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# Towards understanding the phenological development of quinoa by expression analysis of putative flowering time genes

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## 1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a highly nutritious crop from the family Amaranthaceae native to the Andean region of South America. The nutritional value of quinoa is exceptionally high due to the presence of all the essential amino acids together with a perfect balance of micronutrients, vitamins, fiber, and carbohydrates. Many quinoa accessions are tolerant to abiotic stresses such as salinity, drought, and short-term frost. Hence it has a high potential for cultivation in diverse climatic regions, particularly marginal lands. The potential of quinoa as an alternative staple food has been realized by European countries. The primary requirement for quinoa cultivation in Northern Europe is the day-length adaptation through modification of flowering time. Therefore, our study aims to understand the flowering time regulation in day-length-sensitive and –insensitive quinoa accessions in response to photoperiod.

## 2. Materials and Methods

### Plant material

- We selected early- and late-flowering accessions from different geographical regions for expression analysis (**Table 1**).
- Plants were grown in climate chamber: 8 h light (SD) and 16 h light (LD) at 22 °C (**Figure 1**).
- We performed leaf sampling every week at ZT-4 until the onset of floral dehiscence. For diurnal expression analysis, we sampled leaves every 4 hours at the bolting stage.
- We phenotyped quinoa accessions for days to bolting and days to flowering under climate chamber and field conditions in Kiel
- To identify the flowering transition stage under LD conditions, we performed histology analysis of the apical shoots of short-day (Ames-13760) and long-day (D-11889) accessions.

**Table 1:** Overview of the accessions used for expression analysis. Phenotypic data obtained from the climate chamber under short and long-day and field trial in Kiel in 2019.

| Seed code | Accession ID | Origin | Days to flowering | | Days to flowering Field (2019) |
|---|---|---|---|---|---|
| | | | SD | LD | |
| 170867 | PI-614886 | Chile | 43±0 | 46±1 | 53±3 |
| 171605 | PI-587173 | Argentina | 51±2 | 71±1 | 86±2 |
| 171230 | Titicaca | Denmark | 38±0 | 41±2 | 52±3 |
| 170876 | CHEN-109 | Peru | 69±0 | 70±1 | 86±3 |
| 182372 | D-11889 | Argentina | 58±3 | 48±2 | 72±3 |
| 182136 | Ames-13760 | USA | 46±1 | 62±3 | 61±3 |

### Expression analysis of flowering time genes

- We analyzed the relative joint expression of *CqFT1, CqFT2, CqRFT, CqCOL2, CqCOL4 and CqCOL5* genes due to their homology to known genes from *B. vulgaris* or *O. sativa.* Conserved primers among the paralogs of these genes were used. Expression values were normalized with two reference genes (*CqIDH-A* and *CqPTB*).

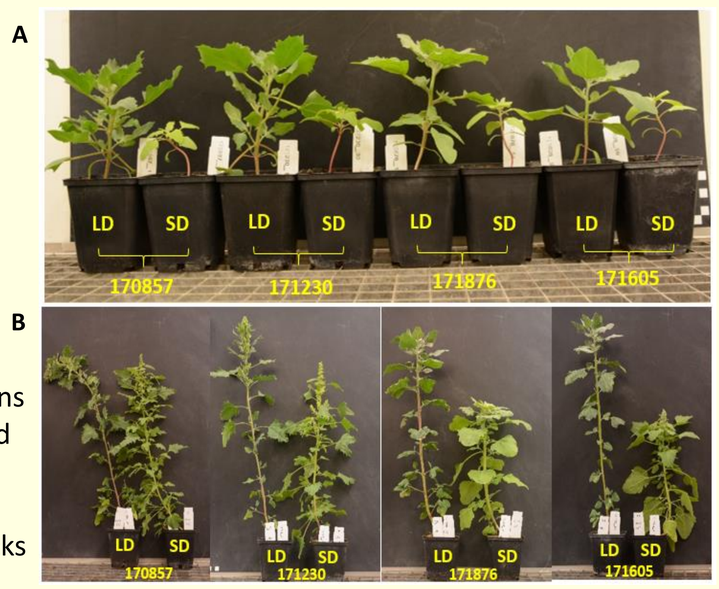


**Figure 1:** Growth of the quinoa accessions under short (SD) and long days (LD). **A:** Three weeks old plants **B:** Seven weeks old plants.

## 3. Results

- A difference in the expression through developmental stages was found for *CqFT2* under SD conditions. Under LD, the expression of the analysed genes was mostly detected in later developmental stages. *CqFT1* is only highly expressed during and after flowering (**Figure 2**).
- *CqFT1* and *CqFT2* in a short-day accession depicted similar patterns as in a day-length insensitive accession under short-day conditions (**Figure 3**).
- *CqRFT1* showed high expression before bolting under SD (**Figure 3**).
- *CqCOL* genes showed higher expression at the reproductive stage under both SD and LD conditions, which might indicate their possible role as floral integrators in quinoa (**Figure 3**).
- The flowering transition in quinoa slowly progresses from early developmental stages as seen by apical dominance release (**Figure 4**). *CqCOL* genes showed higher expression during these stages in a short-day accession.

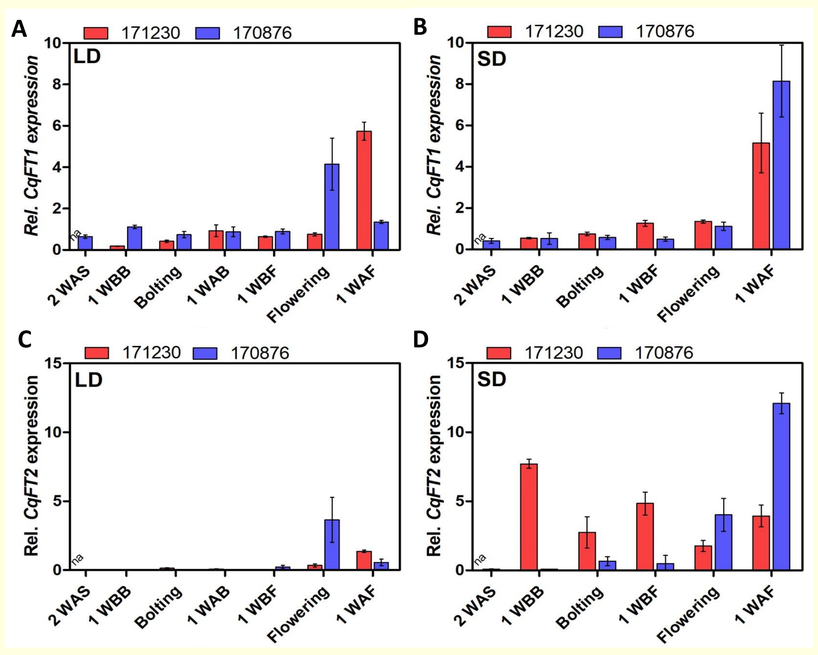


**Figure 2:** Spatial expression of *CqFT1* (**A**,**B**) and *CqFT2* (**C**,**D**) in leaves under short (**B**,**D**) and long-day conditions (**A**,**C**) in 171230 and 170876 during plant development (2 WAS: two weeks after sowing, 1 WBB: one week before bolting, 1 WAB: one week after bolting. 1 WBF: one week before flowering and 1 WAF: one week after flowering).

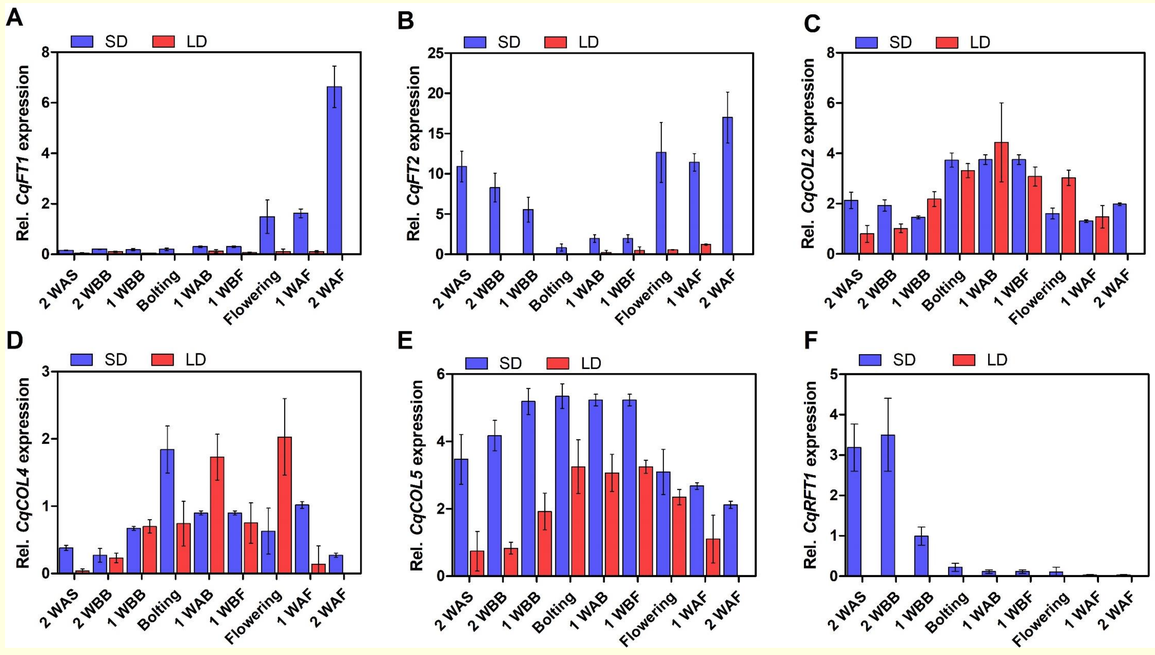


**Figure 3:** Spatial expression of *CqFT1*(**A**), *CqFT2*(**B**), *CqCOL2* (**C**), *CqCOL4* (**D**), *CqCOL5* (**E**) and *CqRFT*(**F**) in leaves under short and long day conditions in 171605 during plant development (2 WAS: two weeks after sowing, 2 WBB: two weeks before bolting, 1 WBB: one week before bolting, 1 WAB: one week after bolting. 1 WBF: one week before flowering, 1 WAF: one week after flowering and 2 WAF: two weeks after flowering).

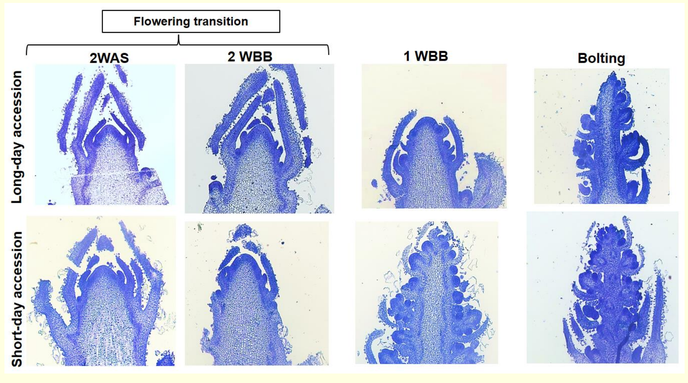


**Figure 4:** Flowering transition in quinoa under long day conditions (2 WAS: two weeks after sowing, 2 WBB: two weeks before bolting, 1 WBB: one week before bolting. Sectioning of tissues at 8 µm and staining with toluidine blue .

## 4. Conclusions and future prospects

The homologs of the main flowering time regulators in sugar beet, *CqFT2* and *CqFT1* genes, are diurnally regulated under SD conditions, however their expression stays undetected under LD. *CqFT2* may act as a repressor of flowering only under SD, while *CqCOL2*, *CqCOL4* and *CqCOL5* seem to induce flowering under both SD and LD in the short-day accession, 171605. Based on our results we expect different regulation of flowering under LD conditions compared to SD. We will gain a broader view of the flowering time regulation in quinoa by combining these results with a genome-wide association study based on a large set of phenotypic data obtained from different environmental conditions in Australia, China, Germany, USA, and Saudi Arabia. Moreover, a QTL mapping in biparental populations and haplotype variation studies are being performed. Our results will be beneficial to develop quinoa cultivars adapted to the Northern European climate.

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