Identification of causal site for an *Arabidopsis* C30-144-as3 mutant

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Mutations are one of the crucial factors for natural selection and species evolution. They occur frequently in nature. With the development of science and technology, we can create artificial mutagenesis and manually search for desirable characters. Among those artificial mutagenesis techniques, heavy-ion beam irradiation has been established as a reliable approach in plant breeding.[1)] An important parameter for ion-beam mutagenesis can be identified as linear energy transfer (LET). The LET implies the amount of energy deposited per unit length of a particle's path (keV/μm). The C-ion irradiation (30 keV/μm) on Arabidopsis seeds has a high mutation frequency[2)] and results mainly in single nucleotide variants (SNVs), small indels (<100 bp), in the responsible genes for mutant phenotypes. The number of homozygous mutated genes per genome is approximately five, making it easy to identify the causative gene.[3)]

In this study, we irradiated Arabidopsis (Col-0) seeds with 400 Gy C-ions (30 keV/μm). The resulting mutant plants were isolated, allowing further generations to be selfed, and screened for interesting phenotypes. The C30-144-as3 mutant was identified as later flowering and round rosette leaves containing plant (Fig. 1). After the whole genome sequencing, mutation calling was conducted using the AMAP: A pipeline for whole-genome mutation detection.[4)] This analysis resulted in homozygous SNVs, a large deletion, and intrachromosomal translocations (ITX) occurred in genic regions (Table 1). In order to identify the candidate gene, we planned to perform a linkage analysis using a backcrossed (BC) population. The BC_1F_2 population consists of 84 plants. Phenotype scoring referred to the perfect C30-144-as3 plant (M_3) with later flowering and round rosette leaves.

Fig. 1. Ion beam mutant C30-144-as3 shows later flowering and round rosette leaves containing plant (b) compared to Col-0 plant (a) on 31 days after germination.

Table 1. Homozygous mutated genes found in C30-144-as3.

Chr.	Position	Type	Size	Affected genes
1	13088360-13167752	ITX	79.39Kb	AT1G06917
2	19600910	SNV	1bp	AT2G47860
3	11316869-12608684	DEL	1.29Mb	111 genes
3	23056674	SNV	1bp	AT3G62300
4	998176	SNV	1bp	AT4G02280
4	9318677	SNV	1bp	AT4G16550

A total of 17 plants showed mutant phenotype. The segregation of the F2 population fitted the 3 : 1 segregation ratio (wild-type: mutant = 67 : 17, χ^2 = 1.02). Therefore, we hypothesize that this mutation was caused by a recessive single gene. Linkage analysis was performed for all the homozygous mutated genes using 17 F_2 plants, which showed the mutated phenotype. The AMAP pipeline revealed large deletion of approximately 1.29 Mb in M_3 plants. This deletion has lost a total of 111 genes, according to the Arabidopsis genome browser. We used two primer pairs and Sanger sequencing of PCR products for genotyping experiments. Linkage analysis resulted in 16 homozygous plants linked with the above large deletion (one of the plants among 17 plants resulted in non-reproducible linkage analysis result, which could be due to contaminated DNA), indicating responsible gene might be included in the deleted fragment. Because among the homozygous mutated genes, only the 1.29 Mb deletion showed the linkage. Next, we checked the gene description of all 111 genes and found a single gene responsible for regulating flowering time and leaf shape. It's the gene AT3G30260 and already identified as *AGAMOUS-LIKE 79* (*AGL79*).[5)] The non-functional mutants were reported to show later flowering and round rosette leaves. Then we presumed *AGL79* gene was the causative gene for the C30-40 mutant.

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References

1) T. Abe *et al.*, *Plant mutation breeding and biotechnology* (Oxfordshire UK: CABI, 2012), p. 99.
2) Y. Kazama *et al.*, Plant Biotechnol. **25**, 113 (2008).
3) Y. Kazama *et al.*, BMC Plant Biol. **11**, 161 (2011).
4) K. Ishii *et al.*, Genes Genet. Syst. **91**, 229 (2016).
5) R. Gao *et al.*, Front. Plant Sci. **8**, 2226 (2018).