3 - 32 Reproductive Growth Index Analysis of a Heavy Ions-induced Mutant of *Arabidopsis thaliana*

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Due to their unique advantages in physics and biology, heavy ion beams, with high mutation rate and wide mutation spectrum, have been widely used in plant breeding as a novel and efficient physical mutagen. In this work, *Arabidopsis thaliana* (197#), which displayed decreased fertility, was induced by carbon ion beams accelerated by the Heavy Ion Research Facility in Lanzhou (HIRFL). In order to determine the discrepancies between WT and mutant plants, a series of development indexes were analyzed during the reproductive growth stage.

Both WT and mutant plants were grown in the greenhouse with the condition of 22 ° C and 70% relative humidity under continuous illumination of 5 000 lux. According to the measurement results, there were significant differences in bolting rate, silique length, siliques number and seed length between WT and mutant seedlings, while there was no obvious discrepancy in seed width between WT and mutants (Table 1). The average bolting rate on 30^{th} day after germination of mutant plants were 18.84% that of WT plants, which indicates that the transition from vegetative growth to reproductive growth of mutants was much later than WT. In addition, the number of siliques and the silique length on the main stem of mutant plants was only 68.64% and 62.17% of WT plants, respectively. Meanwhile, the seed length of mutant plants was 91.18% of WT. All of the results showed that the mutants displayed a lagged reproductive development as well as a decreased fertility.

Table 1 Morphological comparison betweenWT and 197# mutant plants.

Detected indicator	WT	197#	Percentage of WT/ %
Bolting rate/ %	25.86±0.02	5.13±0.01**	18.84
Siliques number	11.83±3.77	8.12±2.8**	68.64
Silique length /mm	9.386±0.95	6.13±0.61**	62.17
Seed length/ mm	0.34±0.04	0.31±0.02*	91.18
Seed width/mm	0.17±0.01	0.16±0.01	94.11

The variations in the phenotypes could be attributed to the changes of gene expression modes. In order to investigate the molecular mechanism that induced the lagged reproductive growth of the mutant plants, both WT and mutants were cultured for 30 d in greenhouse, harvested samples were frozen immediately in liquid nitrogen, and stored at -80 ° C. The total RNA was extracted from the samples by using Plant RNA Kit (Omega, Shanghai). For real-time PCR, Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Shanghai) was used to make cDNA from 2 μg of RNA in a 20 μL reaction volume. Each cDNA sample was diluted 1:9 in water, and 4 μL of that dilution was used as template for real-time PCR. Analysis of the expression level of FLOWERING LOCUS T (FT), FRUITFULL(FUL), CONSTANS (CO) genes which can induce floral development were assayed by real time fluorescence quantitative-PCR. The expression levels of FT and FUL in mutants decreased significantly than those of WT plants, which were 0.184 and 0.596 fold of WT, respectively(Fig. 1). While there was no obvious difference in the expression level of CO gene between mutants and WT. The decreased expression level of genes which can promote flowering might attribute to the late maturation of mutant plants.

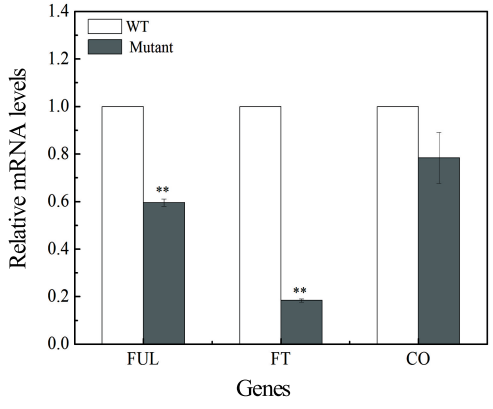

Fig. 1 Analysis of the gene expressionsin WT and 197# mutants Values are the mean ± SE (**$P < 0.01$).