Cauliflower (Brassica oleracea var. botrytis) is a prominent commercial vegetable worldwide. The aesthetic appearance of cauliflower curd is the primary factor determining its market value. The curd is composed of undifferentiated inflorescences and an inflorescence meristem that continuously generates replicas (Smyth, 1995). Many Brassica species have chilling requirements and flowering induced by vernalization. After vernalization, cauliflower will exhibit normal curd development under suitable environmental conditions and then begin flowering (Labate et al., 2006). However, unsuitable temperatures produce malformed curds, such as buttoning curds, Riceyness curds, or bolting curd in the small plant stage. Fernández et al. (1997) indicated that low temperature is crucial for the apical growth of cauliflower when transitioning from the vegetative to reproductive stage. In addition, curd initiation and growth are related to effective temperature (Pearson et al., 1994). Therefore, retardation or lack of curd initiation is attributable to insufficiently low temperature (Liptay, 1981). The success of curd initiation is determined by temperature (Rahman et al., 2007) and maturity type (Nowbuth and Fellows, 2000). Wurr and Fellows (2000) suggested that the optimal temperature for curd initiation is lower in cauliflower types that mature in the early summer (9 °C) than in those that mature in winter (13 to 14 °C). One study demonstrated that climate change postpones curd initiation and that more leaves are produced when the temperature is increased by 2.9 °C to reach 17 °C (Wurr et al., 1996). Cauliflower is critical to marketability because less time for harvest corresponds to reduced cost of pest control and increased market value. A high correlation between maturity type and season must exist to meet these targets. The season factor obviously affects early cultivars when they were planted late in autumn–winter (Augustine et al., 1980). Cebula et al. (2005) suggested that the period before curd formation is shorter in early-maturity cultivars than in late-maturity cultivars in moderate climates. Another study reported lower temperature requirements for curd growth in late-maturity varieties (Wurr et al., 2004).

Relevant studies have revealed that curd initiation is correlated with maturity, including duration in the juvenile period and number of leaves at curd initiation (Booj and Struijk, 1990; Wurr and Fellows, 2000). The number of leaves is determined at the end of the juvenile period and is also a stable characteristic (Hand and Atherton, 1987). However, curd initiation sometimes does not occur under high-temperature conditions, even with a sufficient number of leaves. Optimal temperature is a critical factor for curd initiation and development. Williams and Atherton (1990) indicated that curd initiation occurred earlier with fewer leaves at a low temperature (5 °C) and that more leaves were required at a warm temperature (20 °C). However, the environment changes the number of leaves that develop at curd initiation phases, and another study suggested that the apex diameter could be used as an index for the end of juvenileity (Fellows et al., 1999). Curd initiation is determined through visual observation when the apex diameter reaches the initiation point at 0.6 mm (Olesen and Grevsen, 2000; Pearson et al., 1994; Rahman et al., 2013; Wurr, 1981). Tissue dissection is performed under a binocular microscope to accurately determine the amount of time required for curd initiation, with a vertical apex diameter greater than 0.04 mm indicating initiation (Fellows et al., 1999; Wurr et al., 2004). Previous studies showed that BoFLC2 polymorphisms existed in Brassica genus crops. The expression of Flowering Locus C (FLC) in Brassica oleracea includes BoFLC3 in broccoli (Lin et al., 2018) and BoFLC4 in cabbage (Lin et al., 2005). However, in Chinese kale and broccoli, BoFLC5 does not contribute to flowering time because of premature stop codons (Razi et al., 2008). More reports demonstrated that BoFLC2 was associated with cauliflower crops (Matschewegski et al., 2015; Okazaki et al., 2007; Ridge et al., 2015). However, the majority of research was on curd initiation, and development generally has focused on cauliflowers with relatively low ambient temperatures. No study has investigated the effect of temperature on curd initiation and development of tropical cauliflowers. Thus, in this experiment, we investigated the influence of temperatures (18 to 30 °C) by performing tissue dissection and evaluating BoFLC2 expression during the transformative period of curd initiation for two tropical cauliflower cultivars under high temperatures. In addition, we evaluated the apical changes in response to different temperature treatments of tropical cultivars. This research can provide insight into curd initiation and formation in tropical cauliflower species and serve as a reference for cauliflower production in tropical regions.
Materials and Methods

Two cauliflower F1 cultivars, cv. H-37 and cv. H-80, obtained from Ching Long Seed Co. Ltd., Tainan City, Taiwan, were used as the testing materials. The commercial culture guide identifies ‘H-37’ as an early-maturity and heat-tolerant type and ‘H-80’ as a mid- to late-maturity type. Seeds were sown in a 128-plug tray filled with peatmoss and grown in a greenhouse. On day 21, seedlings were transplanted to plastic pots with diameters of 9 cm containing peatmoss with 0.5 g of organic fertilizer No. 1 (5.0% N, 2.5% P2O5, 2.5% K2O, and 65.0% organic material) from Taiwan Fertilizer Co. Ltd. (Taipei, Taiwan). On day 26, we transferred the seedlings of two cauliflower cultivars to a growth chamber and exposed them to 18, 24, and 30°C treatments with a 16/8-h day/night photoperiod. The light intensity was 5150 Lux and relative humidity was 56% to 61% in growth chamber. The experimental design was a randomized complete block split-plot design with temperature treatment as the main plot and cultivar as the subplot.

Nine plants per cultivar were successively sampled for each temperature treatment. The first investigation began 3 d after the seedlings had been transferred to a growth chamber; we evaluated nine plants of each cultivar under different temperature treatments at 7-d intervals. In our experiment, apex and leaf samples were matched and originated from the same plant, and immature leaf is near apex. The apex was used as the dissected observation and the immature leaf used to detect BoFLC2. We performed tissue dissection to assess the apex initiation and development. It can determine whether the apex and leaf originated from the same sampling. Moreover, we sampled cauliflower apex tissue and fixed them in an FAA solution (70% ethanol, formalin, and glacial acetic acid at a volume ratio of 18:1:1). A frozen section machine was used to dissect apical tissue with a thickness of 0.45 mm. We assessed the morphology of apical tissue sections using a microscope at different sampling stages. The apical growth characters (apex elongation and diameter) were investigated during the juvenile, curd initiation, and curd initial development phases. The BoFLC2 transcription levels of immature leaf samples were detected at the same phases for which apical tissue dissections were performed. For the quantification of BoFLC2 transcript analysis, 100 mg of immature leaf samples from each treatment were collected and stored at –80°C. RNA from homogenized tissue samples was extracted using TRizol Reagent (Thermo Fisher Scientific, Waltham, MA). Total RNA (500 ng) quantified with the Qubit RNA BR kit (Thermo Fisher Scientific) was reverse transcribed using random primers and a fast reverse-transcription kit (Topgen Biotech, Kaohsiung City, Taiwan). The following specific BoFLC2 primers were selected based on a report by Ridge et al. (2015): forward 5’-CGAAGTAGTTGTCACACATGAGC-3’ and reverse 5’-CGGAGAGGGCAGTCTCAAGGTGGTT-3’. In addition, we used a SYBR Green Master Mix (Topgen Biotech, TW) to amplify cDNA. Quantitative real-time polymerase chain reaction was performed on the StepOne Plus Real Time PCR System (Applied Biosystems, Foster City, CA) in accordance with manufacturer’s instructions. We used SAS-EG (SAS Enterprise Guide 7.1 system; SAS Institute, Inc., Cary, NC) for data analysis. We performed mean separation using the least significant difference at $P < 0.05$ when significance among treatments was detected.

Results and Discussion

Apex change during curd initiation. Curd initiation was defined as an apex elongation greater than 0.04 mm (Fellows et al., 1999; Wurr et al., 2004). On the basis of this definition, we distinguished the transition of tested materials from vegetative to reproductive growth according to apex dissection measurements (Fig. 1). Curd initiation occurred on day 17 for ‘H-37’ under all three temperature conditions, which was significantly
were correlated with curd diameter growth and (Pearson et al., 1994). Leaf area and dry matter growth rate of curd diameter could be observed mum duration less than 20 d for the requirement autumn cauliflower. When the temperature was temperature elevation effect in the temperate characteristically early-summer maturity types cultivars grown in temperate zones, which are the low-temperature requirement of cauliflower initiation. These temperatures clearly exceeded 18 to 30 temperatures. The aforementioned studies pro- vided the best direction for the cultivation and breeding for vernalized induction condition. However, global warming and climate change could severely affect tropical cauliflower production. In our study, the temperature require- ments for the curd initiation and development phases of tropical cultivars differed significantly from those of cultivars in temperate zones. Table 2 presents the effect of temperature and cultivar response interactions on apex growth in different phases. A cooler temperature (18 °C) promoted faster growth in early-maturity culti- vars (Fig. 3A), and visual curd growth subse- quently was observed. Furthermore, a sharp decrease was observed under 30 °C conditions compared with 18 and 24 °C, which suggested that high-temperature conditions caused a significant decrease in BoFLC2, with an abnormal minimum quantity and delayed apex development. BoFLC2 expression showed a decreasing tendency during days 17 to 24 after curd initiation (Fig. 3A), and visual curd growth subse- quently was observed. Earlier, Flower cultivars at temperatures greater than 18 °C demonstrated significantly greater apex elongation during curd initiation than at 24 and 30 °C. Both apex elongation and diameter increased more quickly at 18 and 24 °C than at 30 °C at both stages of curd initiation and curd initial development (Table 1). As expected, the early-maturity type ‘H-37’ exhibited quicker apex elongation and growth, also demonstrating that tropical cauliflower cultivars at nonvernal- ized temperatures (18 to 30 °C) can induce curd initiation. These temperatures clearly exceeded the low-temperature requirement of cauliflower cultivars grown in temperate zones, which are characteristically early-summer maturity types and undergo optimal curd initiation at a temperature of 9 to 13 °C (Wurr and Fellows, 2000). Most simulative models evaluated the temperature elevation effect in the temperate climate and indicated that the optimum tempera- ture was 14 °C for curd initiation in summer/ autumn cauliflower. When the temperature was greater than 14 °C, significant reduction on the growth rate of curd diameter could be observed (Pearson et al., 1994). Leaf area and dry matter were correlated with curd diameter growth and temperature. Reports also have indicated that a mean temperature of 14.3 °C showed a mini- mum duration less than 20 d for the requirement of curd initiation (Olesen and Grevesen, 2000). Wurr et al. (2004) indicated that juvenility at an apex diameter of 0.2 mm switched to induction and turned to curd growth after curd initiation at 0.6 mm. In the United Kingdom, these phases were shortened by increased temperatures. Uptmoor et al. (2008) used thermal time re- quired to flowering to predict flowering time at 6 to 18 °C conditions. It indicated that curd initiation was accelerated under noninducing temperatures. The aforementioned studies pro- vided the best direction for the cultivation and breeding for vernalized induction condition. However, global warming and climate change could severely affect tropical cauliflower produc- tion. BoFLC2 was present in temperate cauliflower species during curd induction under nonvernalized temperatures. With the exception of the vernalization regulative pathway, the autonomous pathways are preset and down-regulate the floral repressor FLC to induce flowering (Marquard et al., 2006; Reeves and Coupland, 2000). The model and pathway are displayed in Fig. 4. In this study, tropical cauliflower cultivars could induce curd under nonvernalized tempera- tures, which suggested that the autonomous pathways were performed and down- regulated the floral repressor FLC then in- duced curd development. This is different to vernalization induced in Brassica genus crops. Gan et al. (2014) illustrated that the thermosensory qualities of Arabidopsis were modified by regulating FLC at an increased temperature of 29 °C. The autonomous path- ways of FLC is for response to environmental change (Shea et al., 2018). This research suggests that warm temperatures affect FLC expression and that floral induction was not only caused by the vernalized effect. In this study, BoFLC2 expression in ‘H- 37’ demonstrated an increasing tendency before curd initiation (from day 3 to 17) under all temperature treatments. BoFLC2 expression exhibited a decreasing tendency during days 17 to 24 after curd initiation (Fig. 3A), and visual curd growth subse- quently was observed. Furthermore, a sharp decrease was observed under 30 °C conditions compared with 18 and 24 °C, which suggested that high-temperature conditions caused a significant decrease in BoFLC2, with an abnormal minimum quantity and delayed apex development. BoFLC2 expression showed a different pattern in ‘H-80’ (Fig. 3B) and increased from days 17 to 31. However, BoFLC2 expression abnormal de- creased at day 31 to 38 under 30 °C conditions, and BoFLC2 expression continued to increase at day 38 to 45 under 18 to 24 °C conditions. These results coincided with the curd growth of this cultivar. Thus, the afore- mentioned results suggest that temperatures (18 to 24 °C) can induce curding in early-

Table 2. Effects of temperature and cultivar on apical growth and analysis of variance.

| Temperature | Curd initiation | Curd initial development* | Curd initiation | Curd initial development |
|-------------|----------------|---------------------------|----------------|-------------------------|
| 18 °C       | 0.114 a        | 1.417 b                   | 0.511 a        | 1.522 b                 |
| 24 °C       | 0.064 b        | 1.791 a                   | 0.430 b        | 1.810 a                 |
| 30 °C       | 0.073 b        | 0.087 c                   | 0.313 c        | 0.359 c                 |
| Cultivar    |                |                           |                |                         |
| H-37        | 0.100 a        | 2.117 a                   | 0.446 a        | 2.034 a                 |
| H-80        | 0.068 b        | 0.080 b                   | 0.390 a        | 0.426 b                 |
| Variance    |                |                           |                |                         |
| Temperature |                |                           |                |                         |
| Cultivar    |                |                           |                |                         |
| Interaction | **             | **                        | **             | **                      |

*Curd initial development phase was followed by curd initiation after 1 week.

Table 2. Apex elongation and diameter changes of cultivars tested under different temperature treatments.

| Temperature/c cultivar | Apex elongation (mm) | Apex diam (mm) |
|------------------------|----------------------|----------------|
|                        | Curd initiation      | Curd initial development* | Curd initiation | Curd initial development |
| 18 °C/H-37             | 0.175 a             | 2.780 b                | 0.626 a        | 2.640 b                 |
| 18 °C/H-80             | 0.051 b             | 0.054 c                 | 0.396 bc       | 0.405 c                 |
| 24 °C/H-37             | 0.064 b             | 3.483 a                 | 0.412 bc       | 3.143 a                 |
| 24 °C/H-80             | 0.063 b             | 0.099 c                 | 0.448 b        | 0.477 c                 |
| 30 °C/H-37             | 0.061 b             | 0.087 c                 | 0.299 c        | 0.320 c                 |
| 30 °C/H-80             | 0.086 b             | 0.086 c                 | 0.326 c        | 0.398 c                 |

*Curd initial development phase was followed by curd initiation after 1 week.

Mean separation, within columns and followed by different letters, showed significant differences according to Fisher’s least significant difference test at 5% level.

*NS, ** NS, ***NS Significant or significant at P < 0.05 or 0.01, respectively.
maturity cultivars of tropical cauliflower but that mid- to late-maturity cultivars exhibit a different pattern under high temperatures.

Irwin et al. (2016) used two Arabidopsis genotypes and detected that BoFLC2 repressed dynamics at warm conditions. The vernalized and nonvernalized plants showed different environmental sensitivity. Lin et al. (2018) described a BoFLC3 expressive tendency in subtropical broccoli, which showed a nonvernalized response. Sun et al. (2018) also indicated that high temperatures delayed curd development but did not postpone the BoFLC1 expression in cauliflower, and the gene also showed a down-regulation pattern. In our study, the BoFLC2 expressive pattern of the early-maturity cultivar could be differentiated into two sections during curd-transformation periods. Before curd initiation, the BoFLC2 level significantly increased and then BoFLC2 showed positive regulation after reach curd initiation.

The results confirmed the BoFLC2 was acted and apex was amplified at a non-vernalized temperature. The apical morphology and molecular information strongly suggested that BoFLC2 still acted in tropical early maturity cauliflower and did not perform the vernalization pathway.

Development of leaves and stem. The number of leaves developed was significantly greater in ‘H-80’ than in ‘H-37’ at all three phases of curd development (Table 3). Wurr (1981) proposed that the duration of the juvenile phase was related to the characteristics of different cultivars. In ‘H-80’, when comparing both cultivars at 24 °C conditions, temperature treatment produced a greater number of leaves at the end of the juvenile phase and the curd-initiation phase. Conversely, ‘H-37’ produced fewer leaves at both phases at a temperature of 24 °C (Table 4). Wurr et al. (1994) predicted that individual curd initiation occurred between 17 and 21 leaves in four summer–autumn types but also explained that leaf number was affected by the environment. The number of leaves required for curd formation increased from 25 to 43 when the temperature increased from 5 to 20 °C (Williams and Atherton, 1990). In this study, despite producing fewer than nine leaves at 24 °C, tropical cauliflowers were able to induce curd initiation, indicating that the number of leaves required at curd initiation is a far less-critical factor for tropical cauliflower cultivars than it is for temperate cultivars. This result also suggested that the leaf number required for curd induction varies depending on the temperature and type of tropical cultivar.

In the comparison of cultivars, analysis of variance revealed that the stem diameter of ‘H-80’ was significantly longer than that of ‘H-37’ at the three phases. Cebula et al. (2005) indicated that late-maturity types of cauliflower grew thicker stems than early-maturity types. Temperature at 24 °C advanced stem growth and longer than 18 °C and 30 °C. Guo et al. (2004) indicated that stem diameter was associated with vernalization, which suggested that a temperature of 24 °C was perhaps not an effective vernalization temperature for mid- to late-maturity types because more stem growth substituted for apex growth. When both cultivars were subjected to high-temperature conditions (30 °C), apex elongation underwent significant retardation and was accompanied by the continuous growth of stem and leaves. These results also revealed the coexistence of vegetative and reproductive growth during the transformative period of curd initiation, suggesting that stem diameter could not be used as a screening index for curd initiation.

This study provides information concerning apical change in tropical environments. Observation of apex elongation before and after the curd-initiation phase produced an advanced index. At the end of the juvenile phase, the early-maturity cultivar was more responsive to the cooler temperature of...
was advantageous for converting apex to
tation of time to floral induction and flowering
in Arabidopsis. Nat. Commun. 5:5096. doi: 10.1038/ncomms5096.
Guo, D.P., G.A. Shah, G.W. Zeng, and S.J. Zeng. 2004. The Interaction of plant growth regulator
s and vernalization on the growth and flowering of cauliflower (Brassica oleracea var. botrytis). Plant Growth Regulat. 43:163–171.
Hand, D.J. and J.G. Atherton. 1987. Curd initiation in the cauliflower. J. Juvenility. J. Exp. Bot. 38:2050–2058.
Irwin, J.A., E. Soumpourou, C. Lister, J. Ligthart, S. Kennedy, and C. Dean. 2016. Nucleotide polymorphism affecting FLC expression under
nerdphins heading date variation in horticultural brassicas. Plant J. 87:597–605.
Labate, I.A., L.D. Robertson, A.M. Baldo, and T. Björkman. 2006. Inflorescence identity gene alleles are poor predictors of inflorescence type
in broccoli and cauliflower. J. Amer. Soc. Hort. Sci. 131:667–673.
Lin, S.I., J.G. Wang, S.Y. Poon, C.L. Su, S.S. Wang, and T.J. Chiuo. 2005. Differential regulation of the flowering-Locus C expression by vernalization in cabbage and Arabidopsis. Plant Physiol. 137:1037–1048.
Lin, Y.R., Y.J. Lee, M.C. Tseng, C.Y. Lee, C.H. Chen, S.S. Wang, C.C. Liou, L.S. Shuang, A.H. Paterson, and K.K. Hwu. 2018. Subtropical
adaptation of a temperate plant (Brassica oleracea var. italica) utilizing non-vernalexpression-responsive QTLs. Sci. Rep. 8:2–11.
Liptay, A. 1981. Cauliflower: Curd initiation and timing of production in a high-temperature growing season. Acta Hort. 122:47–52.
Marquardt, S., P.K. Boss, J. Hadfield, and C. Dean. 2006. Additional targets of the Arabidopsis autonomous pathway members, FCA and FY.
J. Exp. Bot. 57:3379–3386.
Matschegewski, C., H. Zetsche, Y. Hasan, L. Leibeguth, W. Briggs, F. Ordon, and R. Uptmoor. 2015. Genetic variation of temperature
regulated curd induction in cauliflower: Elucidation of floral transition by genome-wide association
mapping and gene expression analysis. Front. Plant Sci. 6:720, doi: 10.3389/fpls.2015.00720.
Nowbuth, R.D. and S. Pearson. 1998. The effect of temperature and shade on curd initiation in temperate and tropical cauliflower. Acta Hort. 459:79–87.
Okazaki, K., K. Sakamoto, R. Kikuchi, A. Saito, E. Togashi, Y. Kuginuki, S. Matsumoto, and M. Hirai. 2007. Mapping and characterization of
FLC homologs and QTL analysis of flowering time in Brassica oleracea. Theor. Appl. Genet. 114:595–608.
Olesen, J.E. and K. Gregersen. 2000. A simulation model of climate effects on plant productivity and variability in cauliflower (Brassica oleracea var botrytis). Scientia Hort. 83–87–107.
Pearson, S., P. Hadley, and A.E. Wheldon. 1994. A model of the effects of temperature on the growth and development of cauliflower (Brassica oleracea var. botrytis). Scientia Hort. 59:91–106.
Rahman, H.U., P. Hadley, and S. Pearson. 2007. Relationship between temperature and cauliflower growth and development after curd initiation. Plant Growth Regulat. 52:61–72.
Rahman, H.U., P. Hadley, S. Pearson, and M.J. Khan. 2013. Response of cauliflower (Brassica oleracea var. botrytis) growth and development after curd initiation to different day and night temperatures. Pak. J. Bot. 45:411–420.
Rahman, H.U., E.C. Howell, H.J. Newbury, and M.J. Kearsey. 2008. Does sequence polymorphism of FLC paralogues underlie flowering time
in cauliflower (Brassica oleracea L. var. botrytis)? Theor. Appl. Genet. 116:179–192.
Reeves, P.H. and G. Coupland. 2000. Response of plant development to environment: Control of flowering by daylength and temperature. Curr. Opin. Plant Biol. 3:37–42.
Ridge, S., P.H. Brown, V. Hecht, R.G. Driessen, and J.L. Welle. 2015. The role of BoFLC2 in cauliflower (Brassica oleracea var. botrytis L.)
reproductive development. J. Exp. Bot. 66:125–135.
Shea, D.J., E. Itabashi, S. Takada, E. Fukai, T. Kakizaki, R. Fujimoto, and K. Okazaki. 2018. The role of FLOWERING LOCUS C in vernal-
ization of Brassica: The importance of vernalization research in the face of climate change. Crop Pasture Sci. 69:30–39.
Smyth, D.R. 1995. Origin of the cauliflower. Curr. Biol. 5:361–363.
Sun, X.J., B. Bucher, Y. Ji, A.D.J. van Dijk, R.G.H. Immink, and G. Bonnema. 2018. Effect of ambient temperature fluctuation on the timing
of the transition to the generative stage in cauliflower. Environ. Exp. Bot. 155:742–750.
Uptmoor, R., T. Schrag, H. Stützel, and E. Esch. 2008. Crop model based QTL analysis across environments and QTL based estimation of time to floral induction and flowering in Brassica oleracea. Mol. Breed. 21:205–216.
Williams, C.A. and J.G. Atherton. 1990. A role for young leaves in vernalization of cauliflower: I. Analysis of leaf development during curd induction. Physiol. Plant. 78:61–66.
Wurr, D.C.E. 1981. The influence of cold treatments on the uniformity of cauliflower curd initiation and maturity. Acta Hort. 122:107–113.
Wurr, D.C.E., J.R. Fellows, K. Phelps, and R.J. Reader. 1994. Testing a vernalization model on field-grown crops of four cauliflower cultivars. J. Hort. Sci. 69:251–255.
Wurr, D.C.E., J.R. Fellows, and K. Phelps. 1996. Investigating trends in vegetable crop response to increasing temperature associated with climate change. Scientia Hort. 66:255–263.
Wurr, D.C.E. and J.R. Fellows. 2000. Temperature influences on the plant development of different maturity types of cauliflower. Acta Hort. 539:69–74.
Wurr, D.C.E., J.R. Fellows, and M.P. Fuller. 2004. Simulated effects of climate change on the production pattern of winter cauliflower in the UK. Scientia Hort. 101:359–372.
Zenkteler, E., S. Samardakiewicz, A. Kalużewicz, and M. Knaflewski. 2012. Effect of devernalization on the transition from vegetative to prefloral phase of the broccoli (Brassica oleracea var. italica cv. ‘Fiesta’) shoot meristem. Acta Agrobot. 65:29–36.