Abstract

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Regulatory systems to detect the environmental cues should have evolved for plants to control timing of life-stage transitions. Such mechanisms have been often dissected in laboratory experiments but seldom validated for natural habitat conditions, i.e., *in natura*. Vernalization, the promotion of flowering by winter cold, is a prominent naturally occurring phenomenon, so far best characterized using artificial warm and cold treatments. The floral inhibitor *FLOWERING LOCUS C* gene of *Arabidopsis thaliana* (*AtFLC*) has been identified as the central regulator of vernalization. First *in natura* analysis of gene expression was previously conducted for an *FLC* orthologue, *AhgFLC*, in a natural population of perennial *Arabidopsis halleri*. Time-series RT-qPCR analysis revealed a clear seasonal pattern of *AhgFLC* expression and implied the existence of the molecular machinery which functions as the long-term memory of past temperature and filters out short-term noises. However, the precise molecular mechanism for plants monitoring the long-term trend of temperature has not been analysed. In *A. thaliana*, *AtFLC* shows an idiosyncratic pattern of histone modification at different stages of cold exposure, believed to regulate transcriptional responses of *AtFLC*. Chromatin modifications, including H3K4me3 and H3K27me3, are routinely quantified using chromatin immunoprecipitation (ChIP), standardized for laboratory samples. In this study, I modified a ChIP protocol to make it suitable for analysis of field samples. I describe experimental conditions for performing sampling and sample preservation in the field and demonstrate that these conditions give robust results, comparable with those from laboratory samples. The ChIP protocol incorporating these modifications, "Field ChIP", was used to initiate *in natura* chromatin analysis of *AhgFLC*. I revealed the two-year seasonal dynamics of *AhgFLC* expression, H3K4me3 and H3K27me3 levels. The statistical analysis, phase shift analysis and mathematical modeling elucidated how *AhgFLC* is epigenetically regulated in natural conditions. I also discuss the prospect to expand these analyses to the genome-wide level to find the novel memory genes.