(61.2%) and bits of cyaniding(21.0%) and delphinidin(17.8%). On the contrary, the unique pigment can be detected in mutant was cyanidin. The core reason of depigmentation in mutant was the absence of pelargonidin and delphinidin (Fig. 2).

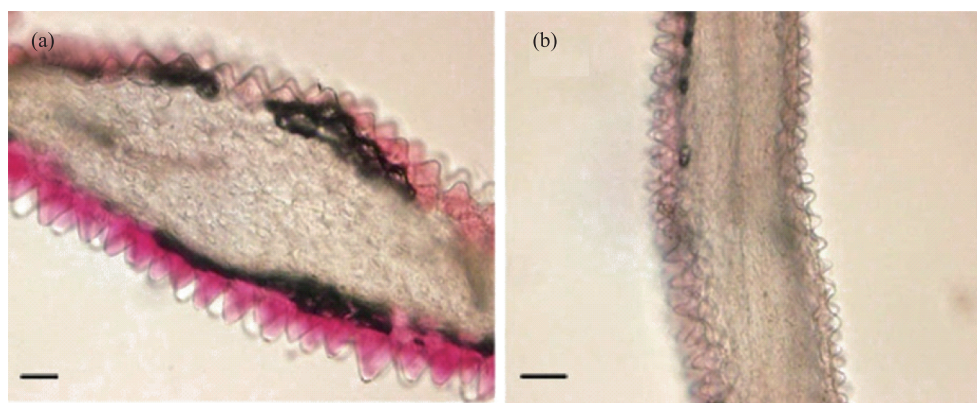


Fig. 1 Microscopic assessment of epidermal cells. (a) wild type, (b) mutant.

Quantitative real-time PCR were executed to analyze the expression profiles of the key structural genes involved in the anthocyanin biosynthetic pathway. The results suggested that most of genes were down-regulated in mutant,

especially early genes *CHS*, *CHI* showed the significantly down-regulated transcriptional level and the expression of late gene *ANS* almost be suppressed. The down-regulated or inhibited transcription of these key genes blocked the biosynthesis of anthocyanin, and that was the crucial reason for the deficiency of anthocyanin which lead to the depigmentation of mutant.

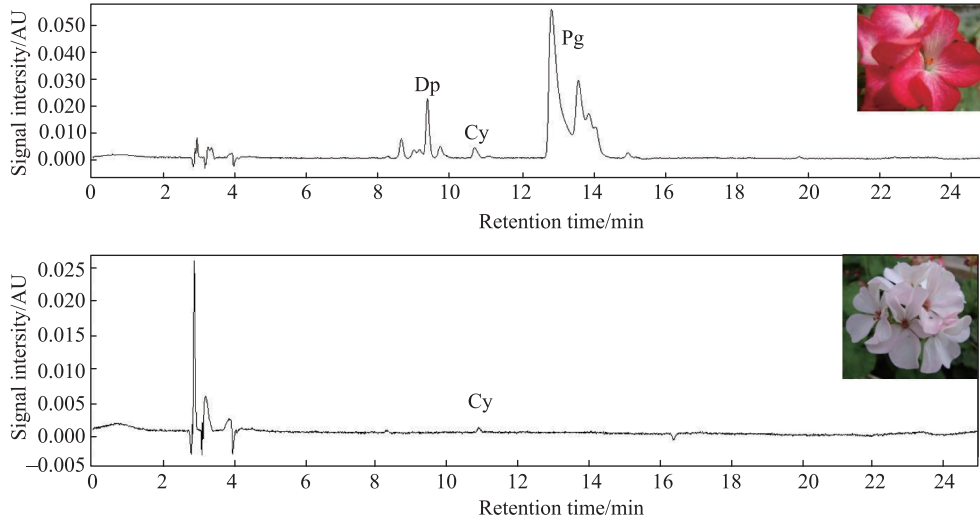


Fig. 2 Comparison the compositions and contents of pigments in flowers of wild type and mutant of geranium. Dp: Delphinidin; Cy: Cyanidin; Pg: Pelargonidin.

These results above provided part of the explanation of the mechanism of coloration change in mutant and established the research foundation for the follow-up design and color improving in geranium. At the same time, we can deduce that heavy ion beam irradiation may be utilized as a mutagen for flower color improvement and may be proposed as an effective approach for mutation breeding of ornamental plants.

## 4 - 43 Effect of Aeration Rate on the Cellulase Production of *Aspergillus niger* and Mixed Fermentation with *Trichoderma reesei*

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In recent years, growing attention has been devoted to maximize the yield for the cellulase production in a bioreactor depending on the specific process. However, there are many factors that affected the fermentation performance and cellulase production, such as aeration rate, agitation speed, pH value, temperature<sup>[1]</sup>. The aeration rate is a key factor for mycelia growth and enzyme production, due to aeration rate conditions influence mass transfer by affecting the bubble size, air hold up and turbulence within the vessel as well as biomass production<sup>[2]</sup>, and provides air flow, oxygen transfer, and removal of generated carbon dioxide under submerged fermentation. Therefore, aeration rate optimization plays a crucial role in cellulase production.

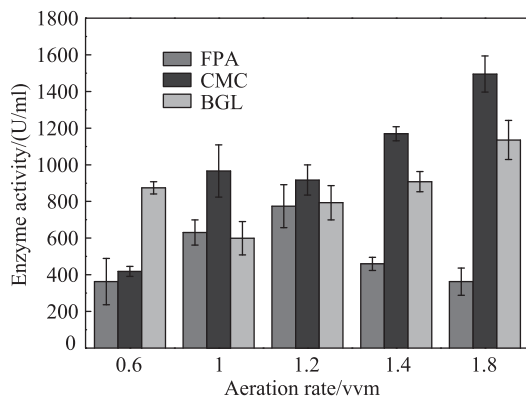


Fig. 1 The effect of aeration rate on the cellulase production.

The aim of this study is to evaluate how aeration rate affect cellulase production of *Aspergillus niger* and mixed fermentation with *Trichoderma reesei*. The fermentation was carried out in a 10 L stirred tank bioreactor, and filter paper assay (FPA), endoglucanase (EG) and β-glucosidase (BGL) activities was analyzed.

Fig. 1 showed that the filter paper assay (FPA) activity increased with increasing aeration rate and reached the maximum enzyme activities (773.85 U/ml) at 1.2 vvm, and then decreased with the increasing of aeration rate. While endoglucanase (CMC) activities correlated positively with increasing aeration rate. The highest endoglucanase (CMC) and β-glucosidase (BGL) activities were obtained at aeration rate (1.8 vvm) after 48 h fermentation, reached 1 495.2 U/ml and 1 135.43 U/ml,