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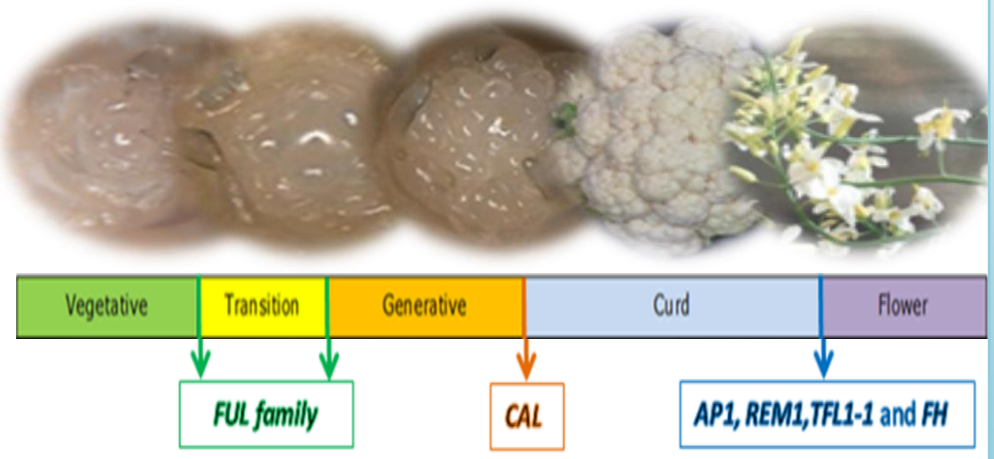
Molecular and morphological characterization of meristems in cauliflower:

Effects of high ambient temperature on switch to generative stage



Thesis of Master of Science

By:

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Department of Plant Breeding

Wageningen University

April, 2013

Molecular and morphological characterization of meristems in cauliflower:

Effects of high ambient temperature on switch to generative stage

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Molecular and morphological characterization of meristems in cauliflower:

Effects of high ambient temperature on switch to generative stage

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Molecular and morphological characterization of meristems in cauliflower:

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Abstract

Flowering time is the most important developmental trait for most crops, including *Brassica* crops (*Brassica oleracea ssp. Botrytis*). It determines where the crops can be cultivated and ensures high agricultural productivity. In many early cauliflower varieties, hot temperature causes a delay in the switch from vegetative to generative meristem and thus a delay in curd formation and harvest which causes economic losses during the cauliflower production. Base on the knowledge from the model plant *A. thaliana* and *Brassica rapa*, the expression of 4 reference genes (*EFα1, YLS8, 18sRNA* and *Actin*) and 17 flowering time candidate genes (*AP1-a, AP1-c, FUL-a, FUL-b, FUL-c, FUL-d, FH, TFL1-1, CAL, REM1, UFO, CCE1, NSN1-1, NSN1-2, NSN1-3, Cry2-1* and *Cry2-2*) were measured to molecularly indicate the phase shifts in cauliflower meristem and changes in gene expression during curd formation and flowering under the plants exposed to high ambient temperature (day/night: 27/22°C) at 5th, 6th and 7th week when still in vegetative stage. Primers could be designed using the sequence of reference genome chiifu (AA). A stable reference gene *Actin* was identified and a set of genes with expression correlating to cauliflower meristem developmental stages was defined (*AP1, REM1, TFL1-1, FH, NSN1, CAL* and *FUL* family). This set of flowering time candidate genes was monitored for expression for several weeks (week 5 to 20) after control and high temperature treatment and 24hr before and after high temperature switch. The results showed that the expressions of *CAL* and *Cry2* were influenced by high ambient temperatures at week 6 after sowing, which causes a delay of the switch from vegetative to generative meristem of cauliflower. At the same time the related morphology traits, leaf number, stem thickness, curd weight and curd diameter are also observed to find the correlation between these trait and the timing of meristem stage switches.

Key words: *Brassica oleracea ssp. Botrytis,* flowering time, meristem switch, curd formation, temperature swap, gene expression

1. Introduction

1.1 Brassicaceae

Brassicaceae family (Cruciferae or mustard family) is one of the largest families in the angiosperm plant kingdom which comprises 338 genera and 3700 species in some 10 poorly defined tribes (Sadowski & Kole,2011). Several species of great scientific, economic and agronomic importance are included, such as model species (e.g., *Arabidopsis),* as well as many widely cultivated species (e.g. cabbage, cauliflower, turnip, radish, etc.). *Brassicaceae* viz. *Arabidopsis* (*Arabidopsis thaliana*) and *Brassica* species as the well-known model plants have revolutionized out knowledge in almost every field of modern plant biology. The genus *Brassica* is a monophyletic group which is evolutionarily closely related to model crucifer plant *Arabidopsis thaliana*, and is reported to have diverged almost for 14.5-20.4 million years (Naser et al.,2012).

The well-known plant from the family *Brassicaceae* viz. *Arabidopsis* lacks its economic value but it is a model for molecular biology and genetics because of the small genome size, short generation time, also the ease of genetic approaches to study development, physiology, and gene function coupled with simple transformation methods.

The genus *Arabidopsis* has nine species and a further eight subspecies which is based on morphological and molecular phylogenies (Koch et al.,2008). The *Arabidopsis* species *Arabidopsis thaliana* (2n=10) is currently used in almost every discipline of biological experiment. In fact, after *Drosophila melanogaster* and *Caenorhabditis elegans*, *A.thaliana* was the first plant, and the third multicellular organism to be completely sequenced (Naser et al.,2012). The completely sequenced genome of *A. thaliana* paved the way to be a better understanding of every aspect in plant biology and presents the way to study gene function in crop plants.

Cultivated *Brassicas* are represented by six interrelated species, each with considerable morphological variation as the result of local selection and breeding. The three diploid species are *Brassica rapa* (2n=20, genome AA), *B.nigra* (2n=16, genome BB) and *B.oleracea* (2n=18, genome CC). From the cytogenetical relationships of the *Brassica* species it is evident that there are three amphidiploid derivatives, *Brassica juncea* (2n=36, genome AABB, brown mustard), *B.carinata* (2n=34, genome BBCC), *B.napus* (2n=38, genome AACC, oilseed rape), derived by hybridization and polyploidization of two of the diploid species (Ashraf & McNeilly,2004). The genetic relationship between species in the *Brassicaceae* family is described by well-known triangle of U **(Figure1)**.

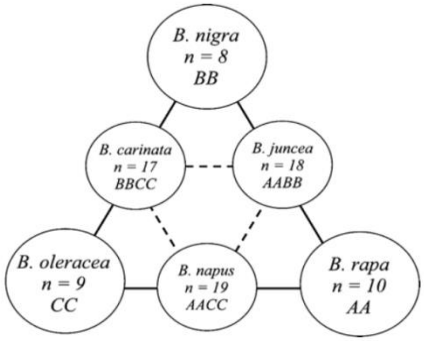


Figure1. *Cytogenetic relationships between the diploid and amphidiploid Brassica species. Adapted from U (1935).*

The phylogenetic relationships within *Brassicaceae,* and of genome structure, gene expression are studied in recent years. Comparison between *A. thaliana* and related species have been used to study genetic control of flowering and leaf form in several Brassicaceae species. (Schranz et al.,2007). Preliminary comparisons between *Brassica* species and *A. thaliana* have indicated that the small genome of *A. thaliana* is syntenic to the three sub-genomes of diploid *Brassica* species (Lagercrantz,1998). So the relative knowledge, which has already been found in *A.thaliana* could be applied in *Brassicas'.*

1.1.1 Brassica oleracea ssp. Botrytis (Cauliflower)

Brassica oleracea in its natural form is called *Wild Cabbage* which is a tall biennial plant growing up to two meters. The wild cabbage is domesticated into a range of cole vegetables (Smyth,1995). *Brassica oleracea* is a diploid species with many subspecies covering a wide range of commercial important vegetable crop forms, also be called cultivar groups, such as broccoli, cauliflower, cabbage, kale, brussels sprouts, savoy and Chinese kale **(Table 1)** (O'neill & Bancroft,2000; Bonnema et al.,2011). *B.oleracea* is tolerant to salt, lime and became established as an important human food crop plant. All of the *B.oleracea* vegetables are powerhouses of nutrition and contain good quantities of many essential vitamins and minerals.

Table 1*. Seven major cultivars groups of B.oleracea*

Cultivar groups	Capitata	Acephal	Alboglabra	Botrytis	Gemmifera	Gongylodes	Italica
Examples	cabbage	kale	Chinese broccoli	cauliflower	brussels sprouts	kohlrabi	broccoli
Phenotypes							

Brassica oleracea ssp. Botrytis (Cauliflower) is a short caulescent plant with shoot tips composed of young leaves and leaf primordia situated around an apical dome which is separated by expanding internodes. The shoot tip components of the cauliflower are large, and easy to be detached, measured and analysed following environmental changes. The grower's cauliflower

consists of a large immature inflorescence (the curd) (Dixon,2007). The curd is now generally considered to be an early, arrested stage of indeterminate inflorescence development, as its formation precedes floral initiation (Anthony et al.,1996). The inflorescence is a raceme, and flowering starts from the bottom to the top of the inflorescence. During the development of the reproductive structures, the inflorescence begins to elongate from the outer flowers of the curd and can be as large as 60 to 75 cm. When the temperature is greater than 20°C, it will stimulate seed stalk elongation and form yellower flowers with 4 petals, 4 sepals, 6 stamens, one pistil contains two carpels with 10 to 15 ovules inside each carpel. After fertilization of the ovules, seeds form inside a pod is called a silique (Rosales). According to the morphology, the growth phases of cauliflower can be divided into five stages, between vegetative growth and flowering: (1) the vegetative stage; (2) initiation of inflorescence resulting in formation of secondary meristems in axils of bracts; (3) curd development by the reiteration of meristems, each new meristem gives rise to a higher order; (4) curd maturity with no flower initials; (5) floral differentiation and elongation in some of the inflorescence branches. (Anthony et al.,1996). Among the many *Brassica* species, the seedling has to pass through a juvenile phase during which it cannot be induced to flower (Guo et al.,2004). Cauliflower plants go through a juvenile stage during which curd initiation does not occur, and cannot be initiated. The end of the juvenile period depends on the variety. At the completion of this stage, the plant has reached a mature vegetative phase when curd initiation can occur, or be induced. (North Willamette Research & Extension Center,2004). More information is needed with regard to the above environmental and juvenile period factors on actual inflorescence initiation and development (Guo et al.,2004). Reproductive development determines the value of the crop, the key steps in this phase of growth remain physiologically and genetically poorly understood (Denise & Thomas,2008). To reduce uncertainties in harvest time predictability and market availabilities, it is necessary to control the timing of floral transition and curd formation during the breeding effort (Jung & Mu¨ller,2009).

1.2 Flowering Time

One of the most important traits for both wild and cultivated plants is flowering time which is also the most important developmental trait for the production of *Brassica* crops (Yuan et al.,2009). Phenotypic flowering time is mostly measured as days after sowing (DAS) until the day on which first flowering appeared on 50% of plants (Liu,2001). It is a complex trait that is mainly regulated by the genotype and by environmental factors such as photoperiod, nitrogen level, ambient temperature and light. Temperature in considered to be one of the major factors (Nowbuth & Pearson, 1998). Change in the flowering time can lead to large reductions in the yield and quality of the harvested products. Therefore, understanding the mechanism of flowering control is important in agronomic practice. In this study we do not consider flowering time, when the first flower opens, but we use the term molecular flowering time, or molecular switch from vegetative to generative meristem. This can be seen by cytological observation of the meristem. This can be measured also by the transcription levels of the flowering time genes in *Arabidopsis*.

1.2.1 Flower time in *Arabidopsis*

Flowering time in *Arabidopsis* has become a model for understanding complex trait genetics in plants. Many genes involved in the regulation of flowering-time have been identified from *Arabidopsis*. Geneticists have identified ~180 genes which implicated in flowering time control in the model plant species *Arabidopsis thaliana* (Fornara et al.,2010). These genes evolve an elaborate genetic network. The flowering time genes function on four major promotion pathways: photoperiod, vernalization, autonomous, and gibberellin **(Figure 2)**.

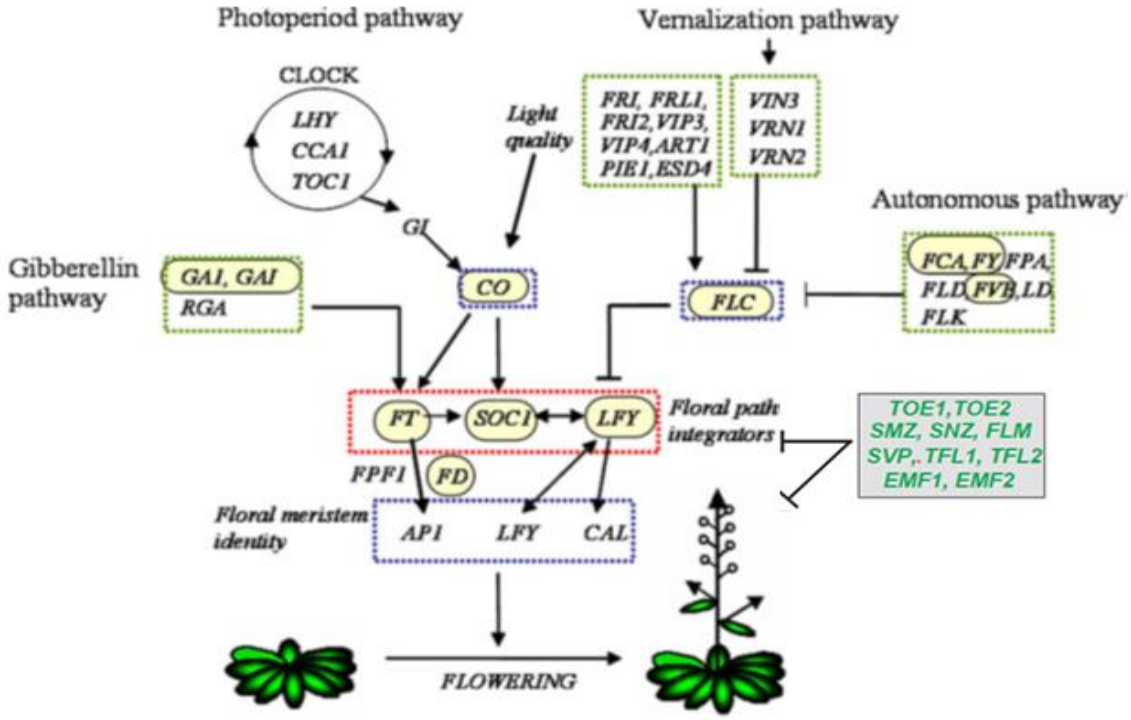


Figure 2. *Pathways controlling flowering time in Arabidopsis (Corbesier & Coupland,2006; Henderson & Dean,2004).*

Molecular studies have conclusively show that *CRYPTOCHROME2 (CRY2), FRIGIDA (FRI), FLOWERING LOCUS C (FLC), FLOWERING LOCUS (FLM), PHYTOCHROME A (PHYA)* all harbor natural polymorphisms that alter flowering time in *Arabidopsis*. During that, *FLC* involved in the convergence of autonomous and vernalization pathways, encodes a MADS-box transcription factor that acts as a repressor of the floral transition in a dosage-dependent manner (Rouse et al.,2002). And *FLC* may also influence the ambient temperature pathway by physically interacting with the *SHORT VEGETATIVE PHASE (SVP)* which is the floral repressor gene under different ambient temperature (Lee et al.,2007). In another study, alternatively splicing was found in *Arabidopsis* that influenced the flowering time. A normal-length *FLC* transcript with nonsense mutation as well as an alternatively spliced transcript lacking exon 6 was found in the early flowering Van-0 *A.thaliana* accessions (Werner et al.,2005).

A number of key findings have emerged in some studies, the sequential action of floral meristem identity genes (switch the fate of meristem from vegetative to floral) and organ identity genes

(direct the formation of the various flower parts) were involved in flowering in *A.thaliana*. Therefore, flowering time control genes can be expected to interact with floral meristem identity genes. And the floral meristem identity genes are themselves capable of influencing flowering time (Levy & Dean,1998). For example, the overexpression of *LFY* and *AP1* causes early formation of determinate floral meristems (Yaron & Caroline,1998). *Ap1* has been identified as playing a role in inflorescence phase switch in *Arabidopsis* which encodes a putative transcription factor that acts locally to specify the identity of the floral meristem and to determine sepal and petal development. RNA tissue in situ hybridization studies show that *AP1* RNA accumulates uniformly throughout young floral primordia, but is absent from the inflorescence meristem (Gustafson-Brown C et al.,1994). On the molecular level, *AP1* was used to identify the flowering in *Arabidopsis* leaf primordia.

1.2.2 Flower time in *Brassica oleracea ssp.* Botrytis (Cauliflower).

It is an annual or biannual plant that reproduces by seed. Typically, flower bud development in cauliflower in arrested. Instead, the inflorescence meristem continuously generates replicas of itself in a spiral on its flanks. Each new meristem can in turn produce more, in a closely packed, geometric cluster of undifferentiated racemose inflorescence meristems (“curd”) (Smyth,1995).

Curd as an inflorescence is the edible part of the cauliflower plant and the phase transition from the vegetative to the generative phase is of crucial importance. There are three distinguished developmental phases between germination of seed and harvest of the curd (Lagercrantz,1998). Time on which plants resume growth after transplanting affects the duration of the juvenile phase most. The juvenile stage in the stage is which the plantlets are not affected by temperature fluctuations (vernalization or high temperatures). Variability in time of curd initiation within a cauliflower crop appears to be mainly due to a variation in time on which the juvenile phase ended. The end of the juvenile phase is another important phase transition in relation to curd induction, as during the juvenile phase the plant is insensitive to curd inducing conditions, such as the ambient temperature. The end of the juvenile phase can be characterized best by the number of initiated leaves.

In several cauliflower cultivars, like *cv. Delira* and *cv. Elgon*, time of curd initiation after the end of the juvenile phase depends among others on temperature; higher temperature delays curd initiation and increases the final number of leaves. The starting point of the temperature-sensitive phase is uncertain. The existence of a juvenile phase has been reported for cauliflower and the end of this phase is characterised by a physiological switch, which makes plants sensitive to environmental signals (Uptmoor et al.,2011). Several studies have tried to elucidate the genetic control of developmental arrest in *B. oleracea* by identifying and characterizing homologues of the *Arabidopsis* floral homeotic genes (Duclos,2008). The main flowering regulators and their response to environmental signals have been identified in *Arabidopsis thaliana* and homologues of flowering genes have been mapped in many crop species.

The range of variation in flowering time can be large, with a significant amount of this diversity arising from heritable genetic variation. This is exploited by breeders to breed spring, summer and autumn cauliflowers. Varieties differ in the length of time they may remain in a mature vegetative phase before curds are initiated and may also differ in the time required to produce harvestable curds after initiation has occurred (North Willamette Research & Extension Center,2004). High temperatures during the development of cauliflower may be considered as a situation of environmental stress which influence curd initiation. Some varieties are not sensitive for high temperature during vegetative stage.

However, some varieties will respond to a high temperature period during vegetative phase by delay switch to generative meristem and initiating curds. This characteristic can cause harvest scheduling problems. However, the genetic background resulting in flowering time variability in *B. oleracea* is currently not completely understood, so that assigning genotype differences to known genes remains difficult (Uptmoor,2011). The flowering time in cauliflower corresponds to developmental characteristics of apices meristems. Some studies have tried to elucidate the genetic control of *B. oleracea* meristem development by identifying several genes expression during the curd proliferation and flower initiation. Duclos and Bjo¨rkman (2007) proposed that some genes in *B. oleracea*, the expression pattern was arrested which is maintained by altered expression of the genes at different meristem developmental stages by varying the temperature regime. The meristem identity genes *LEAFY (LFY), APETALA 2 (AP2),UNUSUAL FLORAL ORGANS (UFO),* and the MADS-box genes *APETALA 1 (AP1), CAULIFLOWER (CAL),* and *FRUITFULL (FUL),* as well as the floral repressor *TERMINAL FLOWER 1 (TFL1),* and two other curd associated genes *CAULIFLOWER CURD EXPRESSION 1 (CCE1) and REPRODUCTIVE MERISTEM 1 (BoREM1)* were examined at different developmental stages. The reproductive stage was determined by dissection and measurement of the apical meristem. *AP1-a* and *AP1-c* transcripts increased at the inflorescence meristem stage. *CAL* and *REM1* had their maximum expression at the inflorescence meristem. However *CAL* expression declined significantly in reproductive meristem. *LFY* reached its maximum expression in the initial stage of reproductive development, the vegetative to reproductive transition. *UFO* was expressed at very low levels at all developmental stages. *CCE1* transcript levels were equally high in inflorescence meristem and floral primordium. All four *FUL* paralogues were expressed at all stages. *FUL-b, FUL-c,* and *FUL-d* had maximum expression at the inflorescence meristem stage.

We harness this gene expression knowledge to identify potential meristem identity genes that play roles in specific pathways. The pathway responsible for meristem development in *B. oleracea*.

1.3 Gene expression profiling using qRT-PCR

The mRNA molecule as the link between DNA and proteins is of central interest in bioscience. Reverse transcription PCR (RT-PCR) represents a sensitive and powerful tool for analysing RNA (Leslie A. Pray,2008). “Due to its outstanding accuracy, broad dynamic range, and sensitivity,

QPCR has become the most emerging method for quantification of mRNA transcription levels in recent years. Moreover, QPCR is fast, easy to use, and highly reproducible, requiring a minimal amount of RNA, no post-PCR handling, and it avoids the use of radioactivity" (Aleksandar et al., 2004). The initial step in RT-PCR is the production of a single-strand complementary DNA copy (cDNA) of the RNA through the action of the retroviral enzyme, reverse transcriptase. An oligonucleotide primer is required to initiate cDNA synthesis. The primer anneals to the RNA, and the cDNA is extended toward the 5' end of the mRNA through the RNA-dependent DNA polymerase activity of reverse transcriptase (Freeman et al., 1999). To compare the different RNA transcription levels the CT values were compared directly. The CT is defined as the number of cycles needed for the fluorescence signal to reach a specific threshold level of detection and is inversely correlated with the amount of template nucleic acid present in the reaction (Aleksandar et al., 2004).

1.4 Scope of thesis and research goals

This Master's thesis puts forward a program for studying *Brassica oleracea ssp. Botrytis* (Cauliflower) flowering time in two different cultivars (temperature sensitive cultivar Lindurian and insensitive cultivar Fremont), with the aim to understand the mechanism of how the high ambient temperature affects timing of cauliflower meristem switch and curd formation.

The morphological characterization of cauliflower is measured to understand the influence of high temperature during the cauliflower growing process. According to that purpose, various types of characteristics are measured in the greenhouse or field, such as leaf number, stem thickness, curd weight and curd diameter while the collected phenotypic data will be used for statistical data analysis methods to find the correlation between leaf number and stem thickness, curd weight and curd diameter. Another aim is to assess the high ambient temperature delay cauliflower meristem switch by observing the meristem transition under the stereomicroscope.

The main aim in this study is to identify genes associated with cauliflower meristem developmental stage identity with a candidate gene approach. Simultaneously, the changes in candidate genes expression which are influenced by high ambient temperature treatment are researched in different treatment groups and different time points.

2. Materials and methods

2.1 Plant materials

Two hybrid *Brassica oleracea ssp. Botrytis* (cauliflower) cv. Fremont and cv. Lindurian which are usually planted in early spring and later summer were used in this study. The main difference between these two cultivars is that Fremont (Monsanto) is not sensitive to ambient temperature changes (read periods of high temperature) while Lindurian (Syngenta) is, as high temperature lead to unpredictable and delayed harvest time.

2.2 Growth conditions

In order to investigate the temperature sensitivity, we measured a set of phenotypic traits on cauliflower plants cultivated under two different environment growth conditions, both field and greenhouse. For the field experiment, plants growing under the natural environment conditions. In contrast to the field experiment, growth conditions in greenhouse were manual controlled which allowed to expose plants to well defined temperature increase during a period of 7 days.

2.2.1 Field experiment

To investigate the flowering time variation, plants were grown in the open field (sandy clay), under natural environmental conditions, with planting data of 3 week old plants in the field at April 19th, April 26th, May 3rd, May 10th, May 17th, May 24th, May 31th, Jun 7th and Jun 14th, 2012 in Wageningen, the Netherlands (51°59′11″N latitude, 05°39′52″E longitude). First 72 seeds of each cultivar were sown into the seeding soil in the greenhouse (day/night: 21/16°C) for one week and then we transplanted 65 best looking seedlings into the press pots. One week later, we moved the two weeks old plants to outside to adapt to the natural environment conditions. After that, three weeks old plants, a total of 45 Fremont and 45 Lindurian best looking seedlings were planted in one block **(Appendix 1**) and there were 9 times repetitions (9 blocks) in the field experiment **(Appendix 2)**. In order to be able to measure the effect of natural ambient temperature in different meristem developmental stages, in each repetition, the cauliflower were sown and transplanted one week later than the previous block (see **Table 2** for sowing dates). Therefore, natural ambient temperature fluctuations will occur in the different cauliflower growth stages in different blocks.

2.2.2 Greenhouse experiment

Temperature swap experiments were conducted in the greenhouse, where temperature was increased for 7 days, either at week 5 after sowing or at week 6 after sowing. During the greenhouse experiments, two different ambient temperature greenhouse rooms (control room and high

temperature room) were applied. In greenhouse ambient temperature swap experiments, when plants in control room (day/night: 21/16°C) reached the appropriate stage for heat treatment, they were placed in a high ambient temperature room for one week at day/night temperature of 27/22°C and then swapped back to the control ambient temperature environmental conditions. The swap was always done from 12.00 o'clock till the next day 12.00 o'clock.

This greenhouse experiment 1: started at February, 2012. It was a test experiment to find when the ambient temperature sensitive cultivar Lindurian was more sensitive to the high ambient temperature, meaning when high temperature treatments result in a delayed meristem switch did. Three hundred seeds of Lindurian were sown in the seeding soil in modular trays on February 27th 2012. Plants were grown in control greenhouse room under the day/night temperature of 21/16°C. After two weeks, 250 best looking seedling were transplanted into 17cm pots followed by continued growing in the greenhouse. When the seedlings were five weeks old, we transferred 72 plants to high ambient temperature greenhouse room (day/night: 27/22°C). One week later, swap 64, 6 weeks old plants were transferred from control room to high ambient temperature room, and 5 weeks high temperature treatment plants were put back to the control temperature greenhouse room. Next week, did the similar thing, swapped 56, 7 weeks plants and put back 6 weeks high temperature treatment plants. And then, after one week heat temperature treatment the 7 weeks old plants, decreased the high ambient temperature greenhouse room (day/night: 27/22°C) to control temperature (day/night: 21/16°C). Three high ambient temperature treatment groups were completed and the plants continued growing in the greenhouse under the day/night temperature of 21/16°C.

For the greenhouse temperature swap experiment 2: two different cultivars were used (*Brassica oleracea ssp. Botrytis* cv. Fremont and cv. Lindurian). Three hundred seeds of Lindurian and Fremont were sown in the seeding soil in modular trays on June 12th 2012. Trays were placed in a greenhouse at day/night temperature of 21/16°C. After two weeks, 250 best looking seedlings for each cultivar were transplanted into 17cm pots (the soil in the pot is "Lentse potgrond") followed by growing in the greenhouse. There were two tables in each greenhouse room and one cultivar was placed on one table. Plants were growing under the control ambient temperature greenhouse conditions (day/night: 21/16°C) and subsets were used for heat treatment. By previous studies, there was an indication of the period that the Lindurian cultivar was more sensitive to the ambient temperature when the plants were 5 and 6 weeks old. When the plants growing to 5 weeks old (after sown 5 weeks), increased the ambient temperature of one greenhouse room from 21/16°C (day/night) to 27/22°C (day/ night) as the high ambient temperature room. After the 5 weeks old plants growing under high temperature for one week, swapped the 5 weeks high temperature treatment plants from high temperature room (day/night: 27/22°C) to the control room (day/night: 21/16°C). At the same time, the 6 weeks old plants (after sown 6 weeks) were replaced to high temperature room. One week later, switched the high ambient temperature to the control temperature. Per temperature treatment group (control, 5th weeks and 6th weeks) 70 plants were used for each cultivar (Fremont and Lindurian). Plants in control group were maintained in the control greenhouse (day/night: 21/16°C) all the time.

2.3 Phenotypes measurements

A total of five phenotypic traits (leaf number, stem thickness, curd weight, curd diameter and apical meristem) were investigated in the field, while 3 traits were measured in greenhouse (Leaf number, stem thickness and apical meristem).

2.3.1 Leaf number measurement

In the greenhouse temperature swap experiment, the numbers of leaves were counted twice a week (Tuesday and Thursday at 12.30pm) in control, 5th weeks and 6th weeks high temperature treatment groups for each cultivar. Every time we randomly choose 5 plants from one cultivar in each group. In the field experiment, 5 consecutive plants in one line for each cultivar were chose to count the leaf number once a week (Friday). Leaf number was counted starting from the first true leaf at the bottom of the plant, until the leaf size was less than 15mm at the top **(Figure 3).**

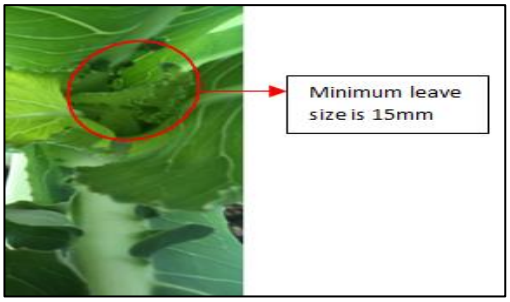


Figure 3. *Minimum measurement size of the leaf number*

2.3.2 Stem thickness measurement

Measure the thickness of the stem (mm) in greenhouse temperature swap experiment twice one week (Tuesday and Thursday at 12.30pm). "Vernier Caliper" was used to measure a portion of the stem above the cotyledon and below the first true leaf of the plant **(Figure 4).** Measurements were conducted to the nearest 0±1 mm.

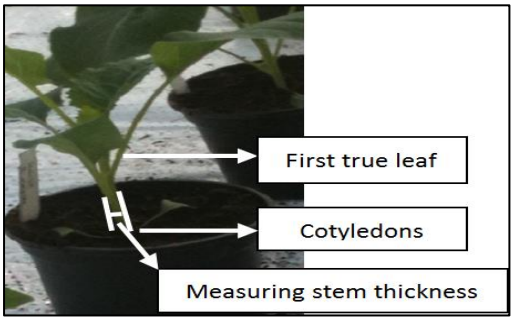


Figure 4. *Plants stem thickness measuring position*

2.3.3 Curd weight and curd diameter measurement

Cauliflower curds in the field from each block were harvested start from July 26th 2012 to 06-09-September 6th 2012. Initial time, 15 weeks old curds (15 weeks after sowing) were harvested from block 1, 2, 3 and 4. But as we saw that the curds were harvested too late which caused they were much older than the normal ones in the supermarket, so we decided to harvest curds two weeks earlier **(Table 2).** 13 weeks old curds (13 weeks after sowing) were harvested from block 5, 6, 7, 8 and 9. Each cultivar (Lindurian and Fremont) in each block, 4 (repeat) x 5 (individual plant) were harvested

one time, removed all leaves and then measured out the curd weight (g) and curd diameter (mm). Measurement of curd diameter was at the widest point of the curd **(Figure 5).**

Table 2. *Cauliflower sowing and harvest time in nine blocks in the field*

Block	Curd old (weeks)	Sowing Time	Harvest Time
1	15	19-4-2012	26-7-2012
2	15	26-4-2012	02-8-2012
3	15	3-5-2012	09-8-2012
4	15	10-5-2012	16-8-2012
5	13	17-5-2012	09-8-2012
6	13	24-5-2012	16-8-2012
7	13	31-5-2012	23-8-2012
8	13	7-6-2012	30-8-2012
9	13	14-6-2012	06-9-2012

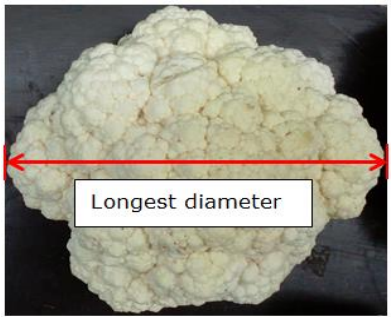


Figure 5. *Measuring the diameter of the longest as the curd diameter*

2.3.4 Apical meristem observation

The developmental stage of the apical meristem was identified by stereomicroscope twice a week (Tuesday and Thursday at 13.30pm) in the greenhouse experiment and once (Friday at 15.30pm) in the field experiment. Under the stereomicroscope, we used forceps and solution planer moving out the leaves around the meristem until the apical meristem was exposed and made pictures for the cauliflower apical meristem under 2.5 times objective lens to identify the plant growth stage. For each cultivar, one meristem was observed for each greenhouse treatment group and field block. Simultaneously, three meristems were harvested and frozen in liquid nitrogen to provide start materials for later gene expression research. Different from greenhouse experiment, one more plant of each cultivar was leaved in the field as the harvested marker.

2.4 RNA isolation, quality control and cDNA synthesis

Total RNA was extracted using the TRIZOL reagent (Invitrogen) starting with frozen cauliflower meristem enriched material. In the previous part of the experiment, each RNA sample was isolated from a single cauliflower meristem. While, in “expression profile of gene before and after temperature swap” experiment, three meristems which used for RNA isolation were harvested for each cultivar in each treatment at the same time point in greenhouse. Genomic DNA contaminations were effectively

removed using RNase-free DNase I treatment (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions, since melting curve gave single peak and genomic amplification was larger than RNA/cDNA amplification(different exons primers). The concentration and quality of the cleaned RNA was measured using the nucleic acid analytic apparatus K6000 (Bio Photometer, Eppendorf, Germany) by 260/280 nm absorption ratio 1.9~2.1. RNA concentration and purity were quantified with Nanodrop measurements and the quality of the total RNA was checked on a 1.5% RNase free agarose gel.

All RNA samples were diluted with nuclease free water (Qiagen) to 100ng/µl in a total volume of 10µl to use for cDNA synthesis. cDNA synthesis was performed with iScriptTM kit (BIO-RAD) according to supplier's instructions and a final volume of 20ul per cDNA sample was obtained. cDNA working dilutions were made at 1:20 with RNA free MQ water.

2.5 Primer design

Specific primer pairs were designed for 4 reference genes *(EFα1, YLS8, 18S and Actin*) **(Table 3)** and 17 candidate genes (*AP1-a, AP1-c, FUL-a, FUL-b, FUL-c, FUL-d, FH, TFL1-1, CAL, REM1, UFO, CCE1, NSN1-1, NSN1-2, NSN1-3, CRY2-1 and CRY2-2*) **(Table 4).** The full cDNA sequences of the genes were retrieved from *Arabidopsis thaliana* (L.) Heynh and blasted against *B. rapa* gene sequences. If for one gene, several orthologous genes were in *B.rapa*, sequence comparisons were done by DNASTAR, Lasergene 9.1 (Lasergene, Madison, Wisconsin, USA), found the specific sequence regions among the orthologous genes. Base on the specific sequence in conserved regions, orthologous specific primers were designed using Primer3Plus website. A total set of 23 primer pairs with Tm 60 ±2 °C , free of dimer structures and with amplified product length no longer than 200bp were designed. Primer specificity and DNA contamination which caused by human operation during primer dilution process were visualised by separating PCR products from cDNA and DNA on 1.5% agarose gel and when only a single band was observed, the band was purified to be template for preparing the standard curves (which takes into account primer efficiency) using qRT–PCR (Ramakers et al.,2003).

Among the 17 candidate genes, we choose gene *Cry2* to test in cauliflower vegetative meristems around the temperature SWAP before and 24hr after. Base on the Arabidopsis results **(Appendix 3)**, temperature increase during the vegetative stage, accelerates flowering. RNA sequence data of RNA isolated from the vegetative meristem before and 24hr after the temperature swap showed a set of genes that was differentially expressed or differentially spliced, even a set of genes that was differentially spliced and expressed. Some genes, like *FLM,* a MADs box transcription factor involved in flowering time regulation, is further analysed. *Cry2* as a gene that was differentially spliced and expressed will be checked in cauliflower meristem materials.

Table 3. *Primers for candidate reference genes and melting peaks cause after qRT-PCT*

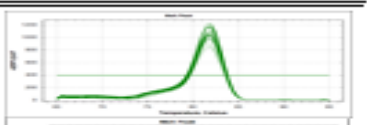
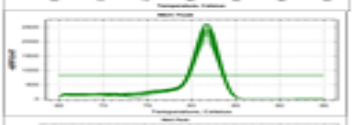
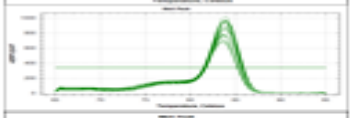
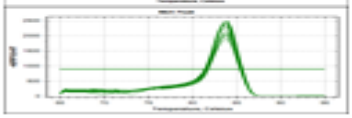
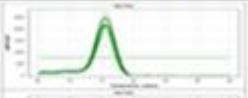
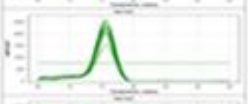
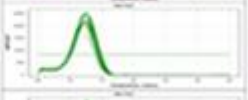
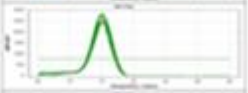
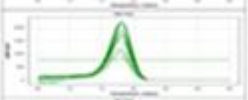
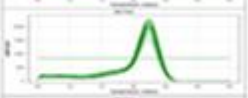
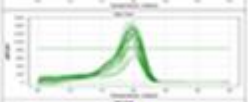
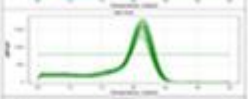
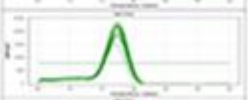
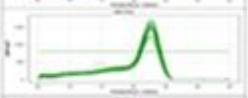
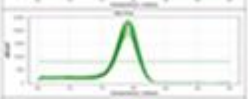
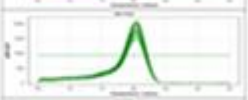
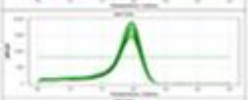
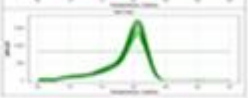
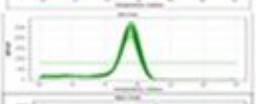
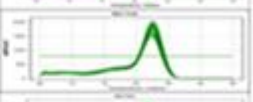
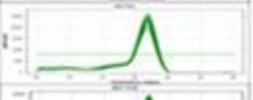
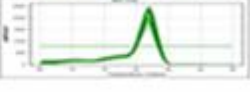
Primer		Sequence	Melting Peak
EFα1	Fw	GTCAGACCCGTGAGCATGCTCTTC	
	Rv	GATCTCATCGTACCTAGCCTTGGAG	
YLS8	Fw	CGGCCATGATTGGGATGAGACTTG	
	Rv	GCTCGTACATGGTGTTGAAGTCTGGAAC	
18S	Fw	CGAGACCTCAGCCTGCTAACTAG	
	Rv	TCAAACTTCCTTGGCCTAAACG	
Actin	Fw	ACGTGGACATCAGGAAGGAC	
	Rv	GAACCACCGATCCAGACACT	

Table 4. *Primers for candidate genes and melting peaks cause after qRT-PCR*

	Primer		Sequence	Tm	GC%	Melting Peak
***AP1* paralogous**	*BoAP1-a*	Fw	CGAGCCCTTCTTATCCAACTAATT	57.1	42	
		Rv	CATACTGAAGCAAAAGAACTTGAGAAA	57.9	33	
	BoAP1-c	Fw	TGGCTAGCTTCTTTCTATCCAATTAATA	58.4	32	
		Rv	CACATACTAGAACCAAAAACTTACAAAGAGA	58.8	32	
***FUL* paralogous**	*BoFUL-a*	Fw	CGCCCTACGACGAATGAATAG	57.5	52	
		Rv	CACAAAAATGCTGAGATACATTATGA	55.3	31	
	BoFUL-b	Fw	CGCCCTACCACGAAAGAATAA	55.7	48	
		Rv	CAGTAAATCCAGAAAAATGCTGATATACA	57.7	31	
	BoFUL-c	Fw	ACGTCCTGCTACCAATGAGTAAAAT	57.8	40	
		Rv	TACGTTCTTGACATTGTAATTCCGTC	57.1	38	
	BoFUL-d	Fw	TCGTCGTTGATTGAACCAAACT	55.2	41	
		Rv	AGTCACCAAAAAAGCTGATACATTATGA	57.8	32	
	BoFH	Fw	AGCGACTTTGGTTGGTGGTATT	56.2	45	
		Rv	AACCACAGCAACTCATGAACTAATTAA	57.2	33	
	BoTFL1-1	Fw	CGTGAATTTGCGATCGAGAAT	56.2	43	
		Rv	TTTCTCTCTGAGCGTTGAAGAAGA	59.3	42	
	BoCAL	Fw	AAACCGCAGCCACCATGTA	55.2	53	
		Rv	AAGGAGATGATGCCATGTAAGGA	56.7	43	
	BoREM1	Fw	CCACGTTAAGTTTCCTTTTCAGTATTT	56.4	33	
		Rv	TGAGCCATGGAACCGAACA	54.8	53	
	UFO	Fw	TTGCGGATATGATCAAAGGAAA	54.0	36	
		Rv	ATTCAAAAGCCCATTGGTTCT	52.6	38	
	CCE1	Fw	TCGTTCCACCACCTTCCAAA	54.3	50	
		Rv	ACGAGCCTGAAATGGTCGTAAT	57.1	45	
***NSN1* paralogous**	*NSN1-1*	Fw	AGGATTGTGTTGCGTGACTG	55.2	50	
		Rv	TTTGACTCTGCGTGTTCACC	54.7	50	
	NSN1-2	Fw	GGATTGTGTTGCATGACTGG	53.2	50	
		Rv	TTTGACTCTGCGTGTTCACC	54.7	50	
	NSN1-3	Fw	TTGTCCTGGAGTTGTGATGC	53.9	50	
		Rv	TGTGGCGTACAAAGCTTGAG	56.2	50	
***CRY2* paralogous**	*CRY2-1-a*	Fw	GCTGTGCAAAGCTACAATGG	55.5	50	
		Rv	GCACAACGGATTCAACAGAC	53.5	50	
	CRY2-1-b	Fw	ACAAGGCGCAAAGTATGACC	55.1	50	
		Rv	TCCATTCAGTTGGCAACCTC	53.9	50	
	CRY2-2-a	Fw	CATTTGGTGCCCTGAAGAAG	54.2	50	
		Rv	ATAGCGGAAACCGTGCTATG	56.1	50	
	CRY2-2-b	Fw	TTGGTGCCCTGAAGAAGAAG	54.8	50	
		Rv	ATAGCGGAAACCGTGCTATG	56.1	50	

Different primers

Different primers

2.6 Quantitative real time PCR

qRT–PCR reactions were performed in 96 position carousel (Light Cycler) with the Light Cycler-RNA amplification kit SYBR Green I (Roche, Mannheim, Germany). A final volume of 10ul per reaction contained 2ul of $cDNA_{1:20\ dilution}$; 5ul of SYBR Green Supermix; 2.4ul of RNA free MQ water; 0.3ul of Forward Primer at 10uM and 0.3ul Reverse Primer at 10uM. The thermal cycling consisted of 95°C for 2 min and 40 cycles of 95°C for 20s, 55°C for 20s and 72°C for 20s **(Appendix 4).** After the PCR a melting curve was generated to check the specificity of the amplification. Data analysis was performed with the Rotor-gene 6 ver. 6.1 software (Applied Biosystems). All the cycle threshold (Ct) values from one gene were determined at the same threshold fluorescence value of 0.2. Two technical replications were made per sample and two biological replications for each time point in greenhouse experiment 1 and 2. In order to avoid misinterpretation of experimental results and erroneous analyses, stable reference gene was applied in each sample every time.

2.7 Data analysis

2.7.1 Phenotypic data analysis

Phenotypic data collected (leaf number and stem thickness, curd weight and curd diameter) were statistically analysed by analysis of variance and correlation.

2.7.1.1 Comparison of leaf number and stem thickness

The Paired Samples T Test in IBM SPSS Statisitcs program version 19th edition was performed to evaluate block (natural environmental) effect in the field or group (temperature swap) effect in the greenhouse as a response of leaf number and stem thickness traits. The difference between two cultivars can also been analysed by this method. It computed the difference between the two variables for each case, and tested to see if the average difference was significantly different from zero.

In SPSS output table **(Table 5),** the significance level of whole entire the Paired Samples T Test was fixed at 95% (P-2tailed= 0.05) which was the criterion the average difference between two variables was significantly or not.

Table 5. *Example for the paired sample T test analysing Lindurian stem thickness in greenhouse experiment*

	Paired Differences					**t[2]**	**Df[3]**	**Sig.(2-tailed)[4]**
	Mean	Std.Deviation	Std.Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair[1] 1 Control-Five	-.27692	3.16153	.87685	-2.18742	1.63357	-.316	12	.758
Pair 2 Control-Six	.51538	3.18299	.88280	-1.40808	2.43884	.584	12	.570
Pair 3 Five-Six	.79231	1.77457	.49218	-.28005	1.86467	1.610	12	.133

[1]*Pairs: Different treatment blocks in field or groups in greenhouse,* [2]*t:T value,* [3]*df: Degrees of freedom,* [4]*Sig: 2-tailed significance value.*

- If the significance value is less than 0.05, there is a significant difference

  = Traits influenced by treatment/ No different between two cultivars

- If the significance value is greater than 0.05, there is no significant difference

  = Traits not influenced by treatment/ No different between two cultivars

2.7.1.2 Correlation analysis

GenStat program version 15th edition was used to analyse the correlation between leaf number and stem thickness, curd weight and curd diameter. The significance level of correlation analysis was fixed at 95% (0.05-2tailed) and 90% (0.1-2tailed).

- If the significance value is less than 0.05, there is a significant correlation between the two variables.
- If the significance value is greater than 0.05 and less than 0.1, there is correlation between the two variables, but less significant than 0.05 level.
- If the significance value is greater than 0.1, there is not significant correlation between the two variables.

2.7.1.3 Distribution pattern of curd weigh and curd diameter analysis

Additionally, to check the distribution pattern of the observed values and normality of the data, Box plots were calculated using the GenStat program 15th edition. All the observed and collected data of cure weight and curd diameter traits were analysed separately.

2.7.2 Real time PCR analysis

The cauliflower meristem samples were divided into four strategic groups (different cultivars, developmental stages and different ambient temperature treatments) for analysis of reference gene stabilities and other candidate genes expression.

2.7.2.1 Reference gene stabilities analysis

The normalised data were imported and analysed by one stability analysis program for reference genes, geNorm ver. 3.4 (Vandesompele et al.,2002) for ranking the reference genes.

geNorm determines the gene stability measure (M) value for each gene, based on the average pair-wise variation for a particular gene with all the other tested genes. Thus, genes can be ranked according to their expression stability through the stepwise exclusion of the least stable gene. The genes with an M value were arbitrarily suggested to be lower than 1.5; genes with the lowest M values have the most stable expression.

2.7.2.2 Expression profile analysis

All the data were an average from two corresponding biological replications (RNA isolated from one meristem, two individual meristem were harvested at the same time point in each treatment group as two corresponding biological replications) done in each experiment. Amplification cycle (Ct) which is analysed with the BioRad CFX software (version 3.0) in gene expression model is the point at which fluorescence increased above a fluorescence threshold above the background fluorescence (Duclos & Bjo¨rkman, 2007). Data or mean Ct values obtained from qRT–PCR were used for expression profile analysis, and fold change relative to the expression of the candidate genes and reference genes is calculated using normalized expression (ΔΔ(Ct)) method with default threshold values using CFX Manager Software (www.bio-rad.com/genomics/pcrsupport).

Kenneth J. Livak and Thomas D. Schmittgen (2001) proposed that the calculate method for Normalized expression (ΔΔ(Ct)). The example of how to calculate the fold change in expression of the target gene relative to the reference gene at various sample groups were studied **(Table 6).** Group x is any experimental group samples ant Group 1 represents the 1xexpression of the target gene normalized to reference gene. A value that is very different from one suggests a calculation error or a very high degree of experimental variation. In this study the default value of efficiency (E) used in the gene expression calculation is 100%. 100% efficiency in this software is equivalent to an efficiency of 2 (perfect doubling with every cycle).

ΔΔ(Ct) formula:

$$\Delta\Delta(Ct) = (CT_{,Target} - CT_{,Reference})_{Group\ N} \times -(CT_{,Target} - CT_{,Reference})_{Group\ 1}.$$

Table 6. *Sample of data analysis using the* $2^{-\Delta\Delta(Ct)}$ *method.*

Sample	Group(3 repeat in 1 group)	Gene	Ct Value	Mean Ct	$2^{-\Delta\Delta(Ct)}$	Mean Fold Change in gene expression
1	**1**	**Target**	**22.3**	**21.9**	**1.023**	**1.02**
2		**Target**	**22.0**		**0.786**	
3		**Target**	**21.5**		**1.243**	
4	**2**	**Target**	**19.8**		**1.845**	**2.00**
5		**Target**	**20.2**		**2.019**	
6		**Target**	**20.0**		**2.149**	
1	**1**	**Reference**	**22.9**	**22.5**	**-**	**-**
2		**Reference**	**22.3**		**-**	**-**
3		**Reference**	**22.4**		**-**	**-**
4	**2**	**Reference**	**21.2**		**-**	**-**
5		**Reference**	**21.7**		**-**	**-**
6		**Reference**	**21.7**		**-**	**-**

$2^{-((20.0-21.7)-(21.9-22.5))}$

Normalized Expression (ΔΔ(Ct)) Formula:

Normalized fold Expression = $E^{\Delta\Delta(Ct)} = 2^{-\Delta\Delta(Ct)}$

- E= Efficiency of primer and probe set. This efficiency is calculated with the formula (%Efficiency*0.01) +1, where 100% efficiency=2 (the default value).

3. Results

3.1 Phenotype

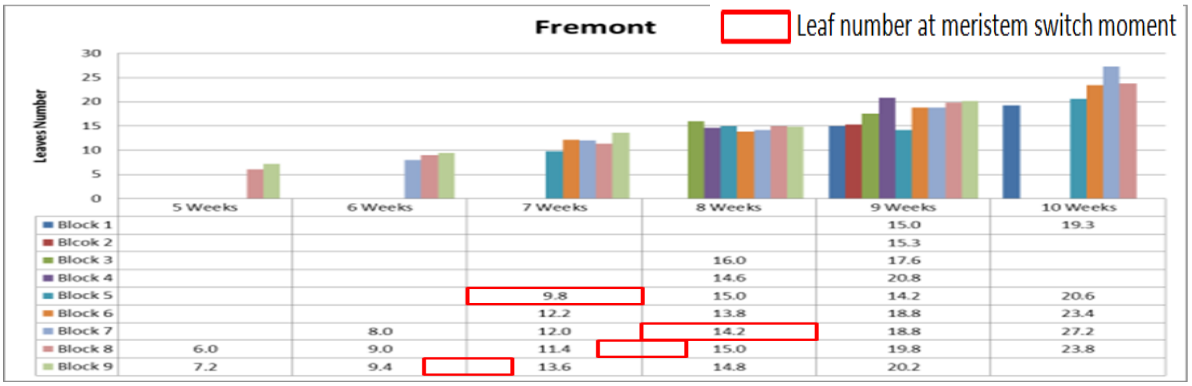
In this study, five traits (leaf number, stem thickness, curd weight, curd diameter and apical meristem) were observed in the field and greenhouse. In addition to the analysis the characteristics of the individual traits, we also tried to find the correlation between them.

3.1.1 Leaf number

For this experiment, the numbers of leaves were measured in the field and greenhouse experimental conditions. Main aim was to see whether the number of leaves at the switch form vegetative to generative meristem differed between cultivars and environments /treatments.

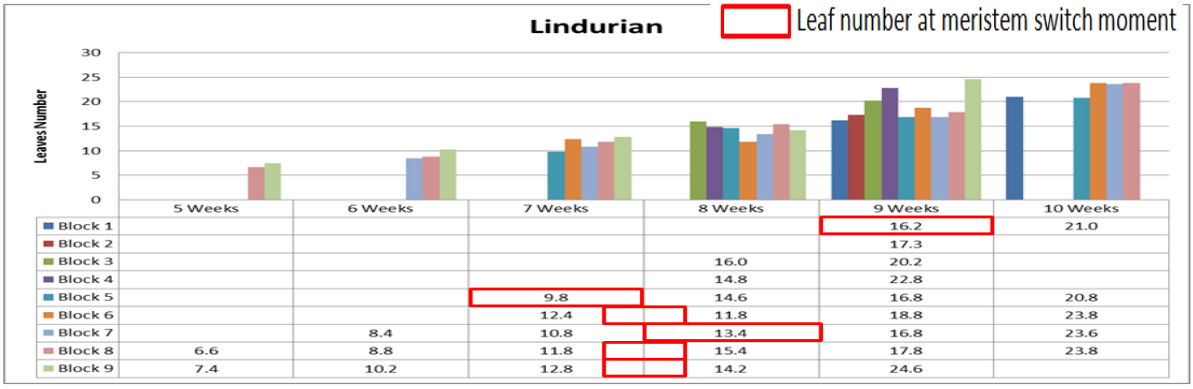
In the field, the sowing time of the seeds in each block was different leaves were accounted each week, however, during the whole field experiment, only leaf numbers of 9 weeks old plants were counted in all nine blocks. For block 1, we started monitoring meristems stage and leaf number at week 9 (after sown 9 weeks) and expected (based on Greenhouse experiment 1) that plants would be in vegetative stage. However, plants were already in generative stage, so we needed to monitor meristem stage and leaf number at earlier time points for remaining blocks. The lowest average count was 6.6 leaves measured on 5^{th} weeks in Block 8. The highest number is average 24.6 on 9^{th} weeks in Block 9.Use the SPASS software analysis the 9 weeks old cauliflower leaf number found that there were no significant differences between Fremont and Lindurian cultivar.

In **Figure 6 and Figure 7**, results are presented of the number of leaves and meristem switch moment. Fremont and Lindurian meristem switched at the same week in Block 5, 7 and 8. In Block 5, meristems switched at week 7 and number of leaves are 9.8. In Block 7, Fremont is 14.2 leaves which week meristem switch and Lindurian is 13.4. In Block 8, Fremont and Lindurian switched between week 7 and week 8, the range of leaf number from 11.4 to 15.0 in Fremont and 11.8 to 15.4 in Lindurian. The meristem switched at different week for these two cultivars in Block 9. For Fremont, meristem switched between week 6 and week7, the leaf number between 9.4 and 13.6. For Lindurian, switch time was the same with Block 8, between week 7 and week 8. In both two cultivars, the blocks without exact switch time (Fremont Block 1, 2, 3, 4, 6 and Lindurian Block 2, 3, 4) is because when we started counting the leaf number in the field, the meristem already switched in these blocks.



	5 Weeks	6 Weeks	7 Weeks	8 Weeks	9 Weeks	10 Weeks
Block 1					15.0	19.3
Blcok 2					15.3	
Block 3				16.0	17.6	
Block 4				14.6	20.8	
Block 5			9.8	15.0	14.2	20.6
Block 6			12.2	13.8	18.8	23.4
Block 7		8.0	12.0	14.2	18.8	27.2
Block 8	6.0	9.0	11.4	15.0	19.8	23.8
Block 9	7.2	9.4	13.6	14.8	20.2	

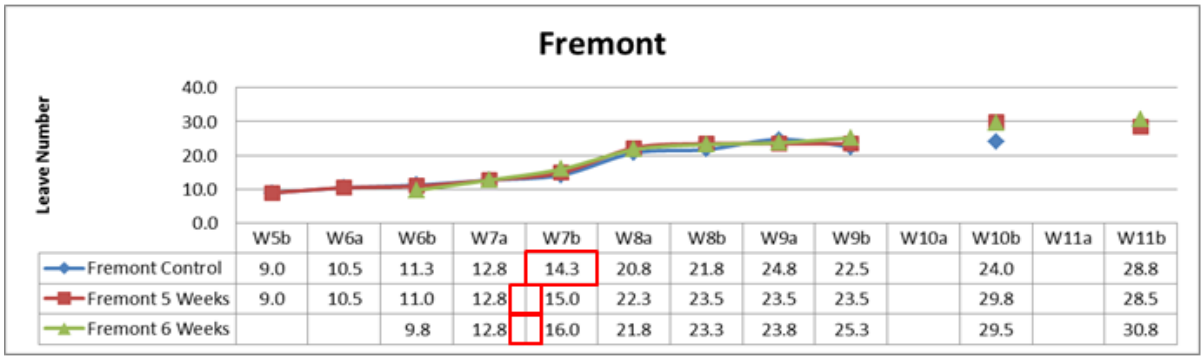
Figure 6. *Fremont leaf number for nine blocks in the field, counted at defined weeks after sowing*



	5 Weeks	6 Weeks	7 Weeks	8 Weeks	9 Weeks	10 Weeks
Block 1					16.2	21.0
Block 2					17.3	
Block 3				16.0	20.2	
Block 4				14.8	22.8	
Block 5			9.8	14.6	16.8	20.8
Block 6			12.4	11.8	18.8	23.8
Block 7		8.4	10.8	13.4	16.8	23.6
Block 8	6.6	8.8	11.8	15.4	17.8	23.8
Block 9	7.4	10.2	12.8	14.2	24.6	

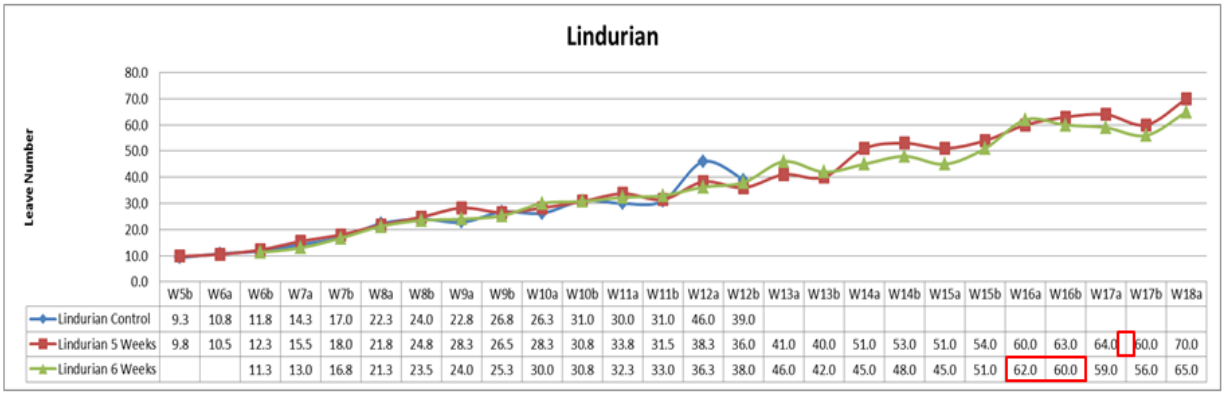
Figure 7. *Lindurian leaf number for nine blocks in the field, counted at defined weeks after sowing*

In **Figure 8 and Figure 9**, results are presented of the number of leaves in the greenhouse temperature swap experiment and marked the leaf number at meristem switch moment in three temperature treatment groups. With the growth of plants, the number of leaves in Lindurian and Fremont groups (control, 5th weeks and 6th week high temperature treatment groups) kept increasing, ranging from 9 to around 60. After comparison the data collection at same time point, Lindurian had more leaves are than Fremont during the plant growth process in both control and high temperature treatment of 5th and 6th week groups.



	W5b	W6a	W6b	W7a	W7b	W8a	W8b	W9a	W9b	W10a	W10b	W11a	W11b
Fremont Control	9.0	10.5	11.3	12.8	14.3	20.8	21.8	24.8	22.5		24.0		28.8
Fremont 5 Weeks	9.0	10.5	11.0	12.8	15.0	22.3	23.5	23.5	23.5		29.8		28.5
Fremont 6 Weeks			9.8	12.8	16.0	21.8	23.3	23.8	25.3		29.5		30.8

Figure 8. *Fremont leaf number for three groups in greenhouse, counted at defined weeks after sowing*



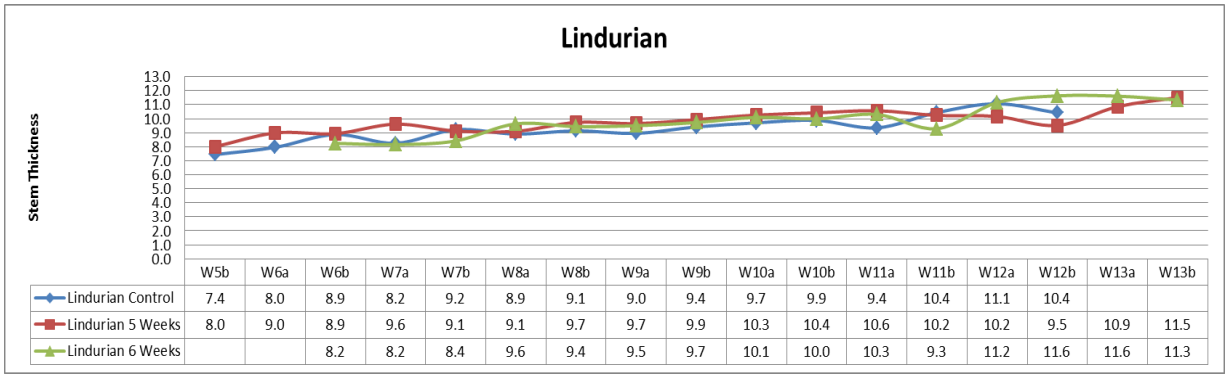
	W5b	W6a	W6b	W7a	W7b	W8a	W8b	W9a	W9b	W10a	W10b	W11a	W11b	W12a	W12b	W13a	W13b	W14a	W14b	W15a	W15b	W16a	W16b	W17a	W17b	W18a
Lindurian Control	9.3	10.8	11.8	14.3	17.0	22.3	24.0	22.8	26.8	26.3	31.0	30.0	31.0	46.0	39.0											
Lindurian 5 Weeks	9.8	10.5	12.3	15.5	18.0	21.8	24.8	28.3	26.5	28.3	30.8	33.8	31.5	38.3	36.0	41.0	40.0	51.0	53.0	51.0	54.0	60.0	63.0	64.0	60.0	70.0
Lindurian 6 Weeks			11.3	13.0	16.8	21.3	23.5	24.0	25.3	30.0	30.8	32.3	33.0	36.3	38.0	46.0	42.0	45.0	48.0	45.0	51.0	62.0	60.0	59.0	56.0	65.0

Figure 9. *Lindurian leaf number for three groups in greenhouse, counted at defined weeks after sowing*

For Lindurian, from week 6 to week 12, three groups leaf number were measured. The range of the numbers was from 11.8 to 39.0 in control group. In 5th and 6th weeks higher temperature treatment groups, the leaf number were from 12.3 to 36.0 and 11.3 to 38.0 from week6 to week 12. For Fremont, from week 6 to week 9, the numbers of leaves in all three treatment groups were obtained. The range numbers are from 11.3 to 22.5 under control condition. 11.0-23.5 and 9.8-25.3 were measured on 5th and 6th week high temperature treatment groups. The week 10 (W10a) and week 11 (W11a) were not measured in Fremont. We analysed whether number of leaves of the three groups of Lindurian and Fremont were different. The comparison between the two groups (control--five weeks, control--six weeks and five weeks--six weeks) showed that there was no significant difference between the different treatment groups in Lindurian or Fremont.

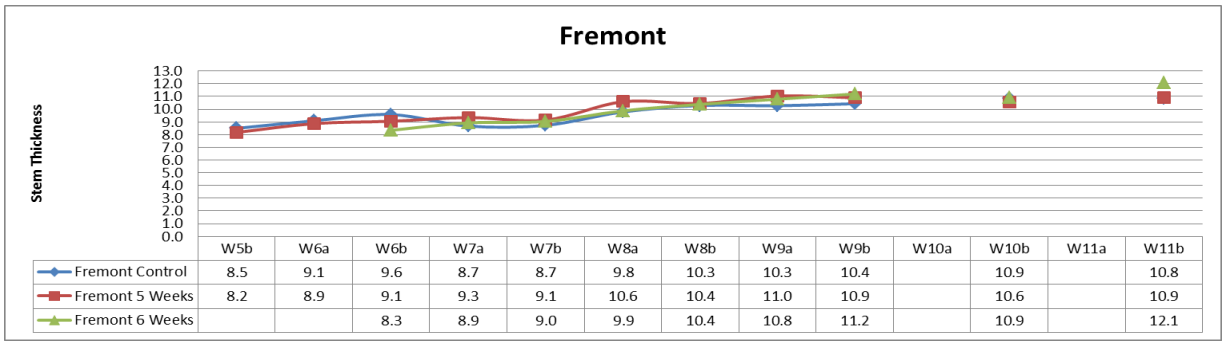
3.1.2 Stem thickness

The stem thickness and the leaf number were measured on the same individual plant when meristems were harvested to visualize in which stage they were. As can be seen from the measurement data of Lindurian **(Figure 10)** and Fremont **(Figure 11)**, the range of the stem thickness in control group were from 8.9 to 10.4 in Lindurian from week 6 to week 12 and from 9.6 to 10.8 in Fremont from week 6 to week 11. In 5th and 6th week higher temperature treatment Lindurian groups, the stem thickness was from 8.9 to 9.5 and 8.2 to 11.6 from week6 to week 12. For Fremont, from week 6 to week 11, the thicknesses of stem in two high treatment groups were obtained. 9.1-10.9 and 8.3-12.1 were measured on 5th and 6th week high temperature treatment groups. The week 10 (W10a) and week 11 (W11a) were not measured in Fremont. The same point in time in the same treatment group, Fremont stems are wider than Lindurian stems. With the time of growth of plant growth, stem width increased.



	W5b	W6a	W6b	W7a	W7b	W8a	W8b	W9a	W9b	W10a	W10b	W11a	W11b	W12a	W12b	W13a	W13b
Lindurian Control	7.4	8.0	8.9	8.2	9.2	8.9	9.1	9.0	9.4	9.7	9.9	9.4	10.4	11.1	10.4		
Lindurian 5 Weeks	8.0	9.0	8.9	9.6	9.1	9.1	9.7	9.7	9.9	10.3	10.4	10.6	10.2	10.2	9.5	10.9	11.5
Lindurian 6 Weeks			8.2	8.2	8.4	9.6	9.4	9.5	9.7	10.1	10.0	10.3	9.3	11.2	11.6	11.6	11.3

Figure 10. *Lindurian stem thickness for three groups in greenhouse*



	W5b	W6a	W6b	W7a	W7b	W8a	W8b	W9a	W9b	W10a	W10b	W11a	W11b
Fremont Control	8.5	9.1	9.6	8.7	8.7	9.8	10.3	10.3	10.4		10.9		10.8
Fremont 5 Weeks	8.2	8.9	9.1	9.3	9.1	10.6	10.4	11.0	10.9		10.6		10.9
Fremont 6 Weeks			8.3	8.9	9.0	9.9	10.4	10.8	11.2		10.9		12.1

Figure 11. *Fremont stem thickness for three groups in greenhouse*

The comparison between the two groups (control--five weeks, control--six weeks and five weeks--six weeks) showed that the stem thickness was no significant difference between the different treatment groups in Lindurian or Fremont.

The detail leaf number and stem thickness data for Lindurian and Fremont are presented in **Appendix 5** and **Appendix 6**.

In the greenhouse experiment, at the same time point, the leaf number and stem thickness were measured for the same individual plant. SPASS software was used to analyse the correlation between leaf numbers and stem thickness. Twenty four time point of the twenty four pairs of data in the three treatment groups for each cultivar were used for analysis. Derived from the analysis results **(Appendix 7),** there were no significant relation between leaf number and stem thickness in control , 5^{th} weeks and 6^{th} weeks groups at most time point by seeing the P value of each pair.

3.1.3 Curd weight and diameter

To estimate curd weight and diameter variation between different sowing time and different cultivars, curd was harvested from the initially at week 15 after sowing for each block. However, the cauliflowers in the blocks that were developed quicker and thus the curd was overripe (loose and start rot) at 15 weeks, which made us decide to harvest week 13 for the later blocks. In order to facilitate the analysis of data, based on the curd age, divided the data to two parts and total nine blocks by harvesting time **(Table 2).** The detail harvesting data for each block is presented in **Appendix 8.** Using box plots, the variation in curd weight and curd diameter in per cultivar was visualized (**Figure 12** and **Figure 13**).

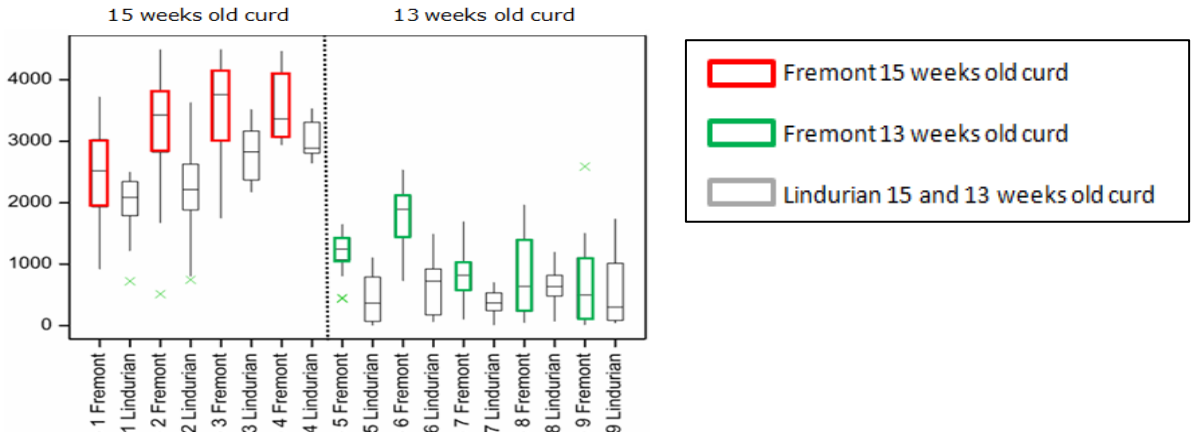


Figure 12. *Box plots showing the distribution of curd weight in different block in the field*

In block 1, 2, 3 and 4, the curds were 15 weeks old. Block 4 to block 9, the curds were 13 weeks old. Whether 15 weeks old curd or 13 weeks old curd, the curd weight of Fremont were larger than Lindurian. According to the curd weight distribution in nine blocks, we found that the variation of the curd weight in Fremont was large than Lindurian expect in block 5 and block 6.

Curd diameter of Fremont was larger than Lindurian from block 1 to block 9, besides block 8. In block 8, when the plants were 13 weeks old, the Lindurian curd diameter was longer than Fremont. However, the meristems of these two cultivars were all switched at week 8 in this block. The distribution of the curd diameter range was similar in these two cultivars when the curd was 15 weeks old. Analysis the 13 weeks old curd, the curd diameter range in Lindurian was more than Fremont in block 5 and block 6.

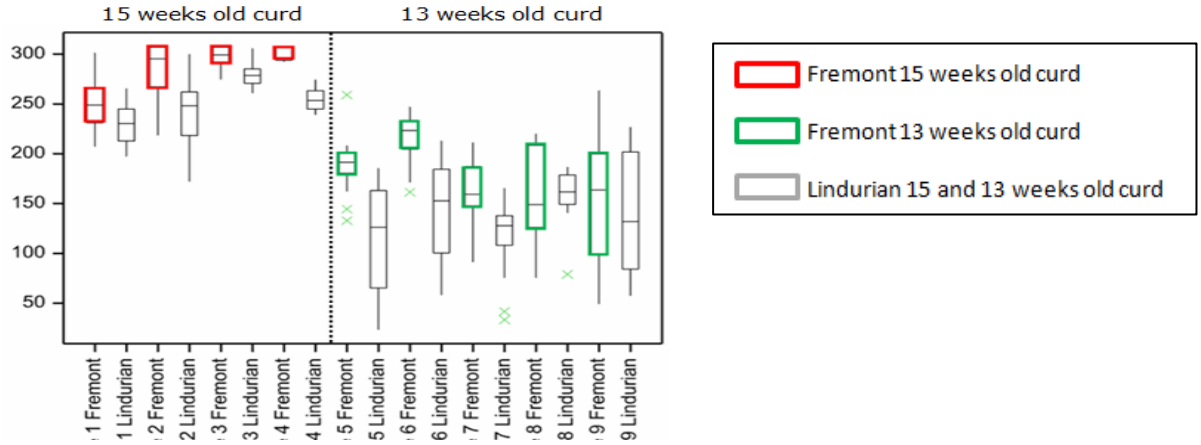


Figure 13. *Box plots showing the distribution of curd diameter in different block in the field*

As can be seen from the data change, the curd weight and curd diameter gaps between Fremont and Lindurian are reduced with the passage of sowing time, which means that Lindurian develops faster than Fremont when later sown, while Fremont maybe develop faster at early sown time.

Correlation analysis was carried out to identify the correlation between curd weight and diameter traits. The correlations computed were considered significant using 0.05 significant levels. From results of

these correlations **(Appendix 9),** curd weight showed a correlation with curd diameter with $P < 0.05$. High value of correlation ($P < 0.01$) was found at some harvest stages.

3.1.4 Apical meristem observation

Based on the apical meristem developmental stage, three meristem developmental growing stages were separated during the cauliflower growing process **(Figure 14),** plus curd and flower stage. About one week to two weeks between the vegetative stage and generative stage was defined as the transition stage, based on the morphology. Cauliflower has a long curd stage, when it is in fact an inflorescence with non-developed flower buds. But after curd ripening, the flower bud develops and some flower truly open. The lengths of each growing stage was significant different for different cultivars.

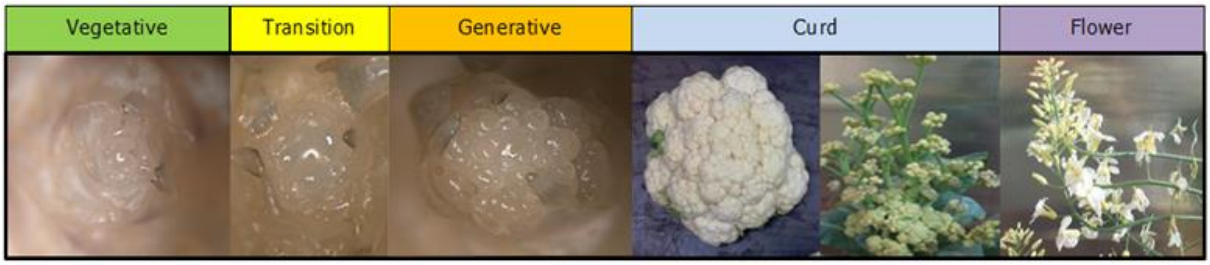


Figure 14. *Cauliflower growing stages*

3.1.4.1 Meristem transition in greenhouse

In the temperature swap experiment 1, in the greenhouse **(Table 7),** the meristems of Lindurian plants in control group transited at week 9 to the generative stage, which means 9 weeks after sowing. Lindurian meristems of 5th week high temperature treatment group plants switched at week 13, 2.5 weeks later than control group. 6th week high temperature treatment plants swapped half weeks earlier than the 5th week plants. The 7th week high temperature treatment plants were in transition again, half week earlier than 6th week swap group. So all temperature swapped Lindurian were delayed in the transition from vegetative to generative meristem.

Lindurian					
Observe Time		Control	5th week swap	6th week swap	7th week swap
Week 5	3-4-2012	Vegetative			
	5-4-2012	Vegetative			
Week 6	10-4-2012	Vegetative	Vegetative		
	12-4-2012	Vegetative	Vegetative		
Week 7	17-4-2012	Vegetative	Vegetative	Vegetative	Vegetative
	19-4-2012	Vegetative	Vegetative	Vegetative	Vegetative
Week 8	24-4-2012	Vegetative	Vegetative	Vegetative	Vegetative
	26-4-2012	Vegetative	Vegetative	Vegetative	Vegetative
Week 9	1-5-2012	Transition	Vegetative	Vegetative	Vegetative
	3-5-2012	Transition	Vegetative	Vegetative	Vegetative
Week 10	8-5-2012	Generative	Vegetative	Vegetative	Vegetative
	10-5-2012	Generative	Vegetative	Vegetative	Vegetative
Week 11	15-5-2012	Generative	Vegetative	Vegetative	Vegetative
	17-5-2012				
Week 12	22-5-2012	Generative	Vegetative	Vegetative	Transition
	24-5-2012	Generative	Vegetative	Transition	Transition
Week 13	29-5-2012	Generative	Transition	Transition	Transition
	31-5-2012	Generative	Transition	Generative	Transition
Week 14	5-6-2012				
	7-6-2012	Generative	Transition	Generative	Transition
Week 18	Nergena	Generative	Generative	Generative	Generative

Table 7. *Greenhouse temperature swaps experiment 1, plants sown on December 21th 2011, Lindurian meristem switch time.*

In the temperature swap experiment 2 (Table 8), when plants were sown in the later season (June, 2012) when day lengths shortened, the transition stage in Lindurian control group was much later compared to experiment one, when plants were sown begin February, 2012. So in the latter part of the trial, there were not enough Lindurian plants in control group. The meristems transition time of Lindurian plants in control group was not known. However, we found that on Sep. 4th, 19 weeks old Lindurian's meristem was in generative stage and the phenotype was abnormal **(Figure 15**). Lindurian meristem of 5th weeks high temperature treatment groups plants switched at week 17. 6th week high temperature treatment plants swapped at week 16.Comparing the transition time in 5th week and 6th week high temperature treatment groups, Lindurian 5th weeks group transitioned half week later than 6th week group.

In Fremont groups, plants in control, 5th week and 6th week groups were transition much early than Lindurian. The meristem of Fremont plants in control group transited at week 7 to the generative stage, which means 7 weeks after sowing. Fremont meristems of 5th week high temperature treatment group plants switched at the same week, week 7. 6th weeks high temperature treatment group plants swapped at the end of week 6, neared control and 5th weeks swap groups. Because temperature insensitive cultivar Fremont is not affected by temperature switch, the Fremont meristem transition time in control, 5th week and 6th week high temperature treatment groups were almost same, at week 7.

Table 8. *Greenhouse temperature swap experiment 2, plants sown on June 12th 2012, Lindurian and Fremont meristem switch time*

		Lindurian			Fremont		
Observe Time		Control	5th week swap	6th week swap	Control	5th week swap	6th week swap
Week 5	17-7-2012	Vegetative	Vegetative		Vegetative	Vegetative	
	19-7-2012	Vegetative	Vegetative		Vegetative	Vegetative	
Week 6	24-7-2012	Vegetative	Vegetative		Vegetative	Vegetative	
	26-7-2012	Vegetative	Vegetative	Vegetative	Vegetative	Vegetative	Vegetative
Week 7	31-7-2012	Vegetative	Vegetative	Vegetative	Transition	Generative	Generative
	2-8-2012	Vegetative	Vegetative	Vegetative	Generative	Vegetative	Generative
Week 8	7-8-2012	Vegetative	Vegetative	Vegetative	Generative	Generative	Generative
	9-8-2012	Vegetative	Vegetative	Vegetative	Generative	Generative	Generative
Week 9	14-8-2012	Vegetative	Vegetative	Vegetative	Generative	Generative	Generative
	16-8-2012	Vegetative	Vegetative	Vegetative	Generative	Generative	Generative
Week 10	21-8-2012	Vegetative	Vegetative	Vegetative			
	23-8-2012	Vegetative	Vegetative	Vegetative	Generative	Generative	Generative
Week 11	28-8-2012	Vegetative	Vegetative	Vegetative			
	30-8-2012	Vegetative	Vegetative	Vegetative	Generative	Generative	Generative
Week 12	4-9-2012	Generative	Vegetative	Vegetative			
	6-9-2012	Vegetative	Vegetative	Vegetative			
Week 13	11-9-2012	No plant	Vegetative	Vegetative			
	13-9-2012	No plant	Vegetative	Vegetative			
Week 14	18-9-2012	No plant	Vegetative	Vegetative			
	20-9-2012	No plant	Vegetative	Vegetative			
Week 15	25-9-2012	No plant	Vegetative	Vegetative			
	27-9-2012	No plant	Vegetative	Vegetative			
Week 16	2-10-2012	No plant	Generative	Transition			
	4-10-2012	No plant	Vegetative	Transition			
Week 17	10-10-2012	No plant	Vegetative	Generative			
	11-10-2012	No plant	Generative	Generative			
Week 18	16-10-2012	No plant	Generative	Generative			
	18-10-2012	No plant	Generative	Generative			

Figure 15. *Sep. 4th, 19 weeks old abnormal Lindurian plant*

3.1.4.2 Meristem transition in field

In the field experiment, Lindurian and Fremont plants were grown under natural environmental conditions. The meristems of Lindurian plants in block 1 transited at week 9. In block 2, block 3 and block 4, we did not get the exact meristems transition time because we did not monitor week 5-8. Lindurian plants in block 5 and block 6 transited at week 7. In block 7, the meristem of Lindurian plants transited at week 8. The meristems transited time for Lindurian plants in block 8 and block 9 was between week 7 and week 8. Comparing the transition time in nine blocks, Lindurian in block 8 and block 9 transitioned two weeks early than block 1 and one week later than block 5 and block 6**.(Table 9)**

In Fremont groups, the transition stages in block 1, 2, 3, 4 and 6 were not been determined in the field experiment. In block 5, the meristem of Fremont plants transited at week 7. The transition stage in block 7 was week 8. Fremont transited in block 8 between week 7 and week 8, in block 9 between week 6 and week 7. Comparing the transition time in nine blocks, Fremont in block 5 and block 9 transitioned one week early than block 7 and block 8**.(Table 9)**

Comparing the transition time in Lindurian and Fremont in the same block, Lindurian in block 1, block 6 and block 9 transitioned later than Fremont. However, in block 5 and block 7, the Lindurian and Fremont plants transited at the same week in the same block.

Table 9. *Field experiment, plants sown on April 19th 2012, Lindurian and Fremont meristem switch time*

Hybrid	Block	Sown time	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13
Lindurian	Block 1	19-4-2012					Transition	Switched	Switched	Switched	Switched
Lindurian	Block 2	26-4-2012					Switched	Switched	Switched	Switched	Switched
Lindurian	Block 3	3-5-2012				Switched	Switched	Switched	Switched	Switched	Switched
Lindurian	Block 4	10-5-2012				Switched	Switched	Switched	Switched	Switched	Switched
Lindurian	Block 5	17-5-2012			Transition	Switched	Switched	Switched	Switched	Switched	Switched
Lindurian	Block 6	24-5-2012			Transition	Transition	Switched	Switched			
Lindurian	Block 7	31-5-2012		Vegetative	Vegetative	Transition	Switched	Switched			
Lindurian	Block 8	7-6-2012	Vegetative	Vegetative	Vegetative	Switched	Switched	Switched			
Lindurian	Block 9	14-6-2012	Vegetative	Vegetative	Vegetative	Switched	Switched				
Fremont	Block 1	19-4-2012					Switched	Switched	Switched	Switched	Switched
Fremont	Block 2	26-4-2012					Switched	Switched	Switched	Switched	Switched
Fremont	Block 3	3-5-2012				Switched	Switched	Switched	Switched	Switched	Switched
Fremont	Block 4	10-5-2012				Switched	Switched	Switched	Switched	Switched	Switched
Fremont	Block 5	17-5-2012			Transition	Switched	Switched	Switched	Switched	Switched	Switched
Fremont	Block 6	24-5-2012			Switched	Switched	Switched	Switched			
Fremont	Block 7	31-5-2012		Vegetative	Vegetative	Transition	Switched	Switched			
Fremont	Block 8	7-6-2012	Vegetative	Vegetative	Vegetative	Switched	Switched	Switched			
Fremont	Block 9	14-6-2012	Vegetative	Vegetative	Switched	Switched	Switched				

3.2 Expression analyses

We wanted to look at expression of flowering related genes around meristem development and curd formation in temperature treated and control meristems. Thus we need a reference gene that was stably expressed in these samples. Based on the candidate genes research in *B.rapa* by Xiao Dong, one stable reference gene was chosen from 4 reference genes (*EFα1, YLS8, 18sRNA* and *Actin*) and 17 flowering time candidate genes (*AP1-a, AP1-c, FUL-a, FUL-b, FUL-c, FUL-d, FH, TFL1-1, CAL, REM1, UFO, CCE1, NSN1-1, NSN1-2, NSN1-3, CRY2-1* and *CRY2-2*) were check by qRT- PCR.

3.2.1 Validation of reference genes

Four reference candidate genes *EFα1, YLS8, 18S* and *Actin* **(Table 3)** were tested in the two cultivars (Lindurian and Fremont) and in two growing stages (vegetative and generative) on four time points for each cultivar after sowing **(Table 10).** *18S* showed Ct values ranging from 7.54 to 7.98 in Fremont and from 7.27 to 8.07 in Lindurian **(Figure16 and Figure17).** The Ct value of 18S is too low, which means it is too high expressed in these samples, as we expected target genes do not have this high expression. Therefore, 18S was not used as reference gene in this research. In the cases of *EFα1, YLS8* and *Actin*, Ct value results were stable in two cultivars **(Figure 18** and **Figure 19).** Using the software geNorm one can determines the gene stability measure (M) value for these three reference genes. The result was that *YLS* and *Actin* are more stable than *EFα1* **(Figure 20** and **Figure 21).** However, when we checked the *YLS* and *Actin* PCR products on a 1.5% RNase free agarose gel **(Figure 22),** the *YLS8* primer has more primer dimer than *Actin, so Actin* was chosen as the reference gene for this study.

Table 10. *Reference gene test materials*

Fremont				
Plant old	5 weeks	6 weeks	8 weeks	9 weeks
Growing stage	Vegetative		Generative	
Plant number	F0111,F0113	F0248,F0249	F0130,F0176	F0139,F0143
Lindurian				
Plant old	6 weeks	7 weeks	11 weeks	12 weeks
Growing stage	Vegetative		Generative	
Plant number	L0198,L0227	L0181,L0210	L0073,L0081	L0083,L0141

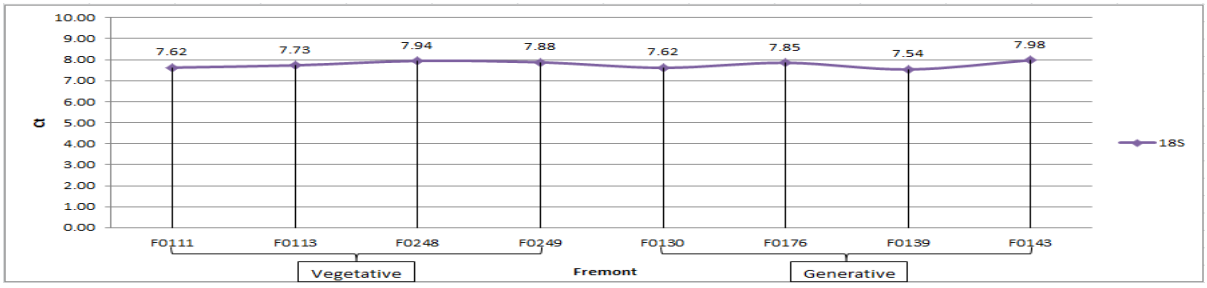


Figure 16. *Reference gene 18S Ct value in Fremont*

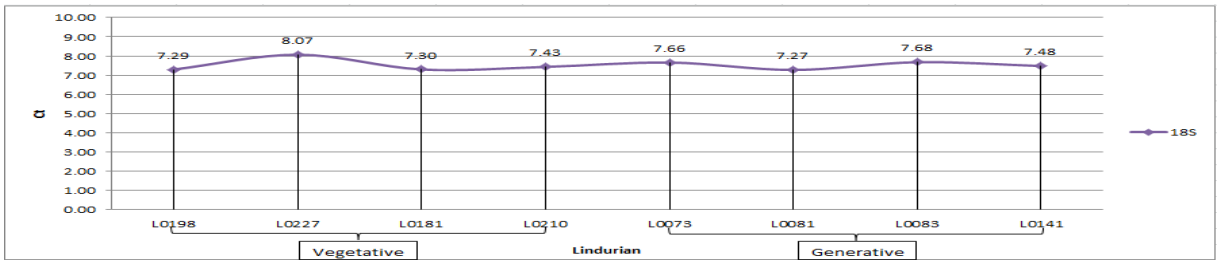


Figure 17. *Reference gene 18S Ct value in Lindurian*

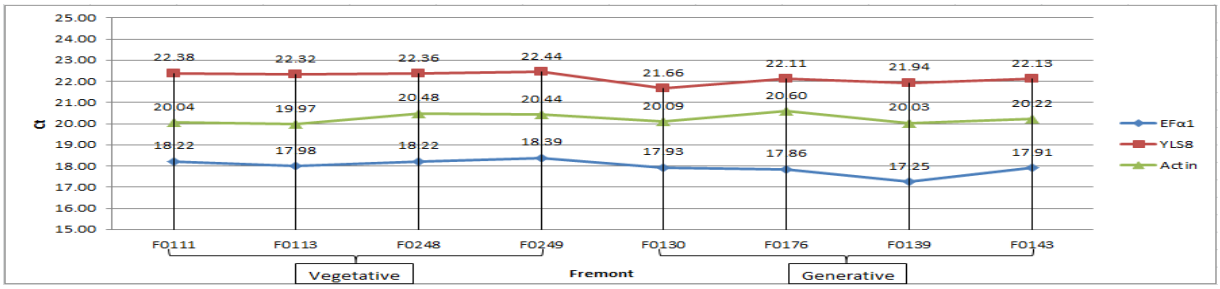


Figure 18. *EFα1, YLS8 and Actin Ct value in Fremont*

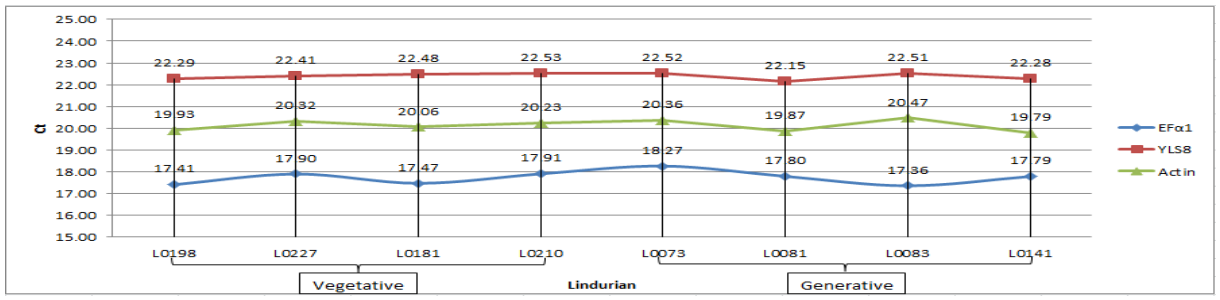


Figure 19. *EFα1, YLS8 and Actin Ct value in Lindurian*

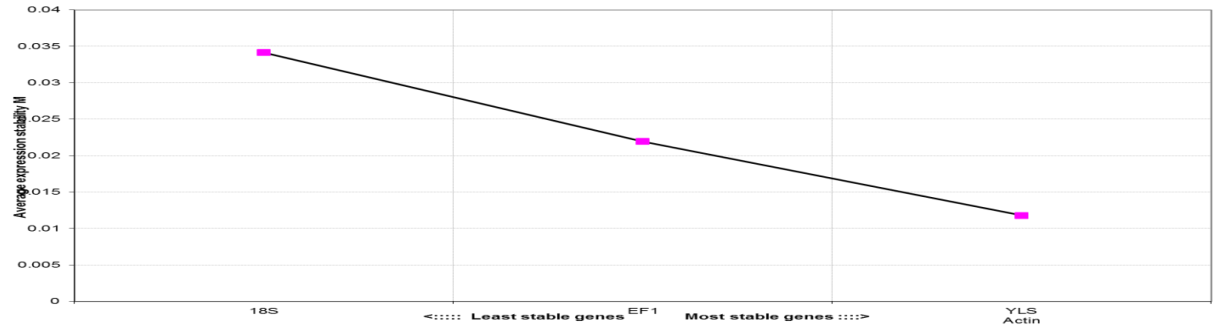


Figure 20. *Use geNorm determines the gene stability in Fremont*

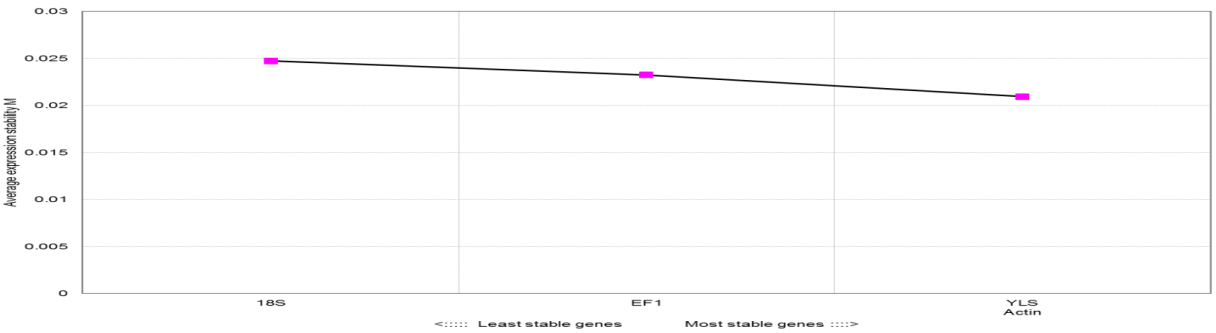


Figure 21. *Use geNorm determines the gene stability in Lindurian*

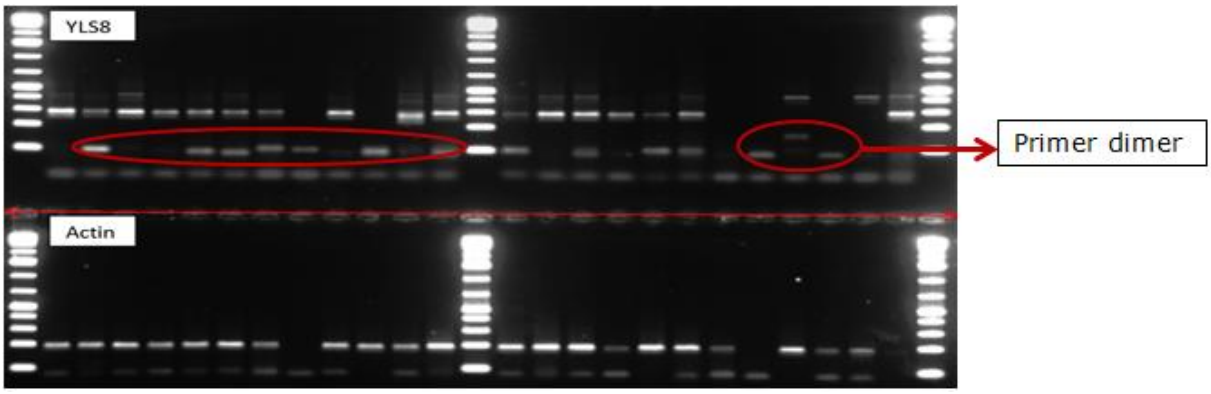


Figure 22. *Use gel tests the primers quality*

In later experiment, reference gene *Actin* was continued testing in Lindurian control group and 6th week high temperature treatment group. The Ct value of *Actin* in these different temperature treated Lindurian samples was still stable, ranging from 19.26 to 20.41 in 22 samples at 6 time points **(Figure 23).**

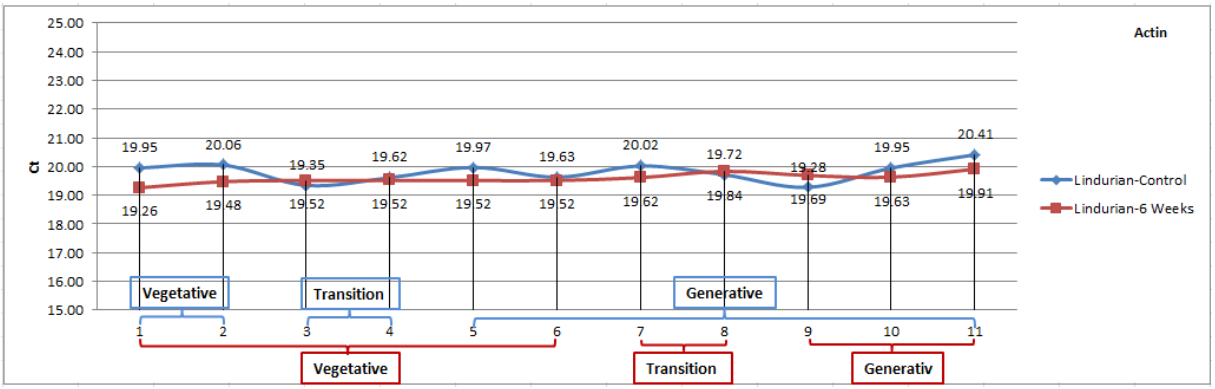


Figure 23. *Ct value of Actin in Lindurian control and 5 weeks high temperature group materials*

3.2.2 Identification of genes that define cauliflower developmental stages in Fremont cultivar

We looked at five development stages (vegetative-transition-generative meristems curd and flower) in the cauliflower. Curd and flower stages can be defined by morphology. The vegetative, transition and generative stages identification need to rely the stereomicroscope. If the meristems are used for RNA extraction, time maybe insufficient to check meristem stage and mistakes may happen, so a molecular expression marker that defines the stage would be handy. On molecular level, several genes were tested for their use to divide the development stages for cauliflower by their normalized fold expression levels. In this part, we focus on the results from Fremont material **(Table 11)** of experiment 2.

Table 11. *Fremont start materials at different time points*

Fremont							
Plant old	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	16 weeks	20 weeks
Growing stage	Vegetative		Transition	Generative		Curd	Flower
Plant number	F0111,F0113	F0248,F0249	F0153,F0157,F0136	F0130,F0143	F0139,F0143	F0076,F0053	F-Small,F-Big

Gene expression analysis showed that at different stages major expression differences occur for several genes:

AP1-a and ***AP1-c*** were not expressed in vegetative, transition, generative and curd stages in Fremont control group. They started expressing in flower stage, the Ct value reduced to 23.82 from 30 or more **(Figure 24).** The normalized fold expression was 2.55 at flower stage **(Figure 25).**

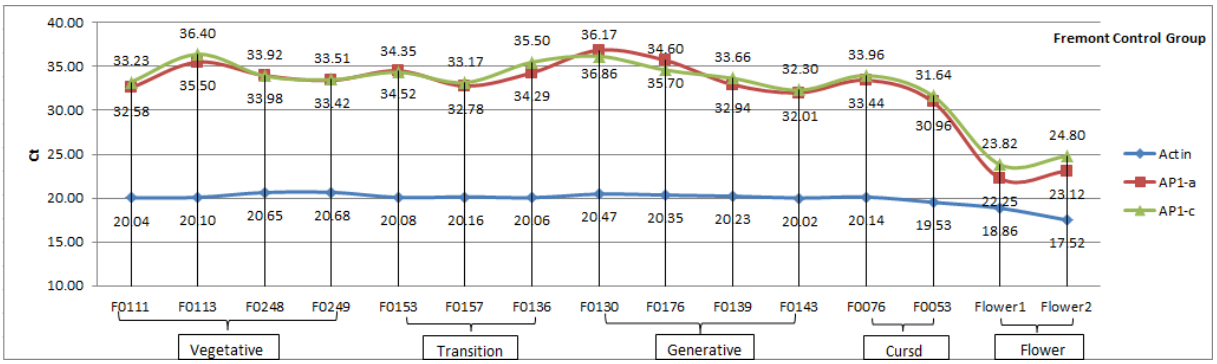


Figure 24. *Ct value for AP1-a and AP1-c in Fremont Cultivar from vegetative stage to flower stage*

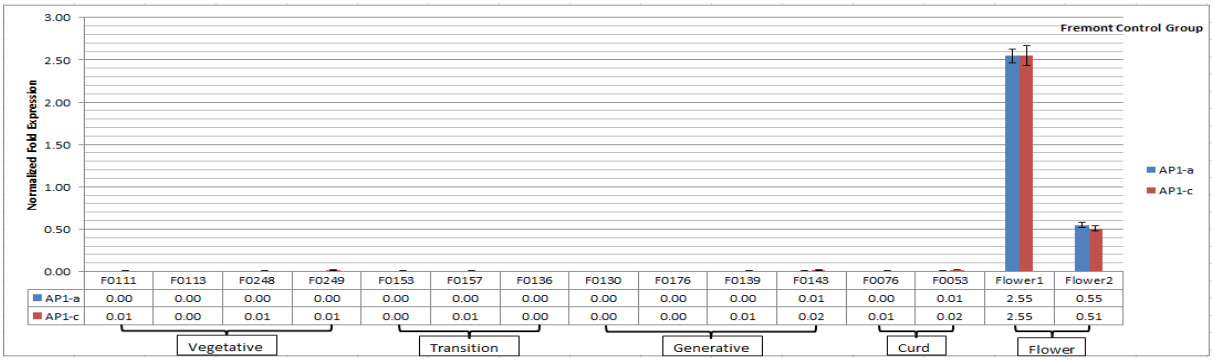


Figure 25. *Normalized against Actin fold expression of AP1-a and AP1-c in Fremont from vegetative to flower stage*

Opposite to *AP1-a* and *AP1-c* results**,** ***REM1, TFL1-1*** and ***FH*** were expressed in all stages tested, except in open flower tissue. The Ct value of *REM1, TFL1-1* and *FH* were around 26 from vegetative stage to curd stage. But in the flowering stage, the Ct value rose sharply to more than 30 **(Figure 26).** During the vegetative to curd developmental stage, the normalized fold expressions ranged from 0.7 to 2.0 for *REM1* and from 2 till 8 for *TFL1-1*, but in flower stage were 0 **(Figure 27).** Vegetative stage to curd stage of *FH* expression ranges from 0.18 to 1.14 and the less expression in flower tissue **(Figure 28).**

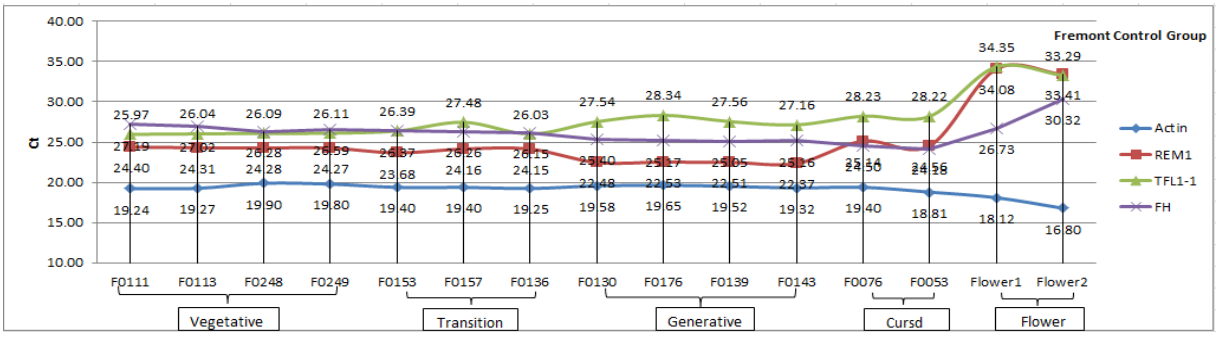
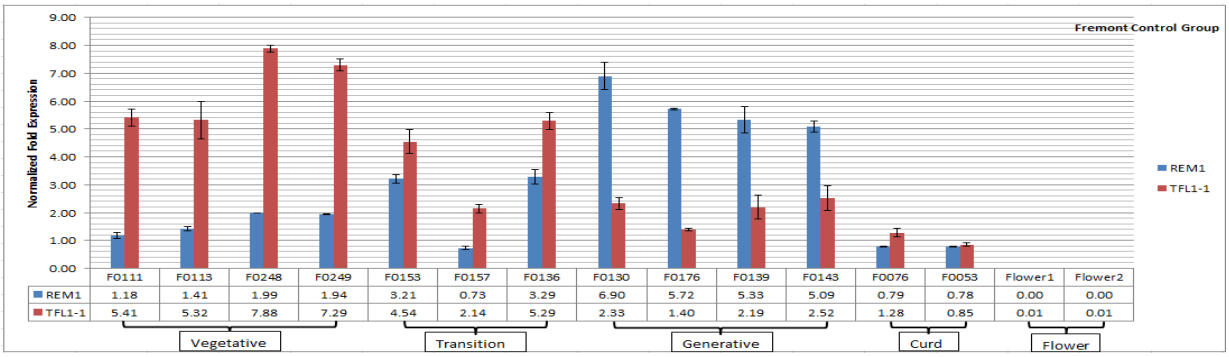
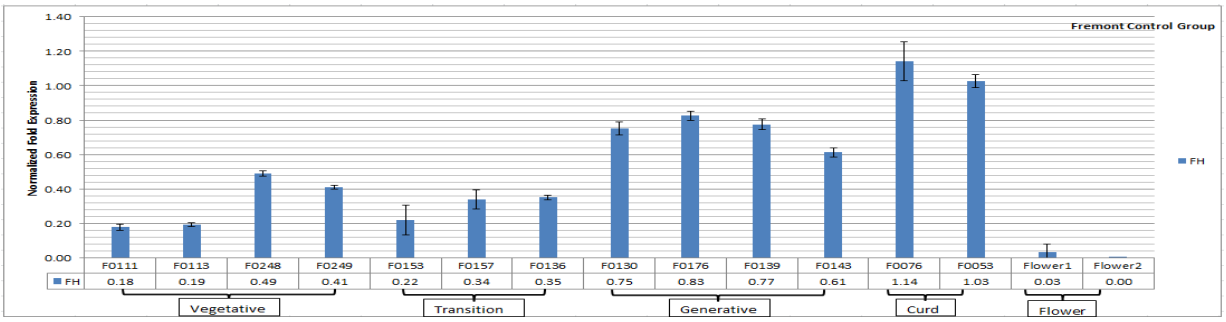


Figure 26. *Ct value for REM1, TFL1-1 and FH in Fremont Cultivar from vegetative stage to flower stage*



	F0111	F0113	F0248	F0249	F0153	F0157	F0136	F0130	F0176	F0139	F0143	F0076	F0053	Flower1	Flower2
REM1	1.18	1.41	1.99	1.94	3.21	0.73	3.29	6.90	5.72	5.33	5.09	0.79	0.78	0.00	0.00
TFL1-1	5.41	5.32	7.88	7.29	4.54	2.14	5.29	2.33	1.40	2.19	2.52	1.28	0.85	0.01	0.01

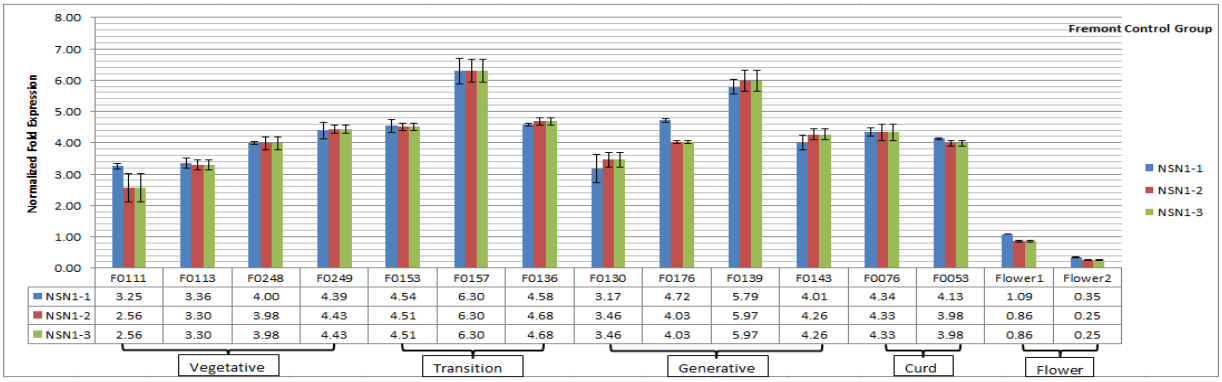
Figure 27. *Normalized against Actin fold expression of REM1 and TFL1-1 in Fremont from vegetative to flower stage*



	F0111	F0113	F0248	F0249	F0153	F0157	F0136	F0130	F0176	F0139	F0143	F0076	F0053	Flower1	Flower2
FH	0.18	0.19	0.49	0.41	0.22	0.34	0.35	0.75	0.83	0.77	0.61	1.14	1.03	0.03	0.00

Figure 28. *Normalized against Actin fold expression of FH in Fremont from vegetative to flower stage*

NSN1 family has high expression level from vegetative to curd stage, expression fluctuated between 2.56 and 6.3, while significant decline was happened in flower stage, with lowest expression level, smaller than 1.00 **(Figure 29).**



	F0111	F0113	F0248	F0249	F0153	F0157	F0136	F0130	F0176	F0139	F0143	F0076	F0053	Flower1	Flower2
NSN1-1	3.25	3.36	4.00	4.39	4.54	6.30	4.58	3.17	4.72	5.79	4.01	4.34	4.13	1.09	0.35
NSN1-2	2.56	3.30	3.98	4.43	4.51	6.30	4.68	3.46	4.03	5.97	4.26	4.33	3.98	0.86	0.25
NSN1-3	2.56	3.30	3.98	4.43	4.51	6.30	4.68	3.46	4.03	5.97	4.26	4.33	3.98	0.86	0.25

Figure 29. *Normalized against Actin fold expression of NSN1 family in Fremont from vegetative to flower stage*

CAL had a low expression from vegetative stage to generative stage (0.06 to 0.59) and its highest expression level ranged from 2.5 to 4.5 which lays in the curd stage which significantly higher than other periods **(Figure 30).**

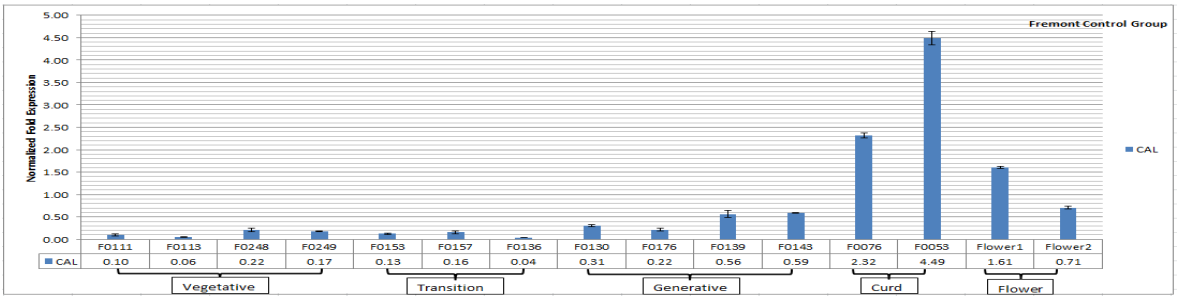


Figure 30. *Normalized against Actin fold expression of CAL in Fremont from vegetative to flower stage*

***FUL* family** was an interesting gene family which can be used to divide the first three growth stages (vegetative, transition and generative stage) in cauliflower. In Fremont, the Ct value of the *FUL-a, FUL-c and FUL-d* were in the range of 21 to 29. But the *FUL-b* had a higher Ct value than the other *FUL* genes, higher than 31 in all five cauliflower growing stages **(Figure 31).** *FUL* family showed almost no normalized expression during the cauliflower meristem vegetative stage, and then the expression level sharply increased to 2.00 in transition stage. In generative and curd stages, the expression level doubled, reaching to the highest value 6.00. However, *FUL-a* significantly lower expressed than the other three through the whole process **(Figure 32).**

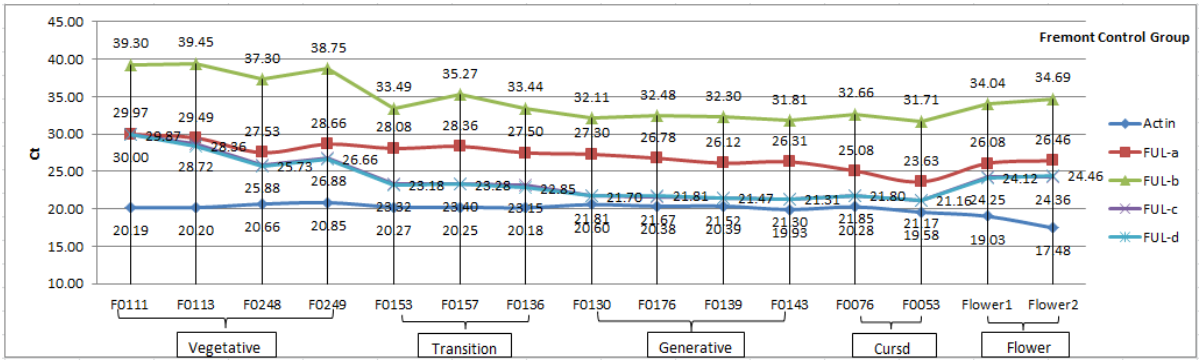


Figure 31. *Ct value for FUL family in Fremont Cultivar from vegetative stage to flower stage*

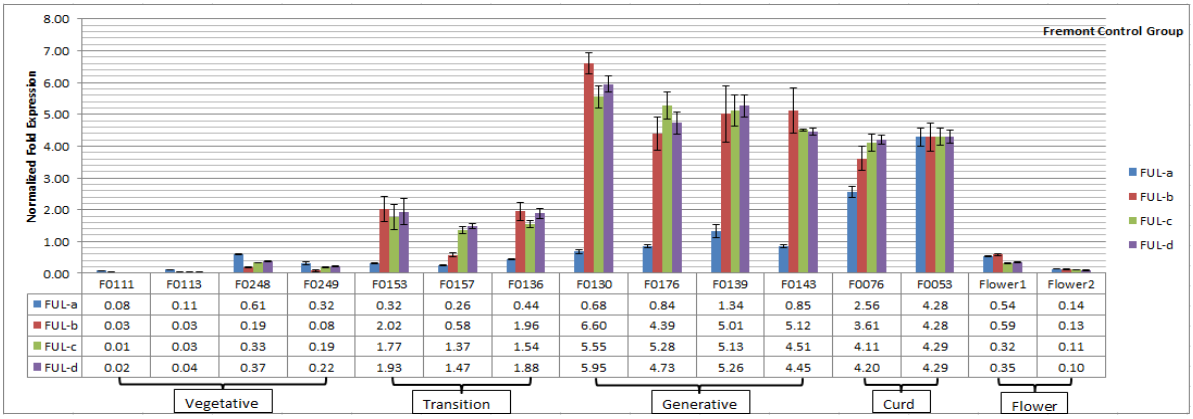


Figure 32. *Normalized fold expression of FUL family in Fremont from vegetative to flower stage*

Several other genes (*UFO, CCE1* and *CRY2* family) were also tested in Fremont materials, but no wishing expression patterns (different expression between different meristem developmental stages) were revealed. The Ct value and expression results of these genes are in the **Appendix 10**.

3.2.3 Effect of temperature swap on gene expression during development

Different developmental stages of the temperature sensitive Lindurian grown under control and 6th week high ambient temperature treatment **(Table 12)** was tested to screen gene expression, in order to test whether expression level was influenced by high ambient temperature. Because the time limit, during this experiment, we did only use meristem and curd tissue, so no flower materials.

Table 12. *Lindurian control and 6th high ambient temperature treatment group*

Lindurian												
Plant old	Week 7		Week 9		Week11		Week 12		Week 13		Week 14	
Control group	Vegetative		Transition		Generative							
Plant number	L0181	L0210	L0241	L0277	L0073	L0081	L0083	L0141	L0040	L0068	L0270	L0273
6 Weeks group	Vegetative						Transition		Generative			
Plant number	L0240	L0242	L0067	L0164	L0037	L0171	L0103	L0167	L0070	L0263	L0005	L0054

CAL had high Ct values in the Lindurian control group at all stages tested, most of the time point the Ct values were greater than 33. However, in 6th week high temperature treated group, almost all time points Ct values were lower than those in the control group**. (Figure 33).** *CAL* expression analysis showed a low expression level from 0.00 to 0.30 in Lindurian control group in vegetative, transition and generative stages. But in Lindurian 6th week high temperature treatment group, from the later vegetative stage to early generative stage, expression increased to 1.10 (point 5) and then decreased to 0.80 (point 9) in early generative stage. All points in time in the whole process, *CAL* expression higher in 6th week high temperature treatment group than in control group. **(Figure 34)**

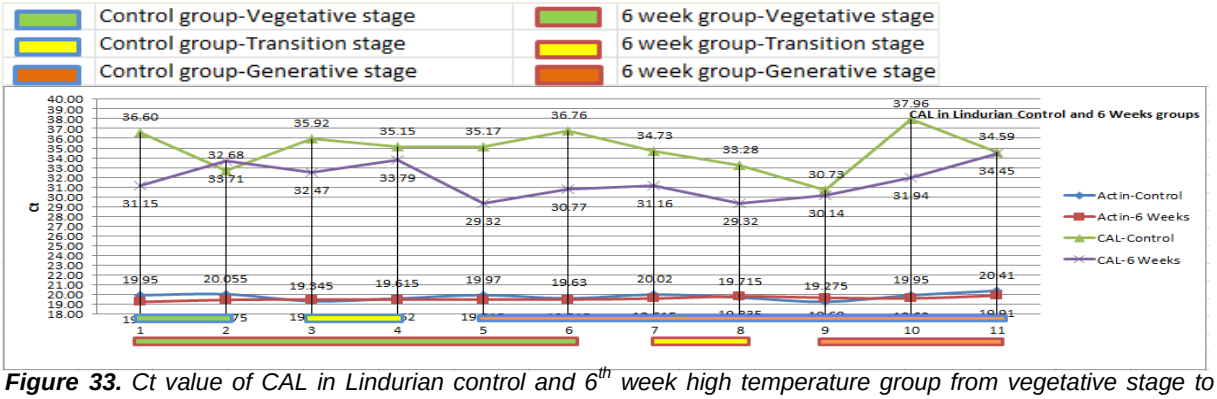


Figure 33. *Ct value of CAL in Lindurian control and 6th week high temperature group from vegetative stage to generative stage*

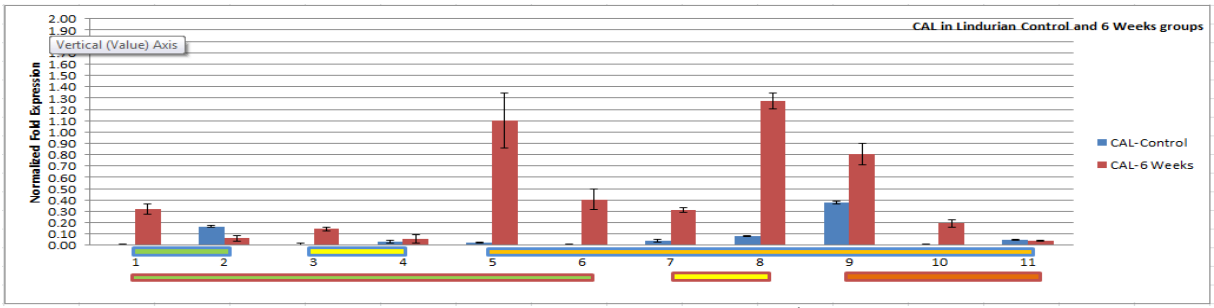


Figure 34. *Normalized fold expression of CAL in Lindurian control and 6th week group from vegetative to flower stage*

Expression of the *FUL* family, *FH, UFO, CCE1, NSN1-1, NSN1-2, NSN1-3, REM1* and *TFL1-1* was also measured in Lindurian control and 6th week high temperature treated group, however, did not see any effect of the temperature treatment. The Ct value and gene normalized fold expression are shown in the **Appendix 11.**

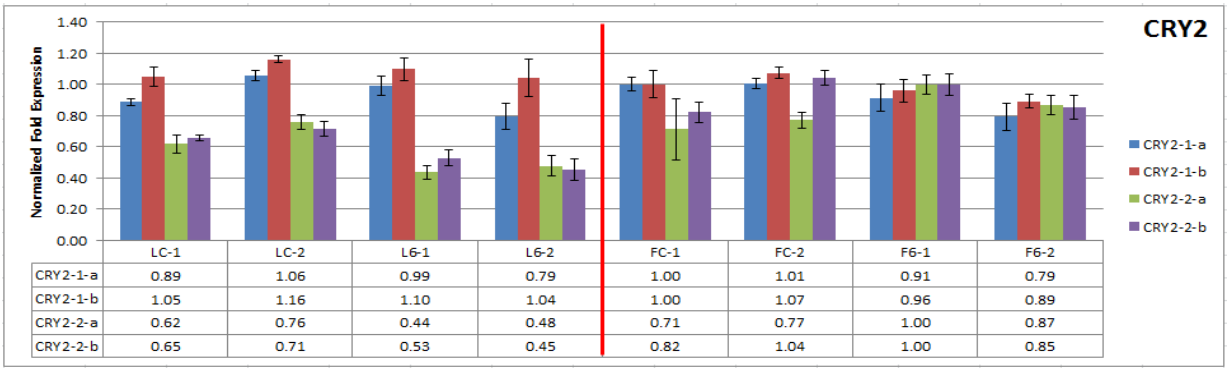
3.2.4 Expression profile of gene before and after temperature swap

We also liked to evaluate whether gene expression changed directly around the temperature swap. Thus we used material harvested in one day (24h) before and after the 6th week high temperature treatment. Meristems of five plants at every time point were cut and pooled, mixed after which RNA was isolated. Two biological repeat were used in this study **(Table 13).**

Table 13. *Start material for one week before and after high temperature treatment experiment*

Treatment	Fremont (1,2-biological repeat)	Lindurian (1,2-biological repeat)
Control group	Control –1, Control – 2	Control – 1, Control – 2
6 Weeks high temperature	6 Weeks – 1, 6 Weeks – 2	6 Weeks – 1, 6 Weeks – 2

CRY2 has two paralogy were *CRY2-1* and *CRY2-2* on chromosome A10 in *Brassica rapa*. So we predicted that there will also be two paraogues in *Brassica loeracea*. *CRY2-1* expressed stable before and after high temperature treatment in two cultivars. For *CRY2-2*, the average of normalized expression decreased after high temperature treatment in Lindurian from 0.70 to 0.45. However, in Fremont, the normalized expression level increased from 0.74 to 0.98. **(Figure 35)**



	LC-1	LC-2	L6-1	L6-2	FC-1	FC-2	F6-1	F6-2
CRY2-1-a	0.89	1.06	0.99	0.79	1.00	1.01	0.91	0.79
CRY2-1-b	1.05	1.16	1.10	1.04	1.00	1.07	0.96	0.89
CRY2-2-a	0.62	0.76	0.44	0.48	0.71	0.77	1.00	0.87
CRY2-2-b	0.65	0.71	0.53	0.45	0.82	1.04	1.00	0.85

Figure 35. *CRY2 family Normalized against Actin fold expression in Lindurian and Fremont 24hr before and after high temperature treatment*

Several other genes (*AP1, CAL*, *REM1, TEL1-1, CCE1, FH*, *NSN1* family and *FUL* family) were also test in these materials, but expression patterns were similar in control and 6th week high temperature treatment samples. The Ct value and expression results of these genes are in the **Appendix 12**.

4. Discussion

4.1 Morphological characterization and correlation

The Morphology of the cauliflower plant has been investigated repeatedly during the past two centuries (Sadik,2007). In this study, we focus on leaf number, stem thickness, curd weight and curd diameter. Simultaneously, the correlation between traits is also involved. The study of correlation between the dimensions with different traits of an organism, has been a long important theme in plants. For example, the relationships between the total leaf area (TLA) and height has been found in 12 tree species (D. A. Coomes† & P. J. Grubb, 1998). In previous study, if there were developmental correlation between dimensions of these organs, it might limit the ability of each organ to evolve an independent response. Hereby, correlations between different organs are viewed as a potential source of further evolutionary constraints which is resistant to evolutionary changes (BROUAT et al., 1998). Two cauliflower cultivars were observed during the study, we focus on a relationship between the diameter of a stem and the number of the leaf, curd weight and curd diameter as well.

4.1.1 Leaf number and stem thickness

Leaf number of cauliflower is a “marker” which is used by many workers to predict curd initiation (NOWBUTH & PEARSON,1998). In Booij and Struik study (1990), the number of leaves below the curd is mainly determined by the temperature before curd initiation and increases at increasing temperature. The similar result was found in Wurrte al. (1996) research. Increasing the ambient temperature during the experiment increased the final number of leaves in cauliflower. However, these results came out from the experiment when cauliflowers were grown with different temperatures during the whole developmental stages. Comparing with our study, only one week with high temperature treatment in the two cauliflower cultivars were growing under process. Even though Lindurian is temperature sensitive cultivar and Fremont is not temperature sensitive cultivar, there is no significant difference for leaf number among the three temperature treatment groups with these two cultivars **(Figure 8 and Figure 9).** In other words, the leaf number is not influenced by a short time ambient temperature change. We also want to find the relationship between leaf number and meristem switch time by analysing the leaf number around the meristem switch moment in the field and greenhouse. However, the data of leaf number around meristem switch moment in field and greenhouse are not comprehensive and accurate. Because plants in some blocks were already in generative stages in the field when we started monitoring and Lindurian in control group were not enough in greenhouse.

The plant has been given better development as expressed by greater stem thickness, height, leaf thickness and so on (Zeng et al.,2009).The stem thickness was also measured in this experiment as there were not all plants in the greenhouse compartment developed uniformed, this is because plants were very close and some plants were in the shade of other plants. This speed of development can also influence the meristem switch and thus can be used as correction factor. At the same time, we found that cauliflower stem thickness was also not influenced by one week high temperature treatment in Lindurian and Fremont.

There is no significant difference in different treatment groups because of only one week high temperature treatment in our experiment. If we increase the temperature for a long time, the leaf number and stem thickness maybe changed in different treatment groups. Another reason is that we stopped counting the leaf at week 10 in field and week 13 in greenhouse. The leaf number maybe increase after week 11 or 13 until the curd mature.

As previous studies, we wanted to find the relationship between two morphological traits, leaf number and stem thickness in cauliflower as well as the comparisons of the relationships between the different temperature treatment groups (control, 5th weeks, 6th weeks high temperature treatment groups). We found that there was no correlation between the number of leaves and the stem thickness in both cases **(Appendix 7).** However, a larger sample of cultivar is necessary for firm conclusion, correlation of leaf number-stem might thus be generally different from what we got from this study.

4.1.2 Curd weight and curd diameter

The production of cauliflower is recorded by curd weight and curd diameter (Kieffer et al.,2001). Curd initiation is influenced by both temperature and shade (Booij & Struik,1990). The heritability of the curd (inflorescence) size of cauliflower has been studied in particular detail. Curd weight is closely correlated with curd diameter, an easily measurable trait (Kage et al., 2004). In this study, Fremont curds were heavier and larger than Lindurian whether 15 weeks old plants or 13 weeks old plants. Fremont meristem switched much earlier than Lindurian which could lead to this phenotype. Meristem switched early applies a longer generative process for plant reproduction which caused the curd weightier to become larger. During the long generative process, some functional genes could be changed by some conditions which influence the curd formation. According to the analysis of the correlation between curd weight and curd diameter, we found that it was significant in this research **(Appendix 9).** However, we could not ignore that the number of curds we harvested from the field was not large. So the correlation between curd weight and curd diameter still need to be further confirmed.

4.2 Meristem switch

During the flowering process, the shoot apical meristem is converted to an inflorescence meristem that forms flowers (Torti,2012). Environmental conditions play a major role in the timing of the floral transition. These has been a concerted effort to understand the mechanisms of controlling flowering time and floral development utilizing a molecular genetic approach in *Arabidopsis* (Mouradov et al.,2002). The transition from vegetative to reproductive phases during *Arabidopsis* development is the result of a complex interaction of environmental and endogenous factors. A long, cold temperature treatment (i.e., a winter season) induces or accelerates *Arabidopsis* flowering (Macknight et al., 1997). *Brassica* species have related to genome of *A. thaliana* (Lagercrantz, 1998). So the relative knowledge which has been already found in *A.thaliana* could be applied in *Brassicas'.*

Since early time it has been known that flowering in *Arabidopsis* is under the control of the temperature, we focus on high temperature influence on the *Brassica oleracea ssp. Botrytis* (cauliflower) flowering. It is well known that high temperature delays curd formation and the harvest time. In this study, high ambient temperature factor in the temperature sensitive cultivar delayed the switch from vegetative to generative meristem, so delayed the onset of curd formation. In the temperature sensitive cultivar Lindurian, different degrees of meristem switch postponed happened in 5^{th}, 6^{th} and 7^{th} week high temperature treatment groups. Flowering time of Lindurian in 5^{th} week high temperature treatment group is postponed one week later than 6^{th} week and 7^{th} week groups **(Table 7 and Table 8).** However, based on the greenhouse experiment 2, the time of meristem switch for Fremont was not different among these three temperature groups and many weeks earlier than Lindurian groups. This is because the characteristics of cauliflower cultivar. Comparing with the Lindurian, Fremont is not temperature sensitive cultivar whose meristem switch is not influenced by ambient temperature.

Comparing two temperature swap experiments in greenhouse, Lindurian in two experiments were almost under the same ambient temperature condition, in addition to sowing time. Greenhouse experiment 1 started sowing on February 27^{th}, 2011, while Lindurian in experiment two was sown on June 12^{th} 2012. However, meristem switch of Lindurian was much later (nearly four weeks later) in experiment 2 than in experiment one in the same temperature group **(Table 7 and Table 8).** According to this result, it can be assumed that the day length is also a main factor which influences the cauliflower meristem switch time. We did not use temperature insensitive cultivar Fremont in greenhouse experiment one. Thus, we could not compare Fremont meristem switch between two greenhouse experiments. The day length influence on Fremont cannot be analysed here. But we can see that Fremont in experiment two switched earlier than Lindurian in experiment one. If we want to analyze the high temperature influence on the cauliflower meristem switch, in the future study, it is better to sow the seeds at the same date to exclude the impact of day length factors. If we want to study the day length influence, more cultivars and more sowing periods should be used.

4.3 Identification of genes that define cauliflower developmental stages

Flowering induction and flower development are highly complex processes that require the coordinate expression of numerous genes throughout the different stages of development (Ferrandiz et al., 2000; Vivian F. Irish, 2009; Frank Wellmer & José L. Riechmann, 2010). Based on morphological criteria, cauliflower development has been broken down into a number of distinct stages. We would like to have in addition to defining development stage just by morphological criteria, molecular criteria. With the aim of identifying genes specifically expressed in the cauliflower meristem development, we isolated and characterized cDNAs corresponding to genes specifically expressed in the meristems of the cauliflower plant. This study has identified several different stage acting genes that determine the stage of organ primordial.

The first step in flower development is the transition from a vegetative to an inflorescence meristem, while the second step is transition of inflorescence meristem into a floral meristem (Mandel et al.,1992). In *Arabidopsis*, *APETALA 1* as a floral meristem identity gene (Tränkner et al.,2010), is required for the transition of an inflorescence meristem into a floral meristem and for normal sepal and petal development (Vivian F. Irish.,2010). *Ap1* expression induces the development of terminal flowers and early flowering phenotypes. In addition, previous studies have revealed that *AP1* can serve as a good marker to determine whether *Arabidopsis* is at the flowering stage (Jaya et al.,2010). Thus we analyse the expression of the AP1 in cauliflower in this study. In Arabidopsis, loss of function mutations in *AP1* show a partial conversion of floral meristems to a more inflorescence (Vivian F. Irish.,2010). Overexpression of *AP1* is sufficient to convert the inflorescence meristem to a terminal flower in *Arabidopsis* (Mougiou et al.,2012). The *AP1* gene is uniformly expressed early in flower development. That means *AP1* RNA accumulates uniformly throughout young floral primordia (Ferrandiz et al.,2000). However, the inflorescence meristem stage in cauliflower is much longer than in *Arabidopsis*. In cauliflower, the inflorescence meristem means the stage from generative meristem to full grown curd and then into floral primordial. From the result we get, in cauliflower Fremont, *AP1* is only expressed in open flower tissue. Therefore in essence, *AP1* expression at *Arabidopsis* and cauliflower support the conclusion that *AP1* acts locally to specify the identity of floral meristems, but the time between vegetative meristem and flowers is very long in cauliflower.

The expression patterns of *REM1, TFL1-1 and FH* expression are opposite pattern compared to *AP1* expression. *REPRODUCTIVE MERISTEM 1 (REM1)* could break the arrest in *Arabidopsis* to allow continued reproductive development. It may also be a transcription factor that up-regulates floral meristem genes. The expression of *REM1* is highest at the inflorescence meristem stage, and transcripts are detectable in floral primordium and floral bud stages in *Arabidopsis* (Duclos,2008). *REM1* expresses specifically in cauliflower reproductive meristems. It expression is detected in vegetative apices of cauliflower and absent from the youngest apical domes (Franco-Zorrilla et al.,1999). *TERMINAL FLOWER 1 (TFL1),* as a strong repressor of flowering, is

a key regulator of flowering time and the development of the inflorescence meristem in *Arabidopsis* (Hanano & Goto,2011). *TFL1* is expressed during the vegetative phase, where it delays the commitment to inflorescence development and thus affects the timing of the formation of the inflorescence meristem as well as its identity (Bradley et al., 1997). In *Arabidopsis*, *TFL1* loss of function causes the terminal flowers in the shoot apex. In contrast with the loss of function phenotypes, overexpression of *TFL1* delayed flowering (Hanano & Goto,2011). This study is showing that *REM1* and *TFL1* are expressed in cauliflower meristem from vegetative stage to flower stage in Fremont. Both *REM1* and *TFL1* expression were zero in floral meristem (flower tissue) and low in inflorescence meristem (vegetative, transition, generative and curd stages) **(Figure 27).** The expression of *FH* reaches a peak level at curd maturity in cauliflower (Anthony et al., 1996). *FH* expression patterns in this study give similar result. Similarly, *FH* also has a low expression level in floral meristem (flower).

The MADS box proteins *FRUITFULL (FUL)* not only control flowering time, but also affect determinacy of all meristems (Melzer et al., 2008) *and* plays a role in promoting the floral transition. It is closely related to the meristem identity genes *AP1* and *CAL* (Ferrandiz et al.,2000). Since *FUL i*s a multifunctional gene in *Arabidopsis*, multiple copies of this gene in other species could increase specialization through subfunctionalization. Four *FUL* paralogues (*FUL-a, FUL-b, FUL-c, and FUL-d*) are present in cauliflower. All four *FUL* paralogues are expressed at all stages. *FUL-c* and *FUL-d* are the most abundant transcripts. However, compared with *FUL-b, FUL-c,* and *FUL-d, FUL-a* has a slightly different expression level during the whole cauliflower meristem developmental stage (Denise & Thomas, 2008). The similar different expression level of FUL family as the above conclusions also appeared in this study **(Figure 32).** *FUL-c* and *FUL-d* have a higher expression level than *FUL-a, FUL-b*. The expression level of the *FUL-c* and *FUL-d* during the cauliflower meristerm development has significant difference in vegetative, transition and generative meristem stages. They peaked at generative stage. Hence, the *FUL-c* and *FUL-d* may be used as the genes that can define cauliflower meristem developmental stages from transition to generative. It is regrettable that the fold expression of the *FUL-c* and *FUL-d* were neither black nor white.

4.4 CAL expression during developmental influence by temperature swap

In *Arabidopsis*, flower-meristem-identity genes *CAULIFLOWER(CAL)* at the shoot apex is important for flower initiation (Kaufmann et al.,2010). *CAL* encodes a protein with a MADS-domain and probably functions as a transcriptional activator. In this study, high temperature which can influence the cauliflower meristem development was considered as a factor. In Lindurian control group, *CAL* had a low expression from vegetative stage to generative stage. Comparing to the control group, *CAL* normalized fold expression in Lindurian high temperature treated 6th week was significant higher at transition stage and generative stage **(Figure 34).** At the same time point,

CAL expression in 6th weeks high temperature treatment group had higher expression level than plant in control group. According to the above experimental results, high temperature may up regulate the *CAL* expression in cauliflower meristem from vegetative stage to generative stage. Thus, *CAL* which is as a transcriptional activator may be influenced by high temperature and its function could relate to the delay of curd formation.

4.5 Gene before and after temperature swap

Changes in gene expression occur without a long period in some plants. In *Arabidopsis*, after 24hr temperature treatment, *hos1-1* plants mutate and flower early. This is due to the expression of RD29A gene and other stress responsive genes 24hr before and after treatment in *hos1-1* plant (Ishitani et al.,1998). Therefore, we chose 24hr before and after high temperature treatment cauliflower plants in this study, in order to find some genes with expression changes during one day time. The *Arabidopsis* photoreceptors *CYR2* is known to play roles in the regulation of flowering time (Mockler et al., 1999). Reduce the light induced down regulation of *CRY2* in Arabidopsis leading to early flowering (El-Assal et al., 2001). We consider that other conditions could also regulate the *CRY2* expression so that regulation of flowering time or meristem transition.

Temperature signals are known to impose strong regulation on flowering time (Chew et al.,2012). To understand how temperature influence *CYR2* to regulate the meristem transition from vegetative growth to reproductive development, influence of cool temperature treatment was investigated by group of R.Immink who illustrated that *CRY2* had differentially expressed and also are differentially spliced when vegetative Arabidopsis plant are exposed to cold and heat temperature environment. From one of the two *CYR2* paralogues, expression decreased after one week high temperature treatment in Lindurian cultivar **(Figure 35).** But in temperature insensitive cultivar Fremont, the expression level of *CRY2* was nearly stable before and after high temperature treatment. Further research should reveal whether in Lindurian, temperature treatments also cause differentially splicing.

The differences of expression pattern between *Arabidopsis* and cauliflower are discussed. *Brassica* and *Arabidopsis* are members of the same family, as well as the Brassicaceae. Indeed, counterparts of essentially all the cloned genes exist in both species and corresponding to expression patterns and mutant phenotypes are similar. This study has tested a model of arrest in *Brassica oleracea ssp. Botrytis* (Cauliflower) that incorporated homologues of the key genes involved in the *Arabidopsis* floral transition*: AP1, CAL, FUL, UFO, FH, TFL1, REM1, CCE1, NSN1 and CRY2*. A number of key findings have been emerged. Flowering involves the sequential action of three groups of genes: those that switch the fate of the meristem from vegetative to floral (floral meristem identity genes), those expressions influenced by high temperature during cauliflower development from vegetative stage to generative stage and those that have different

expression or different spliced before immediately after one day high temperature treatment. The same candidate genes were tested in meristem indentifies experiment and high temperature treatment influence meristem switch experiment. By this way, we want to find some key genes which play an important role during these two pathways in cauliflower.

5. Conclusion

The initially purpose of this study if to research the effect of high ambient temperature in the delay of flowering time of *Brassica oleracea ssp. Botrytis* (Cauliflower), and investigate the expression level on several genes'. At the meanwhile, morphological characterization of cauliflower is observed in the greenhouse and field.

Through the morphological of the meristems observed in cauliflower, high temperature delaying meristem switch in temperature sensitive cultivar Lindurian need to be confirmed in this study. Treatment occurring at 5^{th} weeks old plant has longer delay than treating plant at 6^{th} and 7^{th} weeks old in Lindurian. In addition, insensitive cultivar Fremont is confirmed that its meristem switches are not influenced by high ambient temperature. Leaf number and stem thickness increase during the cauliflower grown process. However, high temperature does not influence them in temperature sensitive cultivar Lindurian and insensitive cultivar Fremont. And correlation does not exist between leaf numbers and stem thickness. For curd weight and curd diameter, Fremont has larger curd (weightier and longer diameter) than Lindurian. This could be attributed to meristem switch early in Fremont than Lindurian. The correlation between crud weight and curd diameter has been found in field experiment. But the correlation coefficient not calculated out is this study.

On molecular experiment part, we focus on the expression of flowering time genes in cauliflower. However, before we study cauliflower flowering time, accurate determination of the meristem developmental stage is necessary. Only according to the morphological characteristics divided developmental phase is accurate, but not always possible, when meristems need to be harvested and immediately frozen for RNA analysis. Therefore, several genes were found in this study to define the growth stages of cauliflower. After stable reference gene *Actin* is determined in cauliflower curd development, some cauliflower developmental stage divided genes are found in this study **(Figure 36).** *AP1* gene is just expressed very late in flower tissue in cauliflower. The opposite result is *REM1, TFL1-1* and *FH* which are not expressed in flower stage but have high expression level from vegetative stage to generative stage. The most interesting marker genes which can be divided into the vegetative stage, transition stage and generative are *FRUITFULL (FUL)* family genes, especially *FUL-c* and *FUL-d* are the most abundant transcripts. In the cauliflower, *FUL-c* and *FUL-d*'s expression level show significant growth from vegetative to generative meristem stage.

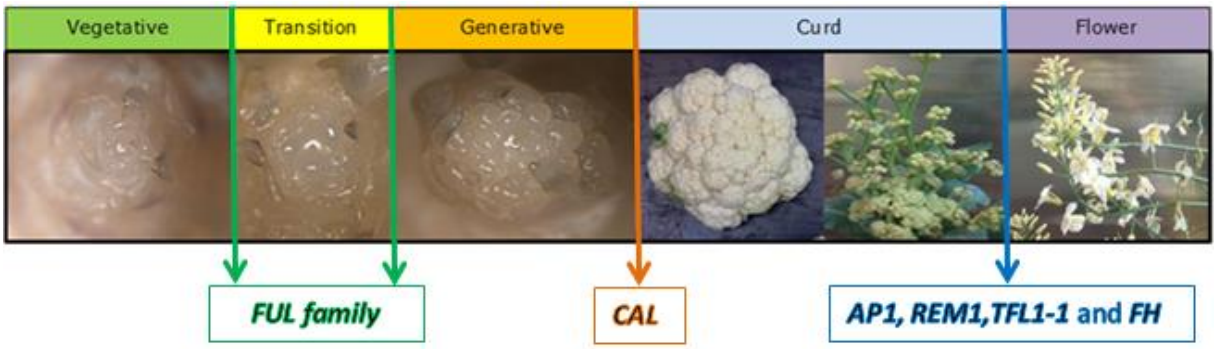


Figure 36. *Cauliflower developmental stages divide genes*

Longer inflorescence meristem stage has been found in cauliflower growth stage by dividing the growth phase with marker genes, comparing with the *Arabidopsis* growth stage. This is because cauliflower has a long curd stage which is the arrested inflorescence phase.

On high ambient temperature influence gene expression level study, preliminary finding is that flower-meristem-identity genes *CAULIFLOWER (CAL)* express differently after high temperature treatment. However, the reason and the regulator of this situation still need more experiments to be discovered.

One week before and after temperature swap is the important time during the cauliflower life, as this may trigger gene expression that will influence meristem transition. Base on the result from *Arabidopsis, Cry2* gene was tested and it was found with expression level decreased after one week high temperature treatment in temperature sensitive cultivar Lindurian, but stable in less sensitive cultivar Fremont. Thus, *Cry2* may have differentially expressed and also are differentially spliced which may be influenced by the high temperature.

This experiment is like a pre-experiment, has increased our understanding of cauliflower curd development. More molecular experiments can be done base on this pre-experiment in the future to find out the mechanism for high ambient temperature delaying the cauliflower flowering time.

6. Further study

As an exploratory experiment, lots of problems happened during the whole experimental process and some of the results we got need to be re-authentication with more experimental units in the future. On the other hand, we did not get much information closely associated with the cauliflower flowering time. So in the future we could choose not do the field experiment.

For morphological traits, a large amount of curd weight and curd diameter data need to be collected to verify the correlation between curd weight and curd diameter in order to test the correlation coefficient between them. By counting the leaf number when we harvest the curd and when meristem switched, a new relationship between leaf number and curd weight could be considered in the further study.

For molecular characterization, fortunately, several cauliflower meristem developmental stage divide genes have been found during this experiment. It is regrettable that these genes do not provide block and white results. Thus, based on the confirm experiments in *Arabidopsis* or other *Brassica* species, finding several accurate meristem identified genes which could define the cauliflower meristem developmental stages with black and white results is important in the further study. At the same time, when we redo the greenhouse experiment, more biological repeat could be used. This is can provide a good research base on cauliflower flowering time research.

Because of time constraints, few flowering time candidate genes were tested in this experiment. In further experiments, a candidate gene could be tested starting from vegetative meristem which will be not treated by high ambient temperature to later floral meristem in cauliflower. Through this process, a whole gene expression process can be described in cauliflower meristem tissue.

Based on these pre-experimental results, I believe that the later experiment will be able to be clearer and get good results.

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Appendix

Time Schedule

Plant breeding master thesis time schedule (07.2012 – 01.2013) I

Week	Day	Time	Theme	Explaination	Remark	Feeling
1	Monday	09-7-2012	Read papers	Read papers about the *Brassicas* , like the classfication of *Brassicas* .		Although I already study agriculture for four years, *Brassicas* still are new cultivars to me. First time try to know the *Brassica* is a little bit hard for me to understand some information. When I went to the field on Friday, the weather was rainy and soaked to the skin. The typical weather in Netherland. Lucky, I did not catch a cold.
	Tuesday	10-7-2012	First meeting	With Guusje, Johan and Verhage Leonie, introduce information about the thesis.	Verhage Leonie is a PHD student who studies the Arabidopsis' flowering time in another group.	
	Wednesday	11-7-2012	Morning meeting	A final presentation about a kind of *Brassica rapa*.		
	Thursday	12-7-2012	Read papers	Read papers about the *Brassicoceae, Brassica, Brassica oleraceo(cauliflower)* and *Arabidopsis (A. thaliana)*.		
	Friday	13-7-2012	Go to the field	With Johan. (1)Count the leaves number for 5,6,7,8,9 weeks(after sowing, the seed grows for 5 weeks) cauliflowers. (2)Cut the meristems(each block,each specie cut three plants) for frizing. (3)Remove the second plant which we want to make pictures of meristem and bring them back to the lab.	(1)There are two kinds cauliflowers: Lindurian(L) and Fremont(F). Linfluenced by temperature, F not. Grow same time, L curd is smaller than F. (2)Did not cut the meristem which we want to make pictures in the field. Beacuse when we go back to the lab, the meristem will be dead or damaged.	
2	Monday	16-7-2012	Read papers	Read papers about the relationship between *Brassica* and *Arbidopsis* and flower time.		This is the second week of my master thesis. Just try to understand more information about this project. Read some papers, but there are so many papers here, I really do not know which one need I carefully read. So still amazing about what I will do in the future. Maybe last week I need find a complete line of my thought.
	Tuesday	17-7-2012	Read papers	Read papers about the flower time relative genes and genetic methods		
	Wednesday	18-7-2012	Morning meeting	Title "Mapping genes for leaf traits in *Brassica rapa* " present by Xiaodong	Many questions be discussed, most of them are about the gene mapping and gene QTL. I know the phenoQTL and the expressionQTL after this presentation. This case forces on the candidate genes, but is better to find novel genes.	
	Thursday	19-7-2012	Go to the greenhouse	With Johan. Cut the meriterm from two species cauliflowers. Three for mRNA, one for picture.	There are two greenhous rooms, one is control, the other one is high temp(5 weeks). Each room has both Fremont and Lindurian.	
	Friday	20-7-2012	Go to the field and do the safety test	(1) Do the same thing with last Friday. (2) Cut three plants back to use for my practices. Practice make pictures for meristem from 5 and 9 weeks cauliflowers under microscope.	(1) Use 1X and 2.5X times to take the meristem picture. When the tissue is big, first use the 1X and then use the 2.5X. If is small, just use the 2.5X. (2) Do not damage the meristem.	
3	Monday	23-7-2012	Start to write proposal	Have a stucture of the proposal and add some general information.		Actually, it is the first week I began do the experiment. I start to learn fome technologies which I will use in the thesis from Johan. And after Thursday, helped Johan collecting the seeds, I feel tired...when I go back home, I derictly go to bed after shower. Studying *Brassica* is a hard work, I known. But after I do something by myself, I know more about the Brassica which I will learn for 6 months in the future.
	Tuesday	24-7-2012	Go to the greenhouse and write proposal	With Johan and Leonie, cut the meristem and swap the plants.	(1) Swap partial 5 weeks plants from high temp to control temp.(2) Swap some 6 weeks plants from control temp to high temp. In the high temp, plants just stay for one week, and then go back to the control temp. (3) Swap for 70 for each caultivar. Switch plants with the green labels.	
	Wednesday	25-7-2012	Morning meeting	Title "Hormone effect and sugar storage in turnip Genotypes" by Temesgen Menamo		
	Thursday	26-7-2012	Write proposal, collect seeds in Negerna	Help Johan collect Brassica seeds, for more than hundred plants.	For each plant put the label in the bag and cut the plant, put it in a big paper bag. Be careful with the seeds, do not loss them. We work for 3 hours under the 37 degree, Johan is a hard work man.	
	Friday	27-7-2012	Go to the field, first time make the picture by myself	With Johan. (1)Count the leaves number for sowing 6,7,8,9 weeks cauliflowers. (2) Cut the meristem(each block,each kind of species cut three plants) to frize.(3)Remove the second plant which we want to make pictures of meristem and bring them to lab for making pictures.	This time we did not cut the cauliflowers in block 6, because the two cultivars' meristems in that block already switched.	
4	Monday	30-7-2012	Write proposal	General finish the proposal.		One month already finished. I writed the proposal. But writing is hard for me. It is a challenge. First time I make many mistakes in my proposal, even I modified my proposal by myself for many times. I have no courage let Johan help me to check it. Johan encourages me and give me lots suggestions. I think, in the future, I can improve my writing by other people's help. I need more confidence.
	Tuesday	31-7-2012	Go to the Negerna greenhouse and the Unifarm greenhouse	(1) Go to the Negerna greenhouse to help Ningwen to sow the Brassica seeds. (2) Go the the Unifarm greenhouse to cut the cauliflowers' meristems with the Johan and make the meristems' pictures by myself. The variation temperature treatments already finished for 5 or 6 weeks cauliflowers.	(1) After sow the Brassica seeds in the soil, use some soil cover the seeds and then put some fine sand on the surface to make sure the seeds can get suitable water and air. Because the Brassica seeds are small. If seeds are big, we do not use find sand. (2) The temperature in the high temperature greenhouse room 5 (6 weeks) turn down to the control temperature.	
	Wednesday	01-8-2012	Finish the first draft of my proposal	Send the first draft to Johan.	I forgot the morning meeting today. That is a pity.	
	Thursday	02-8-2012	Go to the greenhouse	With Johan. Cut the meriterm from two species cauliflowers.	The 5 weeks and 6 weeks cauliflowers already finished high themperature treatment and growing under the control temperature condition.	
	Friday	03-8-2012	Go to the field	With Johan. Cut cauliflowers from the block 7,8,9.	Today I cut the meristems by myself. Although slowly, I can do it by myself. All the cauliflowers in the field switched.	

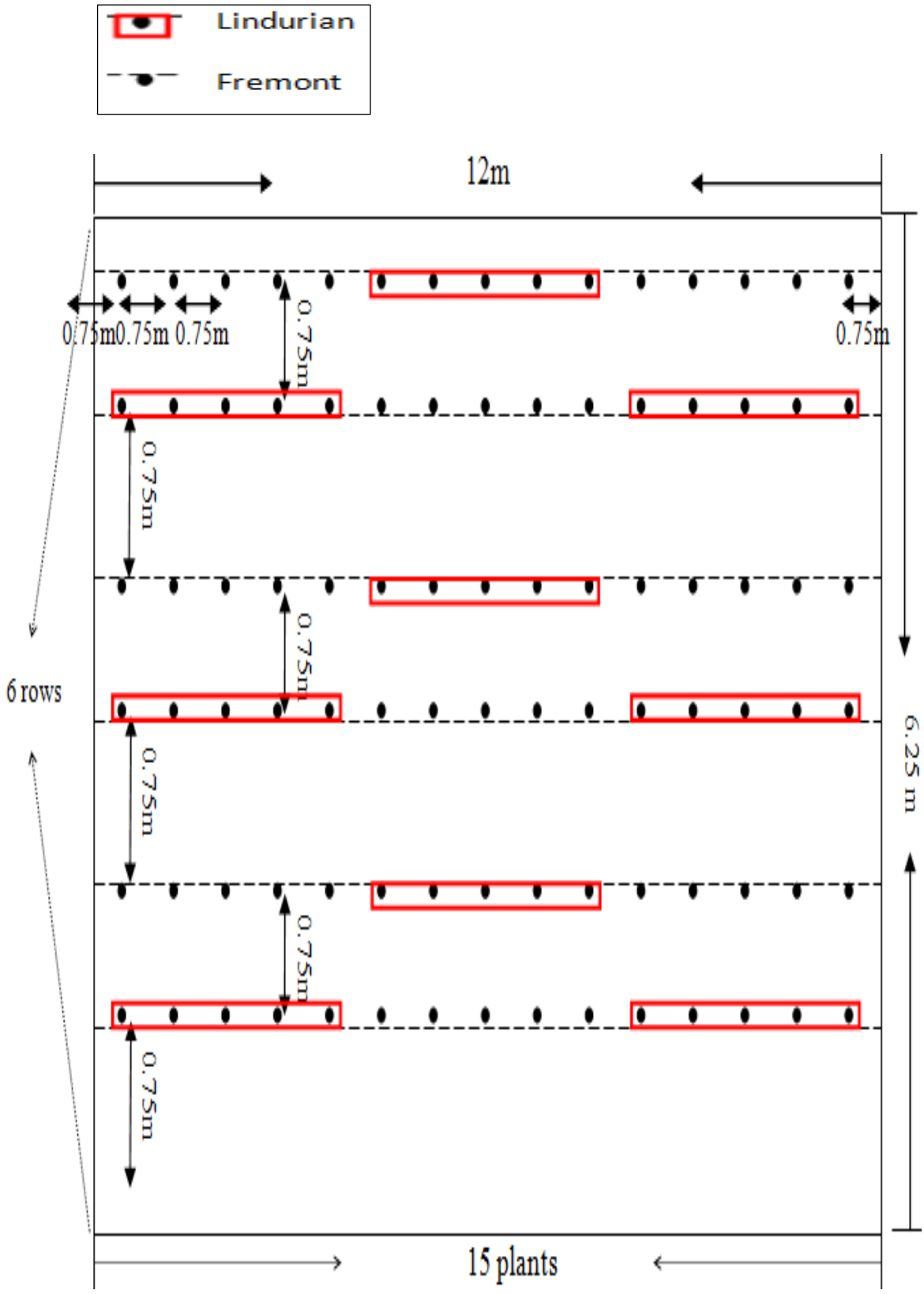
Week	Day	Time	Theme	Explaination	Remark	Feeling
1	Monday	06-8-2012	Change proposal	Find flowering time candidate genes which I interested.		This week I do a lot of work, espesily on Thursday. Johan and me work all the day, until 5 o'clock, we just finish the measure. The large number of cauliflowers been cut from the field. When I go back home, my arms and legs are pain. Because I stand for a all day. But is good that the work for cut meristem from the field is finished this week. Hope next week the lab work will start.
	Tuesday	07-8-2012	Biweekly TTI meeting	With Guusje, Johan, Verhage Leonie and Immink, Richard or Dijk, Aalt-Jan van.	Not cut the meristem in greenhouse. Will do it tomorrow.	
	Wednesday	08-8-2012	Morning meeting and cut the meristem in greenhouse	TTI Green Genetics Thermoflow project: Ambient temperature (in)sensitivity in cauliflower by Johan.	Better understanding the project (about the cauliflower flowering time).	
	Thursday	09-8-2012	Cut the meristem and harvest the curds.	With Johan hand Wins. Cut curd from the blcok 3,4 and 5. And then weight them. (120 cauliflowers). Cut the meristem in the greenhouse.	Each block cut 4x5 cultivars. So for each blcok, there are 20 Fremont and 20 Lindurian. Size their wide and weight them.	
	Friday	10-8-2012	Go to the field (The last time)	With Johan. Do the same thing as before for the block 8,9. And cut some cauliflowers in protection line to use for priacticing RNA extraction.	This is the last time for this experiment cut the meristem from the field. Because all the meristems are already switched laste week. We cut more week to make sure they all switched.	
2	Monday	13-8-2012	Go to the Negema greenhouse	Help Ningwen transplant the Brassica rapa seedings from the tray to the polt for the full day.	Know how to transplants the seedings of Brassica.	First I did not know what I need to write in the proposal. Writing is a big problen for me. So after Guusje checked my proposal, there were so many comments. I lose some important part in my proposal, feel not good. But on the other side, the master thesis is a learning process. So if I can learn something I did not know before, this process is valuable. Hope everything goes well. But I still not feel I start the experiment, just prepare the start material. Worried about it.
	Tuesday	14-8-2012	Cut the meristem	Just myself cut the meristems		
	Wednesday	15-8-2012	Morning meeting	Title "Genome comparison of three morphotypes in Brassica rapa" present by Lin Ke.	Different knowledge be introduced in this presentation. Use the software to analysis the Brassica genome. Based on the genome information publiced in the database.	
	Thursday	16-8-2012	Go to the greenhouse and the field to harvest the curds	(1)In the greenhouse cut the meristem by myself.(2) Harvest the curds in the field for block 6 and block 4.	For the curds harvest, we also harvest the block 4. Because the block 1 and 2 were harvested when the plants were 15 weeks old. But last time, the block 4 was harvest for 14 weeks old. So we harvset the block 4 this week when the plant also 15 weeks old to compare with the block 1 and 2.	
	Friday	17-8-2012	Change proposal after Guusje comment.			
3	Monday	20-8-2012	Reading papers			This week I find a big problem in this project. The Lindurian control plants are not enough but the meristem still not switch. So we change the program, one time just use one Lindurian control plant. And for the Fremont, just cut them on Thursday.
	Tuesday	21-8-2012	Go to the greenhouse	Cut the meristem for swap 2. Just cut Lindurian plants and not cut Fremont plants. Because the Frenomt already switch, I do not need cut the Fremont twitch one week.	Today I find a problem, the Lindurian control plants are not enough, just have 7 plants. So I send an email to the Johan to find a way to solve the problem.	
	Wednesday	22-8-2012	Learn primer desigen	Lear qRT-PCR desigen with Ningwen	Use the Brassica rapa data base, not the Brassica oleracea.	
	Thursday	23-8-2012	Cut the meristem and harvest the curds.	Harvest the curds in block 7 with adriver and cuting, measuring them by myself. In the greenhouse, just use one Lindurian control plant.	Change the meathod in the greenhouse. For the Lindurian control, I just use one plant to use the microscope to observe the meristem transition result.	
	Friday	24-8-2012	Reading papers			
4	Monday	27-8-2012	Reading papers			This week John go to the holiday. I just continue the project. And discuss with Guusje to chang my thesis project. Because the Lindurian plants are not enough. So I have to chang the proposal a lot. I need do the swap 1, not do the swap 2.
	Tuesday	28-8-2012	Cut the meristem in the greenhouse.	No Fremont. Lindurian control just observe one plant.		
	Wednesday	29-8-2012	Morning meeting	Short discuss with Guusje. Want the slove the problem in the project.		
	Thursday	30-8-2012	Cut the meristem and harvest the curds.	Harvest the curds in block 8 and measuring them. Cut the Fremont and Lindurian plant.	Just observe one Lindurian control plant. All the Fremont are switched. The Fremont curds become big.	
	Friday	31-8-2012	Radix Open Day			

Week	Day	Time	Theme	Explaination	Remark	Feeling
1	Monday	03-9-2012	Sort out data	Sort out my experimental nodebook.		This is the first week I try to do the lad experiment--qRT-PCR. Although just learn the steps of the whole process, still feel grade. In the future, more qRT-PCR related experiments will be done, hope I can get accurated results.
	Tuesday	04-9-2012	Greenhouse work, Meeting	With Guusje, Verhage Leonie discuss experiment. Cut the Lindurian meristem.	Change the experiment method.	
	Wednesday	05-9-2012	Learn primer test, Agarose gel and qRT-PCR	With Xiaodong	Missing the morning meet. The qRT-PCR is along process.	
	Thursday	06-9-2012	Hravest curds and make picture for meristem in greenhouse	The Lindurian in field is not enough 20 plants, just has 14 plants. In the greenhouse, just make picture for Lindurian without cut meristem.	(1) This is the last time harvest curds.(2) This is the last Lindurian control plant in the greenhouse.(3) Just make pictures for Lindurian, no Fremont and no meristem cutting.	
	Friday	07-9-2012	Learn qRT-PCR	From Xiaodong		
2	Monday	10-9-2012	Read papers			Greenhouse work was the main thing I did during this week. Because the materials which I will use and the candidate genes still not determin. Last week, the main thing is prepared the material for qRT-PCR.
	Tuesday	11-9-2012	Greenhouse work	Cut the meristem for swap 2. Just cut Lindurian plants (without Control group) and not cut Fremont plants. Make pictures for curds.	Just one plant for 5th and 6th weeks Lindurian groups.	
	Wednesday	12-9-2012	Morning meeting	"Pollen FISH: a cytogenetical technique exploring on Brassica rapa" by Xuan Shuxin .		
	Thursday	13-9-2012	Greenhouse work	Cut the meristem for swap 2. Just cut Lindurian plants (without Control group) and not cut Fremont plants.	Lindurian meristem still in vegetative stage.	
	Friday	14-9-2012	Read papers	Lear information about the rRT-PCR.	Prepare for next week work.	
3	Monday	17-9-2012	Read papers			Fist week do the lab experiment. Prepare the start material. Because it is the first time, most time Johan help me. I think next time I can do by myself.
	Tuesday	18-9-2012	Greenhouse work	Cut the meristem for swap 2. Just cut Lindurian plants (without Control group) and not cut Fremont plants.	Lindurian 6th week group 0473 maybe in early transition stage. But not quite sure about this.	
	Wednesday	19-9-2012	Make sure start materials and isolate RNA from meristem tissue	Cultivar: Fremont+Lindurian. Detail: Fremont-week 5,6(vegetative) and 8,9(generative). Lindurian-6,7(vegetative) and 11,12(generative).	Each week choose two individual plant's meristem. Just have vegetative and generative stage.	
	Thursday	20-9-2012	Greenhouse work	Cut the meristem for swap 2. Just cut Lindurian plants (without Control group) and not cut Fremont plants.	More leaves come out at the top of plant(with picture).	
	Friday	21-9-2012	RNA synthesis	Clean up RNA and reverse RNA to cDNA.	First time synthesis cDNA, help by Johan.	
4	Monday	24-9-2012	Read papers			The first week do qRT-PCR by myself. Test the reference genes. Find a stable reference was the the first step before I start test the other candidate genes. Luck, the qRT-PCR result I do have high accuracy.
	Tuesday	25-9-2012	Greenhouse work and reference genes test.	Cut the meristem for swap 2. Just cut Lindurian plants (without Control group) and not cut Fremont plants. Reference genes test. Cultivar:Fremont+Lindurian. Reference genes:YLS and EF1.	After qRT-PCR, test RNA part by gel to check the purity of RNA.	
	Wednesday	26-9-2012	Morning meeting and test reference genes	"The genetic analysis of leaf architecture in Brassica rapa" by Xiao Dong. Reference genes test:YLS and 18S.		
	Thursday	27-9-2012	Analysis data	Analysis which reference genes more stable.		
	Friday	28-9-2012	Greenhouse work	Cut the meristem for swap 2. Just cut Lindurian plants (without Control group) and not cut Fremont plants.	Not Thursday, one day later.	

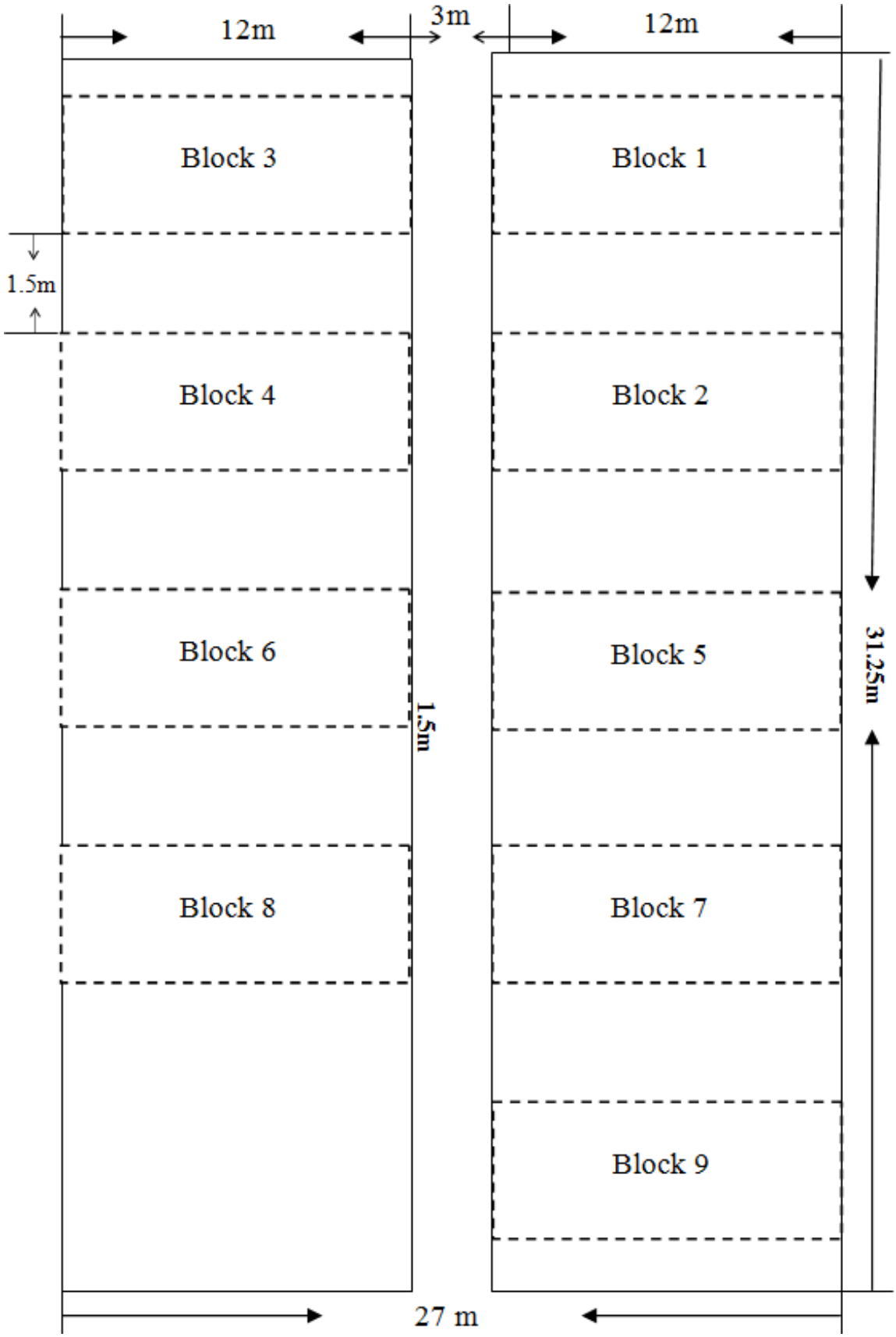
Week	Day	Time	Theme	Explaination	Remark	Feeling
1	Monday	1-10-2012	Read papers			Without reference genes, other candidate genes also be tested in this week. A big problem is AP1 not expression in any materials. We need to think why this situation happened. Hope next week can find the answer.
	Tuesday	2-10-2012	Greenhouse work and reference test	Cut the meristem for swap 2. Just cut Lindurian plants (without Control group) and not cut Fremont plants. Reference genes test: YLS and Actin.	Lindurian 5th week plant already in generative. 6th week plant in transition stage.	
	Wednesday	3-10-2012	Morning meeting and RNA isolate.	"Gene expression analysis during turnip formation in Brassica rapa" by Habtanmesh. Isolate RNA from curd issue.	RNA isolate from Lindurian and Fremont control group curd tissue which nearly come out flower.	
	Thursday	4-10-2012	Greenhouse work;RNA deanup and cDNA sythesis	Cut the meristem for swap 2. Just cut Lindurian plants (without Control group) and not cut Fremont plants. RNA cleanup and cDNA snythesis.	This time, Lindurian meristem all in transition stage. Last time maybe one plant strange.	
	Friday	5-10-2012	Find information about the candidate genes	Use website to find expressiong information(expression stage).	Candidate genes:AP1,FUL family, TFL1, CAL, REM1, UFO and AP2.	
2	Monday	8-10-2012	qRT-PCR for reference gene	Cultivar:Fremont+Lindurian.Test reference genes:A(YLS,AP1);B(YLS,Actin)	Use RNA and cDNA of Fremont and Lindurian materials.	The best result in this week was that make sure the stable reference gene for cauliflower. And then I can start testing the candidates.
	Tuesday	9-10-2012	Gel test quality of RNA	Test the qRT-PCR plate for 08-10-2012.		
	Wednesday	10-10-2012	Greenhouse work;Isolate RNA from flower tissue	Flower tissue comes from Hans group and do not know the cultivar name.		
	Thursday	11-10-2012	Greenhouse work(last time);qRT-PCR	Cultivar:Fremont control group+another cultivar flower. Candidate genes:AP1(primer comes from Leonie)	The last time to make pictures for Lindurian. Because there is no 6 weeks Lindurian plant in the greenhouse, but still has 5 weeks Lindurian	
	Friday	12-10-2012	Primers diluted; qRT-PCR	Cultivar:Fremont control group+another cultivar flower. Candidate genes:(AP1-a, AP1-c); Cultivar:Lindurian control group. Candidate genes:(AP1-a, AP1-c and FUL-a).	Just test candidate genes in Lindurian control group cannot compare with the high temperature group. So in this stage, I will just test candidate genes in Fremont.	
3	Monday	15-10-2012	qRT-PCR	Cultivar:Fremont control group+another cultivar flower. Candidate genes:A(FUL-a,FUL-b);B(FUL-c,FUL-d).		Finished the candidate genes test with another cauliflower flower tissue. Several interested genes were found. But the flower tissue was not our experimental cultivar, so futher experient need be done to verify the results.
	Tuesday	16-10-2012	qRT-PCR	Cultivar:Fremont control group+another cultivar flower. Candidate genes:A(FH,TFL1-1);B(CAL,REM1).		
	Wednesday	17-10-2012	qRT-PCR	Cultivar:Fremont control group+another cultivar flower. Candidate gene	Second time test candidate genes: FUL-a, FUL-b.	
	Thursday	18-10-2012	Check the plants in the greenhouse.	After finished the meristem observe, transfer all the plants to one greenhouse and check wether the Fremont come out open flowers.	Some 6 weeks high temperature treatment Fremont plants already came out open flowers. Control Fremont group not.	
	Friday	19-10-2012	Isolate RNA; Synthesis cDNA.	Cultivar: Lindurian control and 6 weeks high temperature treatment groups.	12 Lindurian 6 weeks high temperature treatment group' plants. 4 Lindurian control gourp's plants.	
4	Monday	22-10-2012	qRT-PCR	Cultivar: Lindurian(control group and 6 weeks high temperature treatment group). Candidate genes: A(AP1-a);B(AP1-c).		Do the experiment as before. But now is test the candidate genes which may influence the cauliflower flower time when change the temperature. But the result is not good enough.
	Tuesday	23-10-2012	qRT-PCR	Cultivar: Lindurian(control group and 6 weeks high temperature treatment group). Candidate genes: A(FUL-a);B(FUL-b).		
	Wednesday	24-10-2012	qRT-PCR	Cultivar: Lindurian(control group and 6 weeks high temperature treatment group). Candidate genes: A(FUL-c);B(FUL-d).		
	Thursday	25-10-2012	qRT-PCR	Cultivar: Lindurian(control group and 6 weeks high temperature treatment group). Candidate genes: A(FH);FB(TFL1-1).		

Week	Day	Time	Theme	Explaination	Remark	Feeling
1	Monday	29-10-2012	qRT-PCR	Cultivar: Lindurian(control group and 6 weeks high temperature treatment group). Candidate genes:A(UFO); B(REM1).		This week finished the candidate genes' test in Lindurian. Just found one gene maybe related to the temperature in Lindurian influence the flower time. Not so good.
	Tuesday	30-10-2012	qRT-PCR	Cultivar: Lindurian(control group and 6 weeks high temperature treatment group). Candidate genes:A(NSN1-1); B(NSN1-2).	Dilute Lindurian cDNA materials.	
	Wednesday	31-10-2012	qRT-PCR	Cultivar: Lindurian(control 6 weeks high temperature treatment group).Candidate genes:A(NSN1-3).		
	Thursday	1-11-2012	Analysis data	Data is about candidate genes expression in cultivar Lindurian control and 6 weeks high temperature treatment group.		
	Friday	2-11-2012	Isolate RNA; Synthesis cDNA.	Cultivar: Fremont Flower-6 weeks high temperature treatment(F1 and F2).	Control Fremont group has no open flowers, more later than 6 weeks Fremont groups.	
2	Monday	5-11-2012	qRT-PCR	Cultivar: Fremont+another kind cauliflower Flower . Candidate genes:A(FUL-a,FUL-b); B(FUL-c,FUL-d).		Almost finished the experiment, but the data is hard to analysis. Because I do not know use which method to analysis them.
	Tuesday	6-11-2012	qRT-PCR	Cultivar: Fremont+Fremont Flower. Candidate genes:A(AP1-a, AP1-c); B(NSN1-1, NSN1-2).		
	Wednesday	7-11-2012	Morning meeting; qRT-PCR	Cultivar: Fremong+Fremont Flower. Candidate genes: A(NSN1-3,CAL); B(REM1, TFL1).	The Fremont samples are not enough. I will sythesis cDNA again when start the new experiment.	
	Thursday	8-11-2012	Analysis data			
	Friday	9-11-2012	Analysis data			
3	Monday	12-11-2012	Design the primer	Design the primer for AT1G04400, which is different expressed and spliced.	Use to test in Lindurian control and 6 weeks groups and Fremont caultivar.	I need to add one week experiment to test a new candidate gene in Lindurian and Fremont. Hope I can finish the experiment quickly and have more time write my report.
	Tuesday	13-11-2012	Analysis data			
	to	to				
	Friday	16-11-2012	Analysis data			
4	Monday	19-11-2012	Analysis data	Beacuse the q RT-PCR machine's oreder was full, so I have to wait.		If every day I can use the qRT-PCR machine like Sunday, I can get more results!
	to	to				
	Friday	23-11-2012	Analysis data			
	Sunday	25-11-2012	qRT-PCR	Cultivar:Lindurian. Candidate genes:A(CRY2-1-a); B(CRY2-2-a); C(CRY2-1-b); D(CRY2-2-b).	No body use the machine today, so I do the whole day experiment.	
Week	**Day**	**Time**	**Theme**	**Explaination**	**Remark**	**Feeling**
1	Monday	26-11-2012	Test new primers	Cultivar:Lindurian. Primers:CRY2-1-a, CRY2-1-b, CRY2-2-a, CRY2-2b	Several Lindurian RNA materials.	This week, based on the resultes we already get, tested new candidate genes used new materials. Because the time limited, just test several interesting genes. But, a new reserach direction was found.
	Tuesday	27-11-2012	qRT-PCR	Cultivar:Fremont. Candidate genes: CRY2-1-a and CRY2-2-a.	New primers CRY2-1-a, CRY2-1-b, CRY2-2-a and CRY2-2b were used in old materials.	
	Wednesday	28-11-2012	qRT-PCR	New RNA materials. Cultivar:Fremont+Lindurian. Candidate genes:CRY2-1-a, CRY2-1-b, CRY2-2-a, CRY2-2b and CAL	New RNA materials were one week before and after high temperature treatment plants. One sample was 5 plants mixed isolating RNA. Help by Johan.	
	Thursday	29-11-2012	qRT-PCR	Cultivar:Fremont+Lindurian. Candidate genes:A(FUL-a,FUL-b,FUL-c,FUL-d and REM1); B(TFL1-1, FH, AP1-a, NSN1-1 and CCE1).	Test other candidate genes which already tested in early experiment.	
	Friday	30-11-2012	Analysis data			

Appendix 1: One block layout in the field experiment.



Appendix 2: Night blocks layout in the field experiment.

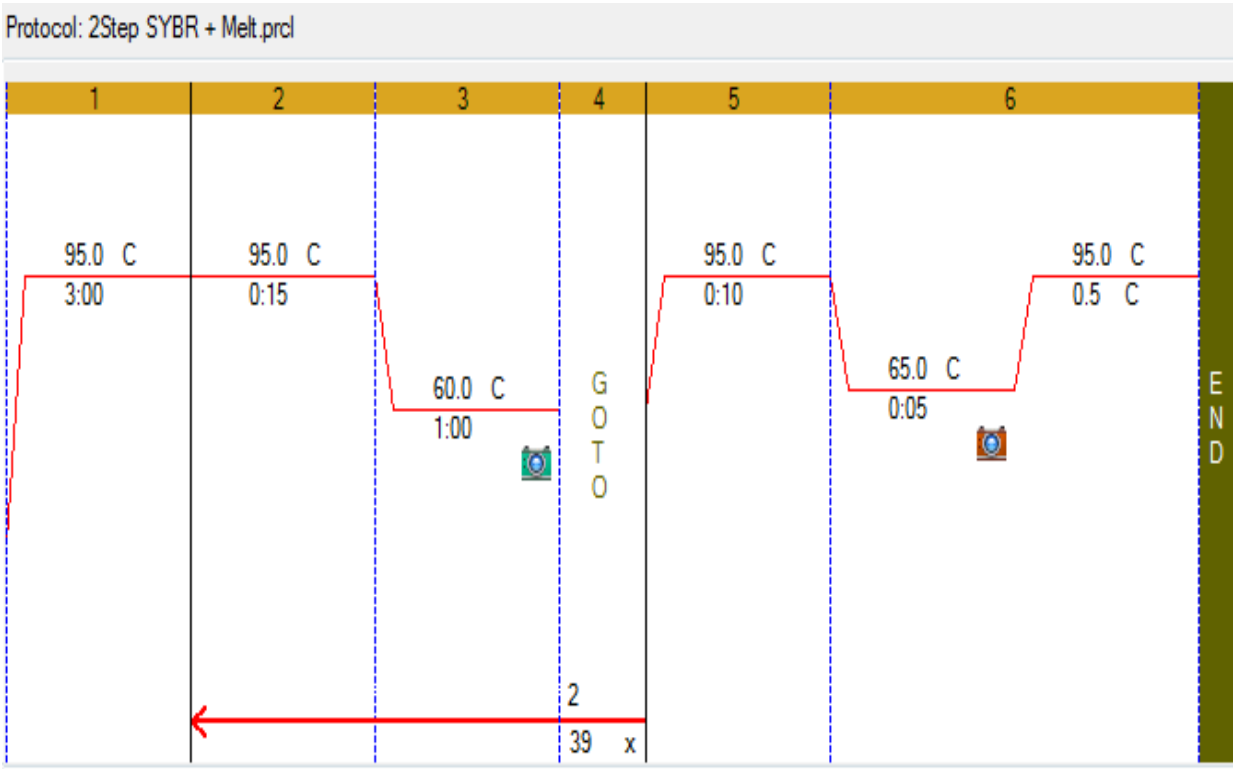


Appendix 3: Temperature treatment (heat or cool) influences flowering genes expression in *Arabidopsis*

Cold Treatment	
Gene	**Symbols**
AT1G30970	Symbols: SUF4 | zinc finger (C2H2 type) family protein | chr1:11040613-11043593 REVERSE LENGTH=367
AT1G51430	Symbols: | unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT3G28370.1); Has 13 Blast hits to 13 proteins in 4 species: Archae - 0; Bacteria - 0; Metazoa - 0; Fungi - 0; Plants - 13; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLink). | chr1:19068241-19069366 FORWARD LENGTH=160
AT1G54920	Symbols: | unknown protein; FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; LOCATED IN: membrane; EXPRESSED IN: 23 plant structures; EXPRESSED DURING: 15 growth stages; Has 35333 Blast hits to 34131 proteins in 2444 species: Archae - 798; Bacteria - 22429; Metazoa - 974; Fungi - 991; Plants - 531; Viruses - 0; Other Eukaryotes - 9610 (source: NCBI BLink). | chr1:20470916-20474898 FORWARD LENGTH=890
AT1G04400	Symbols: CRY2, FHA, AT-PHH1, PHH1, ATCRY2 | cryptochrome 2 | chr1:1185719-1187901 REVERSE LENGTH=612
AT1G04750	Symbols: VAMP7B, VAMP721, ATVAMP721, AT VAMP7B | vesicle-associated membrane protein 721 | chr1:1331857-1333426 REVERSE LENGTH=219
AT1G45332	Symbols: | Translation elongation factor EFG/EF2 protein | chr1:17172507-17176683 REVERSE LENGTH=754
AT1G48740	Symbols: | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | chr1:18023590-18025960 REVERSE LENGTH=393
AT1G69450	Symbols: | Early-responsive to dehydration stress protein (ERD4) | chr1:26107120-26110006 REVERSE LENGTH=711
AT1G47840	Symbols: HXK3 | hexokinase 3 | chr1:17616243-17618859 REVERSE LENGTH=493
AT1G09530	Symbols: PIF3, POC1, PAP3 | phytochrome interacting factor 3 | chr1:3077216-3079367 FORWARD LENGTH=524
AT1G24070	Symbols: ATCSLA10, CSLA10 | cellulose synthase-like A10 | chr1:8516437-8519734 REVERSE LENGTH=552
AT1G48410	Symbols: AGO1 | Stabilizer of iron transporter SufD / Polynucleotidyl transferase | chr1:17886285-17891892 REVERSE LENGTH=1048
AT1G67300	Symbols: | Major facilitator superfamily protein | chr1:25193832-25196751 REVERSE LENGTH=494
AT1G12920	Symbols: ERF1-2 | eukaryotic release factor 1-2 | chr1:4396555-4397859 REVERSE LENGTH=434
AT1G53770	Symbols: | O-fucosyltransferase family protein | chr1:20071460-20073708 REVERSE LENGTH=563
AT1G55250	Symbols: HUB2 | histone mono-ubiquitination 2 | chr1:20607214-20612302 FORWARD LENGTH=900
AT1G77080	Symbols: MAF1, FLM, AGL27 | K-box region and MADS-box transcription factor family protein | chr1:28958311-28959054 FORWARD LENGTH=141
AT1G79730	Symbols: ELF7 | hydroxyproline-rich glycoprotein family protein | chr1:30000743-30003969 REVERSE LENGTH=589
AT2G21070	Symbols: FIO1 | methyltransferases | chr2:9040940-9043136 FORWARD LENGTH=483
AT2G25930	Symbols: ELF3, PYK20 | hydroxyproline-rich glycoprotein family protein | chr2:11059459-11063178 FORWARD LENGTH=695
AT2G27990	Symbols: BLH8, PNF | BEL1-like homeodomain 8 | chr2:11921540-11923902 REVERSE LENGTH=584
AT2G44950	Symbols: RDO4, HUB1 | histone mono-ubiquitination 1 | chr2:18542602-18548247 REVERSE LENGTH=878
AT3G10390	Symbols: FLD | Flavin containing amine oxidoreductase family protein | chr3:3229293-3232345 FORWARD LENGTH=884
AT3G11440	Symbols: ATMYB65, MYB65 | myb domain protein 65 | chr3:3603056-3604929 FORWARD LENGTH=553
AT3G15354	Symbols: SPA3 | SPA1-related 3 | chr3:5169327-5172480 REVERSE LENGTH=837
AT3G60250	Symbols: CKB3 | casein kinase II beta chain 3 | chr3:22270714-22271962 REVERSE LENGTH=276
AT4G11110	Symbols: SPA2 | SPA1-related 2 | chr4:6772163-6776675 FORWARD LENGTH=1036
AT4G31877	
AT4G32980	Symbols: ATH1 | homeobox gene 1 | chr4:15914865-15916873 REVERSE LENGTH=473
AT5G02810	Symbols: PRR7, APRR7 | pseudo-response regulator 7 | chr5:638283-641461 REVERSE LENGTH=727
AT5G13480	Symbols: FY | Transducin/WD40 repeat-like superfamily protein | chr5:4326638-4331557 REVERSE LENGTH=647
AT5G23150	Symbols: HUA2 | Tudor/PWWP/MBT domain-containing protein | chr5:7786173-7792080 FORWARD LENGTH=1392
AT5G35840	Symbols: PHYC | phytochrome C | chr5:14008049-14011619 FORWARD LENGTH=1111
AT5G65050	Symbols: AGL31, MAF2 | AGAMOUS-like 31 | chr5:25982415-25986114 FORWARD LENGTH=182
AT5G65060	Symbols: MAF3, FCL3, AGL70 | K-box region and MADS-box transcription factor family protein | chr5:25987527-25991065 FORWARD LENGTH=196

Heat Treatment	
Gene	**Symbols**
AT1G26930	Symbols: | Galactose oxidase/kelch repeat superfamily protein | chr1:9336211-9337476 REVERSE LENGTH=421
AT1G45332	Symbols: | Translation elongation factor EFG/EF2 protein | chr1:17172507-17176683 REVERSE LENGTH=754
AT1G48740	Symbols: | 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein | chr1:18023590-18025960 REVERSE LENGTH=393
AT1G58400	Symbols: | Disease resistance protein (CC-NBS-LRR class) family | chr1:21696165-21699118 REVERSE LENGTH=900
AT1G04400	Symbols: CRY2, FHA, AT-PHH1, PHH1, ATCRY2 | cryptochrome 2 | chr1:1185719-1187901 REVERSE LENGTH=612
AT1G08970	Symbols: HAP5C, NF-YC9 | nuclear factor Y, subunit C9 | chr1:2883144-2883839 FORWARD LENGTH=231
AT1G09530	Symbols: PIF3, POC1, PAP3 | phytochrome interacting factor 3 | chr1:3077216-3079367 FORWARD LENGTH=524
AT1G67690	Symbols: | Zincin-like metalloproteases family protein | chr1:25369086-25373730 FORWARD LENGTH=710
AT1G48410	Symbols: AGO1 | Stabilizer of iron transporter SufD / Polynucleotidyl transferase | chr1:17886285-17891892 REVERSE LENGTH=1048
AT1G09570	Symbols: PHYA, FHY2, FRE1, HY8 | phytochrome A | chr1:3095498-3099216 REVERSE LENGTH=1122
AT1G22770	Symbols: GI, FB | gigantea protein (GI) | chr1:8062398-8067447 FORWARD LENGTH=1173
AT1G24260	Symbols: SEP3, AGL9 | K-box region and MADS-box transcription factor family protein | chr1:8593790-8595862 REVERSE LENGTH=250
AT1G77080	Symbols: MAF1, FLM, AGL27 | K-box region and MADS-box transcription factor family protein | chr1:28958311-28959054 FORWARD LENGTH=141
AT1G79730	Symbols: ELF7 | hydroxyproline-rich glycoprotein family protein | chr1:30000743-30003969 REVERSE LENGTH=589
AT2G23380	Symbols: CLF, ICU1, SDG1, SET1 | SET domain-containing protein | chr2:9955570-9960117 FORWARD LENGTH=902
AT2G25930	Symbols: ELF3, PYK20 | hydroxyproline-rich glycoprotein family protein | chr2:11059459-11063178 FORWARD LENGTH=695
AT2G27990	Symbols: BLH8, PNF | BEL1-like homeodomain 8 | chr2:11921540-11923902 REVERSE LENGTH=584
AT2G44950	Symbols: RDO4, HUB1 | histone mono-ubiquitination 1 | chr2:18542602-18548247 REVERSE LENGTH=878
AT2G46830	Symbols: CCA1 | circadian clock associated 1 | chr2:19246005-19248717 FORWARD LENGTH=608
AT3G05120	Symbols: ATGID1A, GID1A | alpha/beta-Hydrolases superfamily protein | chr3:1430682-1432287 FORWARD LENGTH=345
AT3G11440	Symbols: ATMYB65, MYB65 | myb domain protein 65 | chr3:3603056-3604929 FORWARD LENGTH=553
AT3G12810	Symbols: PIE1, SRCAP, chr13 | SNF2 domain-containing protein / helicase domain-containing protein | chr3:4065636-4073992 FORWARD LENGTH=2055
AT3G15354	Symbols: SPA3 | SPA1-related 3 | chr3:5169327-5172480 REVERSE LENGTH=837
AT3G18990	Symbols: VRN1, REM39 | AP2/B3-like transcriptional factor family protein | chr3:6549077-6551568 REVERSE LENGTH=341
AT4G00650	Symbols: FRI, FLA | FRIGIDA-like protein | chr4:269026-270363 FORWARD LENGTH=314
AT4G11110	Symbols: SPA2 | SPA1-related 2 | chr4:6772163-6776675 FORWARD LENGTH=1036
AT4G16250	Symbols: PHYD | phytochrome D | chr4:9195602-9199486 REVERSE LENGTH=1164
AT4G16280	Symbols: FCA | RNA binding;abscisic acid binding | chr4:9207164-9214412 REVERSE LENGTH=747
AT4G31877	
AT4G32980	Symbols: ATH1 | homeobox gene 1 | chr4:15914865-15916873 REVERSE LENGTH=473
AT5G02810	Symbols: PRR7, APRR7 | pseudo-response regulator 7 | chr5:638283-641461 REVERSE LENGTH=727
AT5G12840	Symbols: HAP2A, EMB2220, ATHAP2A, NF-YA1 | nuclear factor Y, subunit A1 | chr5:4051147-4052961 REVERSE LENGTH=272
AT5G13480	Symbols: FY | Transducin/WD40 repeat-like superfamily protein | chr5:4326638-4331557 REVERSE LENGTH=647
AT5G23150	Symbols: HUA2 | Tudor/PWWP/MBT domain-containing protein | chr5:7786173-7792080 FORWARD LENGTH=1392
AT5G35840	Symbols: PHYC | phytochrome C | chr5:14008049-14011619 FORWARD LENGTH=1111
AT5G37260	Symbols: RVE2, CIR1 | Homeodomain-like superfamily protein | chr5:14751344-14752972 REVERSE LENGTH=287
AT5G60100	Symbols: APRR3, PRR3 | pseudo-response regulator 3 | chr5:24198215-24200502 REVERSE LENGTH=495
AT5G65060	Symbols: MAF3, FCL3, AGL70 | K-box region and MADS-box transcription factor family protein | chr5:25987527-25991065 FORWARD LENGTH=196

Appendix 4: qRT-PCR thermal cycling



1	95.0 C for 3:00
2	95.0 C for 0:15
3	60.0 C for 1:00
	+ Plate Read
4	GOTO 2 , 39 more times
5	95.0 C for 0:10
6	Melt Curve 65.0 to 95.0 C, increment 0.5 C,
	for 0:05 + Plate Read
	END

Appendix 5: Leaf Number of Lindurian & Fremont in nine blocks in field

Block	Weeks	Hybrid	Date	Purpose	Field Number	Number of Leaves	Picture	Curd
1		Lindurian	15-6-2012	Maintain	0001	17		
1		Lindurian	15-6-2012	Binoculair	0002	16	Yes	Transition
1		Lindurian	15-6-2012	Freezer	0003	16		
1		Lindurian	15-6-2012	Freezer	0004	17		
1		Lindurian	15-6-2012	Freezer	0005	15		
1	9	Fremont	15-6-2012	Maintain	0006	14		
1		Fremont	15-6-2012	Binoculair	0007	15	Yes	Generative
1		Fremont	15-6-2012	Freezer	0008	15		
1		Fremont	15-6-2012	Freezer	0009	16		
1		Fremont	15-6-2012	Freezer	0010	15		
1		Lindurian	22-6-2012	Maintain	0011			
1		Lindurian	22-6-2012	Binoculair	0012	22	Yes	Generative
1		Lindurian	22-6-2012	Freezer	0013	20		
1		Lindurian	22-6-2012	Freezer	0014	22		
1		Lindurian	22-6-2012	Freezer	0015	20		
1	10	Fremont	22-6-2012	Maintain	0016			
1		Fremont	22-6-2012	Binoculair	0017	21	Yes	Generative
1		Fremont	22-6-2012	Freezer	0018	20		
1		Fremont	22-6-2012	Freezer	0019	18		
1		Fremont	22-6-2012	Freezer	0020	18		
2		Lindurian	22-6-2012	Maintain	0091			
2		Lindurian	22-6-2012	Binoculair	0092		Yes	Generative
2		Lindurian	22-6-2012	Freezer	0093	18		
2		Lindurian	22-6-2012	Freezer	0094	17		
2		Lindurian	22-6-2012	Freezer	0095	17		
2	9	Fremont	22-6-2012	Maintain	0096			
2		Fremont	22-6-2012	Binoculair	0097		Yes	Generative
2		Fremont	22-6-2012	Freezer	0098	14		
2		Fremont	22-6-2012	Freezer	0099	15		
2		Fremont	22-6-2012	Freezer	0100	17		
3		Lindurian	22-6-2012	Maintain	0181			
3		Lindurian	22-6-2012	Binoculair	0182		Yes	Generative
3		Lindurian	22-6-2012	Freezer	0183	16		
3		Lindurian	22-6-2012	Freezer	0184	16		
3		Lindurian	22-6-2012	Freezer	0185	16		
3	8	Fremont	22-6-2012	Maintain	0186			
3		Fremont	22-6-2012	Binoculair	0187		Yes	Generative
3		Fremont	22-6-2012	Freezer	0188	17		
3		Fremont	22-6-2012	Freezer	0189	15		
3		Fremont	22-6-2012	Freezer	0190	16		
3		Lindurian	28-6-2012	Maintain	0191	21		
3		Lindurian	28-6-2012	Binoculair	0192	20	Yes	Generative
3		Lindurian	28-6-2012	Freezer	0193	21		
3		Lindurian	28-6-2012	Freezer	0194	19		
3		Lindurian	28-6-2012	Freezer	0195	20		
3	9	Fremont	28-6-2012	Maintain	0196	18		
3		Fremont	28-6-2012	Binoculair	0197	18	Yes	Generative
3		Fremont	28-6-2012	Freezer	0198	17		
3		Fremont	28-6-2012	Freezer	0199	17		
3		Fremont	28-6-2012	Freezer	0200	18		

Block	Weeks	Hybrid	Date	Purpose	Field Number	Number of Leaves	Picture	Curd
4		Lindurian	28-6-2012	Maintain	0271	15		
4		Lindurian	28-6-2012	Binoculair	0272	14	Yes	Generative
4		Lindurian	28-6-2012	Freezer	0273	15		
4		Lindurian	28-6-2012	Freezer	0274	15		
4	8	Lindurian	28-6-2012	Freezer	0275	15		
4		Fremont	28-6-2012	Maintain	0276	17		
4		Fremont	28-6-2012	Binoculair	0277	15	Yes	Generative
4		Fremont	28-6-2012	Freezer	0278	14		
4		Fremont	28-6-2012	Freezer	0279	13		
4		Fremont	28-6-2012	Freezer	0280	14		
4		Lindurian	6-7-2012	Maintain	0281	23		
4		Lindurian	6-7-2012	Binoculair	0282	22	Yes	Generative
4		Lindurian	6-7-2012	Freezer	0283	24		
4		Lindurian	6-7-2012	Freezer	0284	23		
4		Lindurian	6-7-2012	Freezer	0285	22		
4	9	Fremont	6-7-2012	Maintain	0286	21		
4		Fremont	6-7-2012	Binoculair	0287	20	Yes	Generative
4		Fremont	6-7-2012	Freezer	0288	20		
4		Fremont	6-7-2012	Freezer	0289	21		
4		Fremont	6-7-2012	Freezer	0290	22		
5		Lindurian	28-6-2012	Maintain	0361	11		
5		Lindurian	28-6-2012	Binoculair	0362	9	Yes	Transition
5		Lindurian	28-6-2012	Freezer	0363	10		
5		Lindurian	28-6-2012	Freezer	0364	9		
5	7	Lindurian	28-6-2012	Freezer	0365	10		
5		Fremont	28-6-2012	Maintain	0366	10		
5		Fremont	28-6-2012	Binoculair	0367	9	Yes	Transition
5		Fremont	28-6-2012	Freezer	0368	10		
5		Fremont	28-6-2012	Freezer	0369	10		
5		Fremont	28-6-2012	Freezer	0370	10		
5		Lindurian	6-7-2012	Maintain	0371	14		
5		Lindurian	6-7-2012	Binoculair	0372	15	Yes	Generative
5		Lindurian	6-7-2012	Freezer	0373	15		
5		Lindurian	6-7-2012	Freezer	0374	14		
5		Lindurian	6-7-2012	Freezer	0375	15		
5	8	Fremont	6-7-2012	Maintain	0376	16		
5		Fremont	6-7-2012	Binoculair	0377	15	Yes	Generative
5		Fremont	6-7-2012	Freezer	0378	15		
5		Fremont	6-7-2012	Freezer	0379	14		
5		Fremont	6-7-2012	Freezer	0380	15		
5		Lindurian	13-7-2012	Maintain	0381	14		
5		Lindurian	13-7-2012	Binoculair	0382	16	Yes	Generative
5		Lindurian	13-7-2012	Freezer	0383	17		
5		Lindurian	13-7-2012	Freezer	0384	17		
5		Lindurian	13-7-2012	Freezer	0385	20		
5	9	Fremont	13-7-2012	Maintain	0386	16		
5		Fremont	13-7-2012	Binoculair	0387	14	Yes	Generative
5		Fremont	13-7-2012	Freezer	0388	14		
5		Fremont	13-7-2012	Freezer	0389	13		

Block	Weeks	Hybrid	Date	Purpose	Field Number	Number of Leaves	Picture	Curd
5		Lindurian	20-7-2012	Maintain	0391	21		
5		Lindurian	20-7-2012	Binoculair	0392	20	Yes	Generative
5		Lindurian	20-7-2012	Freezer	0393	18		
5		Lindurian	20-7-2012	Freezer	0394	22		
5		Lindurian	20-7-2012	Freezer	0395	23		
5	10	Fremont	20-7-2012	Maintain	0396	22		
5		Fremont	20-7-2012	Binoculair	0397	18	Yes	Generative
5		Fremont	20-7-2012	Freezer	0398	20		
5		Fremont	20-7-2012	Freezer	0399	22		
5		Fremont	20-7-2012	Freezer	0400	21		
6		Lindurian	6-7-2012	Maintain	0451	11		
6		Lindurian	6-7-2012	Binoculair	0452	13	Yes	Transition
6		Lindurian	6-7-2012	Freezer	0453	12		
6		Lindurian	6-7-2012	Freezer	0454	14		
6		Lindurian	6-7-2012	Freezer	0455	12		
6	7	Fremont	6-7-2012	Maintain	0456	13		
6		Fremont	6-7-2012	Binoculair	0457	12	Yes	Generative
6		Fremont	6-7-2012	Freezer	0458	12		
6		Fremont	6-7-2012	Freezer	0459	12		
6		Fremont	6-7-2012	Freezer	0460	12		
6		Lindurian	13-7-2012	Maintain	0461	13		
6		Lindurian	13-7-2012	Binoculair	0462	15	Yes	Transition
6		Lindurian	13-7-2012	Freezer	0463	10		
6		Lindurian	13-7-2012	Freezer	0464	12		
6		Lindurian	13-7-2012	Freezer	0465	9		
6	8	Fremont	13-7-2012	Maintain	0466	15		
6		Fremont	13-7-2012	Binoculair	0467	13	Yes	Generative
6		Fremont	13-7-2012	Freezer	0468	13		
6		Fremont	13-7-2012	Freezer	0469	13		
6		Fremont	13-7-2012	Freezer	0470	15		
6		Lindurian	20-7-2012	Maintain	0471	18		
6		Lindurian	20-7-2012	Binoculair	0472	20	Yes	Generative
6		Lindurian	20-7-2012	Freezer	0473	20		
6		Lindurian	20-7-2012	Freezer	0474	18		
6		Lindurian	20-7-2012	Freezer	0475	18		
6	9	Fremont	20-7-2012	Maintain	0476	17		
6		Fremont	20-7-2012	Binoculair	0477	19	Yes	Generative
6		Fremont	20-7-2012	Freezer	0478	19		
6		Fremont	20-7-2012	Freezer	0479	20		
6		Fremont	20-7-2012	Freezer	0480	19		
6		Lindurian	27-7-2012	Maintain	0481	23		
6		Lindurian	27-7-2012	Binoculair	0482	22	Yes	Generative
6		Lindurian	27-7-2012	Freezer	0483	23		
6		Lindurian	27-7-2012	Freezer	0484	24		
6		Lindurian	27-7-2012	Freezer	0485	27		
6	10	Fremont	27-7-2012	Maintain	0486	24		
6		Fremont	27-7-2012	Binoculair	0487	25	Yes	Generative
6		Fremont	27-7-2012	Freezer	0488	23		
6		Fremont	27-7-2012	Freezer	0489	21		

Block	Weeks	Hybrid	Date	Purpose	Field Number	Number of Leaves	Picture	Curd
7		Lindurian	6-7-2012	Maintain	0541	8		
7		Lindurian	6-7-2012	Binoculair	0542	8	Yes	Vegetative
7		Lindurian	6-7-2012	Freezer	0543	8		
7		Lindurian	6-7-2012	Freezer	0544	9		
7		Lindurian	6-7-2012	Freezer	0545	9		
7	6	Fremont	6-7-2012	Maintain	0546	8		
7		Fremont	6-7-2012	Binoculair	0547	8	Yes	Vegetative
7		Fremont	6-7-2012	Freezer	0548	8		
7		Fremont	6-7-2012	Freezer	0549	8		
7		Fremont	6-7-2012	Freezer	0550	8		
7		Lindurian	13-7-2012	Maintain	0551	8		
7		Lindurian	13-7-2012	Binoculair	0552	11	Yes	Vegetative
7		Lindurian	13-7-2012	Freezer	0553	12		
7		Lindurian	13-7-2012	Freezer	0554	11		
7		Lindurian	13-7-2012	Freezer	0555	12		
7	7	Fremont	13-7-2012	Maintain	0556	12		
7		Fremont	13-7-2012	Binoculair	0557	12	Yes	Vegetative
7		Fremont	13-7-2012	Freezer	0558	11		
7		Fremont	13-7-2012	Freezer	0559	13		
7		Fremont	13-7-2012	Freezer	0560	12		
7		Lindurian	20-7-2012	Maintain	0561	14		
7		Lindurian	20-7-2012	Binoculair	0562	16	Yes	Transition
7		Lindurian	20-7-2012	Freezer	0563	11		
7		Lindurian	20-7-2012	Freezer	0564	14		
7		Lindurian	20-7-2012	Freezer	0565	12		
7	8	Fremont	20-7-2012	Maintain	0566	15		
7		Fremont	20-7-2012	Binoculair	0567	13	Yes	Transition
7		Fremont	20-7-2012	Freezer	0568	16		
7		Fremont	20-7-2012	Freezer	0569	14		
7		Fremont	20-7-2012	Freezer	0570	13		
7		Lindurian	27-7-2012	Maintain	0571	11		
7		Lindurian	27-7-2012	Binoculair	0572	16	Yes	Generative
7		Lindurian	27-7-2012	Freezer	0573	19		
7		Lindurian	27-7-2012	Freezer	0574	20		
7		Lindurian	27-7-2012	Freezer	0575	18		
7	9	Fremont	27-7-2012	Maintain	0576	17		
7		Fremont	27-7-2012	Binoculair	0577	17	Yes	Generative
7		Fremont	27-7-2012	Freezer	0578	21		
7		Fremont	27-7-2012	Freezer	0579	21		
7		Fremont	27-7-2012	Freezer	0580	18		
7		Lindurian	3-8-2012	Maintain	0581	23		
7		Lindurian	3-8-2012	Binoculair	0582	27	Yes	Generative
7		Lindurian	3-8-2012	Freezer	0583	24		
7		Lindurian	3-8-2012	Freezer	0584	23		
7		Lindurian	3-8-2012	Freezer	0585	21		
7	10	Fremont	3-8-2012	Maintain	0586	27		
7		Fremont	3-8-2012	Binoculair	0587	28	Yes	Generative
7		Fremont	3-8-2012	Freezer	0588	26		
7		Fremont	3-8-2012	Freezer	0589	27		
7		Fremont	3-8-2012	Freezer	0590	28		

Block	Weeks	Hybrid	Date	Purpose	Field Number	Number of Leaves	Picture	Curd
8		Lindurian	6-7-2012	Maintain	0631	7		
8		Lindurian	6-7-2012	Binoculair	0632	6	Yes	Vegetative
8		Lindurian	6-7-2012	Freezer	0633	7		
8		Lindurian	6-7-2012	Freezer	0634	7		
8		Lindurian	6-7-2012	Freezer	0635	6		
8	5	Fremont	6-7-2012	Maintain	0636	6		
8		Fremont	6-7-2012	Binoculair	0637	6	Yes	Vegetative
8		Fremont	6-7-2012	Freezer	0638	6		
8		Fremont	6-7-2012	Freezer	0639	6		
8		Fremont	6-7-2012	Freezer	0640	6		
8		Lindurian	13-7-2012	Maintain	0641	9		
8		Lindurian	13-7-2012	Binoculair	0642	7	Yes	Vegetative
8		Lindurian	13-7-2012	Freezer	0643	10		
8		Lindurian	13-7-2012	Freezer	0644	9		
8		Lindurian	13-7-2012	Freezer	0645	9		
8	6	Fremont	13-7-2012	Maintain	0646	8		
8		Fremont	13-7-2012	Binoculair	0647	10	Yes	Vegetative
8		Fremont	13-7-2012	Freezer	0648	8		
8		Fremont	13-7-2012	Freezer	0649	9		
8		Fremont	13-7-2012	Freezer	0650	10		
8		Lindurian	20-7-2012	Maintain	0651	11		
8		Lindurian	20-7-2012	Binoculair	0652	12	Yes	Vegetative
8		Lindurian	20-7-2012	Freezer	0653	10		
8		Lindurian	20-7-2012	Freezer	0654	14		
8		Lindurian	20-7-2012	Freezer	0655	12		
8	7	Fremont	20-7-2012	Maintain	0656	12		
8		Fremont	20-7-2012	Binoculair	0657	12	Yes	Vegetative
8		Fremont	20-7-2012	Freezer	0658	11		
8		Fremont	20-7-2012	Freezer	0659	11		
8		Fremont	20-7-2012	Freezer	0660	11		
8		Lindurian	27-7-2012	Maintain	0661	17		
8		Lindurian	27-7-2012	Binoculair	0662	18	Yes	Generative
8		Lindurian	27-7-2012	Freezer	0663	16		
8		Lindurian	27-7-2012	Freezer	0664	12		
8		Lindurian	27-7-2012	Freezer	0665	14		
8	8	Fremont	27-7-2012	Maintain	0666	17		
8		Fremont	27-7-2012	Binoculair	0667	14	Yes	Generative
8		Fremont	27-7-2012	Freezer	0668	15		
8		Fremont	27-7-2012	Freezer	0669	15		
8		Fremont	27-7-2012	Freezer	0670	14		
8		Lindurian	3-8-2012	Maintain	0671	16		
8		Lindurian	3-8-2012	Binoculair	0672	18	Yes	Generative
8		Lindurian	3-8-2012	Freezer	0673	18		
8		Lindurian	3-8-2012	Freezer	0674	19		
8		Lindurian	3-8-2012	Freezer	0675	18		
8	9	Fremont	3-8-2012	Maintain	0676	21		
8		Fremont	3-8-2012	Binoculair	0677	19	Yes	Generative
8		Fremont	3-8-2012	Freezer	0678	19		
8		Fremont	3-8-2012	Freezer	0679	19		
8		Fremont	3-8-2012	Freezer	0680	21		

Block	Weeks	Hybrid	Date	Purpose	Field Number	Number of Leaves	Picture	Curd
8		Lindurian	10-8-2012	Maintain	0691	22		
8		Lindurian	10-8-2012	Binoculair	0692	25	Yes	Generative
8		Lindurian	10-8-2012	Freezer	0693	24		
8		Lindurian	10-8-2012	Freezer	0694	23		
8		Lindurian	10-8-2012	Freezer	0695	25		
8	10	Fremont	10-8-2012	Maintain	0696	21		
8		Fremont	10-8-2012	Binoculair	0697	24	Yes	Generative
8		Fremont	10-8-2012	Freezer	0698	25		
8		Fremont	10-8-2012	Freezer	0699	24		
8		Fremont	10-8-2012	Freezer	0700	25		
9		Lindurian	13-7-2012	Maintain	0721	7		
9		Lindurian	13-7-2012	Binoculair	0722	8	Yes	Vegetative
9		Lindurian	13-7-2012	Freezer	0723	7		
9		Lindurian	13-7-2012	Freezer	0724	8		
9		Lindurian	13-7-2012	Freezer	0725	7		
9	5	Fremont	13-7-2012	Maintain	0726	7		
9		Fremont	13-7-2012	Binoculair	0727	8	Yes	Vegetative
9		Fremont	13-7-2012	Freezer	0728	7		
9		Fremont	13-7-2012	Freezer	0729	7		
9		Fremont	13-7-2012	Freezer	0730	7		
9		Lindurian	20-7-2012	Maintain	0731	10		
9		Lindurian	20-7-2012	Binoculair	0732	10	Yes	Vegetative
9		Lindurian	20-7-2012	Freezer	0733	10		
9		Lindurian	20-7-2012	Freezer	0734	10		
9		Lindurian	20-7-2012	Freezer	0735	11		
9	6	Fremont	20-7-2012	Maintain	0736	9		
9		Fremont	20-7-2012	Binoculair	0737	9	Yes	Vegetative
9		Fremont	20-7-2012	Freezer	0738	10		
9		Fremont	20-7-2012	Freezer	0739	11		
9		Fremont	20-7-2012	Freezer	0740	8		
9		Lindurian	27-7-2012	Maintain	0741	14		
9		Lindurian	27-7-2012	Binoculair	0742	10	Yes	Transition
9		Lindurian	27-7-2012	Freezer	0743	13		
9		Lindurian	27-7-2012	Freezer	0744	13		
9		Lindurian	27-7-2012	Freezer	0745	14		
9	7	Fremont	27-7-2012	Maintain	0746	14		
9		Fremont	27-7-2012	Binoculair	0747	13	Yes	Generative
9		Fremont	27-7-2012	Freezer	0748	14		
9		Fremont	27-7-2012	Freezer	0749	14		
9		Fremont	27-7-2012	Freezer	0750	13		
9		Lindurian	3-8-2012	Maintain	0751	15		
9		Lindurian	3-8-2012	Binoculair	0752	13	Yes	Generative
9		Lindurian	3-8-2012	Freezer	0753	15		
9		Lindurian	3-8-2012	Freezer	0754	14		
9		Lindurian	3-8-2012	Freezer	0755	14		
9	8	Fremont	3-8-2012	Maintain	0756	15		
9		Fremont	3-8-2012	Binoculair	0757	14	Yes	Generative
9		Fremont	3-8-2012	Freezer	0758	15		
9		Fremont	3-8-2012	Freezer	0759	15		
9		Fremont	3-8-2012	Freezer	0760	15		

Block	Weeks	Hybrid	Date	Purpose	Field Number	Number of Leaves	Picture	Curd
9	9	Lindurian	10-8-2012	Maintain	0776	25		
9		Lindurian	10-8-2012	Binoculair	0777	25	Yes	Generative
9		Lindurian	10-8-2012	Freezer	0778	24		
9		Lindurian	10-8-2012	Freezer	0779	25		
9		Lindurian	10-8-2012	Freezer	0780	24		
9		Fremont	10-8-2012	Maintain	0781	21		
9		Fremont	10-8-2012	Binoculair	0782	21	Yes	Generative
9		Fremont	10-8-2012	Freezer	0783	18		
9		Fremont	10-8-2012	Freezer	0784	20		
9		Fremont	10-8-2012	Freezer	0785	21		

Appendix 6: Leaf Number of Lindurian & Fremont in the greenhouse

Hybrid	Greenhouse No.	Date	Swap	Purpose	No. of Leaves	Stem (mm)	Picture
Lindurian	0258	19-7-2012	5 Week	Binoculair	10	8.18	Yes
Lindurian	0265	19-7-2012	5 Week	Freezer	9	7.33	
Lindurian	0266	19-7-2012	5 Week	Freezer	10	8.26	
Lindurian	0267	19-7-2012	5 Week	Freezer	10	8.24	
Lindurian	0364	19-7-2012	Control	Binoculair	10	7.50	Yes
Lindurian	0361	19-7-2012	Control	Freezer	9	7.13	
Lindurian	0362	19-7-2012	Control	Freezer	9	7.95	
Lindurian	0363	19-7-2012	Control	Freezer	9	7.17	
Fremont	0018	19-7-2012	5 Week	Binoculair	9	8.36	Yes
Fremont	0015	19-7-2012	5 Week	Freezer	9	8.07	
Fremont	0016	19-7-2012	5 Week	Freezer	9	7.83	
Fremont	0017	19-7-2012	5 Week	Freezer	9	8.43	
Fremont	0114	19-7-2012	Control	Binoculair	9	8.18	Yes
Fremont	0111	19-7-2012	Control	Freezer	9	8.15	
Fremont	0112	19-7-2012	Control	Freezer	9	9.30	
Fremont	0113	19-7-2012	Control	Freezer	9	8.34	
Lindurian	0350	24-7-2012	5 Week	Binoculair	11	9.16	Yes
Lindurian	0349	24-7-2012	5 Week	Freezer	10	8.66	
Lindurian	0348	24-7-2012	5 Week	Freezer	11	8.62	
Lindurian	0347	24-7-2012	5 Week	Freezer	10	9.50	
Lindurian	0500	24-7-2012	Control	Binoculair	10	8.10	Yes
Lindurian	0499	24-7-2012	Control	Freezer	11	8.03	
Lindurian	0498	24-7-2012	Control	Freezer	11	7.60	
Lindurian	0497	24-7-2012	Control	Freezer	11	8.10	
Fremont	0099	24-7-2012	5 Week	Binoculair	11	8.75	Yes
Fremont	0098	24-7-2012	5 Week	Freezer	11	9.60	
Fremont	0097	24-7-2012	5 Week	Freezer	10	9.20	
Fremont	0096	24-7-2012	5 Week	Freezer	10	7.90	
Fremont	0250	24-7-2012	Control	Binoculair	11	9.58	Yes
Fremont	0249	24-7-2012	Control	Freezer	10	8.60	
Fremont	0248	24-7-2012	Control	Freezer	11	9.22	
Fremont	0247	24-7-2012	Control	Freezer	10	8.95	
Lindurian	0447	26-7-2012	6 Week	Binoculair	10	8.45	Yes
Lindurian	0434	26-7-2012	6 Week	Freezer	12	8.13	
Lindurian	0487	26-7-2012	6 Week	Freezer	12	8.15	
Lindurian	0485	26-7-2012	6 Week	Freezer	11	8.25	
Lindurian	0311	26-7-2012	5 Week	Binoculair	12	9.08	Yes
Lindurian	0294	26-7-2012	5 Week	Freezer	12	9.09	
Lindurian	0323	26-7-2012	5 Week	Freezer	12	8.44	
Lindurian	0282	26-7-2012	5 Week	Freezer	13	9.14	
Lindurian	0395	26-7-2012	Control	Binoculair	12	7.96	Yes
Lindurian	0380	26-7-2012	Control	Freezer	11	9.52	
Lindurian	0416	26-7-2012	Control	Freezer	12	8.79	
Lindurian	0401	26-7-2012	Control	Freezer	12	9.22	
Fremont	0220	26-7-2012	6 Week	Binoculair	10	8.71	Yes
Fremont	0218	26-7-2012	6 Week	Freezer	10	8.42	
Fremont	0187	26-7-2012	6 Week	Freezer	9	7.91	
Fremont	0188	26-7-2012	6 Week	Freezer	10	8.32	

Hybrid	Greenhouse No.	Date	Swap	Purpose	No. of Leaves	Stem (mm)	Picture
Fremont	0097	26-7-2012	5 Week	Binoculair	11	9.07	Yes
Fremont	0025	26-7-2012	5 Week	Freezer	10	9.22	
Fremont	0087	26-7-2012	5 Week	Freezer	12	9.20	
Fremont	0085	26-7-2012	5 Week	Freezer	11	8.72	
Fremont	0151	26-7-2012	Control	Binoculair	12	10.15	Yes
Fremont	0135	26-7-2012	Control	Freezer	11	9.17	
Fremont	0148	26-7-2012	Control	Freezer	11	8.81	
Fremont	0141	26-7-2012	Control	Freezer	11	10.17	
Lindurian	0460	31-7-2012	6 Week	Binoculair	15	8.79	Yes
Lindurian	0444	31-7-2012	6 Week	Freezer	13	8.39	
Lindurian	0478	31-7-2012	6 Week	Freezer	11	7.18	
Lindurian	0474	31-7-2012	6 Week	Freezer	13	8.25	
Lindurian	0292	31-7-2012	5 Week	Binoculair	16	9.33	Yes
Lindurian	0391	31-7-2012	5 Week	Freezer	15	9.47	
Lindurian	0281	31-7-2012	5 Week	Freezer	15	9.70	
Lindurian	0304	31-7-2012	5 Week	Freezer	16	9.94	
Lindurian	0393	31-7-2012	Control	Binoculair	14	8.14	Yes
Lindurian	0382	31-7-2012	Control	Freezer	16	8.26	
Lindurian	0414	31-7-2012	Control	Freezer	15	8.76	
Lindurian	0403	31-7-2012	Control	Freezer	12	7.83	
Fremont	0193	31-7-2012	6 Week	Binoculair	14	9.44	Yes
Fremont	0214	31-7-2012	6 Week	Freezer	11	8.60	
Fremont	0198	31-7-2012	6 Week	Freezer	12	8.58	
Fremont	0233	31-7-2012	6 Week	Freezer	14	9.07	
Fremont	0071	31-7-2012	5 Week	Binoculair	15	10.20	Yes
Fremont	0057	31-7-2012	5 Week	Freezer	13	9.76	
Fremont	0031	31-7-2012	5 Week	Freezer	9	7.28	
Fremont	0090	31-7-2012	5 Week	Freezer	14	10.12	
Fremont	0154	31-7-2012	Control	Binoculair	14	9.22	Yes
Fremont	0157	31-7-2012	Control	Freezer	11	7.05	
Fremont	0153	31-7-2012	Control	Freezer	13	8.81	
Fremont	0136	31-7-2012	Control	Freezer	13	9.60	
Lindurian	0445	2-8-2012	6 Week	Binoculair	17	8.17	Yes
Lindurian	0490	2-8-2012	6 Week	Freezer	17	8.72	
Lindurian	0454	2-8-2012	6 Week	Freezer	17	8.58	
Lindurian	0464	2-8-2012	6 Week	Freezer	16	8.20	
Lindurian	0305	2-8-2012	5 Week	Binoculair	18	9.60	Yes
Lindurian	0337	2-8-2012	5 Week	Freezer	17	8.76	
Lindurian	0278	2-8-2012	5 Week	Freezer	17	9.13	
Lindurian	0312	2-8-2012	5 Week	Freezer	20	9.00	
Lindurian	0394	2-8-2012	Control	Binoculair	17	8.96	Yes
Lindurian	0413	2-8-2012	Control	Freezer	16	9.09	
Lindurian	0404	2-8-2012	Control	Freezer	17	9.24	
Lindurian	0379	2-8-2012	Control	Freezer	18	9.58	
Fremont	0212	2-8-2012	6 Week	Binoculair	16	9.20	Yes
Fremont	0182	2-8-2012	6 Week	Freezer	15	8.96	
Fremont	0202	2-8-2012	6 Week	Freezer	16	8.72	
Fremont	0239	2-8-2012	6 Week	Freezer	17	9.27	

Hybrid	Greenhouse No.	Date	Swap	Purpose	No. of Leaves	Stem (mm)	Picture
Fremont	0030	2-8-2012	5 Week	Binoculair	12	9.10	Yes
Fremont	0065	2-8-2012	5 Week	Freezer	17	9.70	
Fremont	0052	2-8-2012	5 Week	Freezer	15	8.49	
Fremont	0064	2-8-2012	5 Week	Freezer	16	9.28	
Fremont	0147	2-8-2012	Control	Binoculair	15	9.71	Yes
Fremont	0131	2-8-2012	Control	Freezer	17	9.74	
Fremont	0152	2-8-2012	Control	Freezer	15	9.30	
Fremont	0173	2-8-2012	Control	Freezer	10	6.18	
Lindurian	0468	8-8-2012	6 Week	Binoculair	21	9.80	Yes
Lindurian	0491	8-8-2012	6 Week	Freezer	21	9.40	
Lindurian	0433	8-8-2012	6 Week	Freezer	22	9.72	
Lindurian	0457	8-8-2012	6 Week	Freezer	21	9.63	
Lindurian	0340	8-8-2012	5 Week	Binoculair	21	9.06	Yes
Lindurian	0295	8-8-2012	5 Week	Freezer	23	9.18	
Lindurian	0306	8-8-2012	5 Week	Freezer	23	9.27	
Lindurian	0302	8-8-2012	5 Week	Freezer	20	8.91	
Lindurian	0402	8-8-2012	Control	Binoculair	22	9.26	Yes
Lindurian	0417	8-8-2012	Control	Freezer	22	9.01	
Lindurian	0396	8-8-2012	Control	Freezer	23	8.53	
Lindurian	0386	8-8-2012	Control	Freezer	22	8.83	
Fremont	0137	8-8-2012	6 Week	Binoculair	22	10.24	Yes
Fremont	0208	8-8-2012	6 Week	Freezer	23	9.43	
Fremont	0238	8-8-2012	6 Week	Freezer	22	9.88	
Fremont	0219	8-8-2012	6 Week	Freezer	20	9.94	
Fremont	0099	8-8-2012	5 Week	Binoculair	25	10.53	Yes
Fremont	0089	8-8-2012	5 Week	Freezer	22	11.27	
Fremont	0047	8-8-2012	5 Week	Freezer	22	10.67	
Fremont	0029	8-8-2012	5 Week	Freezer	20	9.87	
Fremont	0174	8-8-2012	Control	Binoculair	17	9.25	Yes
Fremont	0130	8-8-2012	Control	Freezer	23	10.00	
Fremont	0129	8-8-2012	Control	Freezer	23	10.14	
Fremont	0176	8-8-2012	Control	Freezer	20	9.75	
Lindurian	0496	9-8-2012	6 Week	Binoculair	23	9.38	Yes
Lindurian	0483	9-8-2012	6 Week	Freezer	23	9.35	
Lindurian	0453	9-8-2012	6 Week	Freezer	24	9.15	
Lindurian	0429	9-8-2012	6 Week	Freezer	24	9.86	
Lindurian	0309	9-8-2012	5 Week	Binoculair	25	10.23	Yes
Lindurian	0313	9-8-2012	5 Week	Freezer	26	10.01	
Lindurian	0339	9-8-2012	5 Week	Freezer	24	9.17	
Lindurian	0272	9-8-2012	5 Week	Freezer	24	9.52	
Lindurian	0381	9-8-2012	Control	Binoculair	27	9.71	Yes
Lindurian	0390	9-8-2012	Control	Freezer	24	9.39	
Lindurian	0400	9-8-2012	Control	Freezer	23	8.33	
Lindurian	0412	9-8-2012	Control	Freezer	22	9.10	
Fremont	0235	9-8-2012	6 Week	Binoculair	22	9.93	Yes
Fremont	0243	9-8-2012	6 Week	Freezer	23	10.47	
Fremont	0183	9-8-2012	6 Week	Freezer	25	10.71	
Fremont	0179	9-8-2012	6 Week	Freezer	23	10.41	

Hybrid	Greenhouse No.	Date	Swap	Purpose	No. of Leaves	Stem (mm)	Picture
Fremont	0092	9-8-2012	5 Week	Binoculair	26	9.75	Yes
Fremont	0045	9-8-2012	5 Week	Freezer	21	10.64	
Fremont	0078	9-8-2012	5 Week	Freezer	22	10.66	
Fremont	0072	9-8-2012	5 Week	Freezer	25	10.74	
Fremont	0177	9-8-2012	Control	Binoculair	23	10.32	Yes
Fremont	0166	9-8-2012	Control	Freezer	21	10.36	
Fremont	0146	9-8-2012	Control	Freezer	22	11.25	
Fremont	0165	9-8-2012	Control	Freezer	21	9.22	
Lindurian	0450	14-8-2012	6 Week	Binoculair	24	9.60	Yes
Lindurian	0483	14-8-2012	6 Week	Freezer	27	10.08	
Lindurian	0466	14-8-2012	6 Week	Freezer	23	8.79	
Lindurian	0482	14-8-2012	6 Week	Freezer	22	9.53	
Lindurian	0308	14-8-2012	5 Week	Binoculair	28	9.85	Yes
Lindurian	0279	14-8-2012	5 Week	Freezer	30	9.74	
Lindurian	0288	14-8-2012	5 Week	Freezer	26	8.45	
Lindurian	0303	14-8-2012	5 Week	Freezer	29	10.57	
Lindurian	0398	14-8-2012	Control	Binoculair	22	8.86	Yes
Lindurian	0408	14-8-2012	Control	Freezer	24	9.15	
Lindurian	0411	14-8-2012	Control	Freezer	22	8.56	
Lindurian	0391	14-8-2012	Control	Freezer	23	9.26	
Fremont	0192	14-8-2012	6 Week	Binoculair	23	10.99	Yes
Fremont	0189	14-8-2012	6 Week	Freezer	25	10.38	
Fremont	0222	14-8-2012	6 Week	Freezer	25	10.24	
Fremont	0221	14-8-2012	6 Week	Freezer	22	11.53	
Fremont	0081	14-8-2012	5 Week	Binoculair	25	11.17	Yes
Fremont	0031	14-8-2012	5 Week	Freezer	24	11.04	
Fremont	0026	14-8-2012	5 Week	Freezer	24	11.83	
Fremont	0080	14-8-2012	5 Week	Freezer	21	10.10	
Fremont	0144	14-8-2012	Control	Binoculair	24	9.93	Yes
Fremont	0139	14-8-2012	Control	Freezer	25	11.17	
Fremont	0145	14-8-2012	Control	Freezer	26	10.08	
Fremont	0143	14-8-2012	Control	Freezer	24	9.91	
Lindurian	0431	16-8-2012	6 Week	Binoculair	27	9.83	Yes
Lindurian	0430	16-8-2012	6 Week	Freezer	22	9.59	
Lindurian	0435	16-8-2012	6 Week	Freezer	27	10.43	
Lindurian	0441	16-8-2012	6 Week	Freezer	25	9.06	
Lindurian	0334	16-8-2012	5 Week	Binoculair	28	9.90	Yes
Lindurian	0277	16-8-2012	5 Week	Freezer	26	10.41	
Lindurian	0297	16-8-2012	5 Week	Freezer	25	9.42	
Lindurian	0307	16-8-2012	5 Week	Freezer	27	10.02	
Lindurian	0392	16-8-2012	Control	Binoculair	31	9.84	Yes
Lindurian	0406	16-8-2012	Control	Freezer	25	8.99	
Lindurian	0418	16-8-2012	Control	Freezer	27	8.87	
Lindurian	0419	16-8-2012	Control	Freezer	24	9.96	
Fremont	0232	16-8-2012	6 Week	Binoculair	23	10.38	Yes
Fremont	0242	16-8-2012	6 Week	Freezer	28	11.37	
Fremont	0140	16-8-2012	6 Week	Freezer	25	11.89	
Fremont	0199	16-8-2012	6 Week	Freezer	25	11.15	

Hybrid	Greenhouse No.	Date	Swap	Purpose	No. of Leaves	Stem (mm)	Picture
Fremont	0024	16-8-2012	5 Week	Binoculair	24	11.11	Yes
Fremont	0095	16-8-2012	5 Week	Freezer	26	12.07	
Fremont	0063	16-8-2012	5 Week	Freezer	24	10.88	
Fremont	0059	16-8-2012	5 Week	Freezer	20	9.52	
Fremont	0142	16-8-2012	Control	Binoculair	23	10.58	Yes
Fremont	0172	16-8-2012	Control	Freezer	25	10.50	
Fremont	0178	16-8-2012	Control	Freezer	21	10.26	
Fremont	0164	16-8-2012	Control	Freezer	21	10.38	
Lindurian	0480	21-8-2012	6 Week	Binoculair	31	9.59	Yes
Lindurian	0443	21-8-2012	6 Week	Freezer	30	10.04	
Lindurian	0455	21-8-2012	6 Week	Freezer	30	11.17	
Lindurian	0469	21-8-2012	6 Week	Freezer	29	9.59	
Lindurian	0332	21-8-2012	5 Week	Binoculair	30	10.00	Yes
Lindurian	0270	21-8-2012	5 Week	Freezer	30	10.23	
Lindurian	0341	21-8-2012	5 Week	Freezer	28	10.31	
Lindurian	0336	21-8-2012	5 Week	Freezer	25	10.51	
Lindurian	0420	21-8-2012	Control	Binoculair	25	11.17	Yes
Lindurian	0407	21-8-2012	Control	Freezer	27	9.26	
Lindurian	0423	21-8-2012	Control	Freezer	25	9.82	
Lindurian	0399	21-8-2012	Control	Freezer	28	8.58	
Lindurian	0456	23-8-2012	6 Week	Binoculair	28	10.68	Yes
Lindurian	0446	23-8-2012	6 Week	Freezer	31	9.99	
Lindurian	0432	23-8-2012	6 Week	Freezer	32	9.54	
Lindurian	0452	23-8-2012	6 Week	Freezer	32	9.73	
Lindurian	0326	23-8-2012	5 Week	Binoculair	28	11.08	Yes
Lindurian	0314	23-8-2012	5 Week	Freezer	29	10.04	
Lindurian	0273	23-8-2012	5 Week	Freezer	31	9.94	
Lindurian	0286	23-8-2012	5 Week	Freezer	35	10.62	
Lindurian	0405	23-8-2012	Control	Binoculair	31	9.88	Yes
Lindurian		23-8-2012	Control	Freezer			
Lindurian		23-8-2012	Control	Freezer			
Lindurian		23-8-2012	Control	Freezer			
Fremont	0180	23-8-2012	6 Week	Binoculair	32	10.86	Yes
Fremont	0191	23-8-2012	6 Week	Freezer	28	11.09	
Fremont	0240	23-8-2012	6 Week	Freezer	26	11.01	
Fremont	0223	23-8-2012	6 Week	Freezer	32	10.60	
Fremont	0061	23-8-2012	5 Week	Binoculair	29	10.68	Yes
Fremont	0032	23-8-2012	5 Week	Freezer	28	10.67	
Fremont	0082	23-8-2012	5 Week	Freezer	30	10.72	
Fremont	0085	23-8-2012	5 Week	Freezer	32	10.16	
Fremont	0150	23-8-2012	Control	Binoculair	22	10.89	Yes
Fremont	0134	23-8-2012	Control	Freezer	21	11.18	
Fremont	0159	23-8-2012	Control	Freezer	28	11.26	
Fremont	0156	23-8-2012	Control	Freezer	25	10.12	
Lindurian	0448	28-8-2012	6 Week	Binoculair	32	10.23	Yes
Lindurian	0442	28-8-2012	6 Week	Freezer	33	10.60	
Lindurian	0481	28-8-2012	6 Week	Freezer	36	10.43	
Lindurian	0440	28-8-2012	6 Week	Freezer	28	9.96	

Hybrid	Greenhouse No.	Date	Swap	Purpose	No. of Leaves	Stem (mm)	Picture
Lindurian	0320	28-8-2012	5 Week	Binoculair	34	10.91	Yes
Lindurian	0289	28-8-2012	5 Week	Freezer	35	10.94	
Lindurian	0331	28-8-2012	5 Week	Freezer	31	10.62	
Lindurian	0274	28-8-2012	5 Week	Freezer	35	9.82	
Lindurian	0421	28-8-2012	Control	Binoculair	30	9.35	Yes
Lindurian		28-8-2012	Control	Freezer			
Lindurian		28-8-2012	Control	Freezer			
Lindurian		28-8-2012	Control	Freezer			
Lindurian	0439	30-8-2012	6 Week	Binoculair	34	9.55	Yes
Lindurian	0477	30-8-2012	6 Week	Freezer	30	8.12	
Lindurian	0459	30-8-2012	6 Week	Freezer	32	10.12	
Lindurian	0437	30-8-2012	6 Week	Freezer	36	9.37	
Lindurian	0333	30-8-2012	5 Week	Binoculair	31	10.14	Yes
Lindurian	0290	30-8-2012	5 Week	Freezer	32	10.83	
Lindurian	0275	30-8-2012	5 Week	Freezer	33	9.88	
Lindurian	0422	30-8-2012	5 Week	Freezer	30	10.09	
Lindurian		30-8-2012	Control	Binoculair	31	10.44	Yes
Lindurian		30-8-2012	Control	Freezer			
Lindurian		30-8-2012	Control	Freezer			
Lindurian		30-8-2012	Control	Freezer			
Fremont	0241	30-8-2012	6 Week	Binoculair	30	12.00	Yes
Fremont	0213	30-8-2012	6 Week	Freezer	32	11.53	
Fremont	0235	30-8-2012	6 Week	Freezer	30	13.16	
Fremont	0207	30-8-2012	6 Week	Freezer	31	11.58	
Fremont	0044	30-8-2012	5 Week	Binoculair	28	10.68	Yes
Fremont	0083	30-8-2012	5 Week	Freezer	29	10.46	
Fremont	0093	30-8-2012	5 Week	Freezer	29	11.62	
Fremont	0034	30-8-2012	5 Week	Freezer	28	10.86	
Fremont	0162	30-8-2012	Control	Binoculair	27	10.18	Yes
Fremont	0169	30-8-2012	Control	Freezer	26	10.22	
Fremont	0167	30-8-2012	Control	Freezer	32	9.76	
Fremont	0158	30-8-2012	Control	Freezer	30	13.13	
Lindurian	0492	4-9-2012	6 Week	Binoculair	36	10.72	Yes
Lindurian	0494	4-9-2012	6 Week	Freezer	33	11.23	
Lindurian	0475	4-9-2012	6 Week	Freezer	36	10.96	
Lindurian	0493	4-9-2012	6 Week	Freezer	40	11.71	
Lindurian	0271	4-9-2012	5 Week	Binoculair	36	10.36	Yes
Lindurian	0284	4-9-2012	5 Week	Freezer	39	9.97	
Lindurian	0280	4-9-2012	5 Week	Freezer	38	10.12	
Lindurian	0283	4-9-2012	5 Week	Freezer	40	10.19	
Lindurian	0387	4-9-2012	Control	Binoculair	46	11.07	Yes
Lindurian		4-9-2012	Control	Freezer			
Lindurian		4-9-2012	Control	Freezer			
Lindurian		4-9-2012	Control	Freezer			
Lindurian	0388	6-9-2012	Control	Binoculair	39	10.41	Yes
Lindurian	0330	6-9-2012	5 Week	Binoculair	36	9.52	Yes
Lindurian	0471	6-9-2012	6 Week	Binoculair	38	11.63	Yes

Hybrid	Greenhouse No.	Date	Swap	Purpose	No. of Leaves	Stem (mm)	Picture
Lindurian		11-9-2012	Control	Binoculair			Yes
Lindurian	0344	11-9-2012	5 Week	Binoculair	41	10.86	Yes
Lindurian	0458	11-9-2012	6 Week	Binoculair	46	11.61	Yes
Lindurian		13-9-2012	Control	Binoculair			Yes
Lindurian	0329	13-9-2012	5 Week	Binoculair	40	11.51	Yes
Lindurian	0476	13-9-2012	6 Week	Binoculair	42	11.33	Yes
Lindurian		18-9-2012	Control	Binoculair			Yes
Lindurian	0285	18-9-2012	5 Week	Binoculair	51		Yes
Lindurian	0473	18-9-2012	6 Week	Binoculair	45		Yes
Lindurian		20-9-2012	Control	Binoculair			Yes
Lindurian	0310	20-9-2012	5 Week	Binoculair	53		Yes
Lindurian	0486	20-9-2012	6 Week	Binoculair	48		Yes
Lindurian		25-9-2012	Control	Binoculair			Yes
Lindurian	0298	25-9-2012	5 Week	Binoculair	51		Yes
Lindurian	0461	25-9-2012	6 Week	Binoculair	45		Yes
Lindurian		27-9-2012	Control	Binoculair			Yes
Lindurian	0276	27-9-2012	5 Week	Binoculair	54		Yes
Lindurian	0409	27-9-2012	6 Week	Binoculair	51		Yes
Lindurian		2-10-2012	Control	Binoculair			Yes
Lindurian	0300	2-10-2012	5 Week	Binoculair	60		Yes
Lindurian	0436	2-10-2012	6 Week	Binoculair	62		Yes
Lindurian		4-10-2012	Control	Binoculair			Yes
Lindurian	0293	4-10-2012	5 Week	Binoculair	63		Yes
Lindurian	0438	4-10-2012	6 Week	Binoculair	60		Yes
Lindurian		10-10-2012	Control	Binoculair			Yes
Lindurian	0291	10-10-2012	5 Week	Binoculair	64		Yes
Lindurian	0479	10-10-2012	6 Week	Binoculair	59		Yes
Lindurian		11-10-2012	Control	Binoculair			Yes
Lindurian	0324	11-10-2012	5 Week	Binoculair	60		Yes
Lindurian	0465	11-10-2012	6 Week	Binoculair	56		Yes
Lindurian		16-10-2012	Control	Binoculair			Yes
Lindurian	0335	16-10-2012	5 Week	Binoculair	70		Yes
Lindurian	0470	16-10-2012	6 Week	Binoculair	65		Yes

Appendix 7: Correlations between leaf number and stem thickness

Remark: No correlation between leaf number and stem thickness

Has correlation between leaf number and stem thickness

Abbreviations :L – Lindurian

F – Fremont

L+N(Number:7,8,9 et.al) – Leaf number when plant was N weeks old.

S+N(Number:7,8,9 et.al) – Stem thickness when plant was N weeks old.

Table 1. *Correlations between leaf number and stem thickness in control*

Correlations between Leave Number and Stem Thinkness in Control Group

	L-L7	F-L7	L-S7	F-S7	L-L8	F-L8	L-S8	F-S8	L-L9	F-L9	L-S9	F-S9	L-L10	F-L10	L-S10	F-S10	L-L11	F-L11	L-S11	F-S11	L-L12	F-L12	L-S12	F-S12
L-L7	1	.333	-.637	.391	-.683	.927	-.022	.958*	.333	-.522	-.222	-.366	-.870	-.174	-.409	-.992**	-.333	.632	.271	-.407	-1.000**	.666	-1.000**	.259
F-L7	.333	1	-.897	.554	-.098	.662	-.185	.325	-.333	-.870	.765	-.911	-.522	-.522	-.207	-.379	-.556	-.422	.887	.035	-1.000**	-.424	-1.000**	-.276
L-S7	-.637	-.897	1	-.368	.149	-.862	-.103	-.540	-.081	.759	-.402	.736	.843	.248	.550	.639	.766	-.016	-.906	-.104	1.000**	-.003	1.000**	.344
F-S7	.391	.554	-.368	1	-.781	.531	-.882	.607	-.738	-.879	.634	-.846	-.108	-.974*	.537	-.503	.313	-.366	.116	-.796	-1.000**	-.286	-1.000**	.640
L-L8	-.683	-.098	.149	-.781	1	-.082	.887*	-.452	.360	.498	.153	.280	.549	-.528	.436	.552	.376	-.247	.252	.945	1.000**	-.337	1.000**	-.865
F-L8	.927	.662	-.862	.531	-.082	1	.074	.880*	.565	-.092	.209	.576	.397	.153	-.346	-.194	.393	.335	.471	-.310	-1.000**	.361	-1.000**	.096
L-S8	-.022	-.185	-.103	-.882	.887*	.074	1	-.276	.328	.386	.014	.305	.433	-.558	.340	.193	.321	.493	.263	.853	1.000**	.406	1.000**	-.803
F-S8	.958*	.325	-.540	.607	-.452	.880*	-.276	1	.482	-.178	.090	.454	.273	.251	-.391	-.447	.325	.491	.155	-.645	-1.000**	.549	-1.000**	.499
L-L9	.333	-.333	-.081	-.738	.360	.565	.328	.482	1	.433	.551	.425	.858**	-.028	.328	.216	.778	.843	.093	.510	a	.787	a	-.456
F-L9	-.522	-.870	.759	-.879	.498	-.092	.386	-.178	.433	1	-.166	.565	.474	.066	.128	.603	.404	.330	-.496	.460	1.000**	.284	1.000**	-.229
L-S9	-.222	.765	-.402	.634	.153	.209	.014	.090	.551	-.166	1	-.371	.401	.089	.565	.294	.679	-.892	.366	-.105	-1.000**	-.875	-1.000**	-.046
F-S9	-.366	-.911	.736	-.846	.280	.576	.305	.454	.425	.565	-.371	1	.547	-.115	-.320	.198	.571	.487	-.458	.361	1.000**	.450	1.000**	-.134
L-L10	-.870	-.522	.843	-.108	.549	.397	.433	.273	.858**	.474	.401	.547	1	-.430	.511	.378	.919*	-.550	-.061	-.072	1.000**	-.537	1.000**	.247
F-L10	-.174	-.522	.248	-.974*	-.528	.153	-.558	.251	-.028	.066	.089	-.115	-.430	1	-.556	.072	-.740	.550	-.107	.745	1.000**	.474	1.000**	-.612
L-S10	-.409	-.207	.550	.537	.436	-.346	.340	-.391	.328	.128	.565	-.320	.511	-.556	1	.294	.973**	-.560	-.269	-.662	1.000**	-.490	1.000**	.729
F-S10	-.992**	-.379	.639	-.503	.552	-.194	.193	-.447	.216	.603	.294	.198	.378	.072	.294	1	.349	-.549	-.229	.498	1.000**	-.592	1.000**	-.339
L-L11	-.333	-.556	.766	.313	.376	.393	.321	.325	.778	.404	.679	.571	.919*	-.740	.973**	.349	1	-.211	-.381	-.681	1.000**	-.141	1.000**	.818
F-L11	.632	-.422	-.016	-.366	-.247	.335	.493	.491	.843	.330	-.892	.487	-.550	.550	-.560	-.549	-.211	1	-.217	-.032	-1.000**	.995**	-1.000**	.078
L-S11	.271	.887	-.906	.116	.252	.471	.263	.155	.093	-.496	.366	-.458	-.061	-.107	-.269	-.229	-.381	-.217	1	.455	-1.000**	-.261	-1.000**	-.663
F-S11	-.407	.035	-.104	-.796	.945	-.310	.853	-.645	.510	.460	-.105	.361	-.072	.745	-.662	.498	-.681	-.032	.455	1	1.000**	-.130	1.000**	-.968*
L-L12	-1.000**	-1.000**	1.000**	-1.000**	1.000**	-1.000**	1.000**	-1.000**	a	1.000**	-1.000**	1.000**	1.000**	1.000**	1.000**	1.000**	1.000**	-1.000**	-1.000**	1.000**	1	-1.000**	1.000**	1.000**
F-L12	.666	-.424	-.003	-.286	-.337	.361	.406	.549	.787	.284	-.875	.450	-.537	.474	-.490	-.592	-.141	.995**	-.261	-.130	-1.000**	1	-1.000**	.173
L-S12	-1.000**	-1.000**	1.000**	-1.000**	1.000**	-1.000**	1.000**	-1.000**	a	1.000**	-1.000**	1.000**	1.000**	1.000**	1.000**	1.000**	1.000**	-1.000**	-1.000**	1.000**	1.000**	-1.000**	1	1.000**
F-S12	.259	-.276	.344	.640	-.865	.096	-.803	.499	-.456	-.229	-.046	-.134	.247	-.612	.729	-.339	.818	.078	-.663	-.968*	1.000**	.173	1.000**	1

*. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).a. Cannot be computed because at least one of the variables is constant.

Table 2. *Correlations between leaf number and stem thickness in 5 weeks high temperature treatment group*

Correlations between Leaves Number and Stem Thinkness in 5 weeks High Temperature Treatment Group

	L-L7	F-L7	L-S7	F-S7	L-L8	F-L8	L-S8	F-S8	L-L9	F-L9	L-S9	F-S9	L-L10	F-L10	L-S10	F-S10	L-L11	F-L11	L-S11	F-S11	L-L12	F-L12	L-S12	F-S12
L-L7	1	.000	.406	-.958*	.577	.317	.822	.375	-.778	-.728	-.834	-.829	.293	-.962*	.693	-.874	-.917	.878	.782	-.997**	.440	-.577	-.961*	-.060
F-L7	.000	1	-.798	-.035	.000	-.621	.351	-.730	.000	.000	.236	-.426	-.956*	.000	-.597	.452	-.346	.478	.155	.077	-.863	.000	-.250	.940
L-S7	.406	-.798	1	-.488	.599	.944	-.147	.989*	-.596	.011	-.749	-.158	.881	-.269	.938	-.794	-.007	-.025	-.075	-.478	.978*	-.599	-.138	-.924
F-S7	-.958*	-.035	-.488	1	-.786	-.486	-.671	-.496	.924	.501	.933	.912	-.247	.844	-.758	.872	.834	-.858	-.583	.960*	-.462	.786	.896	.120
L-L8	.577	.000	.599	-.786	1	.499	-.500	.090	.453	.543	.502	-.223	-.267	-.471	.360	-.555	.624	.507	.453	-.597	-.657	-1.000**	-.478	-.309
F-L8	.317	-.621	.944	-.486	.499	1	-.733*	.652	.178	-.029	.087	.113	-.121	-.042	.737*	-.280	.421	-.019	-.338	-.383	.106	-.768	-.055	-.844
L-S8	.822	.351	-.147	-.671	-.500	-.733*	1	-.105	-.666	-.138	-.390	-.457	.522	-.276	-.198	-.129	-.695	.889	.415	-.780	.360	-.108	-.238	.432
F-S8	.375	-.730	.989*	-.496	.090	.652	-.105	1	-.310	.014	-.086	-.120	.623	-.067	.877**	-.304	-.062	-.020	-.040	-.445	.565	-.683	.137	-.901
L-L9	-.778	.000	-.596	.924	.453	.178	-.666	-.310	1	.208	.842*	.164	-.503	.360	-.058	.368	.376	-.683	.118	.792	-.647	.962*	.100	.258
F-L9	-.728	.000	.011	.501	.543	-.029	-.138	.014	.208	1	.407	-.159	.054	-.049	-.091	-.199	.492	-.639	.469	.707	-.493	-.140	.037	-.188
L-S9	-.834	.236	-.749	.933	.502	.087	-.390	-.086	.842*	.407	1	-.273	-.221	.296	.169	.315	.093	-.620	.488	.863	-.644	.889	.059	.439
F-S9	-.829	-.426	-.158	.912	-.223	.113	-.457	-.120	.164	-.159	-.273	1	-.060	.190	-.337	.154	.566	-.932	-.607	.804	.100	.774	.575	-.230
L-L10	.293	-.956*	.881	-.247	-.267	-.121	.522	.623	-.503	.054	-.221	-.060	1	-.233	.392	-.300	-.280	-.200	.339	-.365	.579	-.169	.196	-.916
F-L10	-.962*	.000	-.269	.844	-.471	-.042	-.276	-.067	.360	-.049	.296	.190	-.233	1	-.212	.923*	-.223	-.845	-.477	.952*	.130	.333	.661	-.034
L-S10	.693	-.597	.938	-.758	.360	.737*	-.198	.877**	-.058	-.091	.169	-.337	.392	-.212	1	-.373	-.096	.324	.151	-.748	.305	-.730	-.178	-.738
F-S10	-.874	.452	-.794	.872	-.555	-.280	-.129	-.304	.368	-.199	.315	.154	-.300	.923*	-.373	1	-.363	-.552	-.377	.911	-.015	.648	.641	.536
L-L11	-.917	-.346	-.007	.834	.624	.421	-.695	-.062	.376	.492	.093	.566	-.280	-.223	-.096	-.363	1	-.971*	-.125	.882	-.452	.367	.052	-.337
F-L11	.878	.478	-.025	-.858	.507	-.019	.889	-.020	-.683	-.639	-.620	-.932	-.200	-.845	.324	-.552	-.971*	1	.761	-.839	-.026	-.507	-.964*	.397
L-S11	.782	.155	-.075	-.583	.453	-.338	.415	-.040	.118	.469	.488	-.607	.339	-.477	.151	-.377	-.125	.761	1	-.752	-.421	.041	-.425	.302
F-S11	-.997**	.077	-.478	.960*	-.597	-.383	-.780	-.445	.792	.707	.863	.804	-.365	.952*	-.748	.911	.882	-.839	-.752	1	-.512	.597	.936	.140
L-L12	.440	-.863	.978*	-.462	-.657	.106	.360	.565	-.647	-.493	-.644	.100	.579	.130	.305	-.015	-.452	-.026	-.421	-.512	1	-.457	.161	-.921
F-L12	-.577	.000	-.599	.786	-1.000**	-.768	-.108	-.683	.962*	-.140	.889	.774	-.169	.333	-.730	.648	.367	-.507	.041	.597	-.457	1	.459	.309
L-S12	-.961*	-.250	-.138	.896	-.478	-.055	-.238	.137	.100	.037	.059	.575	.196	.661	-.178	.641	.052	-.964*	-.425	.936	.161	.459	1	-.217
F-S12	-.060	.940	-.924	.120	-.309	-.844	.432	-.901	.258	-.188	.439	-.230	-.916	-.034	-.738	.536	-.337	.397	.302	.140	-.921	.309	-.217	1

*. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).

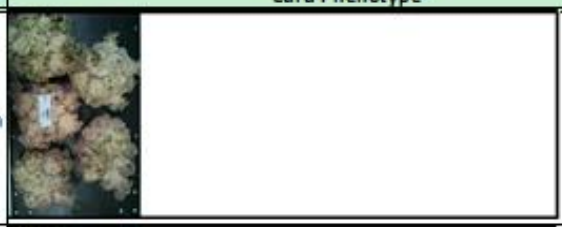
Table 3. *Correlations between leaf number and stem thickness in 6 weeks high temperature treatment group*

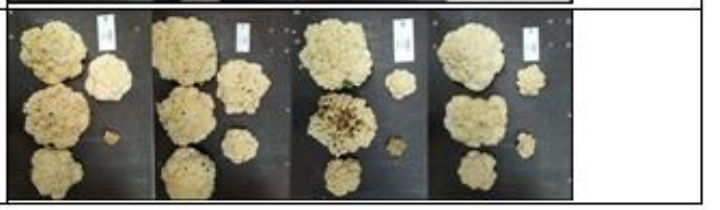
Correlations between Leaves Number and Stem Thinknees in 6 weeks High Temperature Treatment Group																							
	L-L7	F-L7	L-S7	F-S7	L-L8	F-L8	L-S8	F-S8	L-L9	F-L9	L-S9	F-S9	L-L10	F-L10	L-S10	F-S10	L-L11	F-L11	L-S11	F-S11	L-L12	F-L12	L-S12	F-S12
L-L7	1	-.522	-.987*	-.757	-.853	-.870	-.695	-.998**	.522	.346	-.639	-.836	.322	.754	-.150	-.677	-.426	-.870	.711	.565	.501	.455	.626	.286
F-L7	-.522	1	.433	.866	.816	.333	.943	.553	-1.000**	-.132	-.318	-.015	.309	-.556	.889	.612	.000	.778	-.959*	-.371	-.757	.522	-.303	-.961*
L-S7	-.987*	.433	1	.739	.837	.812	.650	.974*	-.433	-.226	.695	.857	-.274	-.645	.091	.552	.558	.782	-.616	-.444	-.355	-.535	-.520	-.175
F-S7	-.757	.866	.739	1	.987*	.416	.983*	.757	-.866	.096	.034	.289	.331	-.457	.734	.446	.481	.759	-.869	-.191	-.476	.147	-.187	-.701
L-L8	-.853	.816	.837	.987*	1	.825*	.669	.455	.687	.506	-.441	.684	.329	.252	.474	.476	.282	.816	-.395	-.284	-.121	.000	-.634	-.625
F-L8	-.870	.333	.812	.416	.825*	1	.333	.582	.790*	.269	-.071	.769*	.130	.019	.083	.543	.289	.852	-.347	-.866	.028	-.522	-.564	-.168
L-S8	-.695	.943	.650	.983*	.669	.333	1	.436	.036	.282	-.328	.403	.138	.096	.652	.575	.327	.789	-.803*	-.262	-.573	.290	-.346	-.819
F-S8	-.998**	.553	.974*	.757	.455	.582	.436	1	.031	-.336	.479	.303	-.143	-.405	-.158	.184	-.009	.901	-.389	-.614	-.112	-.420	-.320	-.328
L-L9	.522	-1.000**	-.433	-.866	.687	.790*	.036	.031	1	.659	-.300	.711*	.305	.444	.073	.354	.458	-.778	-.025	.371	.361	-.522	-.488	.961*
F-L9	.346	-.132	-.226	.096	.506	.269	.282	-.336	.659	1	-.540	.508	.574	.600	.483	.257	.682	-.574	-.165	.959*	.310	.208	-.084	.153
L-S9	-.639	-.318	.695	.034	-.441	-.071	-.328	.479	-.300	-.540	1	-.145	-.441	-.323	-.812*	-.257	.069	.292	.047	-.360	.323	-.972*	.160	.535
F-S9	-.836	-.015	.857	.289	.684	.769*	.403	.303	.711*	.508	-.145	1	-.113	.331	.107	.765*	.688	.573	-.454	-.547	-.111	-.851	-.508	.248
L-L10	.322	.309	-.274	.331	.329	.130	.138	-.143	.305	.574	-.441	-.113	1	-.123	.617	-.203	.006	-.309	-.003	.759	.418	.645	.376	-.352
F-L10	.754	-.556	-.645	-.457	.252	.019	.096	-.405	.444	.600	-.323	.331	-.123	1	-.033	.095	.521	-.926	.108	.959*	.125	.174	-.572	.468
L-S10	-.150	.889	.091	.734	.474	.083	.652	-.158	.073	.483	-.812*	.107	.617	-.033	1	.356	.027	.405	-.454	.055	-.415	.778	.110	-.915
F-S10	-.677	.612	.552	.446	.476	.543	.575	.184	.354	.257	-.257	.765*	-.203	.095	.356	1	.531	.915	-.797*	-.960*	-.604	-.038	-.338	-.559
L-L11	-.426	.000	.558	.481	.282	.289	.327	-.009	.458	.682	.069	.688	.006	.521	.027	.531	1	.000	-.508	.492	.151	-.426	-.143	.226
F-L11	-.870	.778	.782	.759	.816	.852	.789	.901	-.778	-.574	.292	.573	-.309	-.926	.405	.915	.000	1	-.924	-.783	-.858	-.058	-.779	-.646
L-S11	.711	-.959*	-.616	-.869	-.395	-.347	-.803*	-.389	-.025	-.165	.047	-.454	-.003	.108	-.454	-.797*	-.508	-.924	1	.580	.563	-.291	.105	.875
F-S11	.565	-.371	-.444	-.191	-.284	-.866	-.262	-.614	.371	.959*	-.360	-.547	.759	.959*	.055	-.960*	.492	-.783	.580	1	.861	.178	.990**	.341
L-L12	.501	-.757	-.355	-.476	-.121	.028	-.573	-.112	.361	.310	.323	-.111	.418	.125	-.415	-.604	.151	-.858	.563	.861	1	-.290	.248	.769
F-L12	.455	.522	-.535	.147	.000	-.522	.290	-.420	-.522	.208	-.972*	-.851	.645	.174	.778	-.038	-.426	-.058	-.291	.178	-.290	1	.310	-.717
L-S12	.626	-.303	-.520	-.187	-.634	-.564	-.346	-.320	-.488	-.084	.160	-.508	.376	-.572	.110	-.338	-.143	-.779	.105	.990**	.248	.310	1	.242
F-S12	.286	-.961*	-.175	-.701	-.625	-.168	-.819	-.328	.961*	.153	.535	.248	-.352	.468	-.915	-.559	.226	-.646	.875	.341	.769	-.717	.242	1

*. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 8: Curd harvest stages in the field

Block	Harvest Time	Curd Old(Weeks)	Cultivar	Curd Phenotype
1	26-7-2012	15	Lindurian	
			Fremont	
2	2-8-2012	15	Lindurian	
			Fremont	
3	9-8-2012	15	Lindurian	
			Fremont	
4	9-8-2012	14	Lindurian	
			Fremont	

Block	Harvest Time	Curd Old(Weeks)	Cultivar	Curd Phenotype
4	16-8-2012	15	Undurian	
			Fremont	
5	9-8-2012	13	Undurian	
			Fremont	
6	16-8-2012	13	Undurian	
			Fremont	
7	23-8-2012	13	Undurian	
			Fremont	

Block	Harvest Time	Curd Old(Weeks)	Cultivar	Curd Phenotype
8	30-8-2012	13	Lindurian	
			Fremont	
9	6-9-2012	13	Lindurian	
			Fremont	

Curd Measured in the Field

Block 1-15 Weeks

Block	Curd Old (weeks)	Date	Hybrid	Field No.	Weight (kg)	Diameter (mm)
Block1	15	26-7-2012	Lindurian	0021	1.788	212.82
Block1	15	26-7-2012	Lindurian	0022	2.506	244.91
Block1	15	26-7-2012	Lindurian	0023	2.345	231.51
Block1	15	26-7-2012	Lindurian	0024	2.399	265.93
Block1	15	26-7-2012	Lindurian	0031	1.708	205.99
Block1	15	26-7-2012	Lindurian	0032	2.132	244.49
Block1	15	26-7-2012	Lindurian	0033	1.932	229.28
Block1	15	26-7-2012	Lindurian	0034	1.217	197.3
Block1	15	26-7-2012	Lindurian	0035	2.041	224.54
Block1	15	26-7-2012	Lindurian	0051	1.959	257.35
Block1	15	26-7-2012	Lindurian	0052	2.47	245.74
Block1	15	26-7-2012	Lindurian	0053	2.252	243.85
Block1	15	26-7-2012	Lindurian	0054	0.725	203.13
Block1	15	26-7-2012	Lindurian	0055	2.129	228.51
Block1	15	26-7-2012	Fremont	0026	2.923	251.94
Block1	15	26-7-2012	Fremont	0027	3.623	269.27
Block1	15	26-7-2012	Fremont	0028	2.311	233.96
Block1	15	26-7-2012	Fremont	0029	2.675	248.93
Block1	15	26-7-2012	Fremont	0030	3.73	301.64
Block1	15	26-7-2012	Fremont	0036	3.245	277.71
Block1	15	26-7-2012	Fremont	0037	3.069	264.32
Block1	15	26-7-2012	Fremont	0038	2.519	236.77
Block1	15	26-7-2012	Fremont	0039	2.926	239.45
Block1	15	26-7-2012	Fremont	0040	2.513	262.91
Block1	15	26-7-2012	Fremont	0041	1.416	218.1
Block1	15	26-7-2012	Fremont	0042	1.977	247.09
Block1	15	26-7-2012	Fremont	0043	1.936	222.75
Block1	15	26-7-2012	Fremont	0044	1.847	215.45
Block1	15	26-7-2012	Fremont	0045	1.93	263.04
Block1	15	26-7-2012	Fremont	0046	2.487	229.82
Block1	15	26-7-2012	Fremont	0047	3.012	274
Block1	15	26-7-2012	Fremont	0048	2.199	239.99
Block1	15	26-7-2012	Fremont	0049	1.592	231.54
Block1	15	26-7-2012	Fremont	0050	2.892	255.44
Block1	15	26-7-2012	Fremont	0056	3.151	283.01
Block1	15	26-7-2012	Fremont	0057	1.697	217.07
Block1	15	26-7-2012	Fremont	0058	3.006	256.94
Block1	15	26-7-2012	Fremont	0059	0.923	207.4
Block1	15	26-7-2012	Fremont	0060	3.226	274.05

Average			Average		Weight (kg)	Diameter (mm)
Lindurian	15	26-7-2012	Lindurian		1.972	231.10
Fremont	15	26-7-2012	Fremont		2.513	248.90

Block 2-15 Weeks						
Block	**Curd Old (weeks)**	**Date**	**Hybrid**	**Field No.**	**Weight (kg)**	**Diameter (mm)**
Block2	15	2-8-2012	Lindurian	0111	3.637	284.21
Block2	15	2-8-2012	Lindurian	0112	2.685	275.82
Block2	15	2-8-2012	Lindurian	0113	3.475	269
Block2	15	2-8-2012	Lindurian	0114	2.365	259.35
Block2	15	2-8-2012	Lindurian	0115	2.061	248.86
Block2	15	2-8-2012	Lindurian	0121	1.859	220.9
Block2	15	2-8-2012	Lindurian	0122	2.04	247.83
Block2	15	2-8-2012	Lindurian	0123	2.643	253.25
Block2	15	2-8-2012	Lindurian	0124	0.945	185.39
Block2	15	2-8-2012	Lindurian	0125	2.5	248.49
Block2	15	2-8-2012	Lindurian	0141	2.382	236.37
Block2	15	2-8-2012	Lindurian	0142	1.934	233.28
Block2	15	2-8-2012	Lindurian	0143	1.972	240.18
Block2	15	2-8-2012	Lindurian	0144	2.614	262.71
Block2	15	2-8-2012	Lindurian	0145	3.063	300.31
Block2	15	2-8-2012	Lindurian	0151	2.568	261.41
Block2	15	2-8-2012	Lindurian	0152	1.907	210.9
Block2	15	2-8-2012	Lindurian	0153	0.806	172.42
Block2	15	2-8-2012	Lindurian	0154	1.198	215.05
Block2	15	2-8-2012	Lindurian	0155	0.748	215.73
Block2	15	2-8-2012	Fremont	0106	4.498	307.55
Block2	15	2-8-2012	Fremont	0107	3.135	307.55
Block2	15	2-8-2012	Fremont	0108	4.04	301.12
Block2	15	2-8-2012	Fremont	0109	3.787	291.34
Block2	15	2-8-2012	Fremont	0110	4.135	307.55
Block2	15	2-8-2012	Fremont	0116	3.803	301.8
Block2	15	2-8-2012	Fremont	0117	2.683	259.63
Block2	15	2-8-2012	Fremont	0118	3.395	297.35
Block2	15	2-8-2012	Fremont	0119	3.036	293.31
Block2	15	2-8-2012	Fremont	0120	1.673	286.13
Block2	15	2-8-2012	Fremont	0126	3.461	264.78
Block2	15	2-8-2012	Fremont	0127	2.599	258.73
Block2	15	2-8-2012	Fremont	0128	4.07	307
Block2	15	2-8-2012	Fremont	0129	0.515	218.64
Block2	15	2-8-2012	Fremont	0130	2.854	260.41
Block2	15	2-8-2012	Fremont	0136	3.08	269.33
Block2	15	2-8-2012	Fremont	0137	3.499	307.55
Block2	15	2-8-2012	Fremont	0138	3.824	301.95
Block2	15	2-8-2012	Fremont	0139	3.731	307.05
Block2	15	2-8-2012	Fremont	0140	2.791	277.67

Average			**Average**		**Weight (kg)**	**Diameter (mm)**
Lindurian	15	2-8-2012	Lindurian		2.170	242.07
Fremont	15	2-8-2012	Fremont		3.230	286.32

Block 3-15 Weeks						
Block	**Curd Old (weeks)**	**Date**	**Hybrid**	**Field No.**	**Weight (kg)**	**Diameter (mm)**
Block3	15	9-8-2012	Lindurian	0201	3.222	274.40
Block3	15	9-8-2012	Lindurian	0202	2.331	285.48
Block3	15	9-8-2012	Lindurian	0203	2.826	269.70
Block3	15	9-8-2012	Lindurian	0204	2.558	261.15
Block3	15	9-8-2012	Lindurian	0205	3.525	289.28
Block3	15	9-8-2012	Lindurian	0221	2.784	273.52
Block3	15	9-8-2012	Lindurian	0222	3.487	284.10
Block3	15	9-8-2012	Lindurian	0223	2.839	269.36
Block3	15	9-8-2012	Lindurian	0224	2.229	267.59
Block3	15	9-8-2012	Lindurian	0225	2.846	280.46
Block3	15	9-8-2012	Lindurian	0241	2.177	295.19
Block3	15	9-8-2012	Lindurian	0242	3.296	306.21
Block3	15	9-8-2012	Lindurian	0243	2.181	278.69
Block3	15	9-8-2012	Lindurian	0244	2.486	278.23
Block3	15	9-8-2012	Lindurian	0245	2.997	283.47
Block3	15	9-8-2012	Fremont	0216	3.898	305.78
Block3	15	9-8-2012	Fremont	0217	3.638	307.00
Block3	15	9-8-2012	Fremont	0218	3.552	298.54
Block3	15	9-8-2012	Fremont	0219	4.164	302.03
Block3	15	9-8-2012	Fremont	0220	4.500	285.45
Block3	15	9-8-2012	Fremont	0226	4.145	288.49
Block3	15	9-8-2012	Fremont	0227	2.982	279.12
Block3	15	9-8-2012	Fremont	0228	2.762	307.04
Block3	15	9-8-2012	Fremont	0229	3.841	294.84
Block3	15	9-8-2012	Fremont	0230	4.500	292.95
Block3	15	9-8-2012	Fremont	0236	1.751	285.21
Block3	15	9-8-2012	Fremont	0237	3.759	304.50
Block3	15	9-8-2012	Fremont	0238	3.455	307.00
Block3	15	9-8-2012	Fremont	0239	4.235	307.00
Block3	15	9-8-2012	Fremont	0240	3.969	307.00
Block3	15	9-8-2012	Fremont	0246	3.921	296.19
Block3	15	9-8-2012	Fremont	0247	4.280	307.04
Block3	15	9-8-2012	Fremont	0248	3.665	300.34
Block3	15	9-8-2012	Fremont	0249	2.369	288.53
Block3	15	9-8-2012	Fremont	0250	2.334	274.90
Block3	15	9-8-2012	Fremont	0256	3.021	299.28
Block3	15	9-8-2012	Fremont	0257	2.779	297.56
Block3	15	9-8-2012	Fremont	0258	4.057	307.03
Block3	15	9-8-2012	Fremont	0259	4.500	307.03
Block3	15	9-8-2012	Fremont	0260	3.383	293.11

Average			**Average**		**Weight (kg)**	**Diameter (mm)**
Lindurian	15	9-8-2012	Lindurian		2.786	279.79
Fremont	15	9-8-2012	Fremont		3.578	297.72

Block 4-15 Weeks						
Block	**Curd Old (weeks)**	**Date**	**Hybrid**	**Field No.**	**Weight (kg)**	**Diameter (mm)**
Block4	15	9-8-2012	Lindurian	0301	2.291	239.52
Block4	15	9-8-2012	Lindurian	0302	2.090	229.97
Block4	15	9-8-2012	Lindurian	0303	1.726	228.21
Block4	15	9-8-2012	Lindurian	0304	2.746	257.48
Block4	15	9-8-2012	Lindurian	0305	1.973	223.68
Block4	15	9-8-2012	Lindurian	0311	1.541	214.34
Block4	15	9-8-2012	Lindurian	0312	1.865	212.43
Block4	15	9-8-2012	Lindurian	0313	1.905	223.13
Block4	15	9-8-2012	Lindurian	0314	0.697	181.24
Block4	15	9-8-2012	Lindurian	0315	2.475	263.08
Block4	15	9-8-2012	Lindurian	0321	2.341	246.31
Block4	15	9-8-2012	Lindurian	0322	2.146	228.31
Block4	15	9-8-2012	Lindurian	0323	1.716	204.96
Block4	15	9-8-2012	Lindurian	0324	1.422	196.39
Block4	15	9-8-2012	Lindurian	0325	0.668	206.86
Block4	15	9-8-2012	Lindurian	0341	2.308	243.47
Block4	15	9-8-2012	Lindurian	0342	1.664	216.19
Block4	15	9-8-2012	Lindurian	0343	1.643	250.86
Block4	15	9-8-2012	Lindurian	0344	0.701	166.05
Block4	15	9-8-2012	Lindurian	0345	0.248	106.48
Block4	15	9-8-2012	Fremont	0306	3.615	307.00
Block4	15	9-8-2012	Fremont	0307	2.658	240.05
Block4	15	9-8-2012	Fremont	0308	2.856	271.63
Block4	15	9-8-2012	Fremont	0309	2.244	267.99
Block4	15	9-8-2012	Fremont	0310	1.449	251.44
Block4	15	9-8-2012	Fremont	0316	2.888	279.62
Block4	15	9-8-2012	Fremont	0317	2.917	275.28
Block4	15	9-8-2012	Fremont	0318	2.291	228.91
Block4	15	9-8-2012	Fremont	0319	3.103	277.92
Block4	15	9-8-2012	Fremont	0320	1.711	212.78
Block4	15	9-8-2012	Fremont	0336	3.499	270.99
Block4	15	9-8-2012	Fremont	0337	2.902	291.25
Block4	15	9-8-2012	Fremont	0338	3.309	299.32
Block4	15	9-8-2012	Fremont	0339	1.018	204.55
Block4	15	9-8-2012	Fremont	0340	2.879	258.65
Block4	15	9-8-2012	Fremont	0346	1.406	273.93
Block4	15	9-8-2012	Fremont	0347	1.825	245.78
Block4	15	9-8-2012	Fremont	0348	3.376	307.07
Block4	15	9-8-2012	Fremont	0349	1.517	190.31
Block4	15	9-8-2012	Fremont	0350	2.979	300.11

Average			**Average**		**Weight (kg)**	**Diameter (mm)**
Lindurian	15	9-8-2012	Lindurian		1.708	216.95
Fremont	15	9-8-2012	Fremont		2.522	262.73

Block 4-14 Weeks						
Block	**Curd Old (weeks)**	**Date**	**Hybrid**	**Field No.**	**Weight (kg)**	**Diameter (mm)**
Block 4	14	16-8-2012	Lindurian	0331	2.646	239.27
Block 4	14	16-8-2012	Lindurian	0332	3.232	253.45
Block 4	14	16-8-2012	Lindurian	0333	3.538	274.75
Block 4	14	16-8-2012	Lindurian	0334	2.857	259.52
Block 4	14	16-8-2012	Lindurian	0335	2.888	246.8
Block 4	14	16-8-2012	Fremont	0326	3.971	307.04
Block 4	14	16-8-2012	Fremont	0327	4.472	307.04
Block 4	14	16-8-2012	Fremont	0328	2.942	294.55
Block 4	14	16-8-2012	Fremont	0329	3.364	292.53
Block 4	14	16-8-2012	Fremont	0330	3.11	306.97
Average			**Average**		**Weight (Kg)**	**Diameter (mm)**
Lindurian	14	16-8-2012	Lindurian		3.0322	254.758
Fremont	14	16-8-2012	Fremont		3.5718	301.626

Block 5-13 Weeks						
Block	**Curd Old (weeks)**	**Date**	**Hybrid**	**Field No.**	**Weight (kg)**	**Diameter (mm)**
Block5	13	9-8-2012	Lindurian	0411	1.113	185.75
Block5	13	9-8-2012	Lindurian	0412	0.853	184.24
Block5	13	9-8-2012	Lindurian	0413	0.769	164.95
Block5	13	9-8-2012	Lindurian	0414	0.417	124.23
Block5	13	9-8-2012	Lindurian	0415	0.022	45.91
Block5	13	9-8-2012	Lindurian	0421	0.393	136.84
Block5	13	9-8-2012	Lindurian	0422	0.339	128.22
Block5	13	9-8-2012	Lindurian	0423	0.262	113.67
Block5	13	9-8-2012	Lindurian	0424	0.207	96.34
Block5	13	9-8-2012	Lindurian	0425	0.008	23.64
Block5	13	9-8-2012	Lindurian	0431	1.007	181.86
Block5	13	9-8-2012	Lindurian	0432	0.825	166.04
Block5	13	9-8-2012	Lindurian	0433	0.423	133.2
Block5	13	9-8-2012	Lindurian	0434	0.164	99.02
Block5	13	9-8-2012	Lindurian	0435	0.015	48.52
Block5	13	9-8-2012	Lindurian	0441	0.815	161.07
Block5	13	9-8-2012	Lindurian	0442	0.7	159.56
Block5	13	9-8-2012	Lindurian	0443	0.109	78.56
Block5	13	9-8-2012	Lindurian	0444	0.03	51.96
Block5	13	9-8-2012	Lindurian	0445	0.014	33.72
Block5	13	9-8-2012	Fremont	0416	1.489	208.54
Block5	13	9-8-2012	Fremont	0417	0.453	144.45
Block5	13	9-8-2012	Fremont	0418	1.334	197.81
Block5	13	9-8-2012	Fremont	0419	1.222	180.29
Block5	13	9-8-2012	Fremont	0420	0.809	168.94
Block5	13	9-8-2012	Fremont	0426	1.244	187.91
Block5	13	9-8-2012	Fremont	0427	1.638	204.22
Block5	13	9-8-2012	Fremont	0428	1.486	186.32
Block5	13	9-8-2012	Fremont	0429	1.149	193.92
Block5	13	9-8-2012	Fremont	0430	0.943	162.33
Block5	13	9-8-2012	Fremont	0436	1.598	203.7
Block5	13	9-8-2012	Fremont	0437	1.655	259.2
Block5	13	9-8-2012	Fremont	0438	1.196	180.42
Block5	13	9-8-2012	Fremont	0439	1.126	197.73
Block5	13	9-8-2012	Fremont	0440	1.011	190.37
Block5	13	9-8-2012	Fremont	0446	1.295	195.05
Block5	13	9-8-2012	Fremont	0447	1.366	207.6
Block5	13	9-8-2012	Fremont	0448	1.244	192.65
Block5	13	9-8-2012	Fremont	0449	1.363	190.04
Block5	13	9-8-2012	Fremont	0450	0.44	132.92
Average			**Average**		**Weight (kg)**	**Diameter (mm)**
Lindurian	13	9-8-2012	Lindurian		0.424	115.87
Fremont	13	9-8-2012	Fremont		1.203	189.22

Block 6-13 Weeks						
Block	**Curd Old (weeks)**	**Date**	**Hybrid**	**Field No.**	**Weight (kg)**	**Diameter (mm)**
Block 6	13	16-8-2012	Lindurian	0501	0.828	183.79
Block 6	13	16-8-2012	Lindurian	0502	1.498	213.4
Block 6	13	16-8-2012	Lindurian	0503	0.871	181.37
Block 6	13	16-8-2012	Lindurian	0504	0.063	58.3
Block 6	13	16-8-2012	Lindurian	0511	0.725	152.78
Block 6	13	16-8-2012	Lindurian	0512	0.332	120.58
Block 6	13	16-8-2012	Lindurian	0513	0.15	95.09
Block 6	13	16-8-2012	Lindurian	0514	0.232	116
Block 6	13	16-8-2012	Lindurian	0515	0.084	74.58
Block 6	13	16-8-2012	Lindurian	0521	0.829	183.5
Block 6	13	16-8-2012	Lindurian	0522	0.994	191.27
Block 6	13	16-8-2012	Lindurian	0523	0.938	188.98
Block 6	13	16-8-2012	Lindurian	0524	0.297	117.77
Block 6	13	16-8-2012	Lindurian	0525	0.297	121.67
Block 6	13	16-8-2012	Lindurian	0531	1.089	195.23
Block 6	13	16-8-2012	Lindurian	0532	1.182	184.7
Block 6	13	16-8-2012	Lindurian	0533	0.873	175.8
Block 6	13	16-8-2012	Lindurian	0534	0.159	88.05
Block 6	13	16-8-2012	Lindurian	0535	0.144	90.07
Block 6	13	16-8-2012	Fremont	0496	2.228	247.35
Block 6	13	16-8-2012	Fremont	0497	1.196	192.25
Block 6	13	16-8-2012	Fremont	0498	1.873	229.62
Block 6	13	16-8-2012	Fremont	0499	1.426	212.78
Block 6	13	16-8-2012	Fremont	0500	1.634	213.9
Block 6	13	16-8-2012	Fremont	0516	1.76	223.31
Block 6	13	16-8-2012	Fremont	0517	2.123	227.13
Block 6	13	16-8-2012	Fremont	0518	1.056	171.38
Block 6	13	16-8-2012	Fremont	0519	1.381	199.13
Block 6	13	16-8-2012	Fremont	0526	1.951	227.44
Block 6	13	16-8-2012	Fremont	0527	1.894	214.54
Block 6	13	16-8-2012	Fremont	0528	2.07	226.84
Block 6	13	16-8-2012	Fremont	0529	1.508	202.09
Block 6	13	16-8-2012	Fremont	0530	0.732	161.53
Block 6	13	16-8-2012	Fremont	0536	2.541	242.26
Block 6	13	16-8-2012	Fremont	0537	1.959	218.88
Block 6	13	16-8-2012	Fremont	0538	2.248	241.4
Block 6	13	16-8-2012	Fremont	0539	2.212	234.37
Block 6	13	16-8-2012	Fremont	0540	2.085	243.15
Average			**Average**		**Weight (Kg)**	**Diameter (mm)**
Lindurian	13	16-8-2012	Lindurian		0.609736842	143.8384211
Fremont	13	16-8-2012	Fremont		1.783	217.3342105

Block 7-13 Weeks						
Block	**Curd Old (weeks)**	**Date**	**Hybrid**	**Field No.**	**Weight (kg)**	**Diameter (mm)**
Block 7	13	23-8-2012	Lindurian	0591	0.71	165.74
Block 7	13	23-8-2012	Lindurian	0592	0.635	156.98
Block 7	13	23-8-2012	Lindurian	0593	0.339	121.69
Block 7	13	23-8-2012	Lindurian	0594	0.105	87.35
Block 7	13	23-8-2012	Lindurian	0595	0.01	33.33
Block 7	13	23-8-2012	Lindurian	0601	0.287	114.46
Block 7	13	23-8-2012	Lindurian	0602	0.584	146.11
Block 7	13	23-8-2012	Lindurian	0603	0.374	131.17
Block 7	13	23-8-2012	Lindurian	0604	0.345	124.89
Block 7	13	23-8-2012	Lindurian	0605	0.091	75.47
Block 7	13	23-8-2012	Lindurian	0611	0.203	101.93
Block 7	13	23-8-2012	Lindurian	0612	0.286	116.17
Block 7	13	23-8-2012	Lindurian	0613	0.538	142.52
Block 7	13	23-8-2012	Lindurian	0614	0.463	130.14
Block 7	13	23-8-2012	Lindurian	0615	0.365	127.13
Block 7	13	23-8-2012	Lindurian	0621	0.562	140.45
Block 7	13	23-8-2012	Lindurian	0622	0.457	128.52
Block 7	13	23-8-2012	Lindurian	0623	0.471	135.21
Block 7	13	23-8-2012	Lindurian	0624	0.529	133.91
Block 7	13	23-8-2012	Lindurian	0625	0.026	41.62
Block 7	13	23-8-2012	Fremont	0596	1.033	181.89
Block 7	13	23-8-2012	Fremont	0597	0.82	156.9
Block 7	13	23-8-2012	Fremont	0598	0.694	153.5
Block 7	13	23-8-2012	Fremont	0599	0.573	150.64
Block 7	13	23-8-2012	Fremont	0600	0.47	130.19
Block 7	13	23-8-2012	Fremont	0606	1.272	202.52
Block 7	13	23-8-2012	Fremont	0607	1.697	211.6
Block 7	13	23-8-2012	Fremont	0608	1.533	197.43
Block 7	13	23-8-2012	Fremont	0609	0.929	175.21
Block 7	13	23-8-2012	Fremont	0610	0.612	147.05
Block 7	13	23-8-2012	Fremont	0616	0.823	167.03
Block 7	13	23-8-2012	Fremont	0617	1.024	190.11
Block 7	13	23-8-2012	Fremont	0618	0.559	142.36
Block 7	13	23-8-2012	Fremont	0619	0.69	148.46
Block 7	13	23-8-2012	Fremont	0620	0.816	161.55
Block 7	13	23-8-2012	Fremont	0626	1.251	191.56
Block 7	13	23-8-2012	Fremont	0627	0.963	183.69
Block 7	13	23-8-2012	Fremont	0628	0.581	148.04
Block 7	13	23-8-2012	Fremont	0629	0.51	143.62
Block 7	13	23-8-2012	Fremont	0630	0.103	91.47
Average			**Average**		**Weight (Kg)**	**Diameter (mm)**
Lindurian	13	23-8-2012	Lindurian		0.369	117.7395
Fremont	13	23-8-2012	Fremont		0.84765	163.741

Block 8-13 Weeks						
Block	**Curd Old (weeks)**	**Date**	**Hybrid**	**Field No.**	**Weight (kg)**	**Diameter (mm)**
Block 8	13	30-8-2012	Lindurian	0691	0.926	186.98
Block 8	13	30-8-2012	Lindurian	0692	0.699	178.82
Block 8	13	30-8-2012	Lindurian	0693	0.819	161.97
Block 8	13	30-8-2012	Lindurian	0694	0.352	140.9
Block 8	13	30-8-2012	Lindurian	0695	0.073	78.99
Block 8	13	30-8-2012	Lindurian	0701	0.663	166.64
Block 8	13	30-8-2012	Lindurian	0702	1.202	186.06
Block 8	13	30-8-2012	Lindurian	0703	0.536	153.03
Block 8	13	30-8-2012	Lindurian	0704	0.612	161.4
Block 8	13	30-8-2012	Lindurian	0705	0.481	149.18
Block 8	13	30-8-2012	Fremont	0686	1.322	194.18
Block 8	13	30-8-2012	Fremont	0687	0.286	130.74
Block 8	13	30-8-2012	Fremont	0688	0.237	136.53
Block 8	13	30-8-2012	Fremont	0689	0.295	125.93
Block 8	13	30-8-2012	Fremont	0690	0.124	101.44
Block 8	13	30-8-2012	Fremont	0706	1.379	218.18
Block 8	13	30-8-2012	Fremont	0707	1.401	208.56
Block 8	13	30-8-2012	Fremont	0708	1.485	196.24
Block 8	13	30-8-2012	Fremont	0709	0.107	86.56
Block 8	13	30-8-2012	Fremont	0716	1.972	220.43
Block 8	13	30-8-2012	Fremont	0717	1.547	213.28
Block 8	13	30-8-2012	Fremont	0718	0.725	161.24
Block 8	13	30-8-2012	Fremont	0719	0.553	132.53
Block 8	13	30-8-2012	Fremont	0720	0.05	75.47

Average			**Average**		**Weight (Kg)**	**Diameter (mm)**
Lindurian	13	30-8-2012	Lindurian		0.6363	156.397
Fremont	13	30-8-2012	Fremont		0.820214286	157.2364286

Block 9-13 Weeks						
Block	**Curd Old (weeks)**	**Date**	**Hybrid**	**Field No.**	**Weight (kg)**	**Diameter (mm)**
Block 9	13	6-9-2012	Lindurian	0761	0.854	187.02
Block 9	13	6-9-2012	Lindurian	0762	0.267	132.73
Block 9	13	6-9-2012	Lindurian	0763	0.085	84.23
Block 9	13	6-9-2012	Lindurian	0764	0.051	75.34
Block 9	13	6-9-2012	Lindurian	0791	1.741	227.19
Block 9	13	6-9-2012	Lindurian	0792	1.027	201.78
Block 9	13	6-9-2012	Lindurian	0793	1.019	205.87
Block 9	13	6-9-2012	Lindurian	0794	0.725	184.44
Block 9	13	6-9-2012	Lindurian	0795	0.078	83.79
Block 9	13	6-9-2012	Lindurian	0801	1.014	213.14
Block 9	13	6-9-2012	Lindurian	0802	0.335	130.99
Block 9	13	6-9-2012	Lindurian	0803	0.182	108.01
Block 9	13	6-9-2012	Lindurian	0804	0.115	87.36
Block 9	13	6-9-2012	Lindurian	0805	0.045	57.64
Block 9	13	6-9-2012	Fremont	0766	0.894	197.59
Block 9	13	6-9-2012	Fremont	0767	1.048	194.39
Block 9	13	6-9-2012	Fremont	0768	0.481	133.07
Block 9	13	6-9-2012	Fremont	0769	0.437	157.81
Block 9	13	6-9-2012	Fremont	0770	0.015	49.39
Block 9	13	6-9-2012	Fremont	0786	2.59	263.65
Block 9	13	6-9-2012	Fremont	0787	1.461	220.18
Block 9	13	6-9-2012	Fremont	0788	1.513	214.52
Block 9	13	6-9-2012	Fremont	0789	1.186	204.91
Block 9	13	6-9-2012	Fremont	0790	0.458	143.43
Block 9	13	6-9-2012	Fremont	0796	0.632	180.8
Block 9	13	6-9-2012	Fremont	0797	0.521	169.29
Block 9	13	6-9-2012	Fremont	0798	0.122	107.09
Block 9	13	6-9-2012	Fremont	0799	0.074	80.38
Block 9	13	6-9-2012	Fremont	0800	0.019	50.11
Block 9	13	6-9-2012	Fremont	0806	1.159	193.86
Block 9	13	6-9-2012	Fremont	0807	1.032	202.18
Block 9	13	6-9-2012	Fremont	0808	0.257	129.19
Block 9	13	6-9-2012	Fremont	0809	0.107	90.7
Block 9	13	6-9-2012	Fremont	0810	0.067	79.65
Average			**Average**		**Weight (Kg)**	**Diameter (mm)**
Lindurian	13	6-9-2012	Lindurian		0.538428571	141.395
Fremont	13	6-9-2012	Fremont		0.70365	153.1095

Appendix 9: Correlation analysis between curd weight & diameter in field

Remark: No correlation between curd weight and curd diameter

Has correlation between curd weight and curd diameter

Correlations between Curds Weight and Diameter

	L-W1	F-W1	L-D1	F-D1	L-W2	F-W2	L-D2	F-D2	L-W3	F-W3	L-D3	F-D3	L-W4	F-W4	L-D4	F-D4	L-W5	F-W5	L-D5	F-D5
L-W1	1	0.052	.780**	0.093	0.108	-0.219	0.064	-0.283	-0.053	0.022	0.163	-0.213	0.629	0.217	0.885	-0.547	0.345	-0.089	0.284	0.108
F-W1	0.052	1	-0.089	.849**	-0.016	0.216	0.025	0.425	0.345	0.22	-0.219	-0.209	0.282	0.446	-0.63	0.81	0.007	0.115	-0.14	0.027
L-D1	.780**	-0.089	1	0.067	-0.046	-0.294	-0.028	-0.238	-0.183	0.14	0.075	-0.171	0.22	0.149	0.487	-0.663	0.137	0.101	0.07	-0.017	0.214	0.182	0.1	0.266	-0.349	-0.192	-0.208	-0.096	-0.021	0.112	0.182	0.186	-0.257	0.056	-0.173	0.209
F-D1	.849**	0.067	1	-0.025	0.191	0.066	0.354	.614**	0.261	0.13	-0.237	0.345	0.337	0.07	-0.367	0.67	-0.186	-0.381	-0.219	-0.268	0.219	0.253	0.184	0.319	-0.287	0.042	-0.383	0.023	-0.365	0.062	-0.503	0.137	.784**	0.221	.725**	0.048
L-W2	0.108	-0.016	-0.046	-0.025	1	0.018	.894**	-0.112	0.207	0.265	-0.104	.460*	0.184	0.135	-0.121	-0.042	.501*	0.18	0.443	0.163	.549*	-0.02	.613**	-0.028	0.439	0.105	-0.013	0.08	0.05	-0.096	0.251	0.151	0.237	0.208	0.001	0.065	0.062	0.085
F-W2	-0.219	0.216	-0.294	0.191	0.018	1	-0.098	.800**	0.086	-0.14	-0.207	-0.015	0.458	-0.492	-0.268	0.026	0.309	0.167	0.29	0.008	-0.458	-0.032	0.323	-0.074	0.439	0.105	-0.013	0.08	0.05	-0.096	-0.017	-0.164	0.147	0.195	0.143	0.191	0.163
L-D2	0.064	0.025	-0.028	0.066	.849**	-0.098	1	-0.259	0.273	0.187	0.008	0.33	0.004	0.668	-0.175	0.242	0.287	-0.074	0.223	-0.05	0.517*	-0.063	.526*	-0.087	0.16	0.166	0.16	0.097	0.156	0.008	0.123	0.063	0.06	-0.095	0.081	-0.143
F-D2	-0.293	0.425	-0.238	0.354	-0.112	.800**	-0.239	1	-0.036	0.059	-0.36	0.064	0.411	-0.537	.918*	0.077	-0.146	0.06	-0.293	-0.045	0.137	-0.086	0.313	0.064	-0.138	0.018	0.018	-0.087	-0.468	0.099	-0.281	0.102	0.277	0.176	0.314	0.186
L-W3	-0.053	0.345	-0.183	.614*	0.207	0.086	0.273	-0.036	1	0.273	0.287	-0.277	-0.447	-0.449	-0.471	0.382	-0.129	0.069	0.514	0.148	0.494	-0.079	0.331	-0.213	0.29	0.121	0.299	-0.266	0.261	.689**	0.023	.638*	-0.093
F-W3	0.022	0.22	0.14	0.261	0.255	-0.14	0.187	0.059	0.273	1	-0.224	.460*	-0.657	-0.352	-0.481	0.159	-0.084	-0.179	-0.06	0.069	0.117	-0.044	0.092	0.036	-0.035	0.011	-0.031	0.114	-0.6	-0.336	-0.511	-0.401	0.217	0.052	0.055	-0.017
L-D3	0.163	-0.219	0.075	0.13	-0.104	-0.207	0.008	-0.36	.393*	-0.224	1	-0.182	0.001	0.301	-0.467	0.86	0.233	0.1	0.109	0.394	.568*	0.344	.603*	0.19	-0.063	-0.011	-0.132	0.036	0.016	0.145	-0.2	0.173	0.165	-0.247	0.181	-0.186
F-D3	-0.213	-0.209	-0.171	-0.237	.461*	-0.015	0.33	0.054	-0.277	.460*	-0.182	1	0.014	0.717	-0.065	-0.065	0.291	0.134	0.36	0.801	0.211	-0.213	0.216	-0.237	.444*	0.121	.557*	0.224	-0.079	-0.327	0.251	-0.359	-.572*	-0.07	-0.527	0.042
L-W4	0.629	-0.282	0.22	-0.337	0.184	-0.458	0.004	-0.041	-0.447	-0.657	0.001	0.014	1	-0.212	0.36	0.65	0.089	0.236	0.243	-0.214	0.423	-0.284	0.37	-0.313	0.01	-0.169	0.115	-0.247	0.249	-0.583	0.154	-0.342	-0.49	0.05	-0.532	0.058
F-W4	0.217	0.446	0.149	0.07	0.135	-0.492	0.668	0.411	-0.449	-0.352	0.352	0.277	-0.212	1	-0.503	0.578	0.383	-0.051	0.122	1	0.395	-0.432	0.602	-0.26	0.451	-0.301	0.675	0.703	0.348	0.418	0.414	0.616	0.415	-0.158	0.865	0.123	0.743
L-D4	0.685	-0.63	0.487	-0.567	0.121	-0.288	-0.176	0.537	-0.471	-0.481	0.467	0.805	0.65	-0.503	1	-0.796	-0.061	0.105	0.128	0.037	-0.103	-0.225	-0.136	-0.163	-0.276	-0.365	-0.064	-0.273	0.116	-0.583	0.065	-0.355	-0.661	-0.165	-0.803	-0.091
F-D4	-0.547	0.81	-0.663	0.67	-0.042	0.026	0.242	.916**	0.392	0.159	-0.066	-0.065	0.578	-0.796	1	0.122	-0.028	-0.383	0.643	0.088	0.651	-0.061	0.432	0.372	0.167	0.194	0.018	0.344	-0.02	0.187	0.675	0.265	0.857	0.005
L-W1	0.345	0.007	0.137	-0.196	.501*	0.309	0.287	0.077	-0.129	-0.084	0.233	0.291	0.089	0.383	-0.051	0.122	1	0.395	.945**	0.429	.545*	0.111	.592*	0.129	0.408	0.345	.458*	.447*	0.544	0.091	0.621	0.256	-0.344	0.314	-0.196	.542*
F-W1	-0.089	-0.442	-0.101	-0.381	0.18	0.167	-0.074	-0.146	0.228	-0.179	0.1	0.134	-0.236	-0.432	0.105	-0.455	0.395	1	.457*	.858**	-0.313	0.328	-0.187	0.252	0.31	.601**	0.443	.700**	0.535	0.421	0.366	0.455	-0.114	0.291	-0.031	0.433
L-D1	0.284	0.04	0.07	-0.219	0.443	0.29	0.223	0.06	-0.216	-0.06	0.109	0.06	0.243	0.596	0.128	-0.023	.945**	.457*	1	.463*	0.367	-0.053	0.343	-0.042	.500*	.495*	.578**	.573**	0.615	-0.021	.685*	0.171	-0.461	.455*	-0.316	.643**
F-D1	0.108	-0.407	-0.017	-0.268	0.163	0.008	-0.05	0.293	0.319	0.069	0.394	0.301	-0.214	-0.419	0.037	-0.303	0.428	.858**	.463*	1	-0.051	0.365	0.055	0.276	0.283	.473*	0.41	.633**	0.555	0.082	0.357	0.13	-0.237	0.14	-0.22	0.28
L-W2	0.399	0.115	0.214	0.219	.541*	-0.032	.517*	-0.045	0.069	0.117	.568*	0.211	0.423	0.602	0.643	-0.103	0.643	.545*	.575*	-0.313	0.367	-0.051	1	0.145	0.077	-0.025	0.07	.447*	0.535	0.421	0.366	0.458	-0.065	0.134	-0.065	0.116
F-W2	0.12	-0.14	0.182	0.263	-0.02	0.323	-0.063	0.137	0.514	-0.044	0.344	-0.213	-0.284	-0.26	0.088	-0.225	0.088	0.111	0.328	-0.053	0.365	0.259	1	.961**	0.094	0.043	0.072	0.171	0.492	0.5	0.245	.608*	0.35	-0.08	0.435	-0.039
L-D2	0.267	0.1	0.184	.612**	-0.074	.526*	-0.086	0.148	0.092	.603*	0.216	0.37	0.451	0.136	0.651	.532*	0.187	0.343	0.29	.961**	0.21	1	0.21	0.062	0.013	0.036	0.045	0.083	0.212	0.095	0.27	0.109	-0.066	0.208	-0.028
F-D2	0.197	-0.031	0.266	0.319	-0.028	0.439	-0.087	0.313	0.494	0.036	0.19	-0.237	-0.313	-0.301	-0.163	-0.061	0.252	-0.042	0.276	0.196	0.283	0.077	0.062	0.057	1	0.057	-0.09	0.017	0.064	0.355	0.359	0.179	0.491	0.324	-0.074	0.376	-0.026
L-W3	-0.2	-0.076	-0.349	-0.287	0.244	0.105	0.16	0.064	-0.079	-0.035	0.064	.444*	0.01	0.777	-0.276	0.432	0.408	0.311	.500*	0.283	0.077	0.094	0.062	0.057	1	.455*	.946**	.462*	.821**	-0.008	.827**	0.024	-0.252	0.241	-0.123	0.315
F-W3	-0.094	0.166	-0.192	0.042	0.299	-0.013	0.166	-0.138	0.331	0.011	-0.011	-0.121	0.169	0.673	-0.365	0.372	0.345	0.601**	.485*	.473*	-0.025	0.043	0.013	-0.09	.455*	1	.547*	.965**	.658*	0.505	0.57	.548*	0.217	.789**	0.397	.755**
L-D3	-0.112	-0.196	-0.208	-0.383	0.304	0.08	0.16	0.018	-0.213	-0.031	-0.132	.557*	0.115	0.703	-0.064	0.167	.458*	0.443	.578**	0.41	0.04	0.072	0.036	0.017	.946**	.547*	1	.593**	.831**	0.059	.923**	0.104	-0.444	0.394	-0.259	0.397	-0.026
F-D3	0.035	0.086	0.023	0.294	0.05	0.097	-0.087	0.29	0.114	0.036	0.224	-0.079	0.247	0.549	-0.273	0.134	.447*	.700**	.573**	.633**	0.007	0.171	0.045	0.094	.462*	.965**	.590**	1	.691*	0.308	.659*	.579**	0.116	.774**	0.307	.792**
L-W4	0.125	-0.193	-0.021	-0.365	0.251	-0.096	0.156	-0.468	0.121	-0.6	0.016	-0.079	0.079	0.418	0.116	0.018	0.544	0.535	0.615	0.555	0.005	0.492	0.083	0.355	.821**	.658*	.831**	.691*	1	0.332	.887**	0.451	-0.22	0.446	-0.202	0.621
F-W4	-0.136	-0.142	0.062	0.151	-0.017	0.008	0.009	0.009	0.299	-0.368	0.145	-0.327	-0.583	0.414	-0.593	0.344	0.091	0.421	-0.021	0.082	0.658	0.5	0.212	0.359	-0.009	0.505	0.659	0.508	0.322	1	0.355	.957**	0.421	0.439	.645*	0.518
L-D4	0.24	-0.258	0.182	-0.503	0.237	-0.164	0.123	-0.281	-0.266	-0.511	-0.2	0.251	0.154	0.616	0.065	-0.02	0.621	0.366	0.665*	0.357	0.063	0.245	0.095	0.179	.827**	0.57	.923**	.659*	.887**	0.355	1	0.45	-0.458	0.545	-0.188	.798**
F-D4	-0.023	-0.075	0.168	0.137	0.208	0.147	0.063	0.102	0.261	-0.401	0.173	-0.359	-0.342	0.416	-0.355	0.187	0.256	0.455	0.171	0.13	0.146	.606*	.527	0.491	0.024	.586*	0.104	.573**	0.451	.957**	0.45	1	0.397	0.528	.633*	.610*
L-W5	-0.226	.580**	-0.257	.784**	0.001	0.195	0.06	0.277	.689**	0.217	0.165	-.572**	-0.49	-0.536	-0.661	0.675	-0.344	-0.114	-0.461	-0.237	0.043	0.35	0.109	0.324	-0.552	0.217	-0.444	0.115	-0.22	0.421	-0.468	0.397	1	0.23	.949**	-0.005
F-W5	0.082	0.439	0.056	0.221	0.065	0.143	-0.095	0.176	0.023	0.052	-0.247	-0.07	0.05	0.865	-0.165	0.265	0.314	0.291	.455*	0.14	-0.065	-0.08	-0.066	-0.074	0.24	.783**	0.334	.774**	0.446	0.439	0.545	0.528	0.23	1	0.403	.910**	
L-D5	-0.196	.541*	-0.173	.725**	0.062	0.191	0.081	0.314	.630*	0.065	0.181	-0.527	-0.532	0.123	-0.803	0.657	-0.196	-0.081	-0.316	-0.22	0.134	0.435	0.208	0.376	-0.123	0.397	-0.259	0.307	-0.102	.645*	-0.188	.633*	.949**	0.403	1	0.249
F-D5	0.192	0.319	0.209	0.048	0.085	0.163	-0.143	0.186	-0.093	-0.017	0.186	0.042	-0.058	0.743	-0.091	0.005	.542*	0.28	0.433	.643**	0.28	-0.016	-0.039	-0.028	-0.026	0.315	.755**	0.436	.792**	0.621	0.518	.798**	.608*	-0.005	.910**	0.249	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 10: Ct value and expression results for *UFO, CCE1* and *CRY2* in Fremont

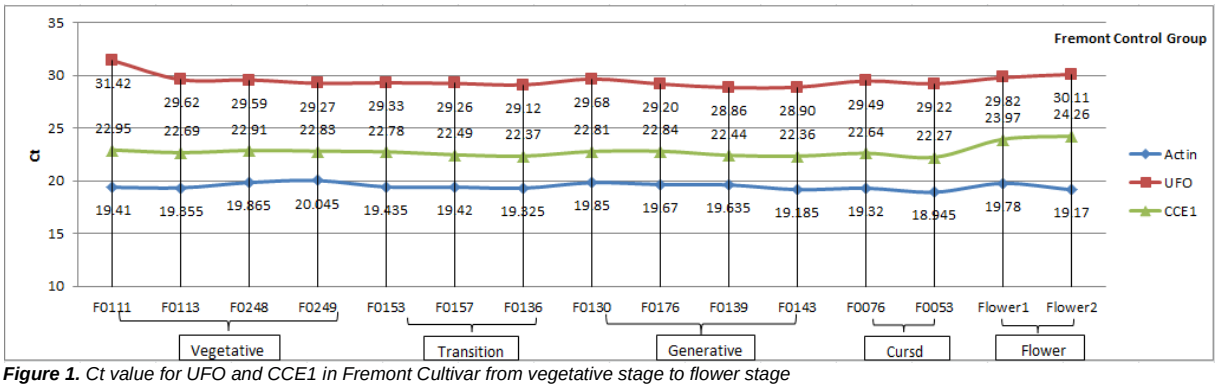


Figure 1. *Ct value for UFO and CCE1 in Fremont Cultivar from vegetative stage to flower stage*

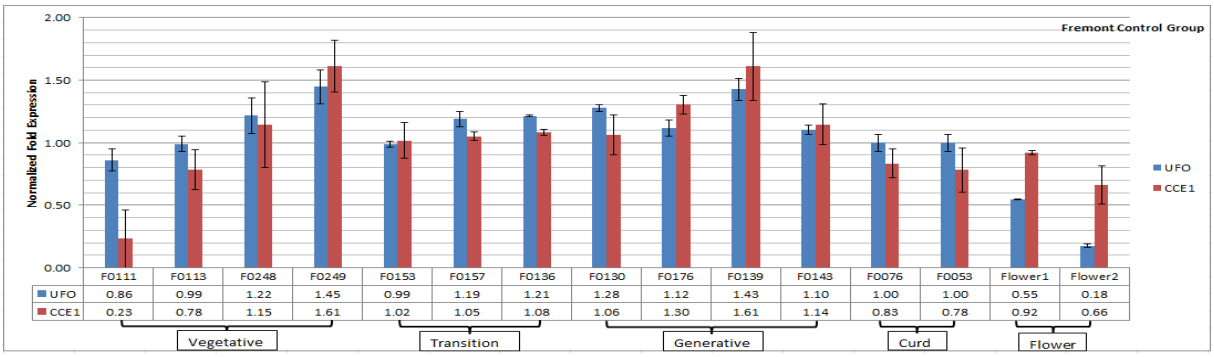


Figure 2. *Normalized fold expression of UFO and CCE1 in Fremont from vegetative to flower stage*

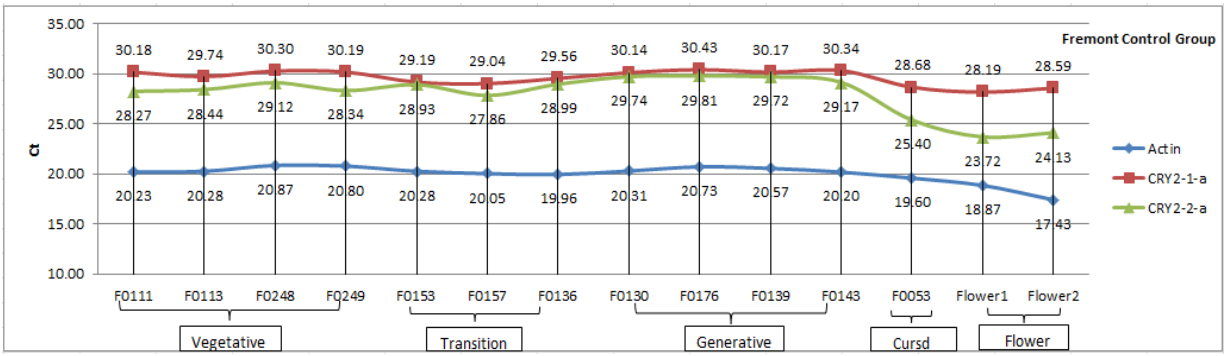


Figure 3. *Ct value for CRY2 family in Fremont Cultivar from vegetative stage to flower stage*

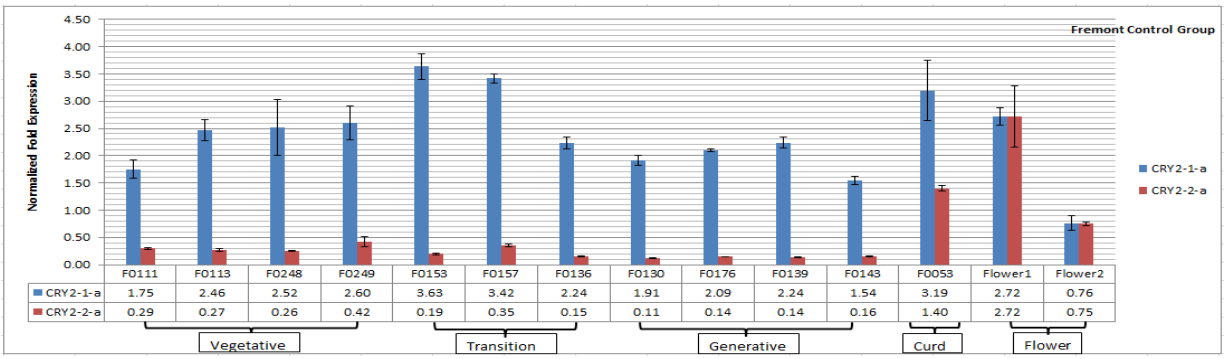


Figure 4. *Normalized fold expression of CRY2 family in Fremont from vegetative to flower stage*

Appendix 11: Ct value and expression results for *FUL family, FH, UFO, CCE1, NSN1-1, NSN1-2, NSN1-3, REM1 and TFL1-1* in Lindurian control and 6th week high temperature treatment group

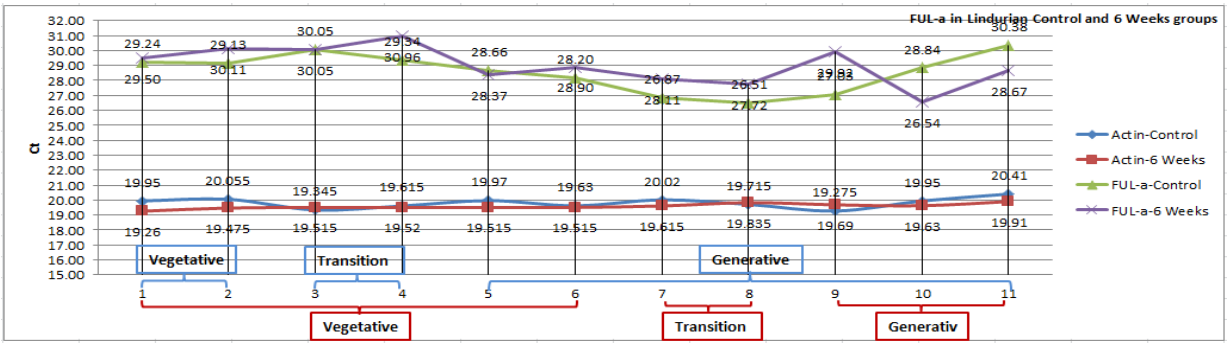


Figure 1. *Ct value for FUL-a in Lindurian cultivar*

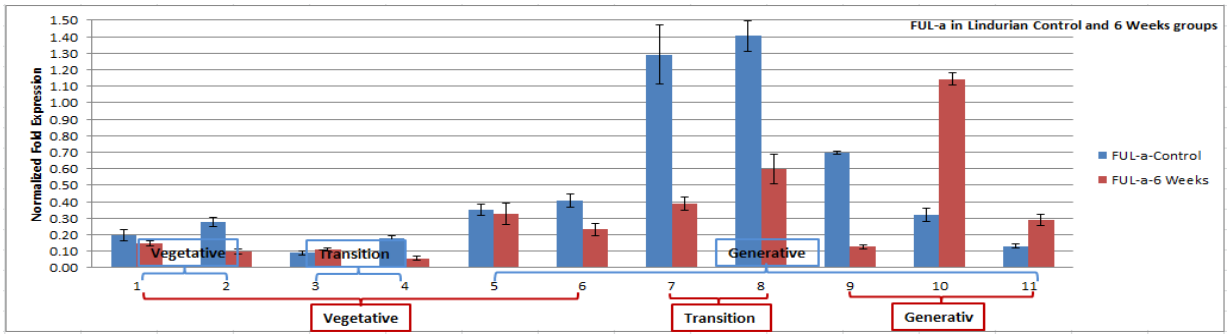


Figure 2. *Normalized fold expression of FUL-a in Lindurian control and 6th week group from vegetative to flower stage*

	Week 7		Week 9		Week11		Week 12		Week 13		Week 14
Control	Vegetative		Transition		Generative						
Plant Num	**L0181**	**L0210**	**L0241**	**L0277**	**L0073**	**L0081**	**L0083**	**L0141**	**L0040**	**L0068**	**L0270**
FUL-b-Ct	--	39.36	38.63	36/04	35.83	35.36	33.23	32.78	32.42	34.42	36.32
6 Weeks G	Vegetative						Transition		Generative		
Plant Num	**L0240**	**L0242**	**L0067**	**L0164**	**L0037**	**L0171**	**L0103**	**L0167**	**L0070**	**L0263**	**L0005**
FUL-b-Ct	--	--	38.35	--	38.16	38.15	37.02	35.41	38.51	34.13	34.44

"--" Means Ct value higher than 40

Figure 3. *Ct value for FUL-b in Lindurian cultivar*

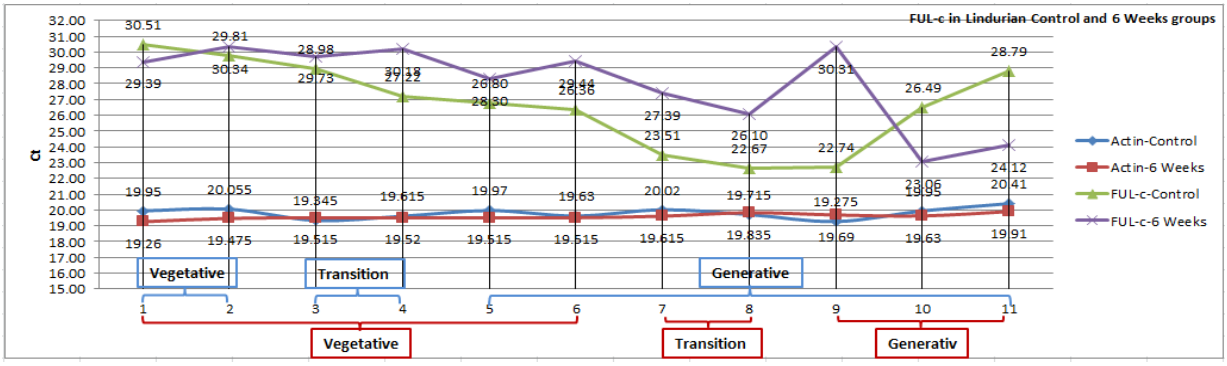


Figure 4. *Ct value for FUL-c in Lindurian cultivar*

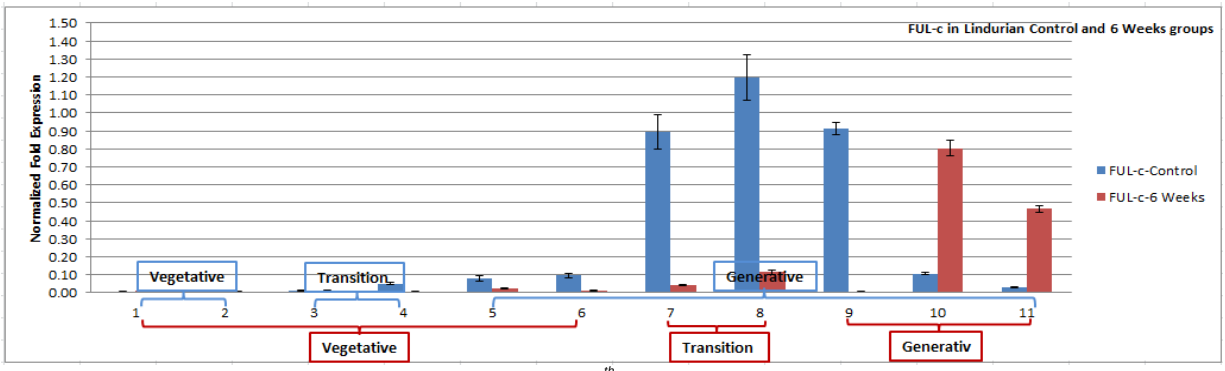


Figure 5. *Normalized fold expression of FUL-c in Lindurian control and 6th week group from vegetative to flower stage*

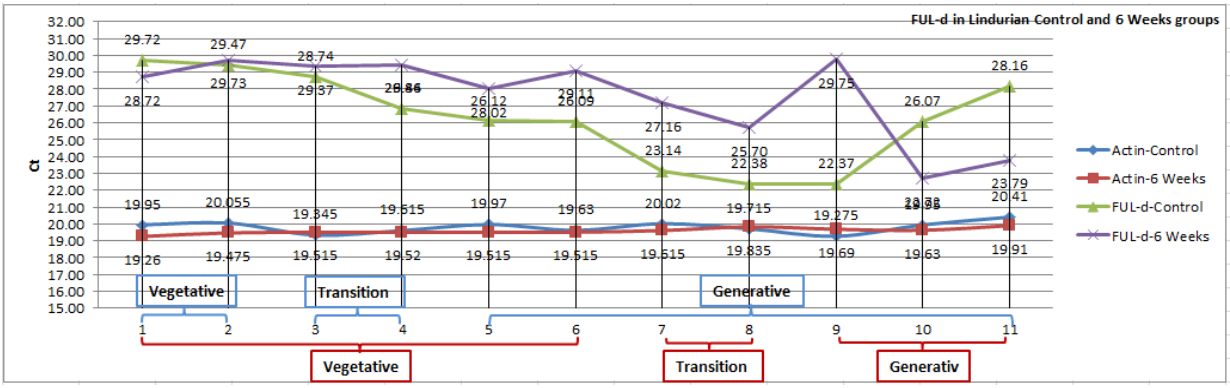


Figure 6. *Ct value for FUL-d in Lindurian cultivar*

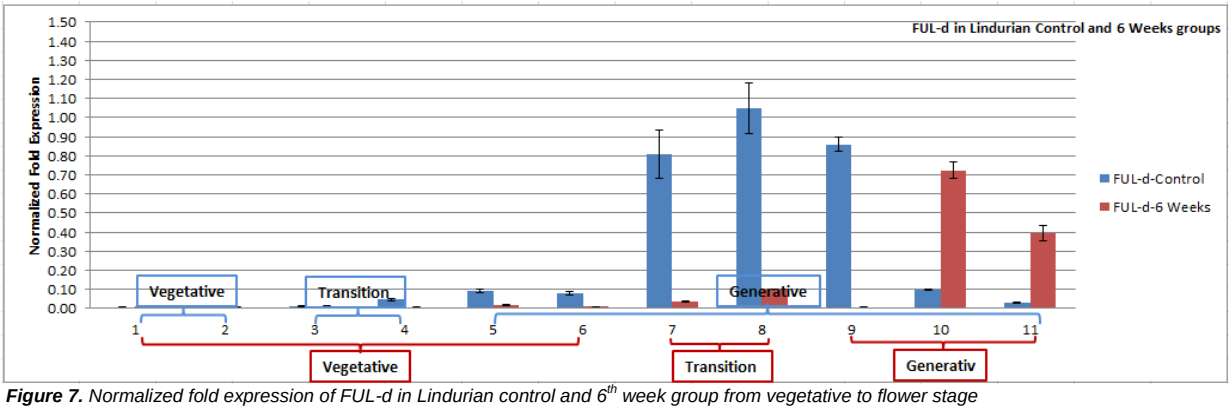


Figure 7. *Normalized fold expression of FUL-d in Lindurian control and 6th week group from vegetative to flower stage*

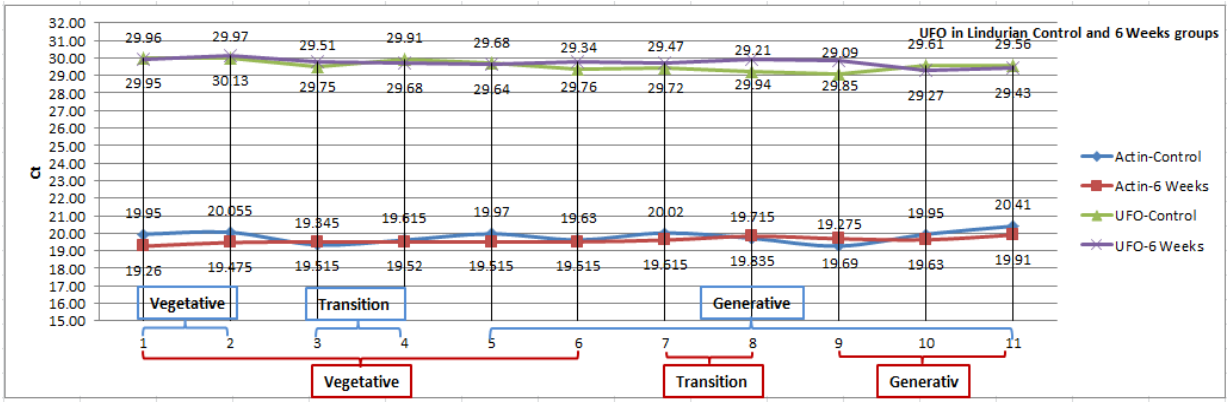


Figure 8. *Ct value for UFO in Lindurian cultivar*

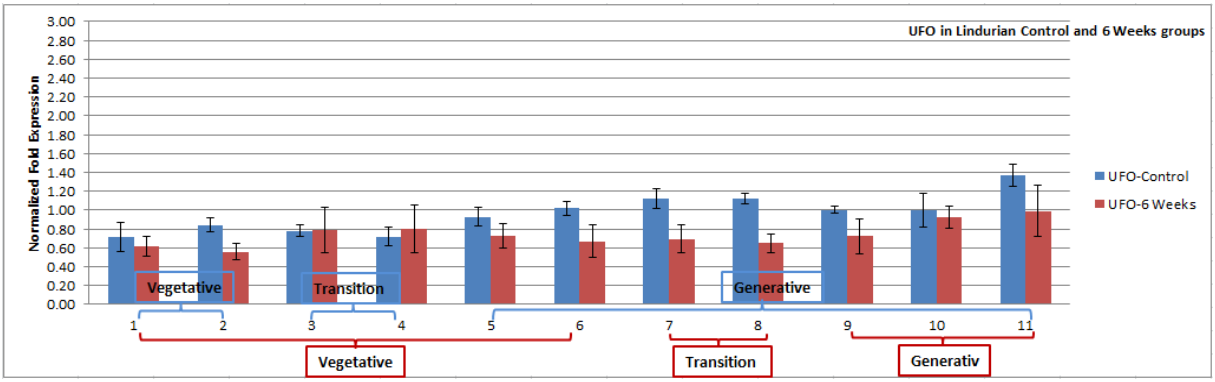


Figure 9. *Normalized fold expression of UFO in Lindurian*

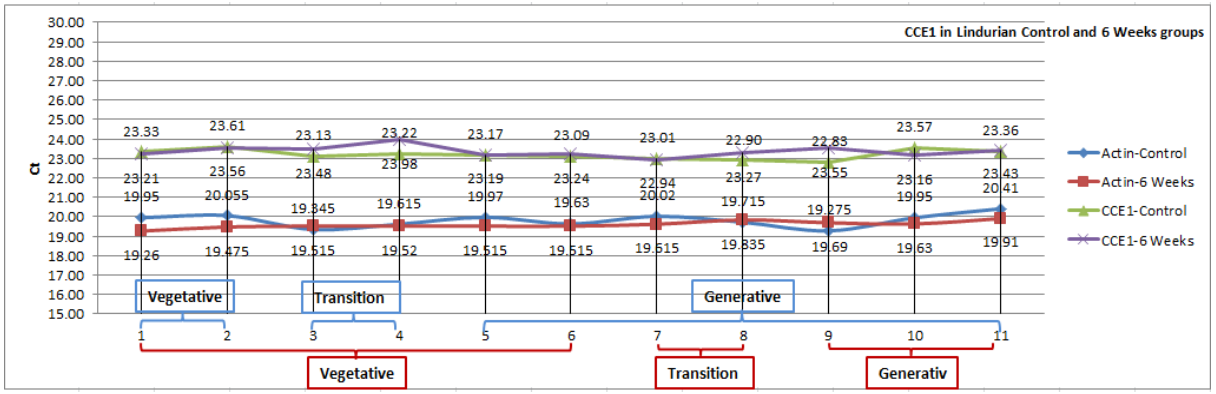


Figure 10. *Ct value for CCE1 in Lindurian cultivar*

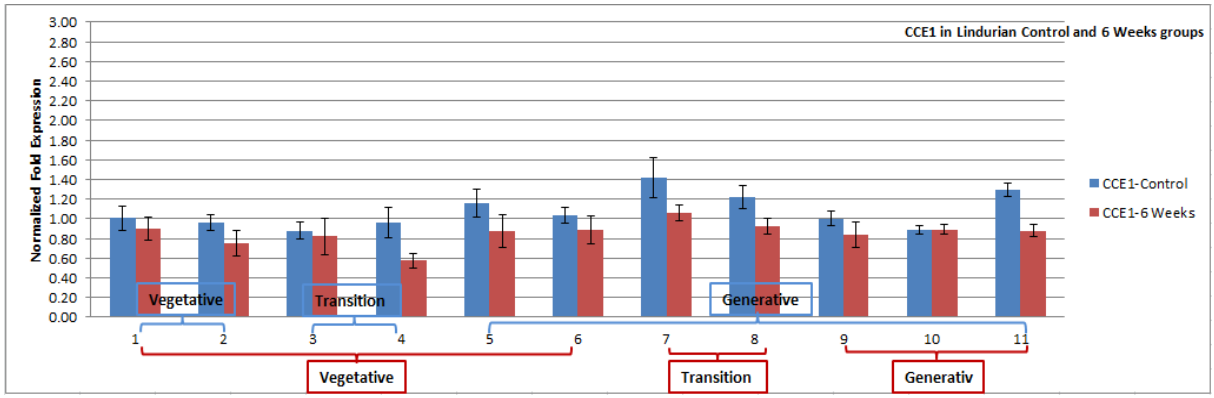


Figure 11. *Normalized fold expression of CCE1 in Lindurian*

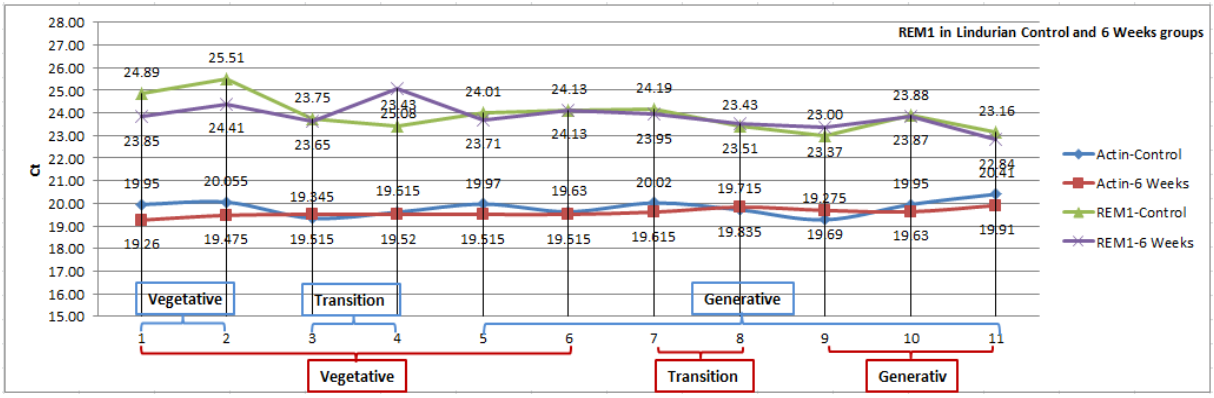


Figure 12*. Ct value for REM1 in Lindurian cultivar*

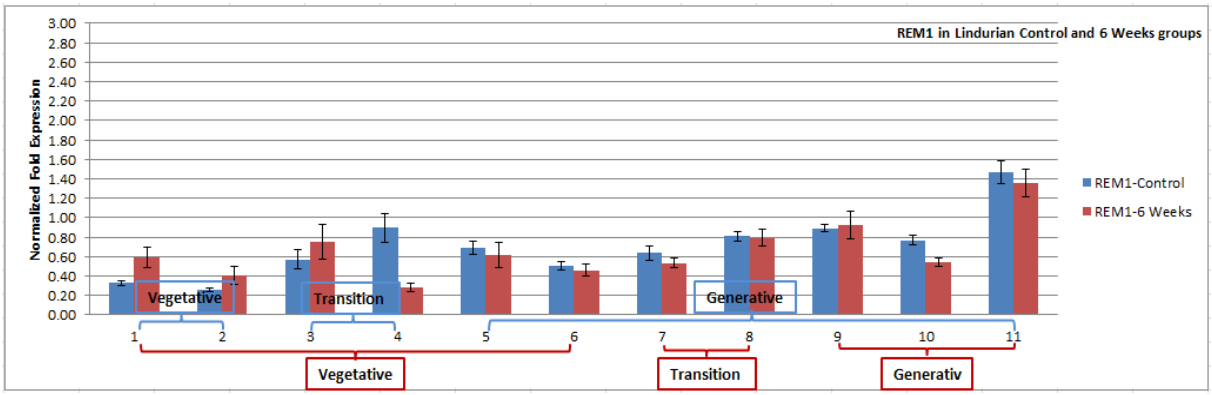


Figure 13. *Normalized fold expression of REM1 in Lindurian*

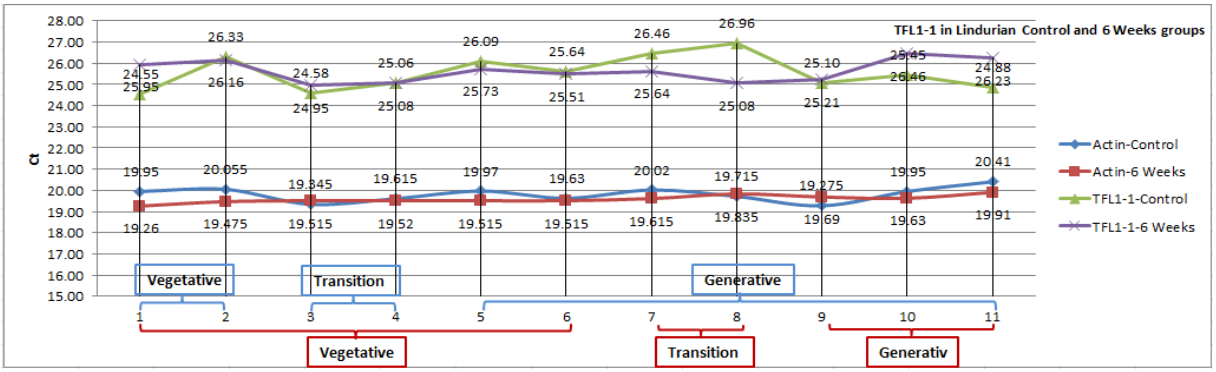


Figure 14. *Ct value for TFL1-1 in Lindurian cultivar*

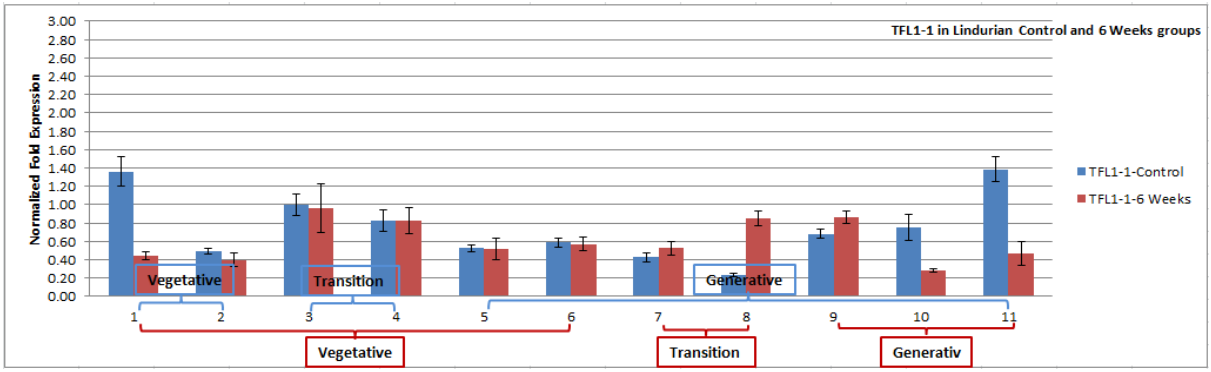


Figure 15. *Normalized fold expression of TFL1-1 in Lindurian*

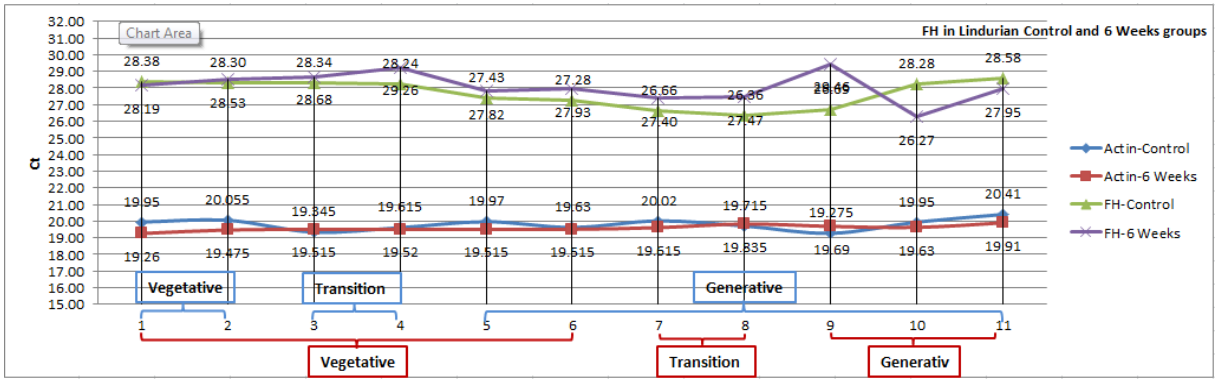


Figure 16. *Ct value of FH in Lindurian control and 6th week high temperature group from vegetative stage to generative stage*

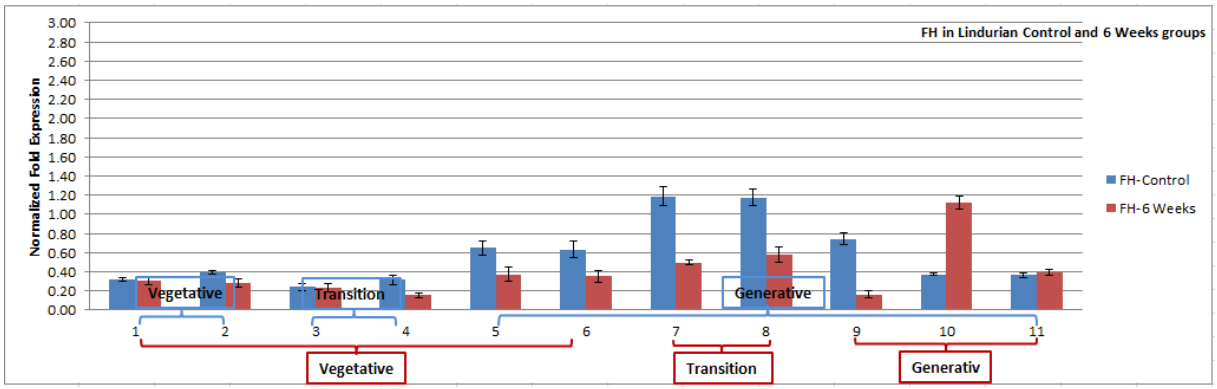


Figure 17. *Normalized fold expression of FH in Lindurian control and 6th week group from vegetative to flower stage*

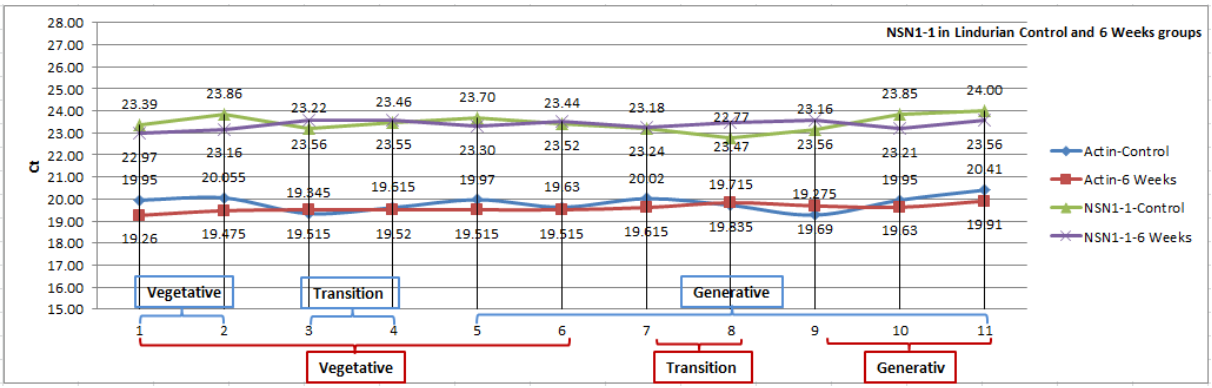


Figure 18. *Ct value for NSN1-1 in Lindurian cultivar*

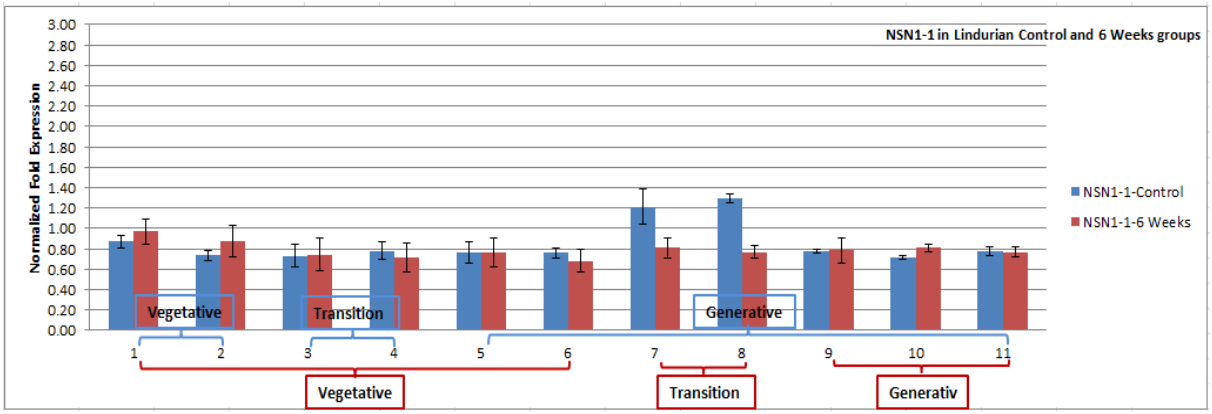


Figure 19. *Normalized fold expression of NSN1-1in Lindurian*

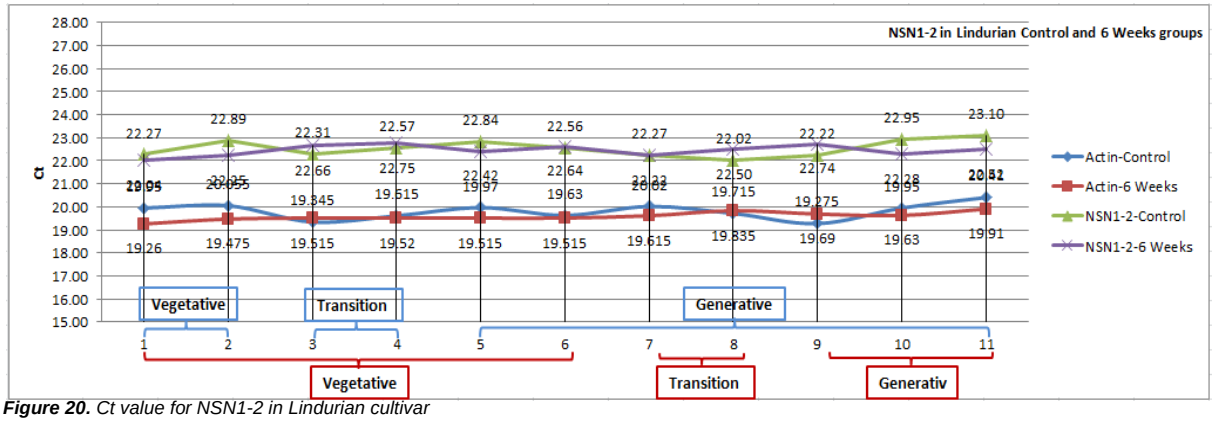


Figure 20. *Ct value for NSN1-2 in Lindurian cultivar*

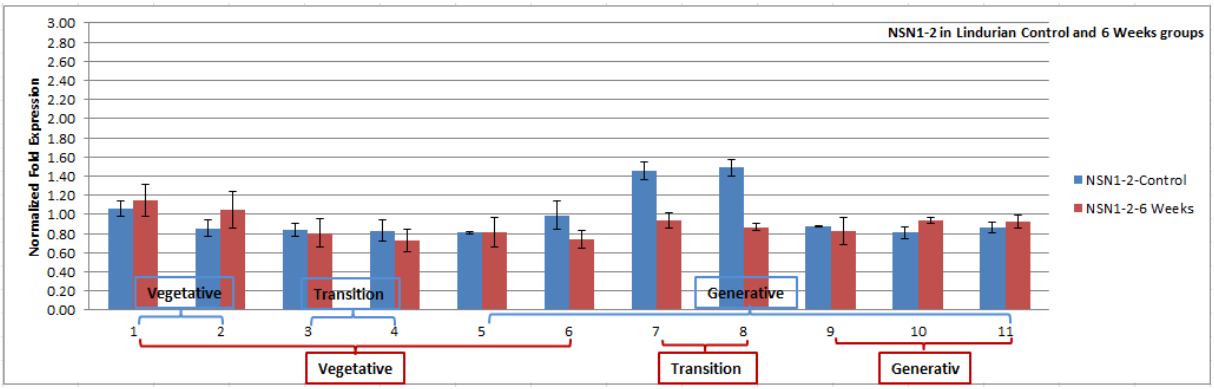


Figure 21. *Normalized fold expression of NSN1-2 in Lindurian*

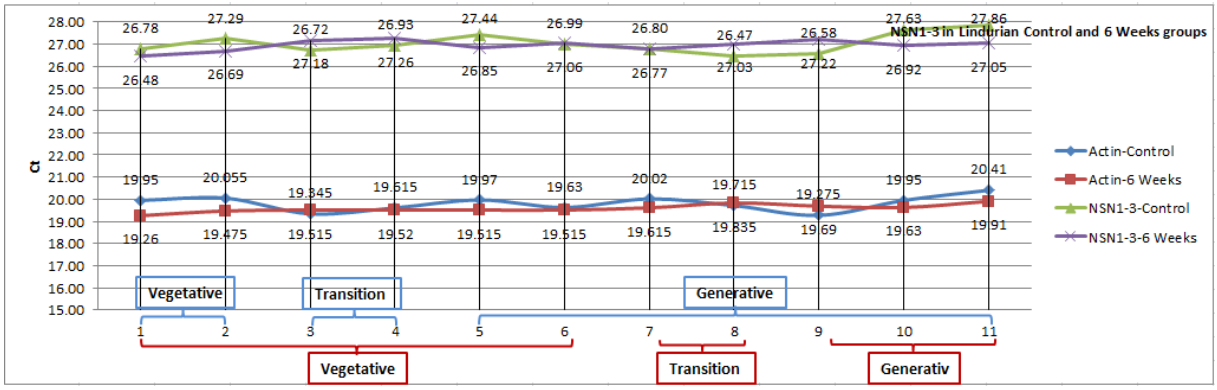


Figure 22. *Ct value for NSN1-3 in Lindurian cultivar*

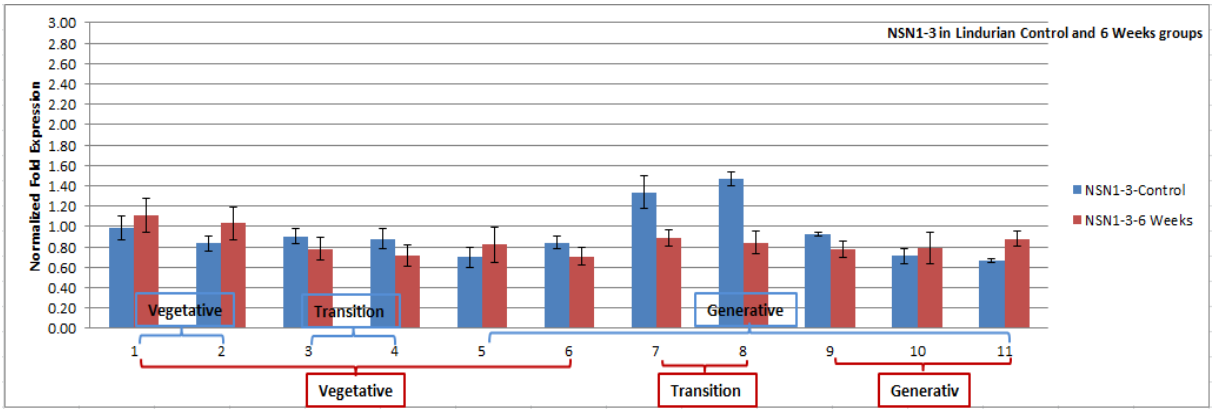


Figure 23. *Normalized fold expression of NSN1-3 in Lindurian*

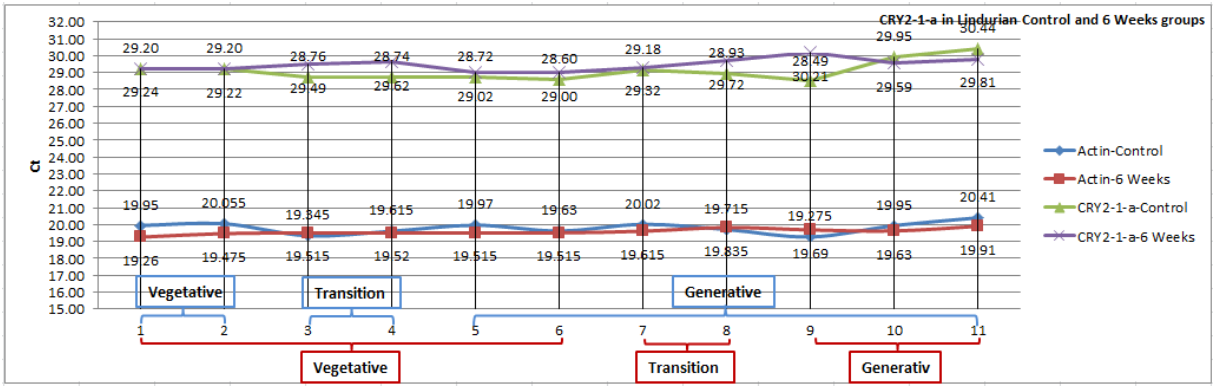


Figure 24. *Ct value for CRY2-1-a in Lindurian cultivar*

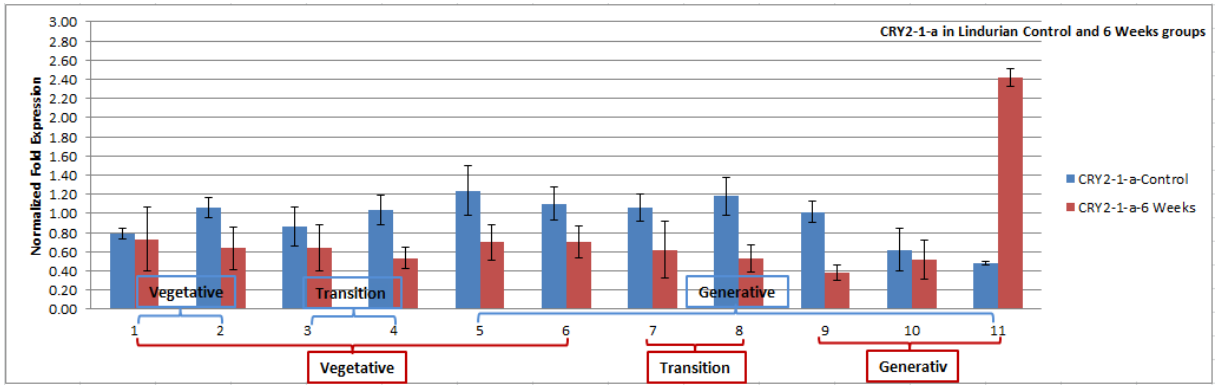


Figure 25. *Normalized fold expression of CRY2-1-a in Lindurian*

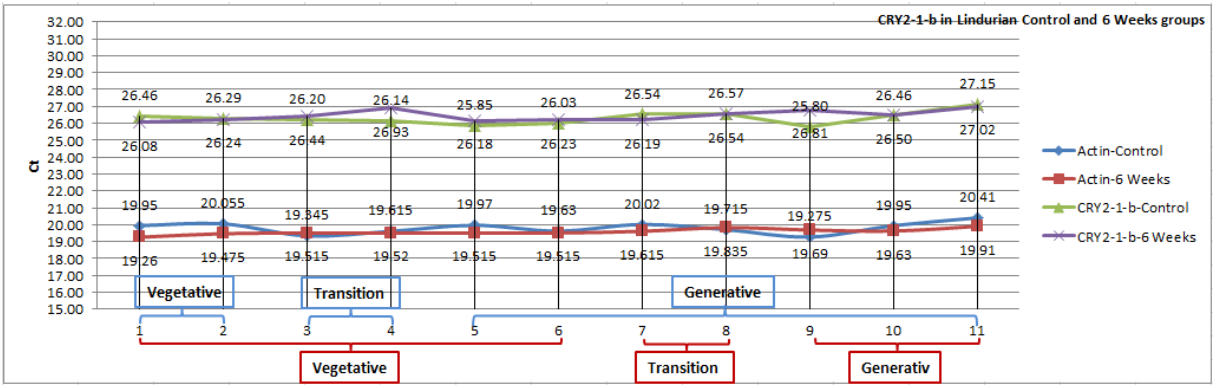


Figure 26. *Ct value for CRY2-1-b in Lindurian cultivar*

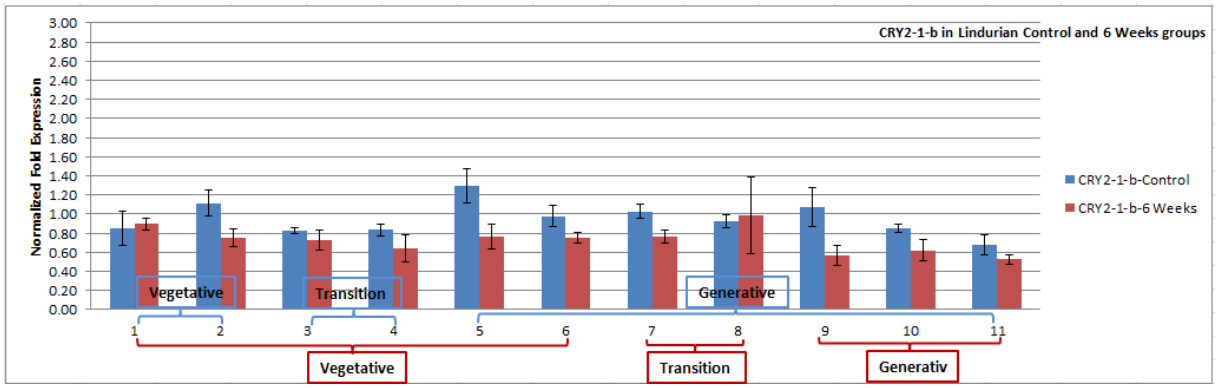


Figure 27. *Normalized fold expression of CRY2-1-b in Lindurian*

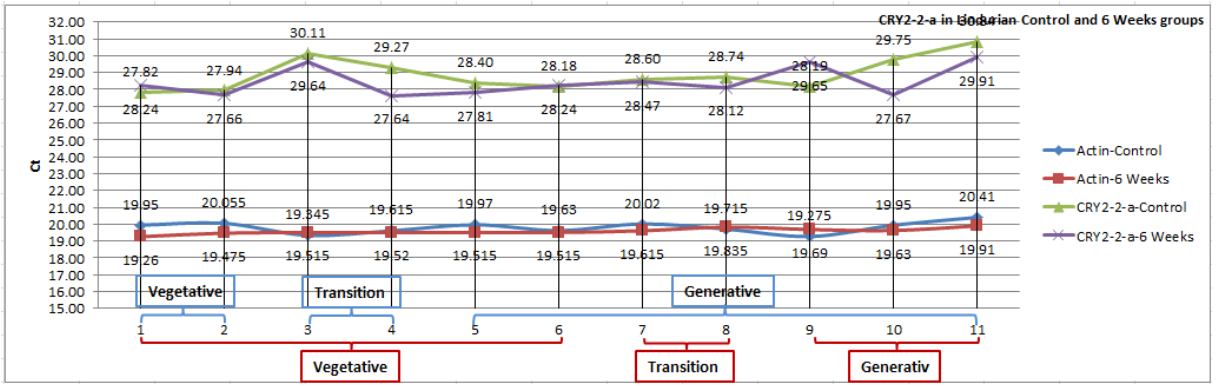


Figure 28. *Ct value for CRY2-2-a in Lindurian cultivar*

Figure 29. *Normalized fold expression of CRY2-2-a in Lindurian*

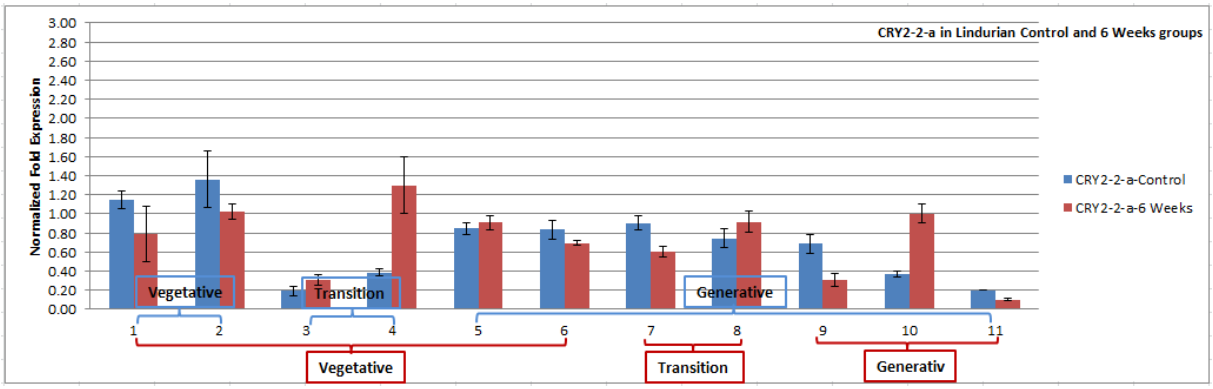


Figure 30. *Ct value for CRY2-2-b in Lindurian cultivar*

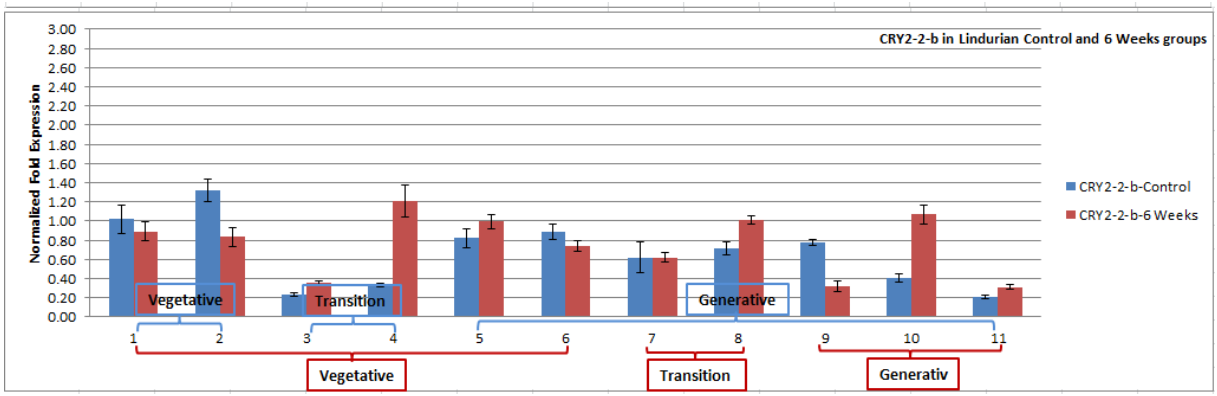


Figure 31. *Normalized fold expression of CRY2-2-b in Lindurian*

Appendix 12: Candidate genes expression level one week before and after high temperature treatment Ct value and normalized fold expression result.

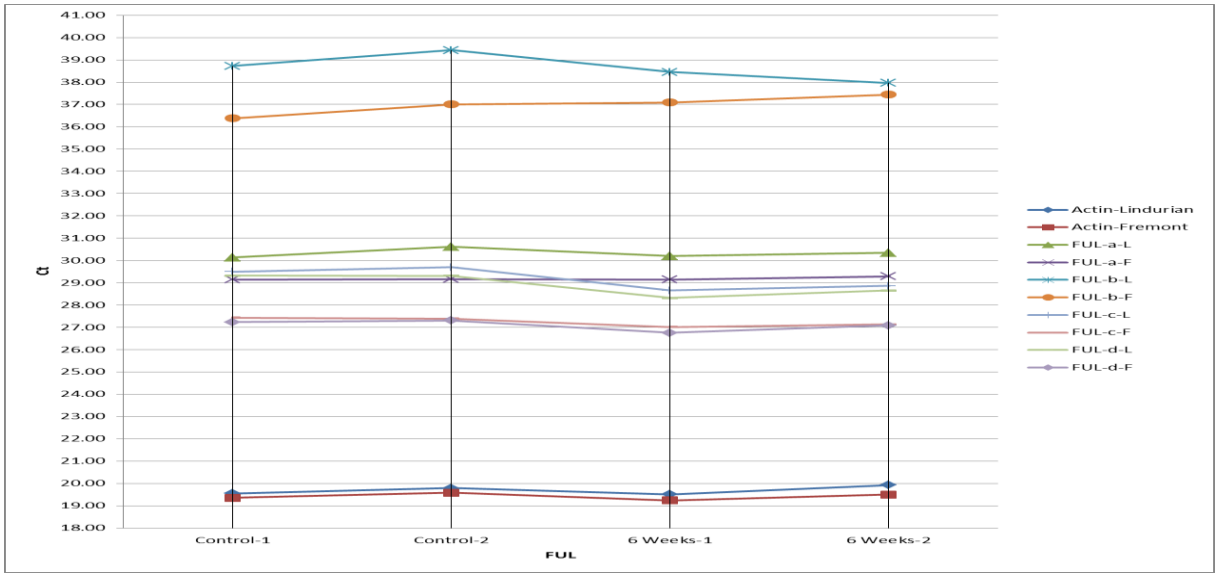
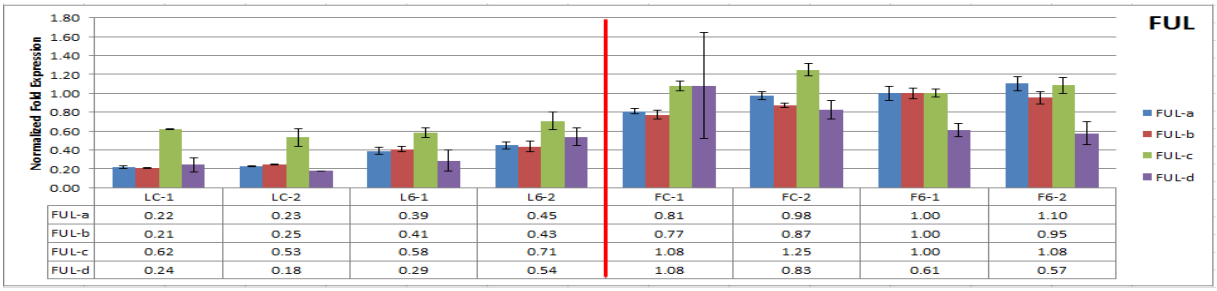


Figure 1. *Ct value for FUL family in Lindurian and Fremont cultivars*



	LC-1	LC-2	L6-1	L6-2	FC-1	FC-2	F6-1	F6-2
FUL-a	0.22	0.23	0.39	0.45	0.81	0.98	1.00	1.10
FUL-b	0.21	0.25	0.41	0.43	0.77	0.87	1.00	0.95
FUL-c	0.62	0.53	0.58	0.71	1.08	1.25	1.00	1.08
FUL-d	0.24	0.18	0.29	0.54	1.08	0.83	0.61	0.57

Figure 2. *Normalized fold expression of FUL family in Lindurian and Fremont cultivars*

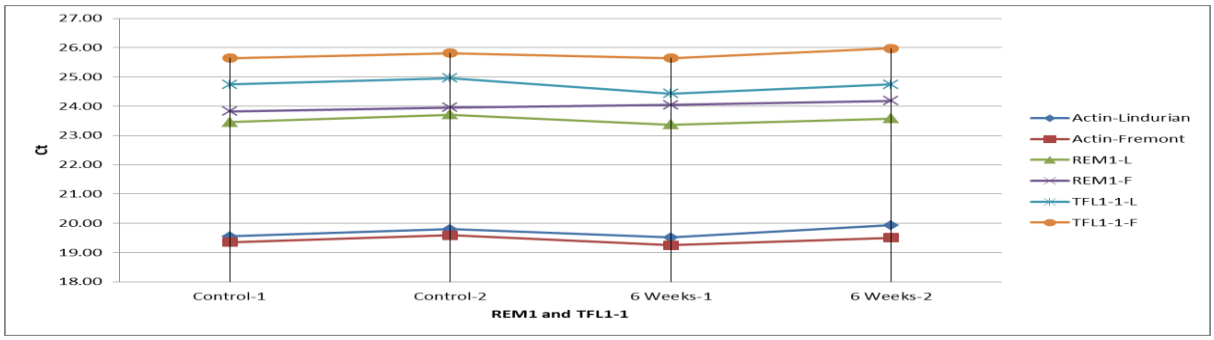
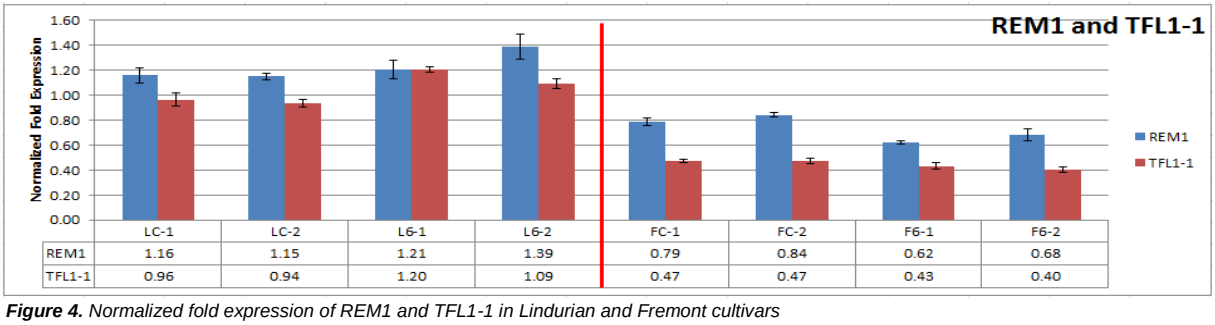


Figure 3. *Ct value for REM1 and TFL1-1 in Lindurian and Fremont cultivars*



	LC-1	LC-2	L6-1	L6-2	FC-1	FC-2	F6-1	F6-2
REM1	1.16	1.15	1.21	1.39	0.79	0.84	0.62	0.68
TFL1-1	0.96	0.94	1.20	1.09	0.47	0.47	0.43	0.40

Figure 4. *Normalized fold expression of REM1 and TFL1-1 in Lindurian and Fremont cultivars*

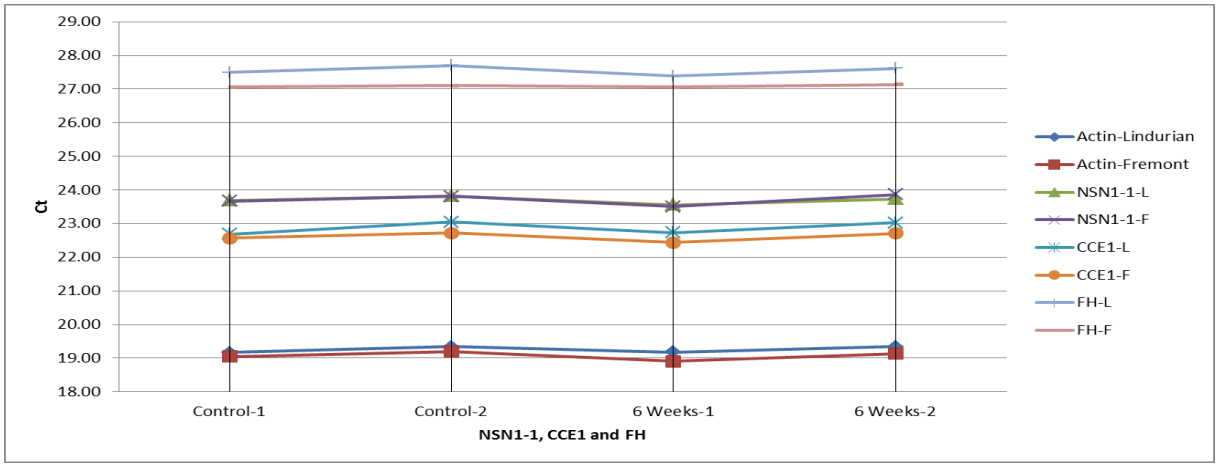
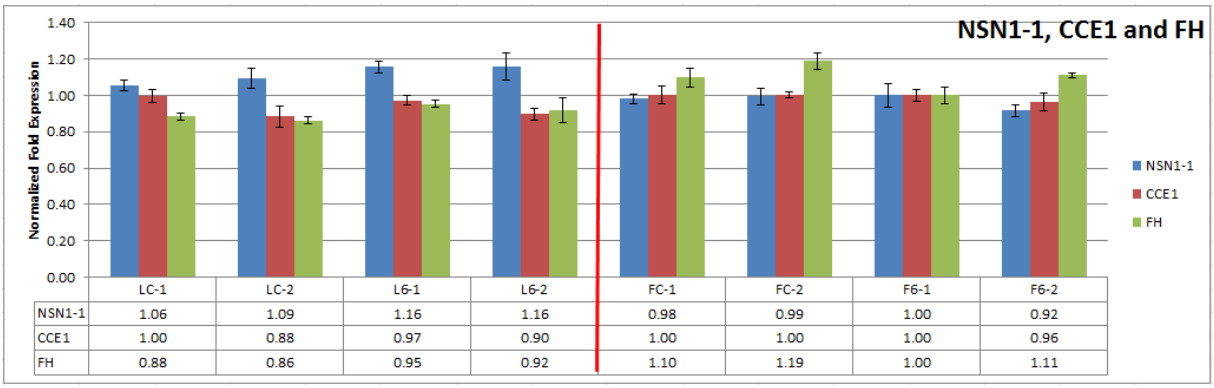


Figure 5. *Ct value for NSN1-1, CCE1 and FH in Lindurian and Fremont cultivars*



	LC-1	LC-2	L6-1	L6-2	FC-1	FC-2	F6-1	F6-2
NSN1-1	1.06	1.09	1.16	1.16	0.98	0.99	1.00	0.92
CCE1	1.00	0.88	0.97	0.90	1.00	1.00	1.00	0.96
FH	0.88	0.86	0.95	0.92	1.10	1.19	1.00	1.11

Figure 6. *Normalized fold expression of NSN1-1, CCE1 and FH in Lindurian and Fremont cultivars*

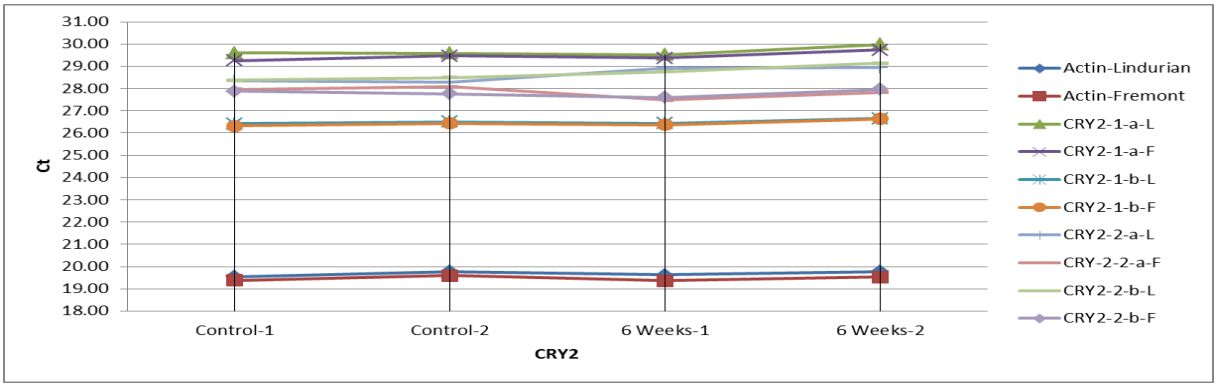


Figure 7. *Ct value for CRY2-1 and CRY2-2 in Lindurian and Fremont cultivars*

Table 1. *Ct value for AP1-a and CAL in Lindurian and Fremont cultivars*

	LC-1	LC-2	L6-1	L6-2	FC-1	FC-2	F6-1	F6-2
AP1-a-Ct	--	--	--	--	36.26	--	--	--
CAL-Ct	35.95	--	36.76	37.06	36.91	--	35.68	--

"--" Means Ct value higher than 40