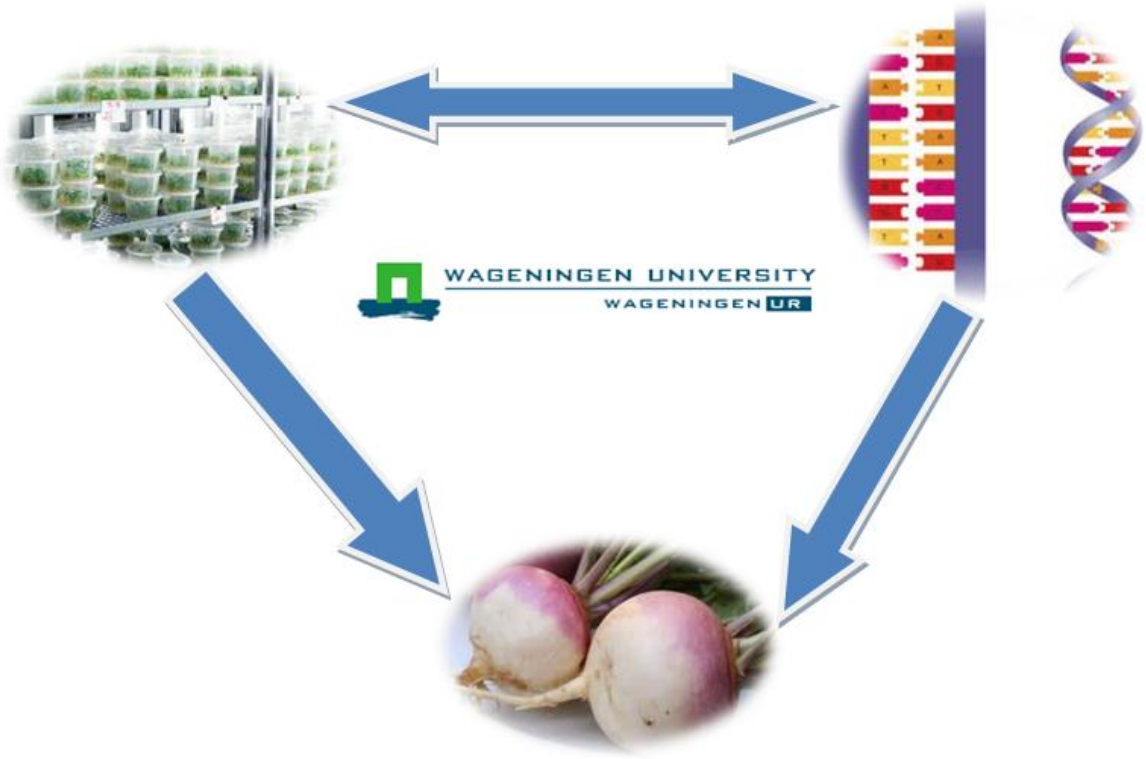
Investigating Hormone Regulation and Sugar Storage during Tuber Development in Turnip Plants (*Brassica rapa*)



MSc Thesis Report (PBR-80436)

Temesgen Menamo (Reg. No. 860717-557-050)

Supervisors: Dr. Ningwen Zhang
Dr. Guusje Bonnema

Laboratory of Plant Breeding, Wageningen University

The Netherlands, Wageningen

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Examiners : Dr. Guusje Bonnema
Dr. Christian Bachem

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Abstract

Brassica rapa belongs to *Brassicaceae* family with diploid (2n=20) genotype and this species consists of morphological diverse crops. It includes leafy vegetables, turnip vegetables, and vegetable oil. This study focused on investigation of hormones and sugar storage during turnip tuber formation in turnip plants. First, sugar and starch content in mature turnip tubers of 19 turnip accessions from different geographical origin and growth conditions (Greenhouse and field) were measured by enzymatic analysis. Glucose and fructose content were higher than sucrose content in most of the accessions and the total sugar and starch content in tubers grown in greenhouse were higher than the ones grown in the field. VT-053 was unique from all accessions, as it had high sucrose content in both field and greenhouse grown conditions. For sugar translocation study, four (SM-17, FT-004, FT-086 & DH-VT-117) accessions were used which collected at different growth stage. Average total sugar content was lower in leaf tissues than in turnip tissues in all tested growth stages. In addition, the average total glucose content was also lower at later growth stages (4^{th} & 5^{th}) in shoot and tuber tissues. This might show the flow of sugar from the leaves (source) to storage organ. Based on these data, VT-115, VT-117, VT-012, VT-053, VT-052, FT-002, YS-143 and red round turnip accessions were selected and grown in vitro condition to assess the effect of sucrose (5, 6, 7, & 8%), medium (Standard MS-medium and Tuber Inducing Medium), photoperiod (long day (LD16) & the shortest day (SD8)) and hormones (BAP and GA_3) *in vitro* condition. The result of this part showed that six percent sucrose with the standard MS - medium in the long day condition induced turnip tuber formation. GA_3 hormone did not show a significant difference in turnip formation for all (0, 3.5, 7.5 and 11.5mg/l) tested concentrations. Application of exogenous BAP (5, 10, & 15mg/l) hormone inhibited turnip tuber formation and turnip growth. Third, gene expression patterns of 21 selected genes in two tissue types (shoot and tuber) from six different growth stages of VT-117 were quantified using qRT-PCR. The result of this part indicated that Out of 21 genes *VTuni_02*, *VTuni_05*, *VTuni_09*, *VTuni_14*, *VTuni_30*, *BraDDF1*, *BraARF1* and *BraARF3_2* might be involved in turnip tuber formation.

Keywords: Brassica rapa, BAP, GA_3, in vitro, sugar storage, Turnip formation genes

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1. Introduction

1.1. *Brassica rapa* background

Brassicaceae is a large plant family with over 338 genera and 3,700 species. It has a wide range of horticulture crops which have great economic value and are grown as vegetables, fodders, sources of oils and condiment ((Al-Shehbaz *et al.*, 2006; Zhao *et al.*, 2010). *Brassica* is a genus which found in Brasscaceae family. The model plant *Arabidopsis* is also closely related to the genus *Brassica* (Ostergaard and King, 2008) which can be exploited to find orthologous genes from *Arabidopsis* with known function. The *Brassica* genus has six species including *the B. rapa, B. nigra, B. oleracea, B. juncea, B. napus* and *B. carinata*. The three diploid Brassica (*B. rapa, B. nigra* and *B. oleracea*) species forming the Triangle of U (figure 1), have hybridized in all possible combinations to produce the three allotetraploid species; *B. juncea, B. napus* and *B. carinata* (Ostergaard and King, 2008; U, 1935).

Brassica rapa species has a diploid (2n=20) genotype and consists of morphological diverse crops. It includes leafy vegetables (Chinese cabbage: -*Brassica Rapa* subsp. *pekinensis)*, pak choi*:-Brassica rapa* subsp. *Chinensis)*, turnip type (*Brassica rapa* subsp*. rapa L.)*, and vegetable oils (Sarson:-*Brassica rapa* L. var. *trilocularis* and biannual oiltypes (winter oil and Chinese turnip rapa*)*) (Song *et al.*, 1995). Turnip (*Brassica rapa* subsp*. rapa L.)* is domesticated in very ancient time and was used for human consumption and animal feed (Vogl-Lukasser *et al.*, 2008). It has believed that the turnip was the first domesticated *Brassica* species and naturally grown from the Western Mediterranean region to central Asia (Campo, 1999). Both Europe and Eastern Asia are one the centers of diversity for *B. rapa subsp. rapa*. It is grown popularly in Europe starting from 2500-2000 BC, the Middle East is starting 1000 BC and Asia starts around 1800 B.C (Vogl-Lukasser *et al.*, 2008).

Turnip crops are annual (Asian turnip) or biennial (European turnip) but the European turnip also grown as an annual. The first year, the plants grow to be used for human consumption in their turnip tuber and the second year produce flowers and set seed. Traditionally, turnip is called root crop but actually it is not a root at all. It consists mainly of the hypocotyl and taproot (tuber). Hypocotyl is plant part that lies between the main root and the first seedling leaves (cotyledons) which grows below or above the surface of the soil. It also has little or no neck and a distinct taproot (studies in Brassica group, Wageningen University).

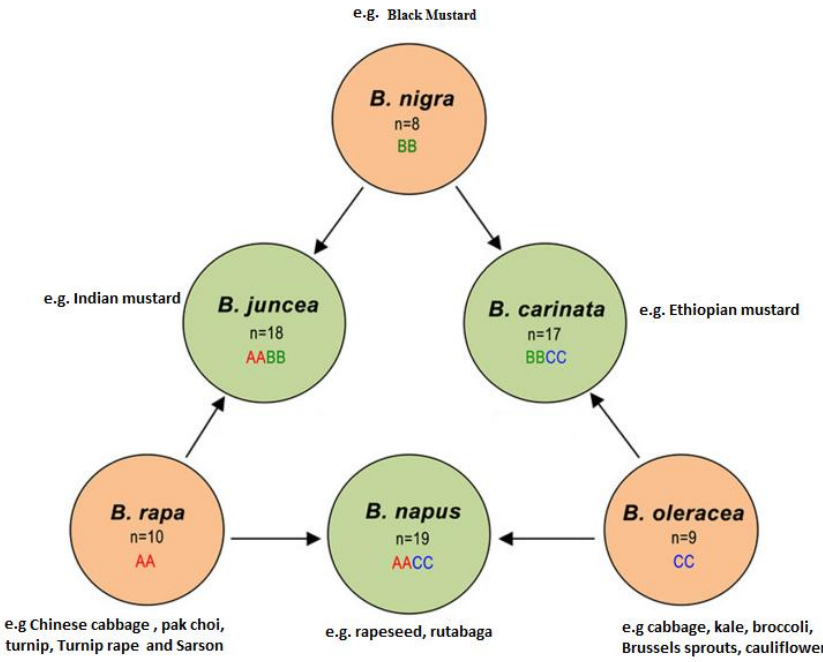


Figure 1. The "Triangle of U" diagram, showing the genetic relationships of six species in the genus Brassica *(Nagaharu, 1935).*

1.2. Economic importance

Brassica rapa is one of important crop among the six economically cultivated Brassica species of U's triangle. It is cultivated all over the world as a vegetable for food and fodder for animals. *Brassica rapa* var. Chinese cabbage and var. *chinensis* (leaf cabbage) are extensively grown as important market garden vegetables in China. The main economic important part of the turnip is hypocotyl with taproot (tuber) but the leaves are also used for fodder as well as for food. The turnip greens (tops) are excellent chopped in salads or cooked briefly as a side dish. Turnip also used for flavor in ingredient soups and a winter salad. It also used in different dishes in some country like India and Pakistan. The nutrient composition of turnip (USDA, 2011), it has more calcium and sodium mineral, particularly rich in vitamin C, very low fat and proteins (USDA, 2011). Brassica vegetables are rich in glucosinolates, a chemical which can block the growth of human cancer cells (He *et al.*, 2012; Kushad *et al.*, 1999; Rosa *et al.*, 1997).

1.3. Sugar storage in turnip tuber

Sugars are synthesized in the mesophyll cells and serve as the major translocation of photosynthetic product for energy source. A vascular network has evolved too long distance transport of sugar from source to sink (Lalonde *et al.*, 1999). Sucrose is the main representative for transporting of photosynthetic assimilated carbon in plants. It synthesized in the leaves and transport long distance via phloem to supply non-photosynthetic organs with energy and carbon resources (Lalonde *et al.*, 1999). Non-photosynthetic tissues and organs also need energy and fixed carbon. In addition to transport metabolite, sucrose contributes osmotic driving force and serves as signal for active or repress expression of specific genes in different tissues (Lalonde *et al.*, 1999).

Glucose and fructose content increase in turnip roots due to acid and alkaline invertases activities increase (Gupta *et al.*, 2001). In contrast, During active root filling period of turnip, a decrease in sucrose synthase, starch content and alkaline invertase activities was also observed in leaf blades (Gupta *et al.*, 2001) which might indicate the transporting starch from leaf to storage organs.

1.4. Researches on *in vitro* turnip formation

Sucrose and GA effect were studied at Wageningen University, laboratory of plant breeding (Guan and Zhang, 2009). It showed that sucrose was main factor to induce turnip tuber formation but GA_3 was depending on sucrose concentration (Part 1.7.4.). However, in tuber crops like potato there are clear researches that have been done on effect hormones, photoperiod, sucrose and medium in *vitro* condition potato tuber formation.

1.5. Potato (*Solanum tuberosum*) and turnip (*Brassica rapa subsp. rapa*)

Potato and Turnip are different crops which potato is a stem tuber crop and turnip is hypocotyl tuber crop. However, they have certain similarities by stolon swelling or thickening of storage organ. Stolon in potato is a differentiated stem, usually arising from underground stem that is developing into tubers under inducting condition. *Brassica rapa subsp. rapa*; thickening usually arises from hypocotyl with taproot which develop to turnip tuber. In potato and turnip during swelling or thickening of storage organs, sugar and starch are translocation from photosynthetic tissues to sink regions. SUTI mRNA is responsible for translocation of sucrose to sink in potato. The SUTI transformants leaves having 100-fold higher amounts of hexoses, and 5- to 10-fold higher levels of sucrose and starch in mature leaves of potato (Riesmeier *et al.*, 1994). During active turnip tuber filling (sink filling; thickening of storage organ), a decrease in sucrose synthase (cleavage) and alkaline invertase activities is observed in leaf blades which

lead to a rapid decline in the starch content in leaf blades and increases hexose content in roots (Gupta *et al.*, 2001).

There are many researches have been done on the potato for sucrose and hormones effects under *in vitro* condition. In turnip also some researches were done on effects of sucrose on turnip tuber formation (Guan and Zhang, 2009b) which shown similar results as potato but it is not sufficient to determine the effect of sucrose, medium and hormones similar in turnip as potato researches. This BSc study also has taken potato as a model plant for tuber formation due to clear research potato in *vitro* culture. Potato and turnip have some similarity based on application of exogenous sucrose concentration study. Sucrose enhances tuber formation for both crops. However, reduce sugars are the main sugar sources for storage organ formation for turnip crop. The differences; turnip is *Brassicaceae* family and potato is *Solanaceae* family. There are also differences and similarity in nutrient compositions. For instance, the nutrient composition of turnip and potato (USDA, 2011), Potato has higher in calories and carbohydrates than turnips; turnip has different in mineral (potato more potassium; turnip more calcium and sodium) contents than potato. Turnips are particularly rich in vitamin C and Potatoes in vitamin B (USDA, 2011). Turnip and potato contain very low fat and proteins and the same amount of fiber.

1.6. Tuberization and turnip tube formation

In potato tuberization is a highly complex developmental process, which starts from the formation of an underground stem with horizontal growth or stolon, which swells in the sub-apical region to form a storage organ or tuber, hence accumulate large amounts of starch (Abelenda *et al.*, 2011). In potato, tuber formation signaling is starts from the leaves and transport to stolons. However, in turnip the main root with hypocotyl forms storage organ with more reduce sugar such as glucose and fructose (Ricardo and Sovia, 1974). Turnip consists of a root, hypocotyl and parts of the shoot. Potato tuberization is caused by secondary xylem growth while Secondary cambial growth starts at an early developmental stage mainly forming secondary xylem and some secondary phloem (Lu *et al.*, 2008). Similarly, development of a turnip storage organ start from thickening of the hypocotyl, brought by a vascular cambium producing cells which differentiate into secondary xylem and phloem (Peterson, 1973).

The physiological and genetic studies on turnip formation are important activities to ensure continual progress of arable farming with high yield and pathogen resistance varieties. However, in the last decades' progress in understanding tuberization has been done particularly on potato by biotechnological approaches, including tissue culture (*in vitro* on hormones and photoperiods test) and RT-PCR (gene expressions). Various genetics researches were done on turnip also such as QTL studies

(Kubo *et al.*, 2010; Lou *et al.*, 2007; Lu *et al.*, 2008; Schranz *et al.*, 2002; Zhao *et al.*, 2009). To study under *in vitro* condition like potato tuberization, turnip tuber formation may also affect by environmental (photoperiods), hormones, sucrose, and nutrient contents.

1.7. Factors affect potato and turnip tuber formation

In potato, clear researches were done on the factors affect tuber formation under *in vitro* condition such as hormones (GA, cytokinins, BAP, and Auxins), photoperiods, sucrose and medium. However, for turnip no clear literatures were done about those factors. As discussed early, in the laboratory of Breeding, Wageningen University, they did on hormones (GA_3) and sucrose effects. They have got similar results as potato researches on exogenous sucrose application in turnip formation *in vitro* condition. Potato researchers show as high concentration of GA_3 had an inhibition effect on tuberization and high sucrose content induced tuber formation (Xu *et al.*, 1998). Similarly, high sucrose concentration was induced turnip tuber formations but GA_3 was depending in sucrose concentration (Part 1.7.4.).

1.7.1. Photoperiod effects

Environmental condition has influence on the formation of vegetative storage organs in plants such as flowers, tubers and bulbs (Abelenda *et al.*, 2011). Potato is originated from short day region; Central America. In the Netherlands, potatoes are earliness gene mutated (CDF interaction with CO and FT) which can grow in long day condition (discussion with Bonnema). Day length is the most prominent morphogenetic factor for the potato tuber formation. It has believed that turnip originated from long day origin. Potato microtuberization is faster (lower tuberization period) in darker than light growth conditions (Hoque, 2010). However, the percentage of nodes tuberization and microtuber fresh weights increased in the light compared with continuous darkness (Donnelly *et al.*, 2003). The photoperiod on growth of plant substitute with different hormones, for instance geberillic acid (Garcia-Martinez and Gil, 2001). The tuber can be induced without sucrose and hormones by using of inductive light length; short day (Seabrook, 2005). In turnip long day condition only tested and the turnip tuber formation started 81 days after sowing of seeds (Aofei and Ningwen, 2009b). Short day had a small storage organ formation than long day condition under high temperature in radish ($25^{O}C$) (Plitt, 1932).

1.7.2. Hormones effect

Plant hormones also have been studied for decades on different crops (e.g. Potato) but no clear studies have be done on turnip (*Brassica rapa subsp. rapa L.*). Plant Hormones were found to affect various aspects of plant development; GA is one of the most candidates' hormones to play a major role for

tuberization in potato. It is reported that exogenously application of GA inhibited or delayed tuberization whereas the application of the gibberellin biosynthesis inhibitor paclobutrazol increased tuberization (Gibson, 2004). In turnip, GA inhibition depends on sucrose concentration (Part 1.7.4). GA application *in vivo* condition was shown areal tuber formation. The tubers formed mainly in the basal portion of the stunted part and on the nodes of elongated parts in *Brassica rapa subsp. rapa L.,* (Nishijima *et al.*, 2005).

Previous researchers show that radish storage root thickening was the involvement of secondary growth by genesis of vascular cambium activity (Ting and Wren, 1980b). In turnip vascular cambium activity attain their large roots radial dimensions (Peterson, 1973). Radish storage organ thickening was the result of cambial activity (Ting and Wren, 1980a). So, Studying on the hormonal control of vascular cambium activation in roots and the factors influencing the formation of the secondary vascular tissues of the root may be important for tuber formation such as carrots, radishes, turnips, etc. Auxins and cytokinins are known to control the secondary growth of radish and carrot. Application of BAP has been observed as effective in inducing cell divisions in the hypocotyl (Ting and Wren, 1980b). MS medium supplemented with BAP has shown that induced the microtubers formation as well as shoot and root length in potato (Rafique *et al.*, 2004).

1.7.3. Sucrose effects

Sucrose concentration is found to show a negative correlation with GA concentration. Exogenous sucrose application causes a decrease of bioactive free gibberellin levels with an increase in levels of inactive conjugated gibberellins (Gibson, 2004). The microtuber induction response is the best when solid or liquid MS medium supplemented with 8% sucrose per liter (ASLAM and IQBAL, 2010). A high percentage of sucrose (5% and 8%) induced turnip formation in VT-115 and VT-012 genotypes (Guan and Zhang, 2009). Sucrose has been also identified as the limiting factors for root cambial activation at the seedling development stages of turnip (Peterson, 1973).

1.7.4. Combination (photoperiod, Hormones and sugar) effect

Phytohormones and sugar have crosstalk interactions. For instance, gibberellins and cytokinins have been shown to help regulate sugar metabolism or transport. Sugars also have been shown to regulate the expression of phytohormones response pathways and phytohormones regulate the expression of some genes encoding possible components of sugar response pathways in potato (Gibson, 2004). The combination of BAP and sucrose in short day condition promotes potato tuber formation (Pelacho and Mingo-Castel, 1991). The combined action of cytokinin and higher sucrose (8%) concentration has

shortened the potato microtuber induction time from 21 & 18 days (8% sucrose alone) to 10 days and stimulated tuber initiation when MS medium supplemented with BA (5mg/l) and 8% of sucrose (ASLAM and IQBAL, 2010). Auxin and GAs hormones crosstalk help for initiation and induction of tubers in potato (Roumeliotis *et al.*, 2012).

In cocoyam (*Xanthosoma sagittifolium L. Schott*) the highest percentage of tuberization (83%) was obtained with 30 mM BAP and 80 g/l sucrose under short day condition. However, highest fresh weight per microtuber was obtained in Long day dark regime with 80 g/l sucrose and 30 µM BAP (Omokolo *et al.*, 2003). Medium with 8% sucrose concentration, 10^{-5}M IAA, $5x10^{-6}$M BAP and $5X10^{-4}$M inositol enhanced root thickening in radish (Ting and Wren, 1980b). The combination of GA_3 (0.02, 0.2 and 0.4mg/l) with high concentration of sucrose (5%) had shown non-significant difference on turnip formation in VT-115, VT-012 and Natu-hakurei accessions. But GA3 (0.02, 0.2 and 0.4mg/l) with the combination of 2% sucrose has been shown decreasing of turnip size (thickness) with increasing of GA_3 levels (Guan and Zhang, 2009).

1.8. Genetic researches on storage organ formation

In *Arabidopsis* FLOWERING LOCUS T (FT) identified as a main component of the foreign or mobile flowering to be promoting signal produced in the leaves (Abelenda *et al.*, 2011). In potato mobile signal or tuberigen has been reported to induce tuber formation that indicated potato has homolog gene of FT encodes this signal (Abelenda *et al.*, 2011). In Sugar beet also two flowering locus T (BvFT1 & BvFT2) gene paralogs to *Arabidopsis* is identified but they have antagonistic functions and expression diurnal oscillations (Pin *et al.*, 2010). BvFT1 is expressed in the morning in short day and repress flowering. BvFT2 expressed in the evening in long day; is functionally conserved with FT and essential for flowering (Pin *et al.*, 2010).

There are also lots of genes which are responsible for tuberization with the presence or absence of GA hormones. Overexpression or downregulation of these genes are possible to control tuberization. In potato *in vitro* culture, nitrogen 10% in medium was used during tuberization which may enhance stress condition and induced tuber formation. The GA metabolism pathway is altered in response to abiotic stresses. For instance, In *Arabidopsis* the DDF1 gene is an AP2 transcription factor that is closely related to the dehydration responsive element–binding proteins (Yamaguchi, 2008). Overexpression of *DDF1* reduces GA4 content and the plant becomes dwarfed (Yamaguchi, 2008). StGA2ox1 is up-regulated during the early stages of potato tuber development before visible swelling. It is expressed in an early tuber initiation stage by modifying GA levels in the sub-apical stolon region at the onset of tuberization, thereby

facilitating normal tuber development and growth (Kloosterman *et al.*, 2007). GA is also affected by phytochrome; in long day endogenous GA is induced but in short day inhibited. Down-regulation of phytochrome B (PhyB) expression strongly induced potato (*S. tuberosum*) tuberization (Martinez-Garcia *et al.*, 2001).

Sucrose synthase (SuSy) is a key enzyme in the development of storage root in radish (Rouhier and Usuda, 2001) and sweet potato (Li and Zhang, 2003). A very low level of SuSy was observed 3 day after sowing and it increases when the radish hypocotyl thickening increases (Rouhier and Usuda, 2001). SuSy was the most actively expressed enzymes in sucrose metabolism in developing storage root in sweet potato. It had correlation with sink strength and the SuSy pathway was predominant for sucrose cleavage related to starch-accumulation (Li and Zhang, 2003).

Gibberellin acid and cytokinin hormones exhibit antagonistic effects on various processes in various species. GA repressed numerous cytokinin responses in different growth stage of Arabidopsis (Weiss and Ori, 2007) and tomato (Fleishon *et al.*, 2011) including shoot and root elongation, cell differentiation, shoot regeneration in culture, and meristem activity. GA_3 inhibited induction of the cytokinin primary-response gene, type-An Arabidopsis response regulator 5 (Greenboim-Wainberg *et al.*, 2005); type A Tomato Response Regulators (Fleishon *et al.*, 2011). Additional study in Arabidopsis, application of exogenous cytokinin inhibits the expression of *GA20ox* and *GA3ox* and promotes that of *RGA* and *GAI (Weiss and Ori, 2007).*

ARF is a plant protein family with roles in Auxin-mediated responses. Exogenous application of Plant hormones can regulate Plant growth and development. The role of ARF genes have been identified in plant growth and development in *A. thaliana (Fukaki et al., 2006; Sorin et al., 2005)*. A total of 31 ARF genes are also identified in *Brassica rapa* genome *(Mun et al., 2012)*.

Many root crops or root like crops can undergo considerable hypocotyl or root radial growths which would be suitable for food. Turnip is one of fleshy and edible hypocotyl crop. So, it is important to know what factors are involved in controlling the activity radial (thickening) turnip hypocotyl/tuber growth.

1.9. Scope of the thesis

Tuberization mechanisms in potato plants have been intensively studied (Abelenda *et al.*, 2011; Ewing and Struik, 1992; Gregory, 1956; Xu *et al.*, 1998; Zhang *et al.*, 2005), while in turnip tuber formation, there is a little research has done on sucrose and hormones *in vitro* condition. This study also was taken potato researches as model for testing turnip tuber formation in tissue culture condition. The aim of this study was to determine factors that affect turnip tuber formation and to study gene expression effect *in vitro* culture. The study also assessed the sugar content of turnip accessions from *in vivo* grew and measured sugar translocation in different tissues of four turnip accessions during plant developmental stages. Effect of BAP hormone and sucrose on expression of turnip tuber formation related genes *in vitro* condition was also assessed.

This study had the following objectives:

- To measure sugar and starch content in turnip tuber
- To investigate the translocation of sucrose, fructose and glucose from the source to sink during turnip formation
- To assess the effect of gibberellin acid (GA_3) and BAP concentrations in *B. rapa subsp. rapa* turnip tuber formation *in vitro*
- To answer whether high sucrose concentration can induce turnip formation like potato?
- To identify the effect of BAP hormone and sucrose on the following genes in VT-117 turnip accession *in vitro* condition:
    - Unique genes for turnip morphotype genotypes
    - Sucrose synthesase (SUS3) and transporter (SUT2)
    - Dwarf and delay flowering 1(DDF1)
    - Flowering locus T(FT)
    - Auxin responsible factor (ARF)

2. Methods and materials

2.1. Total Sugar and starch (Total Carbohydrate) test

Total Carbohydrate interims of Glucose, fructose, sucrose and starch content of 19 turnip accessions which taken from different geographical (Table 1) origin were tested by the microliter plate reader (enzymatic analysis) in Biochemical laboratory, Wageningen university. The reader measures the sugar by using light absorbance at 340nm. The accessions were taken from previous experiments which collected and stored in -20^{O}C (freeze dried) from field and greenhouse conditions. Sugar translocation was also assessed by using of 4 available turnips accessions(SM-17, FT-004, FT-086 & DH-VT-117) freeze-dried material which was stored at-20^{o}C. The samples were collected from five growth stages (1st = 30 days; 2nd = 45 days; 3rd = 60 days; 4th =75 days and 5th = 300 days after sowing) from leaf (young and old)) and turnip (inner (the core of the turnip) and outer (most of the peal)).

2.1.1. Glucose/Fructose/ Sucrose

Those of three sugars were measured with the Boehringer Manneheim kit (cat. No. 10 71 260 035) by the microtiter plate reader. It is enzymatic bioanalysis which used UV method. Freeze dried turnip Powder was weight (5mg) in the epje tube. It suspended in 1ml of 80% ethanol and incubated for 1 hour in a shaking water bath of 70^{O}C. It was vortex very well before, and after incubation. The centrifugation was done for 10 minutes at 13000rpm to get the supernatant. The supernatant was put into the new small centrifuge cap for glucose, fructose and sucrose test. The microtiter plate was divided into five parts (air blank, glucose/fructose blank, sucrose blank, sample for sucrose and sample for glucose/fructose). Ten microliter of sample solution was taken to pipette in the microtiter plate according to kit guide (Appendix 1). The D-glucose concentration was determined before and after the β-fructosidase enzymatic hydrolysis of sucrose. D-glucose catalysis by hexokinase enzyme at pH 7.6 with adenosine-5-triphosphate (ATP) to glucose-6-phosphate (G-6-P) simultaneously adenosine-5-diphosphate formed. G-6-P is oxidized by nicotinamide-adenine dinucleotide phosphate ($NADP^{+}$) to gluconate-6-phosphate with formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) by the enzyme of glucose-6-phosphate dehaydrogenase. The amount of NADP formed is stoichiomertic to the amount of D-glucose which is measured by absorbance at 340nm. D-fructose was determined subsequently after determination of D-glucose by phosphoglucose isomerase (PGI). Hexokinase is also catalysis from D-fructose to fructose-6-phosphate (F-6-P). Subsequently, F-6-P converted to G-6-P by PGI. G-6-P reacts with NADP to form NADPH. Similarly as D-glucose, the amount of NADPH formed is stoichiometric to the amount of D-

fructose. The sucrose concentration was calculated from the difference in D-glucose concentrations before and after hydrolysis by β-fructosidase.

Table 1. *Brassica rapa subsp. rapa* accessions which used for sugar test with different country of origin, morphotype and population structure

Accessions	Gene bank ID	Origin	Mophotype	Population structure
FT-002	CGN06673	United Kingdom	Fodder turnip	European turnips
FT-004	CGN06678	Denmark	Fodder turnip	European turnips
FT-047	CGN06866	Russia	Fodder turnip	European turnips
FT-051	CGN07164	Russia	Fodder turnip	Asian turnips
FT-086	CG07223	Pakistan	Fodder turnip	Asian turnips
SM-15		Russia		
T-738V	738	Russia		
VT-007	CGN06710	Russia	Vegetative turnip	European turnips
VT-008	CGN06711	India	Vegetative turnip	European turnips
VT-009	CGN06717	Japan	Vegetative turnip	Asian turnips
VT-010	CGN06718	Hungary	Vegetative turnip	European turnips
VT-012	CGN06720	Japan	Vegetative turnip	Asian turnips
VT-013	CGN06721	Japan	Vegetative turnip	Asian turnips
VT-044	CGN06859	Soviet Union	Vegetative turnip	European turnips
VT-052	CGN07166	Netherlands	Vegetative turnip	European turnips
VT-053	CGN07167		Vegetative turnip	European turnips
VT-089	CGN10995	France	Vegetative turnip	European turnips
VT-115	CGN15199	Japan	Vegetative turnip	Asian turnips
VT-117	CGN15201	Japan	Vegetative turnip	Asian turnips
VT-120	CGN15210	Netherlands	Vegetative turnip	European turnips

2.1.2. Starch

Starch also measured by Boehringer Mannheim kit (Cat. No. 10 207 748 035) which is enzymatic bioanalysis. Starch measurement was started from weighting of 50mg samples in tubes. The samples were homogenized with 2X250µl 8MHCl (25%) and 2X1ml of DMSO. It incubated for 1 hour at 60^{O}C in

shaking water bath. The tubes with sample cooled down fast in ice and added 3ml H_2O; 0.8ml 5MNaOH and 3.7ml 0.1M citric acid buffer (pH 4.6; 1.05g/50ml citric acid.$1H_2O$ and 1.47g/50ml Na-citrate.$2H_2O$) while shaking with vortex. From tube 1ml of sample was taken and put in a new small centrifuge tube. It centrifuged for 10 minutes at 12000rpm. Twenty microliter samples were pipette with triplication into microtiterplate according to protocol (Appendix 2). Amyloglucosidase and H_2O were added to hydrolysis starch to D-glucose. The addition of hexokinase and glucose-6-phosphate dehdrogenase at pH 7.6 was formed D-glucose. D-Glucose is phosphorelated to D-glucose-6-phosphate (G-6-P) by adding of ATP and hexokinase which stimulate formation of ADP. In the presence of G-6-P-DH, D-Glucose-6-phosphate is oxidized by nicotinamide-adinine dinucleotide phosphate (NADP) to D-gluconate-6phosphate with the formation of reduced nicotinamide-adinine dinucleotide phosphate (NADP). The amount of NADP formed is stoichiomertic to the amount of D-glucose formed by hydrolysis of starch.

2.2. Tissue culture

2.2.1. Plant materials

Two accessions (VT115 and a commercial hybrid= red round turnip) of *B. rapa subsp. rapa L.* were collected from seed storage of plant breeding laboratory, Wageningen University, for testing of sucrose, medium and photoperiod. Five accessions (VT-115, VT-052, VT-012, FT-002 and YS-143) and one line were used for GA_3 and BAP testing which included different morphotype and origin (table 1). YS-143 was included from annual oil type (yellow sarson). Selection of accessions was based on: a) the quantity of sugars; b) previous study on turnip tuber formation *in vitro* condition; and c) uniform germination capacity. The seeds were sown in standard MS media with 2 % sucrose (MS2), Phytoblend agar 8 g/l and pH 5.7 for germination. The seeds were sterilized with 80% (v/v) ethanol for 2 minutes and then transferred to 1 % commercial sodium hypochlorite solution (15 minutes) containing 0.05% Tween20 as a surfactant. The seeds were washed (10 minutes) by sterilized demi-water. The seeds were sown in culture jars, containing 65ml of the medium. The jars sealed and then kept at $24^{O}C$ in long day (LD16) until the seedlings produced true leaves or two weeks.

2.2.2. *In vitro* culture

The experiment was conducted *in vitro* laboratory of plant Breeding, Wageningen University, to check the effect of sucrose, hormones (GA_3 and BAP), medium and photoperiods conditions on the turnip tuber formation. The experiment was divided into two parts because the 2^{nd} part was done based the result of the 1^{st} part. The 1^{st} part was testing of medium (standard MS-medium and tuber inducing medium),

photoperiods (long day and short day) and sucrose (0, 5, 6, 7 and 8%) concentrations. Tuber inducing medium is low nitrogen contain (10%). The photoperiods were long day (LD) with 16 hours light and short day (SD) with 8 hours light. The 2nd part was testing of BAP and GA_3 hormones. The GA_3 (0, 3.5, 7.5 and 11.5mg/l) and BAP (0, 5, 10 and 15mg/l) were applied with filter sterilization when the medium had 40-50^{O}C after autoclaving of it by 121^{O}C for 18 minutes. The plantlets were transferred to the treatments when the plantlets produced true leaves (two weeks after sowing).

2.3. Gene expression

2.3.1. Collection of samples

VT-117 accession was selected for gene expression due to early turnip tuber formation. VT-117 was germinated *in vitro* condition in MS2-medium. The plantlets transferred to 2% sucrose, 6% sucrose and 6% sucrose with 10mg/l BAP at a week after sowing. The samples were collected from turnip tuber (hypocotyl and tap root) and the whole shoot tissues at a week interval until 6 time points. The samples had 2 biological replications which each biological replication had 8 plants. There were 64 samples in total including biological replications. All samples were collected and immediately put into liquid nitrogen then store in -80^{O}C.

2.3.2. RNA extraction and cDNA synthesis

The samples were grinded by mortar and pestle. Some of the leaf samples were grinded by coffee grinder which had the highest quantity of sample (like leaf samples). Some samples were also grinded with extraction buffer which had small sample amounts, especially the early time points (1st and 2nd week's samples). The total RNA was purified by using RNeasy Mini Kit. After purified total RNA, the quantity and quality were checked by Nano-drop and gel, respectively. The total RNA also cleans up by using of Deoxyribonuclease I (Invitrogen) kit. cDNA was synthesized by using iScript™ cDNA synthesis Kit which used reverse transcriptase. The total RNA was diluted to 100ng/l±2 before cDNA synthesis in all samples (appendix 12).

The whole total RNA samples were amplified in qRT-PCR to check the DNA contamination; DNA only amplifies by qRT-PCR. The Ct value should be greater than 38 or non-signal if the RNA samples are no DNA contamination. It also checked on the gel which it couldn't show a band. Re-Dnase treatment was done on those samples which shown DNA contamination and re-RNA extraction also made to be sure free

from DNA contamination. Finally, all samples have been shown non-signal on qRT-PCR or on gel run. There was a chance to lose 50-95% RNA during re-Dnase treatment that why re-RNA extraction made.

2.4. Data analysis

Glucose, fructose, sucrose and starch were measured by microtiter plate reader. The microtiter plant reader was measured the amount of NADPH formed by means of light absorbance (UV) at 340nm. The amount of NADPH formed is stoichiometric to the amount of glucose, fructose and starch. The sucrose concentration was calculated from the difference of D-glucose concentration from glucose/fructose sample and glucose concentration from sucrose sample. The absorbance difference of glucose, fructose, sucrose and starch were calculated according to the following formula:

- $\Delta Abs_{glucose}$ = $sample_{gluc/Fru}$ (A_2-A_1)-$Blank_{glu/fru}$ (A_2-A_1)
- $\Delta Abs_{fructose}$ = $sample_{glu/fru}$ (A_3-A_2) – $Blank_{glu/fru}$ (A_3-A_2)
- $\Delta Abs_{sucrose}$ = ($sample_{suc}$ (A_2-A_1)- $Blank_{sucrose}$ (A_2-A_1)) – $Abs_{glucose}$
- ΔAbs_{starch} = sample(A_2-A_1)- reagent Blank(A_2-A_1)

A1= the 1st absorbance measurement at 3 minutes; A2 = the 2nd absorbance measurement at 15 minutes; A3 = the 3rd absorbance measurement at 15 minutes
$Blank_{gluc/fruc}$ = Blank Glucose and Fructose
ΔAbs = Absorbance change

The absorbance difference was changed to concentration of each sugar by the following formula:

$$concentration(C) = \frac{VXMW}{\varepsilon XdXvX1000}\ X\ \Delta Abs$$

$$light\ path(d) = \frac{V}{surface}$$

Surface= Πr^2 ; Diameter =0.66cm; Surface = 0.342cm^2

V= final volume (cm^3); MW= molecular weight
d= light path ; v= sample volume (ml)
ε = extinction coefficient of NADPH at:
340nm=6.3[lxmmol^{-1}xcm^{-1}]
Hg 365nm= 3.5[lxmmol^{-1}xcm^{-1}]
Hg 334nm = 6.18[lxmmol^{-1}xcm^{-1}]

$d = \frac{0.304cm3}{0.342cm2} = 0.889cm(Fructose)$ $\quad$ $d = \frac{0.302cm3}{0.342cm2} = 0.883cm(Glucose\ and\ Sucrose)$

$Cglucose = 0.9781X\Delta Absglu(\frac{mg}{ml})$ $\quad$ $Cfructose = 0.9779X\Delta Absfru(\frac{mg}{ml})$

$Csucrose = 1.8583X\Delta Abs\ suc(\frac{mg}{ml})$ $\quad$ $Cstarch = 0.4397Xa??A(\frac{mg}{ml})$

The concentration was changed to g/100g of freeze dried sample:

$$Sugar\ content = \frac{Sugar\ concentration\left(\frac{g}{l}\right)}{Sample\ weight\ \left(\frac{g}{l}\right)} X100(\frac{g}{g})$$

The *in vitro* culture had two parts separately. The 1st part was set up in a completely randomized design with four main factors: photoperiod (long and short day), medium (standard MS-medium and tuber inducing medium) sucrose (0, 5, 6, 7, and 8%) and accessions (VT-115 and RRT). The experiment had 4 replications in 2 accessions (VT-115 and red round turnip). The 2nd part was set up in a completely randomized design with 2 main factors: hormone (GA_3 or BAP) and accessions. It had 5 replications in 6 accessions (VT-115, VT-012, VT-053, VT-052, FT-002 and YS-143) and a line (DHVT-117). The data were destructive (turnip tuber weight, diameter, length and shoot biomass) and non-destructive measurements (leaf number (dead and alive); turnip tuber diameter scale (table 2)). Turnip diameter (thickness) scale was observed a weekly interval from 4 (for RRT due to late germination) to 8 weeks and the rest parameters such as number of leaf (alive and dead), fresh shoot biomass, turnip tuber weight, length and diameter were recorded around 2 months after transplanting of the plantlets. Finally, the data were analysed by SPSS Statistics version 19.0 computer software using general liner model univariate and the Duncan's Multiple Range Test at α 0.05.

Table 2. Turnip diameter (thickness) scale

Scale(x)	Turnip thickness	Scale(x)	Turnip thickness
1.	X < 5mm	2.	5mm ≤ X < 10mm
3.	10mm ≤X < 15mm	4.	15mm ≤ X < 20mm
5.	≥ 20mm		

The gene expression data were analysed by relative gene expression manner. The relative gene expression was changed to log10 to see the expression in fold changes.

$\Delta Ct = Ct_{sample\ genes} - Ct_{reference\ gene}$ Relative Gene Expression (RGE) = $2^{(-\Delta Ct)}$

3. Results

3.1. Sugar content and translocation

3.1.1. Total sugar and starch content

Total sugar (Glucose, fructose and sucrose) and starch content were measured from mature turnip tubers grown in field and greenhouse. The total sugar and starch content of 19 turnip accessions were measured, in which12 accessions were grown in both field and greenhouse grown condition . The total sugar content of the rest seven accessions was also measured which the sample was collected only from greenhouse or field condition (Appendix 5). Total sugar and starch content were higher in the greenhouse than field grown turnip plants in all accessions Except for VT-010, a European turnip (Figure 2). Sucrose content was lower when compared to glucose, fructose and starch in all accessions in greenhouse and field condition except VT-008 and VT-53.

There was a significant difference of sugar content found between turnips grown in the greenhouse and in the field, for all tested accessions (P=0.000). In greenhouse condition the maximum concentrations were: glucose contents in VT-010 (28.09g/100g), Fructose contents in VT-010 (31.46g/100g), sucrose contents in VT-053 (29.90g/100g), and starch contents in FT-051 (24.06g/100g). In field condition, the maximum concentrations were: glucose contents also in VT-010 (33.51g/100g) and sucrose contents in VT-053 (23.39g/100g) but fructose contents in VT-004 (17.84g/100g) and starch content in VT-010 (20.84g/100g).

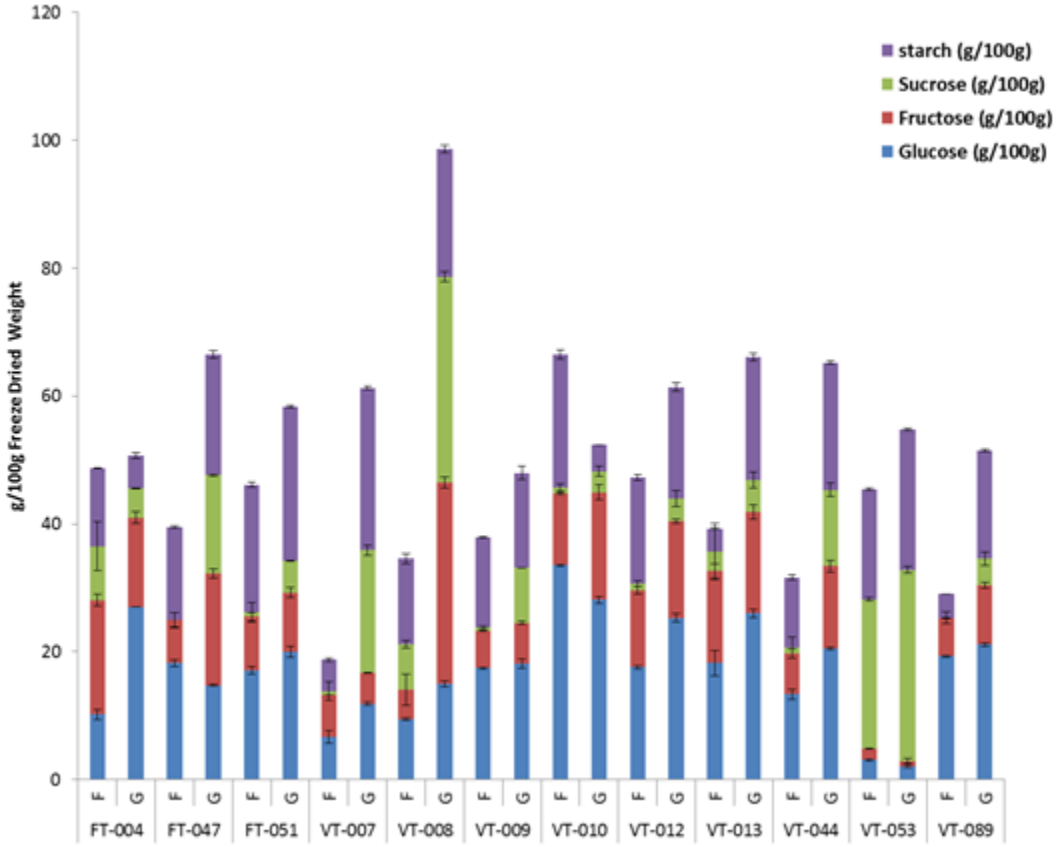


Figure 2. Glucose, fructose, sucrose and starch content in turnip accessions which grown in field (F) and greenhouse (G) conditions.

Correlation

Fructose was negatively correlated to sucrose and starch in greenhouse grown turnip accessions. In field conditions also fructose was negatively correlated with sucrose but positively correlated to starch in field grown turnips (Table 3). Sucrose and starch were positively correlated in greenhouse and field grown turnip genotypes.

Table 3. The correlation between glucose, fructose, sucrose and starch in the field (lower side) greenhouse turnip grown genotypes

Field \greenhouse		glucose	fructose	sucrose	starch
glucose	Pearson Corr.	1	0.444**	-0.843**	-0.273*
	Sig. (1-tailed)		0.000	0.000	0.023
fructose	Pearson Corr.	0.414**	1	-0.031	-0.052
	Sig. (1-tailed)	0.004		0.411	0.355
sucrose	Pearson Corr.	-0.507**	-0.225	1	0.288*
	Sig. (1-tailed)	0.001	0.084		0.017
starch	Pearson Corr.	-0.214*	0.125	0.195	1
	Sig. (1-tailed)	0.095	0.223	0.117	

**Correlation is significant at the 0.01 level (1-tailed). *Correlation is significant at the 0.05 level (1-tailed) Correlation greenhouse (lower) and field (upper). Pearson Corr. = Pearson Correlation

3.1.2. Sugar Translocation

Four turnip accessions (SM-17, FT-004, FT-086 & DH-VT-117) were grown in the greenhouse. Samples were collected from those four accessions for studying glucosinolate metabolism in different growth stages and organs. Figure 3 shows the profile of total sugar content in leaf and tuber tissues in four turnip accessions in different growth stages. It has three main groups; sucrose, fructose and glucose clustered separately. Sucrose and fructose were almost constant throughout of all growth stage and higher in turnip tuber compared to leaf tissues. Glucose content increased from 1st growth stage until the 3rd and decrease after 4th stage in tuber tissues. In leaf tissue glucose content was almost constant throughout of growth stages.

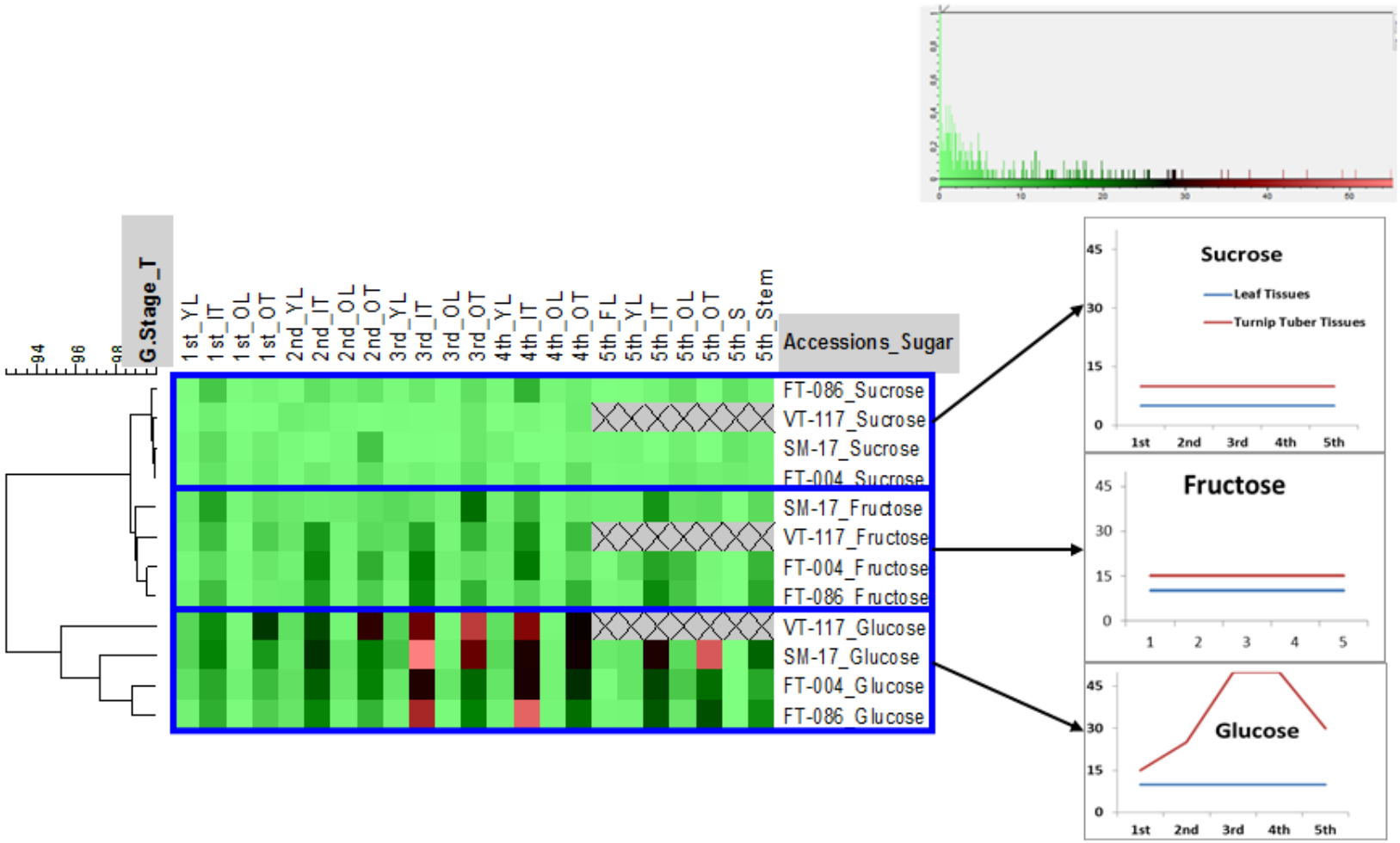


Figure 3. profile of sucrose, glucose and fructose content in four turnip accessions in different time point or different stages (XX indicated as the data missed because the VT-117 decayed by rotten; X-axis indicate growth stage ; 1st=1 month after sowing; 2nd, 3rd & 4th time points had 15 days interval; 5th time point was 9 months after sowing; OL=old leaves; FL=Flower; YL=Young leaves; IT=inner turnip; OT=outer turnip; G.Stage_T= growth stage and tissues; Y-axis shows turnip accessions with sucrose, glucose and fructose content).

Total sugar (glucose, fructose and sucrose) was lower in leaf tissues compared to tuber tissues in all four turnip accessions which were lower than 10g/100g freeze dry weight in all growth stages. The sucrose content was even lower than 5g/100g of freeze dry weight in leaf tissues compared to tuber tissues.

In VT-117, the glucose and fructose content were less than 5g/100g in leaf tissues. In root tissues, glucose and fructose showed an increase with increase of time growth stage except at a 4th growth stage after sowing. Sucrose content was less than 5g/100g at all-time points in all treatments in VT-117 (Appendix 6).

In SM-17 glucose and fructose content was lower than 10g/100g in leaf tissues compared to turnip tuber tissues in development stage depending manner. The sucrose content was less than 2g/100g in young and old leaves. Inner and outer turnip the glucose and fructose content were increased up to 55g/100g. The sucrose content was increased until 9g/100g in the outer root at 45 days after sowing. The sucrose content was also increased in the inner root up to 6.5g/100g until 30 days after sowing. After 30 days of sowing, the sucrose content decreased until 300 days after sowing (Appendix 6).

In fodder type turnips (FT-004 and FT-086) the glucose content was less than 5g/100g in young and old leaf tissues. The glucose content also showed an increasing until 75 days but at 300 days, it decreased in inner and outer part of the turnip. The fructose content was also less than 5g/100g in leaf tissue except at 300 days after sowing in old leaf tissues. The sucrose content was less than 2g/100g in leaf tissues in all growth stages except at 300 days in old leaf tissues (Appendix 6).

3.2. *In vitro* culture

The effects of photoperiodic regime, sucrose level, medium and growth regulator hormones (GA_3 and BAP) on morphogenic and genetic responses of *Brassica rapa subsp. rapa were evaluated*. The accessions were selected based on, previous *in vitro* sucrose test study, endogenous sucrose contents and germination percentage (Figure 4). VT-053, VT-117, VT-115, VT-012 FT-002 and VT-052 accessions were selected based on total sugar content which was comparable endogenous sugar content in greenhouse & field. YS-143 (oil type) and Red Round Turnip (Chinese commercial used) also included in this study. Unfortunately, VT-053 was selected to *in vitro* culture study but the results were excluded due to very low germination percentage (Figure 5). Red round turnip (RRT) was late germinated which transplanting one week later than VT-115 accession.

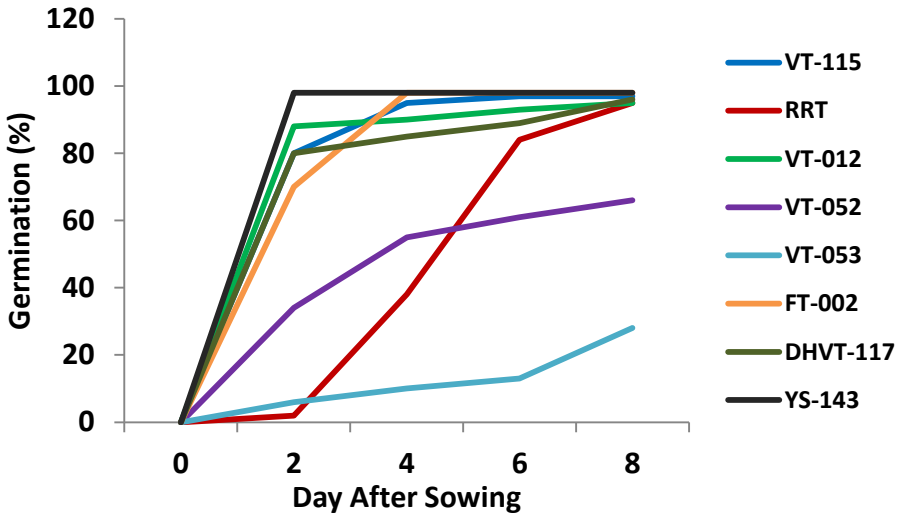


Figure 4. Germination percentage in MS-2 *in vitro* grown turnip accessions.

3.2.1. Interaction sucrose, medium and photoperiod

Turnip tuber formation was affected when turnip grown in different sucrose, medium and photoperiod. Specially, sucrose and photoperiod have high interaction to form turnip tuber. The turnip tuber formation also depended on accession. Some accessions were early to tuber formation and some other accessions were late. VT-115 (accession) and red round turnip (RRT; Chinese commercial used turnip) were used for testing of sucrose, medium and photoperiod.

Turnip tuber diameter scale (thickness)

The turnip tuber diameter scale was semi-quantitative as plantlets were not removed from the tissue culture containers and measured at weekly intervals until 4 or 5 weeks after transplanting. At the end of the experiment real diameter (quantity) was measured (see below turnip tuber diameter). Turnip tuber thickening was clearly affected in short day compared to long day condition. Turnip tuber was also stimulated in 6% sucrose contain Standard MS-medium.

Turnip tuber thickness (≥5mm) was scored from the 2nd week after transplanting from sowing on medium (MS2) under long day condition in both VT-115 and red round turnip. In all treatments under long day condition, turnip tuber thickening started at 2nd week after transplanting except turnip grew on medium without sucrose or high sucrose (8%) under tuber inducing medium (<5mm). Turnip tuber thickening started at 3rd week after transplanting when turnip grew on 8% sucrose with the tuber inducing medium. No turnip tuber thickening was observed on both media (standard MS-medium and tuber inducing medium) without sucrose until the 5th week after transplanting in short and long day conditions. Under short day condition, all treatments didn't show any turnip tuber thickening until 5th weeks after transplanting. Late turnip tuber thickening only observed when VT-115 was grown on 6% sucrose contain standard MS-medium in short day condition. Turnip tuber thickening of red round turnip (RRT) started in standard MS-medium without sucrose at 3rd week in a long day. But tuber inducing medium (TIM) without sucrose content didn't show any thickness until the 5th week after transplanting under long day condition (Figure 5).

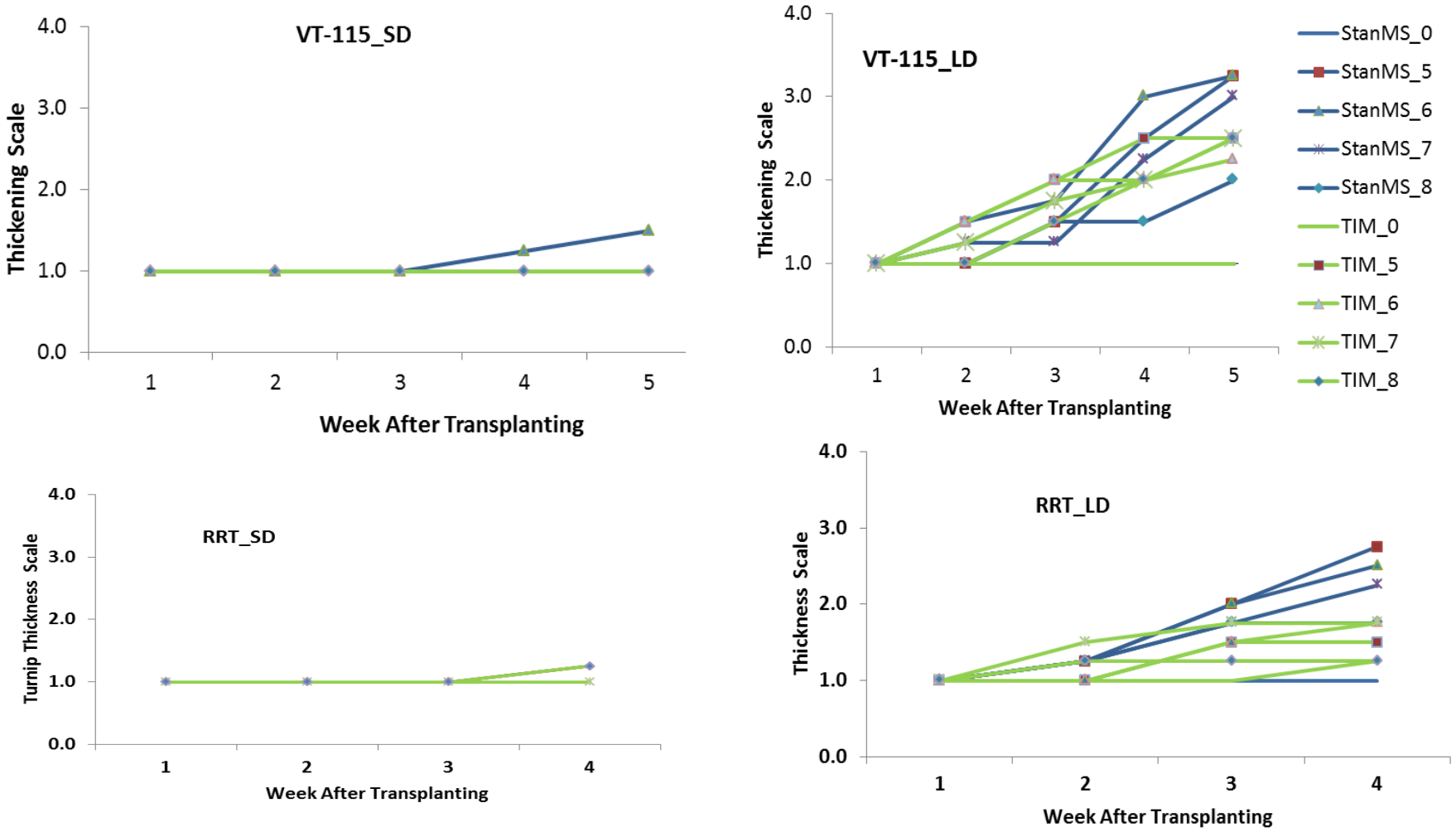


Figure 5. Effect of sucrose, medium and photoperiod on turnip tuber diameter (thickness) with a week interval in VT-115 (upper) and the RRT (lower) accessions *in vitro* condition (SD=short day; LD=long day; RRT= Red Round Turnip; StandMS=standard MS-medium; TIM=Tuber inducing medium; number behind medium type indicates sucrose concentration in percentage, Turnip thickness scale: 1<5mm; 5mm≥2<10mm; 10mm≥3<15mm; 15mm≥4<20mm; 5≥20mm).

Turnip tuber weight

Turnip tuber weight growth was depending in sucrose and nitrogen contains medium as well as photoperiod. Turnip tuber weight was higher on 5 and 6% sucrose concentrations contain standard MS-medium on the long day condition (Figure 6).

Turnip tuber weight showed that a significant difference between VT-115 and RRT (P=0.004). There was a significant differences effect on turnip tuber weight in two way interaction of Sucrose vs. photoperiod (P=0. 000) and Medium vs. photoperiod (P=0. 002) in both VT-115 and RRT. But the sucrose X medium effect was significant (P=0. 022) in VT-115 and a non-significant (P=0. 203) in RRT. The maximum mean of turnip tuber weight was observed from a standard MS-medium with 6% sucrose (3.94g in VT-115; 2.06g in RRT); the second highest tuber weight was observed in standard MS-medium with 5% sucrose (3.92g in VT-115; 1.65 in RRT) sucrose in long day condition.

Turnip tuber length

Some turnip accessions have long tubers at natural condition. The turnip tuber length was not clearly affected by sucrose concentration, medium and photoperiod. It was difficult to define turnip tuber length in short day grown turnips. As above described, turnip tuber thickness was not observed in short day condition. It was better to call hypocotyl length rather than tuber length for short day grew turnip plants. However, I called turnip tuber length for both condition to not make confusion. Comparing within long day condition, the maximum average turnip length was observed for VT-115 grew on 6% sucrose tuber induced medium and for RRT grew on 6% standard MS-medium (Appendix 7).

Turnip tuber diameter

Turnip tuber diameter was inhibited in short day condition (Figure 6). Tuber diameter was higher in standard MS-medium compared to tuber inducing medium. There was a significant difference between turnip tuber diameter (P=0.011) in the interaction of sucrose, medium and photoperiods within VT-115. A non-significant between turnip tuber diameter in turnip grew on sucrose, medium and photoperiods interaction (P=0. 281) in RRT. The maximum mean turnip tuber diameter was observed in tuber inducing medium with 5% sucrose (13.83mm) in VT-115 and standard MS-medium with 5% sucrose concentration (13.38mm) in the RRT under long day condition.

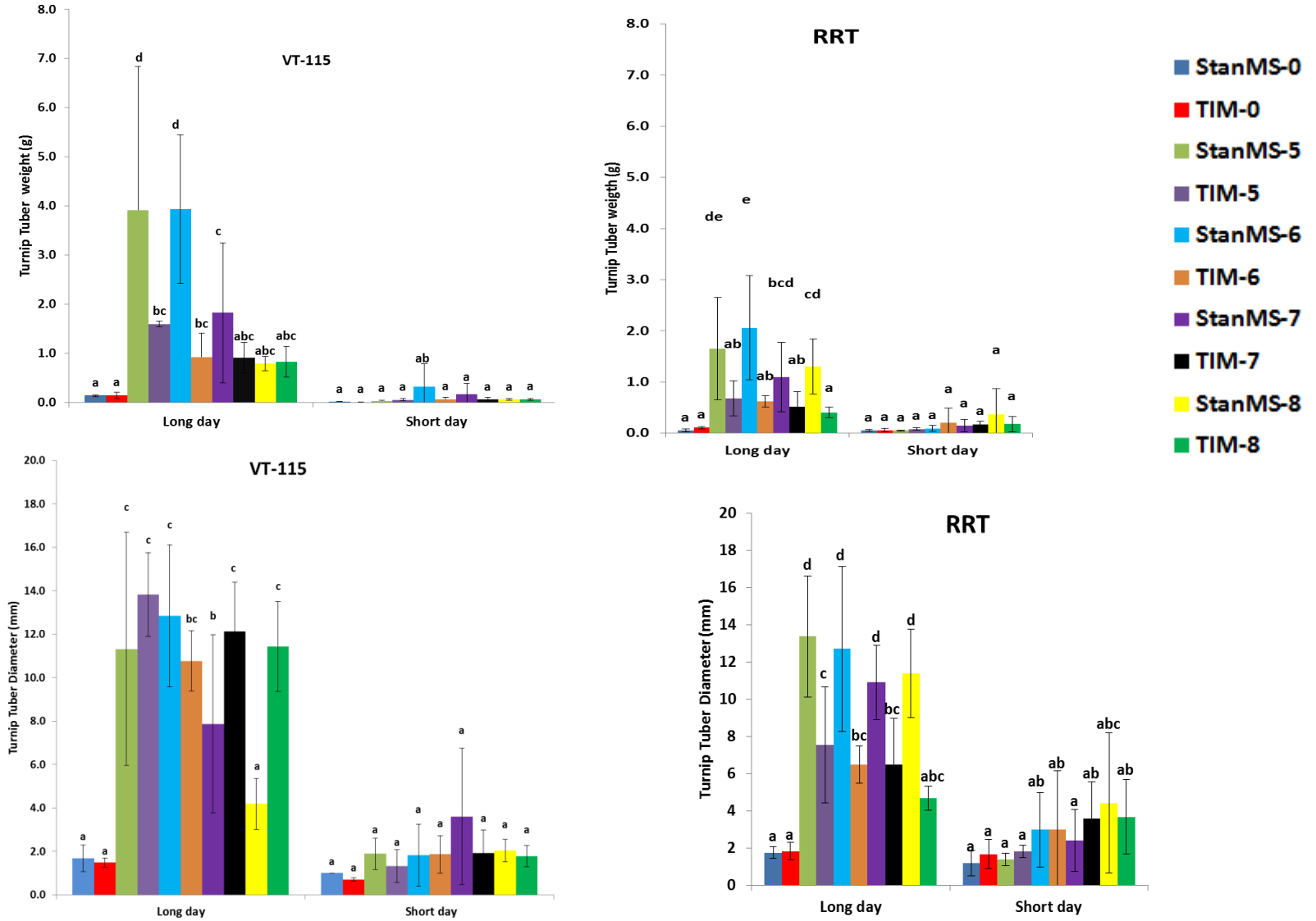


Figure 6. Effect of medium, sucrose and photoperiod in turnip tuber weight (upper) and diameter (lower) *in vitro* grown VT-115 and RRT accessions in 55 days after transplanting (the bar shows the standard deviation; the letters above the standard deviation is significant (all over the treatments) difference at α=0. 05; similar letter shows non-significant; different letter shows a significant difference; TIM= tuber inducing medium; StanMS= standard MS medium; the number shows the sucrose concentration percentage per liter).

Fresh shoot biomass

Fresh shoot biomass includes leaf. Leaf is the power sources of energy. Sugar or energy (photosynthesis) is produced in the leaves and transport to non-photosynthetic region. Sugar is transport interims of sucrose or hydrolysis of sugar (starch) or other reduced sugar. High average fresh shoot biomass was observed in turnip grew on 5% sucrose contain standard MS-medium under long day conditions in both VT-115 and RRT. A significant difference between fresh shoot biomass was observed (P=0.036 in VT-115; P=0.045 in RRT) in the interaction of sucrose, medium and photoperiod. The maximum mean weight of fresh shoot biomass has been observed in standard MS-medium with 5 and 6% sucrose under long day condition within VT-115 (Table 4). Standard MS-medium with 6% & 7% under long day condition has been shown the maximum fresh shoot biomass in RRT.

Leaf number

The numbers of alive and dead leaves were counted separately at the end of the experiment (41 days after transplanting). The maximum averages alive numbers of leaves were observed in all sucrose concentration in short day condition except medium without sucrose concentration in VT-115. Similarly, in RRT also higher alive number of leaf was observed in short day than long day in all sucrose concentration and both media except higher concentration (7% & 8%) sucrose in tuber inducing medium. However, no significant difference was observed between alive number of leaves in interaction of sucrose, medium and photoperiod (P=0.702) in VT-115; while a significant difference (P=0.024) showed in RRT. Higher number of dead leaves were observed in long day compared to short day condition in different sucrose concentration in both media (Table 4).

Table 4. The mean number of leaves and weight of fresh shoot biomass in two mediums with different sucrose concentration in short and long day condition at 55 days after transplanting of the plantlets (StanMS=standard MS-medium; TIM= tuber inducing medium)

Sucrose (%)	medium	photoperiod	The mean of alive leaf number ±std. deviation		Mean of dead leaf number ±std. deviation		Mean of fresh shoot biomass (g) ±std. deviation	
			VT-115	RRT	VT-115	RRT	VT-115	RRT
0	StanMS	Long day	6.0±0.0	6.0±3.0	8.0±1.0	4.0±2.0	2.3±0.5	2.6±0.6
		Short day	0.0±0.0	6.0±0.0	4.0±0.0	2.0±2.0	0.1±0.0	0.9±0.2
	TIM	Long day	6.0±0.0	5.0±1.0	7.0±5.0	5.0±1.0	2.5±0.7	2.5±0.5
		Short day	0.0±0.0	3.0±2.0	4.0±0.0	2.0±3.0	0.1±0.2	0.5±0.3
5	StanMS	Long day	6.0±0.0	8.0±2.0	6.0±0.0	2.0±1.0	5.6±1.0	3.7±1.3
		Short day	12.0±3.0	6.0±1.0	1.0±2.0	1.0±1.0	1.8±0.6	0.8±0.4
	TIM	Long day	3.0±0.0	4.0±0.0	5.0±1.0	5.0±0.0	1.1±0.4	1.4±0.3
		Short day	8.0±1.0	6.0±1.0	4.0±1.0	2.0±2.0	1.3±0.6	1.3±0.3
6	StanMS	Long day	7.0±2.0	6.0±2.0	6.0±1.0	3.0±1.0	5.1±1.1	2.5±1.4
		Short day	10.0±6.0	6.0±2.0	1.0±1.0	0.0±0.0	3.8±2.9	1.1±0.3
	TIM	Long day	5.0±1.0	4.0±3.0	5.0±3.0	4.0±0.0	1.1±0.1	1.2±0.8
		Short day	6.0±3.0	7.0±2.0	6.0±1.0	2.0±1.0	1.2±0.4	1.4±0.4
7	StanMS	Long day	8.0±3.0	6.0±1.0	4.0±1.0	3.0±1.0	4.8±0.6	2.9±0.7
		Short day	11.0±3.0	6.0±1.0	2.0±0.0	1.0±0.0	2.2±1.3	1.1±0.3
	TIM	Long day	4.0±0.00	6.0±2.0	6.0±0.0	4.0±2.0	1.4±0.1	1.5±0.2
		Short day	5.0±3.46	3.0±3.0	6.0±4.0	4.0±2.0	0.7±0.5	1.0±0.8
8	StanMS	Long day	8.0±2.0	8.0±0.0	4.0±0.0	2.0±0.0	3.0±0.4	2.6±0.7
		Short day	9.0±2.0	8.0±2.0	2.0±0.0	1.0±10	1.4±0.8	1.4±0.9
	TIM	Long day	3.0±0.0	3.0±2.0	6.0±1.0	4.0±1.0	1.0±0.2	1.1±0.7
		Short day	5.0±2.0	2.0±2.0	6.0±1.41	3.0±1.0	1.1±0.1	0.6±0.6

Correlation

The number of dead and alive leaves correlated with the turnip formation in VT-115 genotype and RRT. Dead leaf was positively correlated with turnip tuber weight and width in both accessions but a negatively correlated with turnip tuber length in RRT and positive in VT-115. In contrast, the alive leaf number was negatively correlated with turnip tuber length, width and weight in VT-115 (Table 5). This might indicate that when turnip formation increases, the nutrients flow from leaves to the storage organ (turnip tuber) in VT-115. The number of alive leaves in RRT was positively correlated with turnip tuber weight, length and diameter.

Table 5. The correlation between parameters in *vitro* grown VT-115 and RRT genotypes 55 days after transplanting

VT-115 \ RRT		dead leaves	Tuber weight (g)	Tuber length (mm)	Tuber diameter (mm)	alive leave no.	Fresh shoot biomass (g)
dead leaves	Pearson Correlation	1	0.120	-0.022	0.133	-0.359**	0.128
	Sig. (1-tailed)		0.288	0.845	0.240	0.001	0.258
Tuber weight (g)	Pearson Correlation	0.202	1	0.451**	0.915**	0.212	0.609**
	Sig. (1-tailed)	0.072		0.000	0.000	0.059	0.000
Tuber length (mm)	Pearson Correlation	0.263*	0.178	1	0.327**	0.125	0.180
	Sig. (1-tailed)	0.018	0.115		0.003	0.271	0.110
Tuber diameter (mm)	Pearson Correlation	0.236*	0.756**	0.239*	1	0.214	0.604**
	Sig. (1-tailed)	0.035	0.000	0.033		0.057	0.000
alive leaves no.	Pearson Correlation	-0.507**	-0.010	-0.078	-0.137	1	0.547**
	Sig. (1-tailed)	0.000	0.927	0.489	0.227		0.000
Fresh shoot biomass (g)	Pearson Correlation	-0.015	0.672**	-0.023	0.341**	0.352**	1
	Sig. (1-tailed)	0.893	0.000	0.843	0.002	0.001	

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level (upper part for RRT & lower side for VT-115)

3.2.2. Hormones (GA_3 and BAP)

Like sucrose medium and photoperiod test, hormones test also had destructive (turnip tuber diameter scale) and non-destructive (turnip tuber weight, length and diameter) measurement. For destructive measurement, harvesting time was depended on turnip accession. For instance, DHVT-117 was started early turnip thickening. It also harvested before the turnip tuber starting rotten in the medium (Figure legend 8 & 10).

Turnip tuber diameter scale (thickness)

Figure 7 shows the effect GA_3 and BAP on the turnip tuber thickness in 6 turnip accessions during growth stages. Turnip tuber thickening was clearly affected by exogenous BAP application, while the effect of GA_3 was less clear. DHVT-117 has been shown that turnip tuber thickness (≥5mm) within a week after transplanting on medium without hormones and 11.5mg/lGA_3. Turnip tuber thickness was less than 5mm in medium contain 15mg/l and 10mg/l BAP until 7 and 5 weeks after transplanting, respectively in DH-VT-117. VT-115 shows a turnip tuber thickening at the 2nd week after transplanting in all GA_3 concentration treatments but no turnip tuber thickening was observed in BAP concentration.

VT-052 was shown a turnip thickness (≥5mm) on week 4 in without GA_3, week 5 in 7.5mg/l GA_3 and week 7 in 11.5mg/l GA_3 and without BAP concentration. FT-002 was a late turnip tuber thickness (≥5mm) forming accession as the thickness started at 6th and 7th week. YS-143 accession didn't show a turnip tuber thickening (<5mm) until 8 weeks after transplanting; it shows straight line for all BAP and GA_3 concentrations (Figure 7).

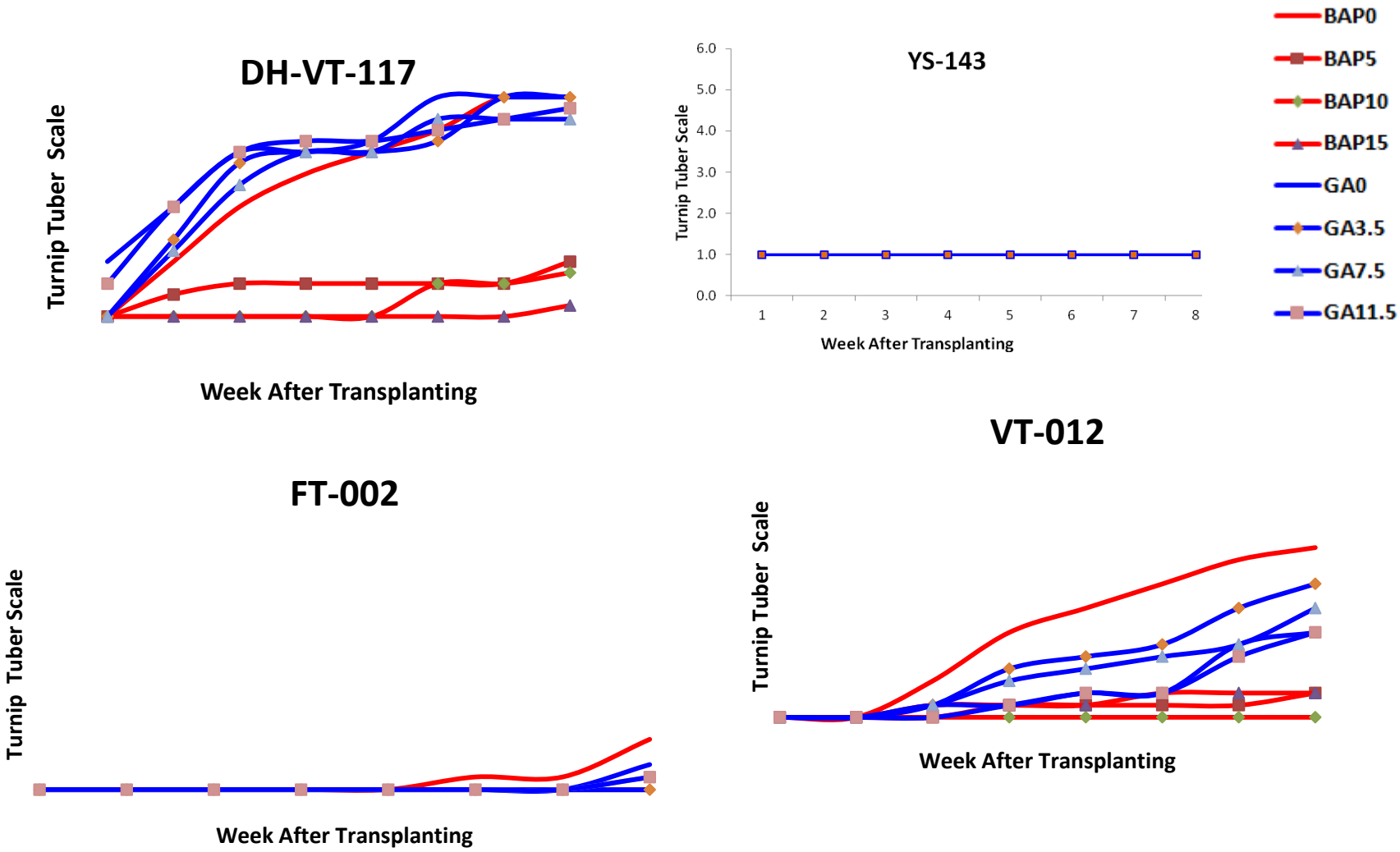
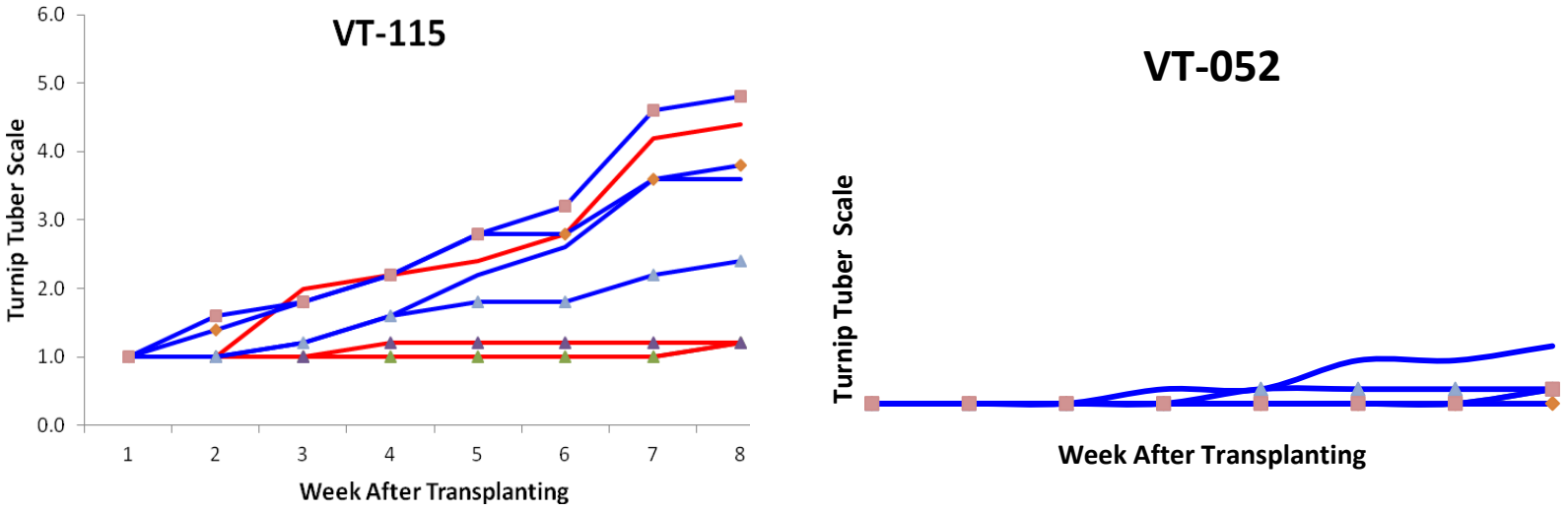


Figure 7. Effect of GA_3 and BAP hormones on turnip thickness in *vitro* grown 6 turnip accessions under *in vitro* condition in a week interval (scale: 1< 5mm; 5mm≥2<10mm; 10mm≥3<15mm; 15mm≥4<20mm; 5≥20mm); YS-134, FT-002 & VT-052 were look like only GA_3 treated but BAP treated plants were showed turnip tuber thickening scale 1.



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Continue Figure 7. Effect of GA_3 and BAP hormones on turnip tuber thickness of 6 turnip accessions under *in vitro* condition in a week interval (scale: 1< 5mm; 5mm≥2<10mm; 10mm≥3<15mm; 15mm≥4<20mm; 5≥20mm); YS-134, FT-002 & VT-052 were look like only GA_3 treated but BAP treated plants were showed turnip tuber thickening scale 1.

Gibberellic acid (GA_3) effect

Application of exogenous GA_3 didn't show a clear effect on turnip tuber diameter, length and weight. Turnip tuber as well as growth didn't inhibit with and without GA_3 concentration medium. The morphological growths of turnip with and without GA_3 concentration also look like normal; no morphological difference observed between with, and without GA_3 grown turnip plant (Appendix 8).

No significant difference was observed between turnip tuber diameter in turnip grown on GA_3 concentration for DHVT-117 (P=0.727), YS-143 (P=0.417), VT-115 (P=0.058) VT-052 (P=0.055) and FT-002 (P=0. 658) accessions. However, turnip tuber diameter was decreased when GA_3 concentrations increased in DHVT-117 and VT-012. VT-012 has been shown a significant (P=0.012) difference between turnip tuber diameter in GA_3 concentrations, while the significant was only without and high concentrations of GA_3 (Figure 8).

Turnip tuber length may help to compare the nature growth of turnip accessions to *in vitro* conditions. Some turnip accessions have long tuber length naturally. Turnip tuber length was increased when the GA_3 concentrations increased in DHVT-117, VT-012, VT-052, FT-002 and YS-143 (Figure 8). It was decreased at the concentration of 11.5mg/l GA_3 in VT-012 and VT-053. Turnip tuber length also has been shown a non-significant difference when turnips are grown on different GA_3 concentrations in DHVT-117 (P=0.181), YS-143 (P=0.071) and VT-052 (P=0.330). The significant of turnip tuber length was observed in VT-115 (P=0.004), VT-012 (P=0.001) and FT-002 (P=0.023) accessions.

Turnip tuber weight was not inhibited or induced when turnip grown in GA_3 contain medium. No significant difference was observed between turnip tuber weight in different GA_3 concentrations within all accessions (Figure 8); DHVT-117 (P=0.913), VT-115 (P=0.829), YS-143 (P=505), VT-012 (0.056), VT-052 (P=0.437) and FT-002 (P=0.291).

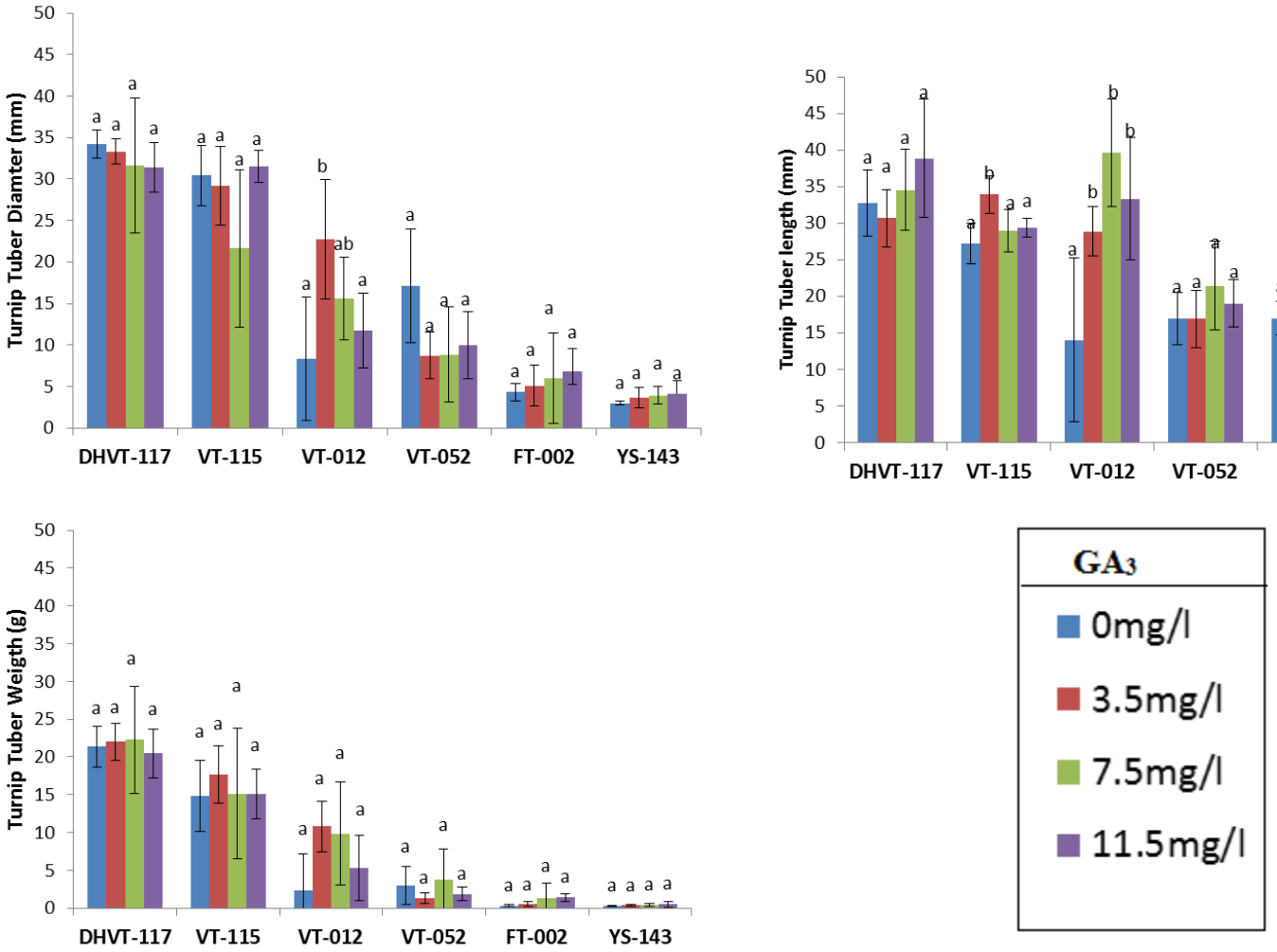

Figure 8. The effect of GA_3 concentration *in vitro* grown condition in 69 days (DHVT-117), 79 days (VT-115& FT-002), 84 days (VT-012), 82 days (VT-052) and 72 days (YS-143) days after transplanting (The bar indicated the standard deviation; letter above the bar indicated the significant difference within genotype α=0.05 and the signifancant was within accessions).

6-Benzylaminopurine (BAP)

Application of exogenous BAP was clearly inhibited turnip tuber diameter, length and weight as well as turnip development (Figure 10). Morphologically, turnip grown with and without BAP contain a big different was observed. A turnip growth was dwarf in all BAP concentration contain medium (Appendix 8). High callus formation was also observed in different BAP concentrations contain medium (Figure 9). Callus formation was higher in 10mg/l when compared to 5mg/l and 15mg/l BAP concentrations. Turnip tuber diameter and weight were decreased when BAP concentration increased in DHVT-117. Significant difference (P=0.000) between turnip tuber diameter, length and weight was observed in all accessions with BAP interaction. Maximum turnip tuber diameter and weight were observed in medium without BAP concentration in VT-115, VT-012, VT-052, FT-002 and YS-143; (Figure 10). The maximum turnip tuber length was also observed in medium without BAP in all accessions except YS-143. The maximum turnip tuber length was observed in DHVT-117 and YS-143 when compared to other accessions.

Figure 9. Callus formation in DHVT-117 when grew on BAP contain standard MS-medium under *in vitro* long day condition in 69 days after transplanting (left side = 5mg/l BAP; middle=10mg/l BAP and right side= 15mg/l BAP).

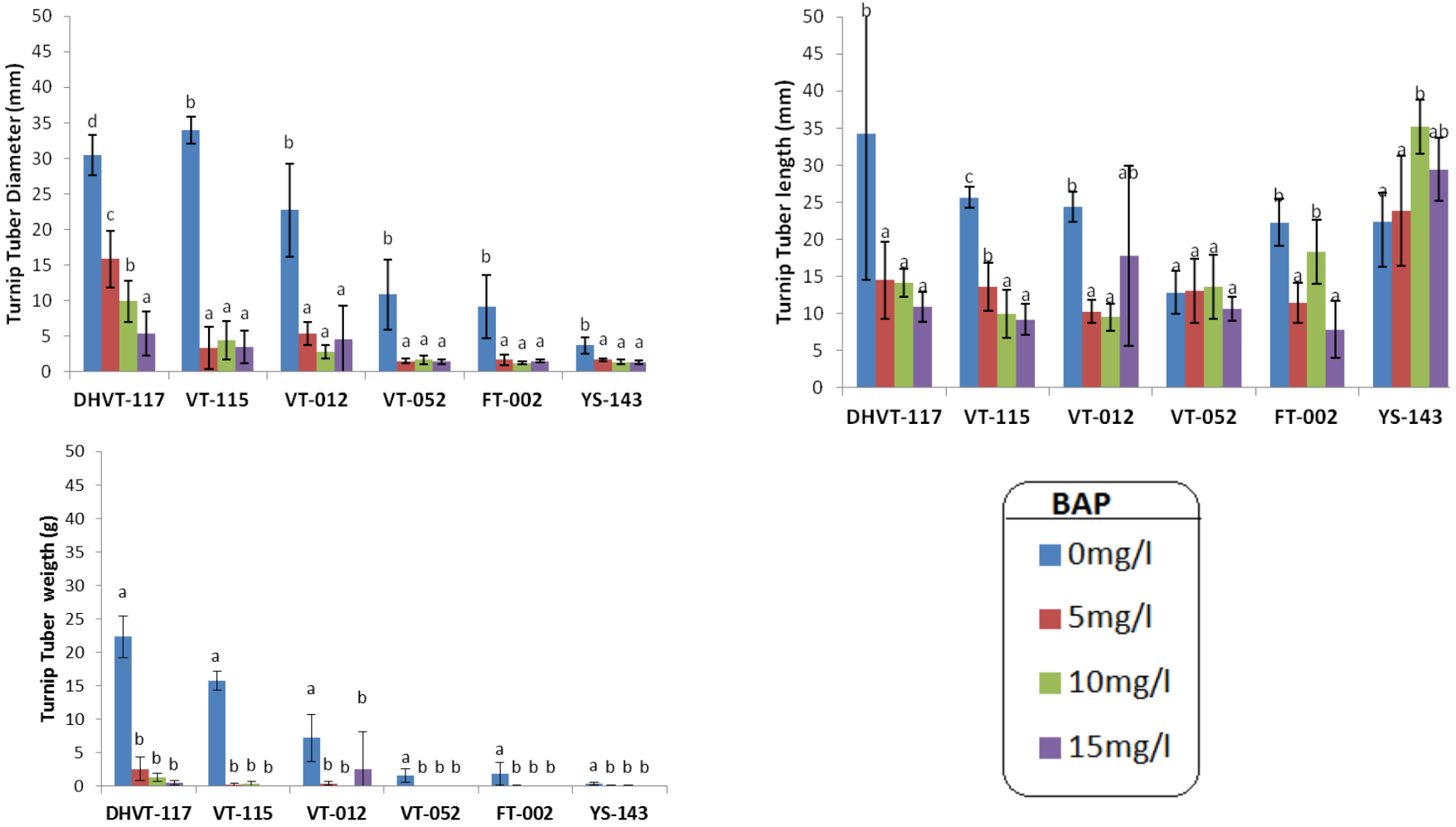

Figure 10. The effect of BAP concentration *in vitro* grown condition in 69 days (DHVT-117), 79 days (VT-115& FT-002), 84 days (VT-012), 82 days (VT-052) and 72 days (YS-143) after transplanting (the bar indicated the standard deviation; letter above the bar indicated the significant difference within accession α=0.05).

3.3. Gene expression

Gene expression study was made on medium contain 2 % sucrose, 6% sucrose with BAP and without BAP. This was selected based on the turnip formation effects *in vitro* condition. Turnip grew on medium contain BAP was inhibited turnip formation. The maximum turnip tuber formation was observed in 6% sucrose. Turnip grew on medium contain 2% sucrose was used as control which gives normal turnip growth and tuber formation.

3.3.1. Reference gene selection

Thirteen candidate reference genes selected on different environmental conditions in non-heading Chinese cabbage (Xiao *et al.*, 2012). Eleven candidate reference genes were tested on different time points and treatment samples from *Xiao et al.,* paper (Table 6). The eleven candidate reference genes were amplified on two qRT-PCR plates in 12 different samples. The first 6 samples which used to amplify the first qRT-PCR (Figure 11) for 8 selected reference genes from turnip grown in: (1) BAP contain medium from leaf tissues at time point 6; (2) BAP contain medium from tuber tissues at time point 6; (3) 6 % sucrose contain medium from leaf tissues at time point 2 (4) 6% sucrose contain medium from tuber tissues at time point 2; (5) 2% sucrose contain medium from leaf tissues at time point 2 and (6) 2% sucrose contain medium from tuber tissues at time point 2.

In the 2nd qRT-PCR amplification (Figure 11) also 6 samples were used which turnip grown in: (1) 2% sucrose contain medium from tuber tissues at time point 6; (2) 2% sucrose contain medium from leaf tissues at time point 6; (3) BAP contain medium from leaf tissues at time point 2; (4) BAP contain medium from tuber tissues at time point 2; (5) 6% sucrose contain medium from leaf tissues at time point 2 and (6) 6% sucrose contain medium from leaf tissues at time point 2. The qRT-PCR software (Bio-radiQ5) gives stability value (M) of the candidate reference genes. Figure 11 shows an average expression stability value of remaining reference genes at each step. During each step the least stable reference gene excluded. The rank starts from the least stable gene at the left and ending with the two most stable genes at the right (the genes are ranked according to increasing expression stability). High M value has low stability and vise verse. First, eight (figure 11 left side) reference genes were tested. As a result, PP2A and Cyp genes were the most stable reference gene. Second, those most stable (PP2A & Cyp) and other four reference genes (figure 11 right side) were tested. Again, PP2A and Cyp were the most stable genes in different *in vitro* samples with different time point (a week interval). However, Cyp had a higher gene expression or less Ct value when compared to PP2A (Table 6). So, PP2A candidate reference gene was selected to assess the expression of the *in vitro* grown turnip plant in different treatments.

Table 6. List of reference genes with mean, minimum, maximum and standard deviation of Ct value.

reference genes	Mean	Number of samples	Minimum	Maximum	Std. Deviation
18SrRNA	9.70	6	8.37	11.07	0.83
Actin-1	24.76	6	24.29	25.44	0.32
Actin-2	24.84	6	23.70	26.09	0.82
CYP	19.25	12	17.65	20.23	0.61
GAPDH	20.02	6	19.22	21.21	1.41
MDHF	19.51	6	16.23	22.71	2.03
PP2A	24.08	12	22.58	24.91	0.65
PPR	31.14	6	29.17	34.19	1.68
Tub_α	24.86	12	23.90	26.25	0.38
UBC18	24.42	6	23.10	25.73	1.22
UBQ	21.94	6	20.76	23.81	1.13

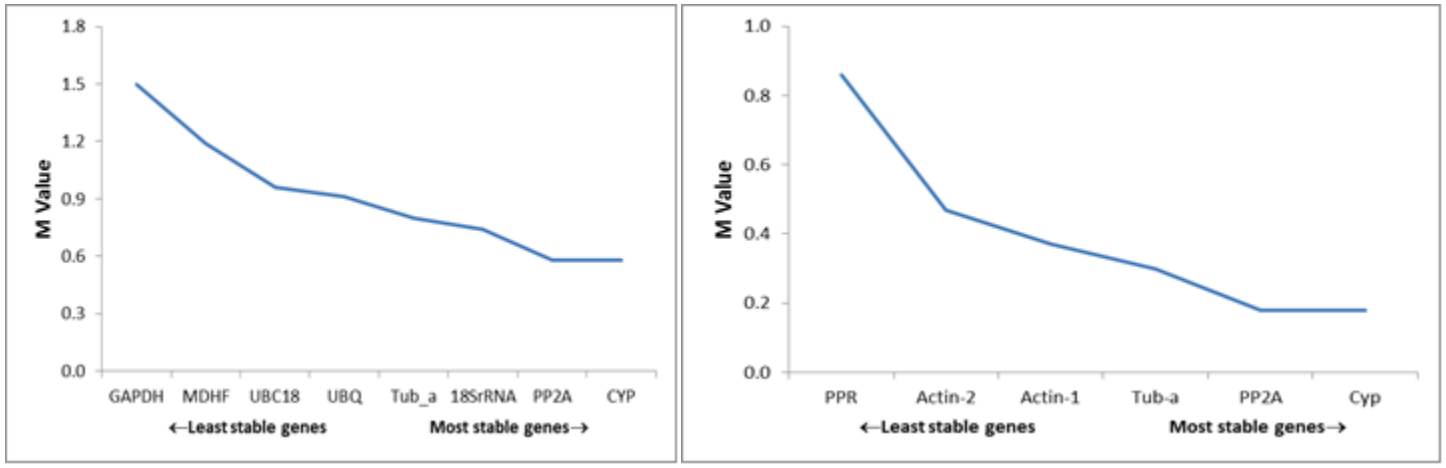

Figure 11. The average expression stability value (M) of the tested reference genes in vitro grown VT-117 turnip accession.

3.3.2. Gene expression profiles during turnip growth

Totally 21 genes were included in this study (Appendix 13). Those genes were selected based on a) the specifically present in DHVT-117 genome based on genome comparison in turnip morphotype, b) previous studies on storage organ turnip or tuber related crops, c) expression in *Arabidopsis thaliana* tissues (especially in hypocotyl) and some genes were not studied due to time limitation.

Out of 21 selected genes, VTuni_02, VTuni_09, VTuni_14 and ARF3_2 genes were more expressed compared to the reference gene. The expression of those four genes were lower expressed in 6% sucrose with BAP contain medium than 6% sucrose without BAP contain medium. Specially, the expression of VTuni_02 and ARF3_2 were showed clearly difference between with, and without BAP contain medium in turnip tuber tissues.

Figure 12 shows profile of all 19 genes relative expression on different turnip growing stages in sucrose and BAP contain medium. The expression level of those genes were almost similar in shoot and tuber tissues except ARF3_2 and BraDDFs (Figure 12). BraARF3_2 gene was lower expression level in tuber tissues than shoot tissues when turnip grown in 6% Sucrose and BAP contain medium. BraDDF1s genes were almost equal expression level in both shoot and tuber tissues when turnip grown in BAP contain medium. In sucrose contain medium, the expression of BraDDF1s genes were more expressed in shoot tissues than tuber tissues. VTuni_02 and VTuni_14 were excluded from clustering due to highly expressed compared to the reference gene (Figure 12 & 11). The gene profiling shows five big groups. Out of 5 groups, 2 groups (ARF3_2 & DDF1s; upper two) were shown that a clear relationships with turnip tuber formation. The expression of those genes were lower in BAP contain medium compared to sucrose content medium in tuber and shoot tissues. The rest groups were showed similar pattern thought out of the 6 weeks. One big group shows low gene expression. The Ct values of lower expressed genes were less than 36 except VTuni_26. VTuni_26 gene had high Ct values which might be lower expressed or no expression *in vitro* condition.

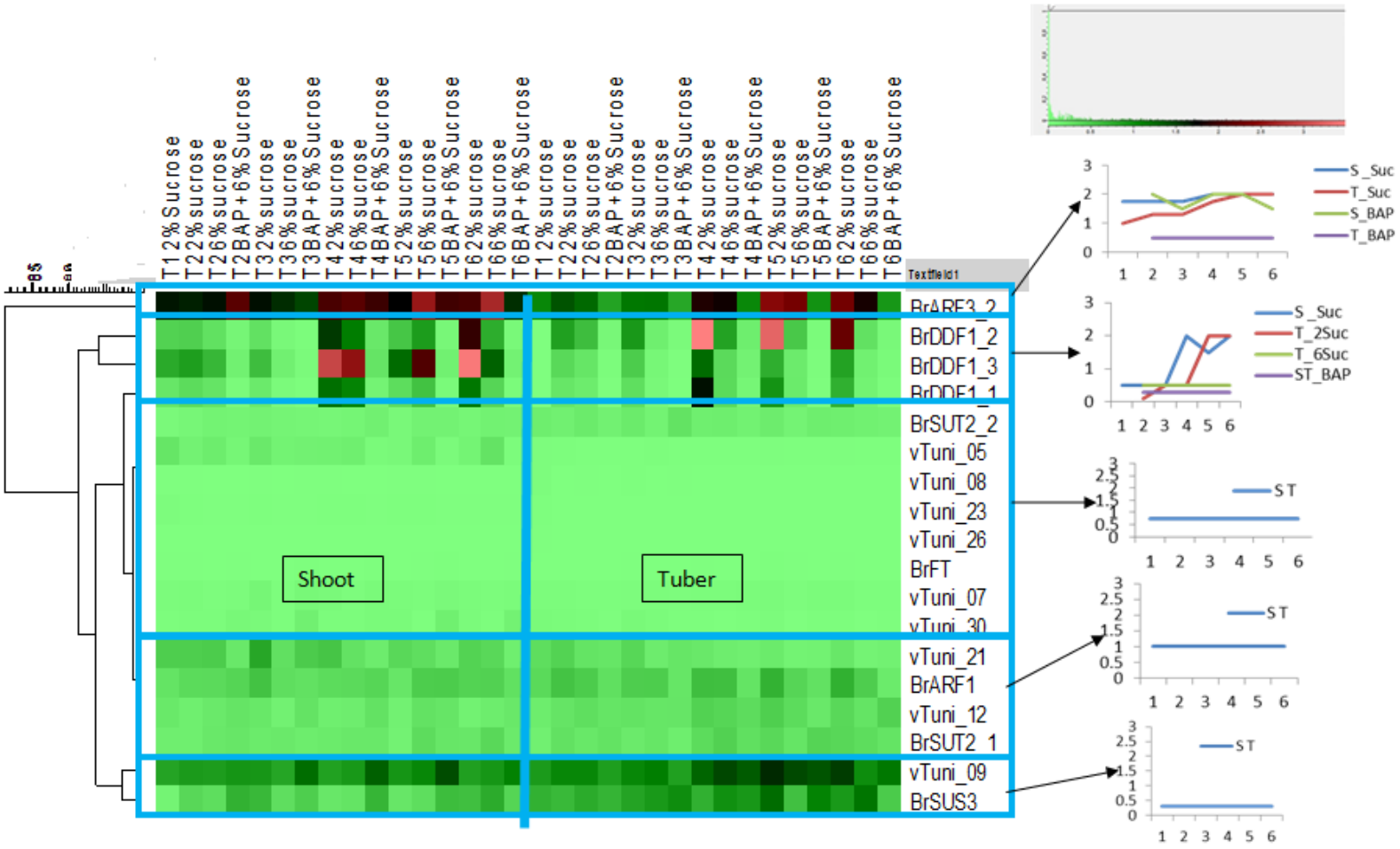

Figure 12. Heat map shows clustering of turnip formation related genes which treated by 2% sucrose, 6% sucrose with and without BAP *in vitro* cultured turnip (excluded VTuni_02 and VTuni_14 genes); S_Suc =shoot tissue in 2 & 6% sucrose; T_Suc = tuber tissues in 2 & 6% sucrose; S_BAP= shoot tissues in BAP; T_BAP= tuber tissues in BAP; T_2Suc= tuber tissues in 2% sucrose; T_6SUc= tuber tissues in 6% sucrose; ST_BAP= shoot and tuber tissues in BAP; ST= Shoot and tuber tissues in 2%, 6% & BAP. X axis is Time point (T1) with treatments (2% & 6% sucrose with and without 10mg/lBAP.

Unique genes for DHVT-117 turnip

ke Lin has identified 30 unique genes through comparing the DHVT-117 genome sequence with the Chiifu genome sequence. From those 30 unique genes, 11 genes were included in this study to see their expression levels differ in turnip formation (Appendix 13). Generally, all genes were lower expressed compared to the reference gene in shoot and tuber tissues except VTuni_02, VTuni_09 and VTuni_14 (described below). VTuni_02 and VTuni_14 genes were very interested gene because; both genes were more expressed compared to the reference gene. In addition to higher expression, both genes were lower expressed when turnip grown on BAP contain medium. VTuni_05 and VTuni_23 genes also interesting, which were lower expressed in turnip grown on BAP contain medium. VTuni_09 and VTuni_30 was 2 to 8 fold more expressed in 6 % sucrose with BAP compared to without BAP in shoot tissues.

VTuni_02 is one unique gene which suggested being plasma membrane intrinsic protein 1c. This protein helps the plant to transport water in the root system. VTuni_02 gene was 5 to 10 fold higher expressed than reference gene in all treatments at all-time points in shoot and tuber tissues. VTuni_02 was up to 5 fold decreased when VT-117 grew on BAP contain medium compared to without BAP at all-time points in turnip tuber tissues (Figure 13).

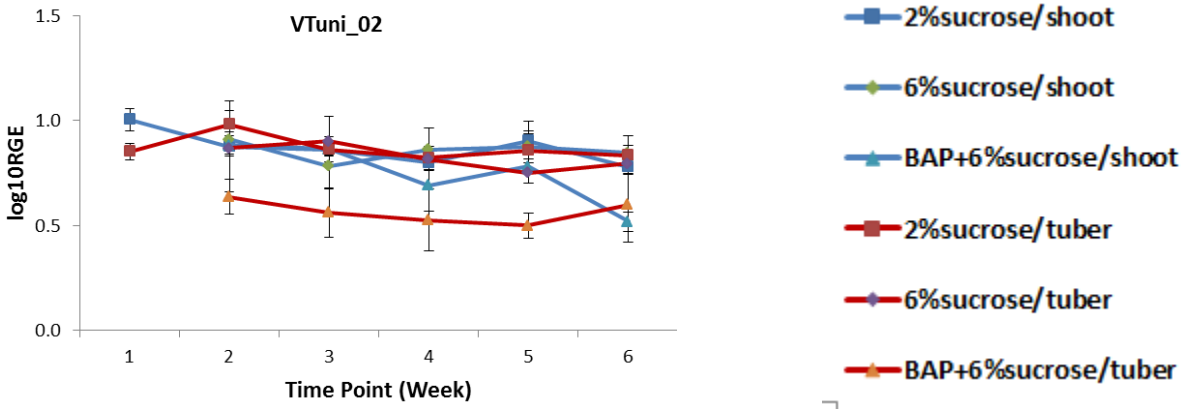

Figure 13. Effect of sucrose and BAP on expression of VTuni_02 gene in VT-117 accession which grown *in vitro* condition within a week interval; the expression was relative to PP2A reference gene; X- axis is time point in week interval and Y-axis log transformation of relative gene expression (RGE) = $2^{-\Delta Ct}$. Log transformation indicate fold change expression. E.g. on Y-axis 0.5 represent 5 fold more expression, 1 represent 10 fold more expression, -0.5 represent 5 fold lower expressions etc.

VTuni_05 gene is identified as to be ATP synthase cf1 alpha subunit which helps the plant to ATP synthases. This gene expression was shown that a significant difference (P=0.000) between the interaction treatments, time points and tissues. The expression was 5 to 23 fold lower compared to PP2A in all treatments in all-time points in both tissues. However, in shoot tissues the gene was 5 to 15 fold higher expressed than turnip tuber tissues. The expression of VTuni_05 gene was lower up to 3 fold when turnip grown in 6% sucrose with BAP contain medium compared to 6% sucrose without BAP in all-time points except time point 5 in turnip tuber tissues. In shoot tissues, 6% sucrose with BAP 3 to 10 folds less expressed than 6% sucrose contain medium in all-time except at time point 3 (Figure 14).

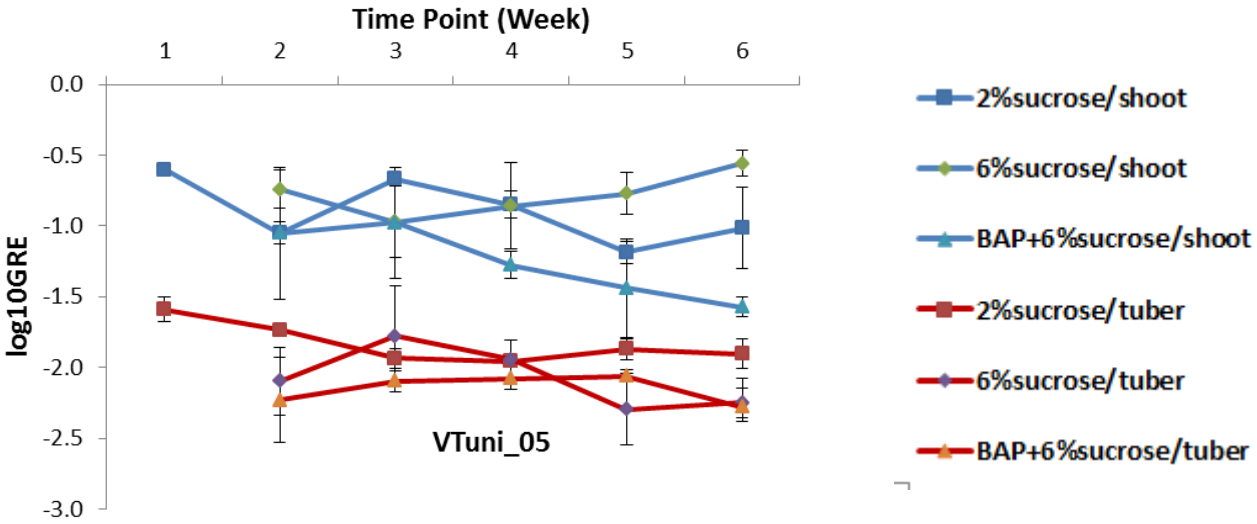

Figure 14. Effect of sucrose and BAP on the expression of VTuni_05 gene in VT-117 accession which grown *in vitro* condition within a week interval; X- axis is time point in week interval and Y-axis log transformation of relative gene expression (RGE) = $2^{-\Delta Ct}$. Log transformation indicate fold change expression. E.g. on Y-axis 0.5 represent 5 fold more expression, 1 represent 10 fold more expression, -0.5 represent 5 fold lower expressions etc.

VTuni_07 gene was up to 20 fold lower expressed compared to the reference gene in time points and tissues depending manner. A significant difference (P=0.004) was observed between expression of VTuni_07 gene in the interaction of time points, treatments and tissues. VTuni_07 gene was 5 fold lower

expressed when turnip (VT-117) grew on medium contain 6% sucrose with BAP than without BAP in turnip tuber tissues at time point 3 (Appendix 14).

VTuni_08 gene was also lower expressed compared to the reference gene in sucrose and BAP contain medium and in all-time points in both tissues. It was more expressed in medium contain 2% sucrose than 6% sucrose with and without BAP (Appendix 14). It also showed a non-significant difference (P=0.651) between gene expression in the interaction of time points, treatments and tissues.

VTuni_09 gene was more expressed up to 2 fold compared to PP2A gene in sucrose and BAP contain medium after time point 3 in tuber tissues (Figure 15). In shoot tissues the expression of this gene was 1 fold lower than the reference gene when turnip grown in sucrose (2% in all-time points & 6% except time point 5) contain medium. There was a non-significant differences (P=0.201) between relative gene expression in the interaction of time points, treatments and tissues.

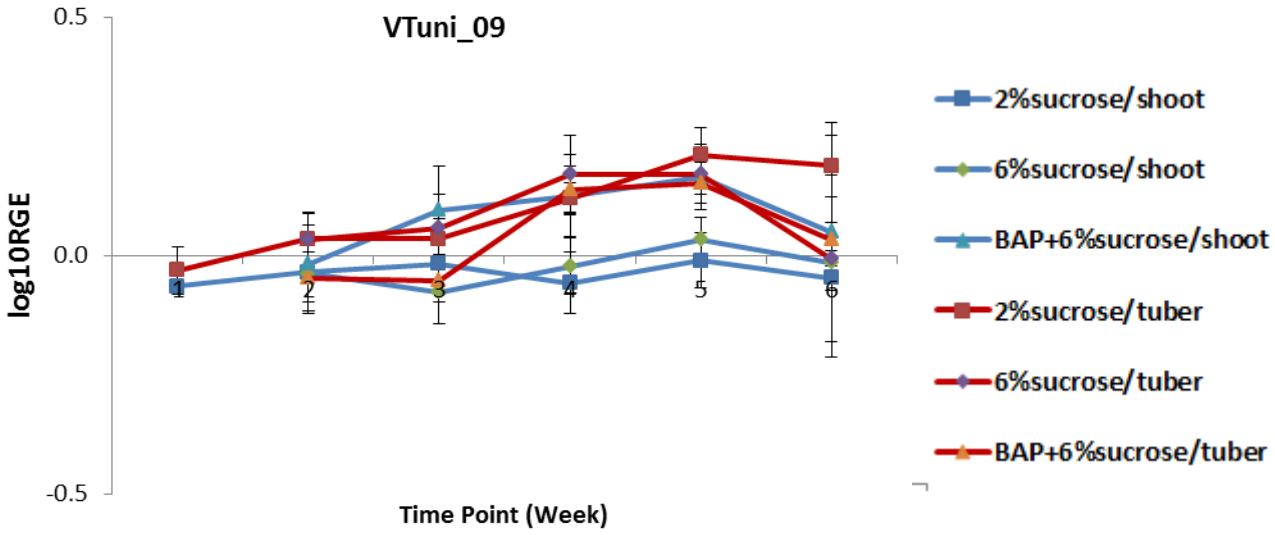

Figure 15. Effect of sucrose and BAP on the expression of VTuni_09 gene in VT-117 accession which grown *in vitro* condition in a week interval; X- axis is time point in week interval and Y-axis log transformation of relative gene expression (RGE) = $2^{-\Delta Ct}$. Log transformation indicate fold change expression. E.g. on Y-axis 0.5 represent 5 fold more expression, 1 represent 10 fold more expression, -0.5 represent 5 fold lower expressions etc.

VTuni_12 gene was 3 to 9 fold lowers expressed compared to PP2A gene in treatment, time point and tissue depending manner. The expression was decreased up to 4 fold in 2% sucrose contain medium in shoot tissues compared to turnip tuber tissues. In turnip tissues the expression was increased up to 5 fold in 2% sucrose contain medium than 6% sucrose with and without BAP. A significance difference has been shown between relative gene expressions in the interaction of time points, treatments and tissues (Appendix 14).

VTuni_14 gene was the only gene among tested DHVT-117 unique genes, which had a highest expression compared to the reference gene. A significant differences (P=0.000) between gene expression was observed in the interaction of time points, treatments and tissues. It has been shown that 8 to 15 fold highly expressed compared to the reference gene in treatment and time point depending manner.

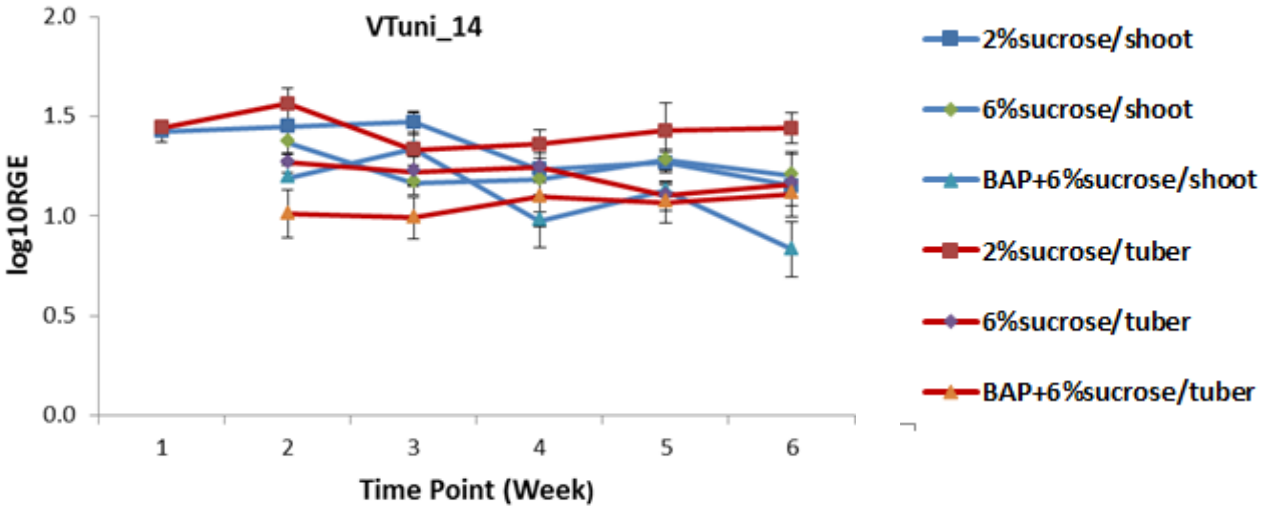

Figure 16. Effect of sucrose and BAP on expression of VTuni_14 gene in VT-117 turnip accession grown *in vitro* condition in weekly intervals; X- axis is time point in week interval and Y-axis log transformation of relative gene expression (RGE) = $2^{-\Delta Ct}$. Log transformation indicate fold change expression. E.g. on Y-axis 0.5 represent 5 fold more expression, 1 represent 10 fold more expression, -0.5 represent 5 fold lower expressions etc.

VTuni_21 gene was 1 to 10 fold lower expressed compared to reference gene in treatment and time point depending manner. In turnip tuber tissues the expression was 2 to 5 fold lower compared to shoot tissues in all-time points depending on the treatments. Specially, in 6% sucrose the expression was 2 fold lower with and without BAP treated samples in turnip tissues compared to shoot tissues. The expression was shown a non-significant difference (P=0.625) between gene expression in the interaction time point, treatment and tissues (Appendix 14).

VTuni_23 also had showed 20 to 50 fold decrease the expression compared to the reference gene in treatments and time points depending manner. There was also a significant difference between relative gene expressions in the interaction of time points, treatments and tissues. This gene was shown that 10 to 30 fold decreased the expression in all treatments at the all-time point in turnip tuber tissues compared to shoot tissues. It was 2 to 9 fold lower expressed in BAP contain medium (except time point 5 root tissues) when compared medium contain sucrose without BAP (Figure 16).

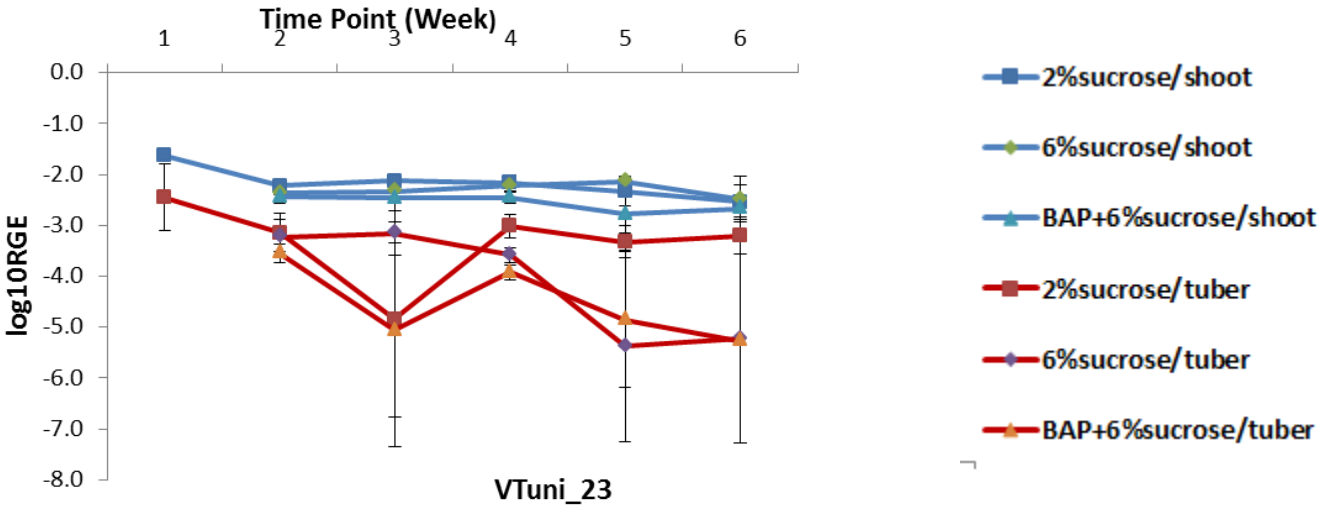

Figure 18. Effect of sucrose and BAP on expression of VTuni_23 gene in VT-117 genotype grown *in vitro* condition within a week interval; X- axis is time point in week interval and Y-axis log transformation of relative gene expression (RGE) = $2^{-\Delta Ct}$. log transformation indicate fold change expression. E.g. on Y-axis 0.5 represent 5 fold more expression, 1 represent 10 fold more expression, -0.5 represent 5 fold lower expressions etc.

VTuni_26 gene was also 21 to 65 fold lower expressed compared to the reference gene in both tissues with treatment and time point depending manner (Appendix 14). The relative gene expression was showed a significant difference (P=0. 004) on the interaction of time point, treatments and tissues. VTuni_30 gene also 8 to 25 fold lower expressed compared to the reference gene. A significant difference (P=0.038) between VTuni_30 gene expression was observed in the interaction of time points, treatments and tissues. The expression of VTuni_30 gene was 2 to 4 fold increase in shoot tissues when turnip grown in BAP contain medium compared sucrose contain medium (Figure 19).

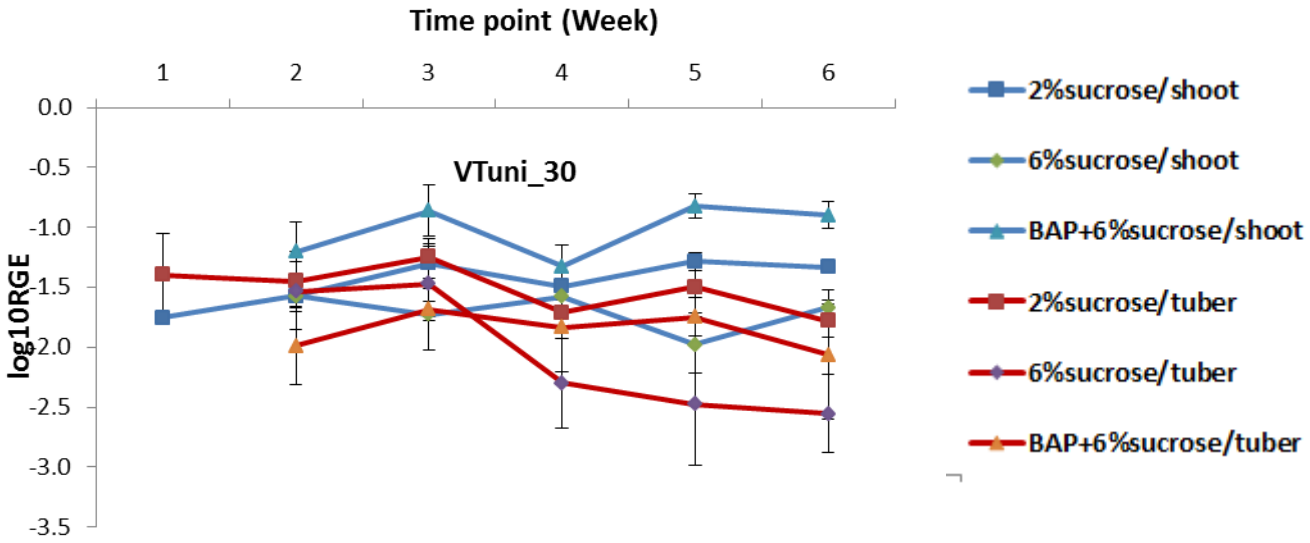

Figure 19. Effect of sucrose and BAP on expression of VTuni_30 gene in VT-117 genotype grown *in vitro* condition within a week interval; X- axis is time point in week interval and Y-axis log transformation of relative gene expression (RGE) = $2^{-\Delta Ct}$. log transformation indicate fold change expression. E.g. on Y-axis 0.5 represent 5 fold more expression, 1 represent 10 fold more expression, -0.5 represent 5 fold lower expressions etc.

SUCROSE SYNTHASE and TRANSPORT (SUS3 & SUT2)

Sucrose synthesis gene (Bra036282/SUS3) was selected to assess the expression in turnip grown on sucrose and BAP contain medium. It was selected the gene which paralog to AT4G02280 in *A. thaliana*. Interestingly, SUS3 gene was up to 10 fold lower expressed in shoot tissues compared to turnip tuber

tissues. This gene also had lower expressed compared to the reference gene in all treatments and all time points. It showed 1 to 9 fold lower expressed compared to PP2A gene in shoot tissues (Appendix 14). There was a significant difference (P=0.000) between relative gene expression in the interaction of time points, treatments and tissues.

In *Arabidopsis thaliana* there are 4 genes identified for sucrose transport annotation. In *Brassica rapa* 6 orthologous are identified for those 4 *A. thaliana* genes. Due to time limit we selected 2 orthologous genes for sucrose transport which were Bra026576 and Bra024802. These two genes are more expressed in cotyledon, hypocotyl and root when compared to other organs in *A. thaliana*. The rest 4 are lower expression in hypocotyl, cotyledon and root compared to other organs.

Bra026576 was lower expressed by 5 to 10 fold than the reference gene in all treatment in all-time points except 6% sucrose treatment in shoot tissues. It was 5 to 10 fold lower expressed in turnip tuber tissues compared to shoot tissues (Appendix 14). Gene expression has also shown a significant difference (P=0.000) in interaction of time point, treatments and tissues.

Bra024802 gene was 5 to 10 fold lowers expressed compared to reference gene in turnip tuber tissues with treatments and time points depending manner. In shoot tissues the expression of this gene (Bra024802) was 8 to 12 fold lower expressed compared to the reference gene. In treatment 2% sucrose also was shown that an increasing of expression of Bra026576 to 4 fold at 5 and 6 time points in turnip tissues (Appendix 14).

DWARF AND DELAYED FLOWERING 1 (DDF1)

There are 3 orthologous genes *B. rapa* to *A. thaliana* which all of them more expressed in hypocotyl and root compare to other organs in *A. thaliana;* Bra026963, Bra019777 & Bra016763. All genes were up to 5 fold more or equal expressed compared to the reference gene after 4 weeks in sucrose contain medium in both tissues. In BAP contain medium, the expressions were lower expressed up to 11 fold in shoot and tuber tissues (Figure 20).

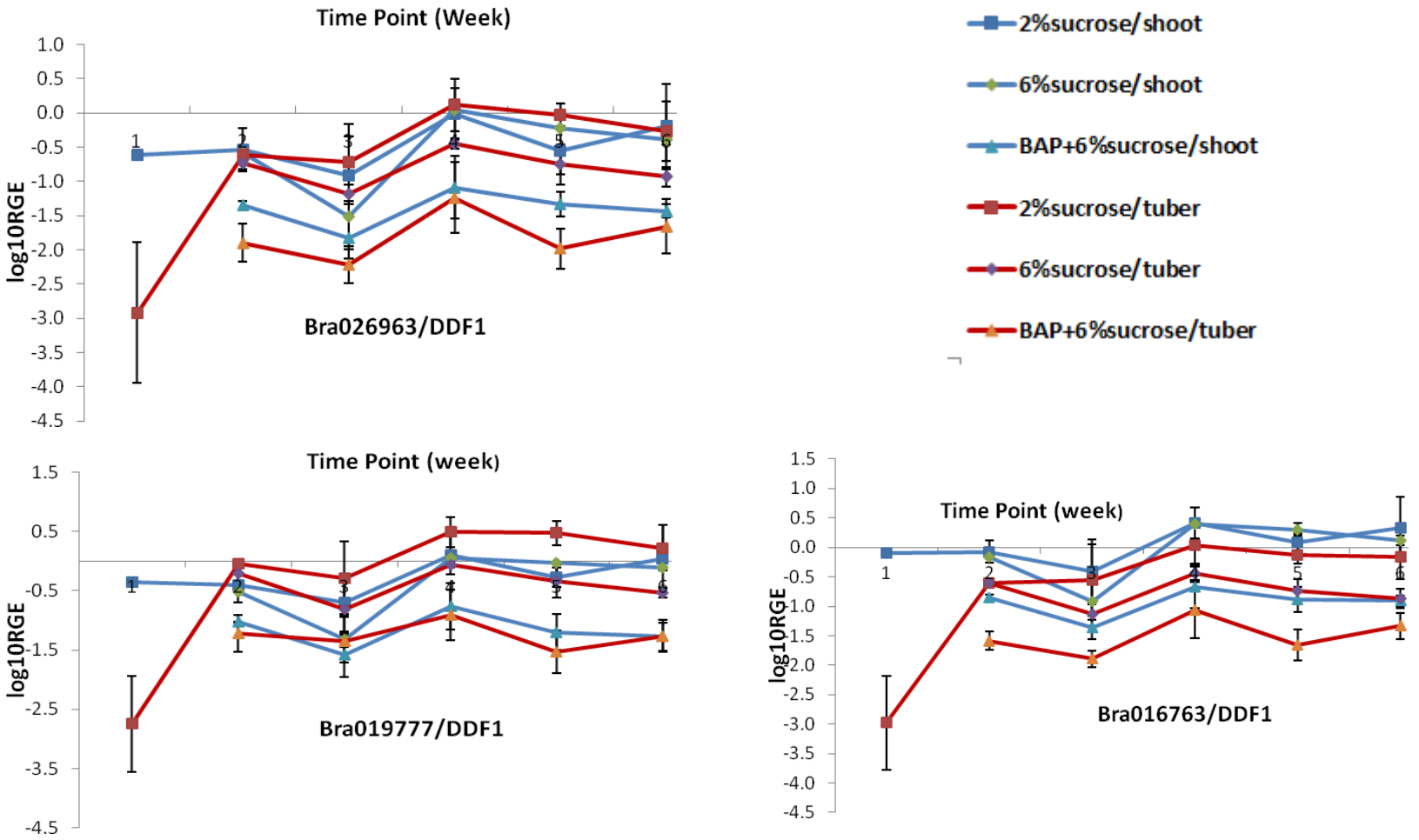

Figure 20. Dwarf and delayed flower genes expression in VT-117 turnip genotype grown under in vitro condition with a weekly interval; X- axis is time point in week interval and Y-axis log transformation of relative gene expression (RGE) = $2^{-\Delta Ct}$. ; log transformation indicate fold change expression. E.g. on Y-axis 0.5 represent 5 fold more expression, 1 represent 10 fold more expression, -0.5 represent 5 fold lower expressions etc.

FLOWERING LOCUS T (FT)

Bra022475 (ortholog to AT1G65480 (*A. thaliana)*) was used to assess the expression patter in turnip grown *in vitro* culture in sucrose and BAP contain medium. This ortholog gene highly expresses in flower and leaf in *A. thaliana*. Bra022475 gene was lower expressed up to 35 fold compared to the reference gene in all treatments at all-time points in the shoot and turnip tissues. The expression of this gene was 5 to 10 fold increase in shoot tissues when turnip grew on BAP contain medium than 6% sucrose contain medium. The expression was increased until week 4 then decreased in shoot tissues. In turnip tissues, it was 5 fold lower expressed in turnip grew on BAP contain medium when compared to 6% sucrose contain medium at all-time points (Appendix 14).

Auxin Responsible Factor (ARF)

A total of 13 ARF genes were identified orthologous *B. rapa* to *A. thaliana* from the brassica database, chiifu genome. BraARF1 and BraARF3-2 were selected based on previous study on BraARFs genes (Mun *et al.*, 2012) which both are expressed in early rooting stage. This gene is more expressed in root and hypocotyl tissues than other organs in *Arabidopsis thaliana*.

Bra035427 (ARF1) gene was up to 7 fold lower expressed compared to the reference gene in all treatments at all-time points in the shoot as well as turnip tuber tissues. Interesting, BAP contain medium was affected the expression of ARF1 gene. ARF1 was 5 to 7 fold more expressed in 6% sucrose without BAP than with BAP in time point depending manner (Figure 21).

Bra021885 (ARF3_2) gene was 1 to 4 fold more expressed than reference gene in turnip grown in sucrose without BAP contain medium except on BAP contain medium. Turnip were grown in medium contain 6% sucrose with BAP have been shown lower expression of AFR3_2 compared to the reference gene in all time points except time point 4 in turnip tissues (Figure 21).

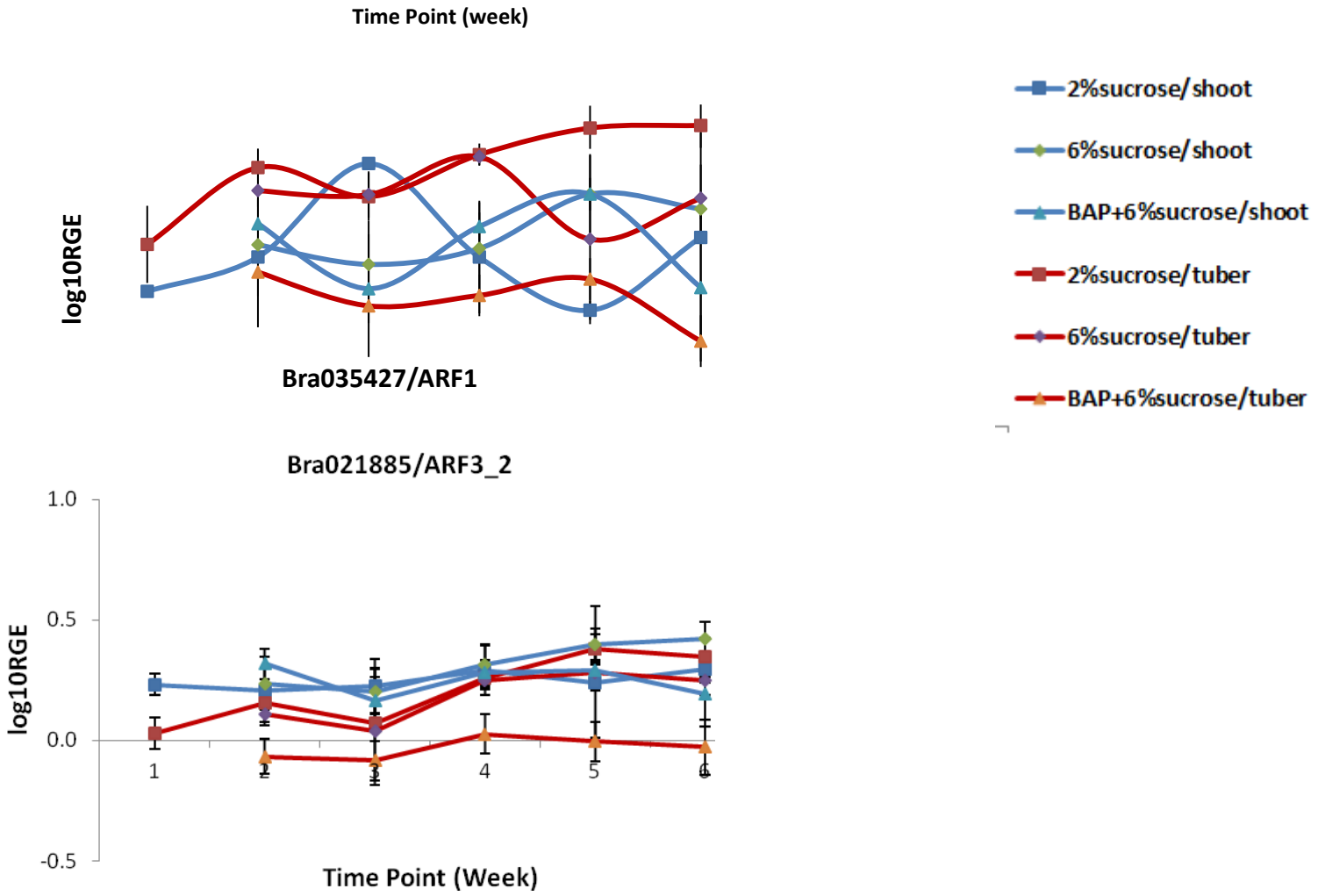

Figure 21. Auxin response factor genes expression in shoot and root of VT-117 turnip genotype grown under *in vitro* condition with a weekly interval; X- axis is time point in week interval and Y-axis log transformation of relative gene expression (RGE) = $2^{-\Delta Ct}$; log transformation indicate fold change expression. E.g. on Y-axis 0.5 represent 5 fold more expression, 1 represent 10 fold more expression, -0.5 represent 5 fold lower expressions etc.

4. Discussion

4.1. Sugar storage and translocation

Turnip tubers of all accessions except VT-008, VT-053 and SM-15 had higher glucose and fructose than sucrose content in both field and greenhouse conditions. Similarly, radish (A*phanus sativus* L.) and turnip *(Brasslca rapa* L.) roots, store less amounts of sucrose but the reduce sugars being the main reserve due to the activity of acid invertase remained high until maturity (Ricardo and Sovia, 1974). High reducing sugar (glucose and fructose) content was observed in the turnip roots which might be due to high acid and alkaline invertases (Gupta *et al.*, 2001). VT-008 had higher sucrose content than the rest accessions under greenhouse condition but it follows VT-053 when they were grown in field condition. VT-053 accession was unique with higher sucrose content in greenhouse and field grown condition.

Some of turnip accessions had higher sugar content in field condition and some others had highest in greenhouse condition. However, total average sugar content was higher in the greenhouse than field grown conditions. There might have several reasons. First, the samples were collected from greenhouse condition most of them were over mature, so turnips were old and plants flowered during harvesting; this was not the case in field condition (discussion with Ningwen and Guusje). Due to this reason, it expected high amount of total carbohydrate in field grown condition than greenhouse. But the result was different from this, and I have no explanation. Second, the grown conditions wouldn't have exact similar environment conditions which also might be affecting the nutrient contents. Another reason, field grown condition might have plenty of water which may be limiting in pot grown turnip in greenhouse condition. Most plants can produce sugars when they get water stress to protect the flow of water from root to soil. For instance, glucose, and fructose concentrations increased as water stress developed are the most important osmotic for adjustment in apple trees (Wang and Stutte, 1992). In potato also sucrose content increased with the severity of stress and Changes in tuber glucose (Bethke *et al.*, 2009). This might be an additional reason for higher content of total sugar content is higher in the greenhouse than field condition. Naturally, turnip has 91-97% water of the fresh weight. The samples which collected from greenhouse had very well grinded than from field collected samples. The carbohydrate might be not easily released from field harvested turnip due to this.

Sucrose and starch were positively correlated in greenhouse and field grown turnip accessions. Negative correlation of fructose and glucose to sucrose is logical. During turnip formation sucrose is converted to reduce sugars (glucose, fructose) that are why most of turnip accessions had higher glucose and fructose. Similarly, glucose and fructose contents have been increase in roots due to an increase in activities of acid and alkaline invertases which the reducing sugar content was about 25 folds higher than sucrose in mature turnip roots (Gupta *et al.*, 2001).

The glucose and fructose content were higher at later stages (4^{th} to 6^{th} time points) in turnip tuber tissues. This might indicate sugar produced in the leaves and transport to turnip tuber tissue for sink development. Glucose and fructose contents were also high in sugar storage study. All accessions have been shown that almost less glucose and fructose content in the leaf tissues. The total sugar content was less than 5g/100g freeze dried weight in the leaf tissues. But the glucose content in the turnip tuber increased in some extent when glucose and fructose content increased in the leaf tissue. The constant content of glucose and fructose in leaf tissues might indicate the leaf produced sugars and transfer for thickening of the storage organ. Glucose content has been shown that a decreased after 4th stages (2 and half months after sowing). Indeed, that was expected because between 4^{th} and 5^{th} stage had 7 months' differences. The 5^{th} stage might be the flowering stage which might be the glucose used for production of reproductive organs and seeds. It was also low content of sucrose, glucose and fructose in leaves in all grown conditions. This also might indicate continued flow of sugars from producing tissue to sink or storage organ.

Sugar testing was time-consuming due to different reasons. The protocol needed some modification on sample amount and time elapsed during absorbance measurement for measuring of total sugar. The protocol is taken from biochemical laboratory used for fruits and vegetables such as tomato, apple, potato and etc. The experiment was started with 50mg turnip powder per 180µl of 80% ethanol. However, the microtiter plate reader couldn't read some of the samples which the absorbance of sucrose, glucose and fructose were the highest number and showed more variability within accession. With a lot of error and try-outs, lastly the test was taken around 5mg turnip powder sample per 1ml of 80% ethanol. Five microgram turnip samples were shown that the reader could read easily without any problems and the difference within accession were less.

The protocol describes that after adding of solution 3 (Appendix 1) shake for 5 seconds in reader and measure after 15 minutes at 340nm to get second absorbance (A_2). Then solution 4 would be added only in glucose/fructose samples and shake for 5 seconds in reader and measure after 15 minutes at 340nm to get absorbance three (A_3). The sucrose sample would be shown approximately similar absorbance's result at A_2 and A_3. But when the elapsed time is only 15 minutes, it wouldn't show similar results. In the kit guide, it described as if the reaction had not stopped after 15 minutes, read observances in 2 minute intervals until the absorbance's increased constantly over 2 minutes. For, turnip 20 minutes was the minimum time to get a constant result but it should be continuing within 2 minutes interval until the absorbance's increase constantly.

4.2. *In vitro* culture

4.2.1. Sucrose, medium and photoperiod effect

The turnip plants were grown in media without sucrose were shown light green color chance compared to plant grown on medium contain sucrose in long as well as short day conditions. The medium without sucrose (0%) resulted in yellow to light green leaf color in VT-115 and red round turnip which indicated that sucrose has large interaction with chlorophyll. I expected a higher turnip tuber formation result from shorter day than long day condition as in potato researches. Previous study on potato showed that in photoperiodic response, the length of the dark period rather than the light period is important. However, the interrupting of an inducing long night with a light treatment (night break) would prevent tuberization, whereas a dark treatment in the middle of a long light period would have no effect (Jackson, 1999). The light was not installed properly for two days under short day condition. There was night break between day two and day three. However, due to late germination of red round turnip, it was not facing the light situation but still turnip tuber formation inhibited under short day condition until the 5^{th} week (Appendix 10).

The interaction sucrose and photoperiod observation result agrees with previous studies. Some genes involved in photosynthesis are strongly induced by Carbon (chlorophyll a/b binding protein, plastocyanin, and small subunit of Rubisco) or both light and sugar (Gln synthetase, nitrate reductase, and Asn synthetase) (Koch, 1996; Thum *et al.*, 2003). In etiolated seedlings, the light is able to override carbon as a major regulator of *ASN1* and *GLN2* expression and in light grown plants; carbon is able to

override light as the major regulator of *GLN2* and *ASN2* expression (Thum *et al.*, 2003). The old study on turnip showed that sucrose was found to be the most effective single substitute for the intact shoot in the dark grown seedlings and limiting factors for root cambial activation at the stage of seedling development (Peterson, 1973).

Another interesting point but unexpected result, due to incorrect installation of light time for the short day, VT-115 accession has been grown under dark conditions for two days. Within these two days all most all treatments Shown as leaf color changes. The treatment without sucrose has been observed yellow leaf color. The rest; 5% sucrose light yellow, 6% sucrose light green; 7 and 8% green leaf color have been observed. However, after 2 days the light installation solved to short day with 8 hours light. Hence, in all treatments with sucrose concentration the leaf color recovered to green but all treatment without sucrose concentration the leaves were dead and the plantlets look like alive but not normal (Appendix 9). As described above, red round turnip was a week late in germination which was transplanting a week later than VT-115. It did not face the light situation. However, the plantlets that treated under short day in different concentration of sucrose the leaf color was green but without sucrose concentration has been shown light green color.

The plantlets which were transplanted to the medium without sucrose there were not getting a chance to recover to grow under short day condition. Why the plantlets had not recovered? It might be with three reasons. The first one is they have not carbon sources from the medium, since the plantlets have not enough carbon and light to undergo photosynthesis. The 2nd one is Light and sugar signalling pathways have studied to regulate the transcription of genes involved in metabolism. For example, the amino acid asparagine genes (*ASN1*, *ASN2* and *ASN3*) serve as an important nitrogen transport compound which regulated by light *(*regulate *ASN2* and repress *ASN1)* and carbon in *A. thaliana (ASN2 and ASN1)* (Lam *et al.*, 1998)*,* so the seedlings might not regulated nitrogen metabolism for growth or regulate of itself. The 3rd sucrose might use for osmotic effect; sucrose solvent flow reduce from plant to medium. In the treatments without sucrose concentration, the sucrose solvent may flow from plant root to the medium. Another study has shown that the supply of exogenous sucrose enhanced Adenosine 5'-diphosphate pyrophosphorylase (AGPase) activity and storage root production in sweet potato (Tsubone *et al.*, 2001). AGPase is a key enzyme for starch synthesis and important for storage organ development in sweet potato.

The maximum means of turnip tuber weight and diameter have been observed in 5 or 6% of sucrose concentration in long day condition. In both accessions (VT-115 & RRT) the turnip tuber thickness (≥5mm) was started at 2nd week in long day condition. Since the turnip tuber thickening was continued until 5 weeks after transplanting. The maximum turnip tuber length was observed under short day condition. Indeed, under short day condition the plant would be etiolated which had longer hypocotyl or stem. Generally, when the medium contained 0% sucrose, there was no turnip tuber thickness (< 5mm) observed during the 5 weeks (after transplanting) observation period except tuber inducing medium in RRT which was started thickness at 3rd week after transplanting. When the Sucrose concentration was increased, the turnip tuber formation increased in a Sucrose concentration depending manner. Similarly, when the medium contained 2% Sucrose or less, no tubers in potato were formed during the 10 day observation period but when the Sucrose concentration was increased beyond 2%, tuberization increased in a Sucrose concentration (Xu *et al.*, 1998). In turnip also has been shown that higher sucrose concentration would lead to higher turnip thickness size. Sucrose concentration less than 5% and sucrose concentration higher than 5% might inhibit the turnip tuber thickness which it had only focused on the concentration of 0, 2, 3, 4, 5, and 8% (Guan and Zhang, 2009). This study was confirmed that 5% and 6% sucrose concentration have been shown a non-significant difference in turnip tuber diameter and weight. However, standard MS-medium with 6% sucrose concentration was shown that the maximum result in a mean of turnip tuber diameter and weight and early turnip tuber thickening under long day condition. Similar results, less thickened hypocotyl has been observed under short day conditions than under long day conditions at the higher temperature (25^{O}C) in radish (Plitt, 1932).

All parameters except number of alive leaves have been shown a maximum under long day conditions in VT-115 and red round turnip accessions. Why in short day condition had maximum alive leaves number? The short day condition might be using the nutrients slowly than long day condition. This result might show short day condition also might give turnip tuber formation after a long period of time. The potential of leaves to export photosynthesis and on the photosynthetic efficiency of the leaves have an influence on Sweet potato storage root growth (Kadowaki *et al.*, 2001). Leaves in short day condition had a less photosynthetic efficiency which had affected the storage root thickness. In addition, radish storage organ development influenced by photoperiod and irradiance level which long photoperiods and high irradiance's producing the largest storage organs (Craker *et al.*, 1983).

The maximum dead leaves have been observed in tuber inducing medium with the long photoperiod condition within VT-115 accession. This is logical because the tuber inducing medium had only 10% of Nitrogen than standard MS-medium. So, it was existed the nitrogen earlier and the leaves were dead more than standard MS-medium. Why it was under long day condition? Long day condition was an early and suitable condition for the turnip tuber formation. So, the plant used most of the nutrients for the turnip tuber formation, hence it was also existed the nutrients early from the medium than short day condition.

4.2.2. Hormones

Gibberellic acid (GA_3)

GA_3 is a plant hormone which promoting growth and elongation of cells. This study has been shown that GA_3 had a non-significant effect on turnip growth and thickening from without until highest concentration (11.5mg/l) of GA_3 in six turnip accessions. It was surprising when compare to potato based on inhibition of tuber formation. In potato, GA_3 has been observed as potato inhibitor. In contrast, GA_3 was not turnip tuber formation inhibitor. The result was showed a non-significant difference between different concentrations of GA_3. However, it showed that increasing of turnip tuber length when increase GA_3 concentration. It is logical because GA_3 is phytohormone which can promote plant elongation.

It was also surprising with interaction of application of exogenous GA_3 and sucrose. The previous study at Wageningen University has been shown that turnip formation inhibited when turnip grown with high concentration of GA_3 with 2% sucrose. But turnip tuber diameter or formation was a non-significant difference between GA_3 concentrations when the sucrose concentration increases to 5% (Guan and Zhang, 2009). This study has also confirmed it; application GA_3 with 6% sucrose had not negative effect on the turnip tuber formation. Interestingly, one old research showed. DHVT-117, VT-012 and YS-143 were shown clearly increases of turnip tuber length and decreasing of turnip tuber diameter when increased GA_3. However, it was a non-significant difference. Indeed, GAs is important for cell elongation and growth that was why the accessions were shown increasing of turnip tuber length.

6-Benzylaminopurine (BAP) effect

There were brown swollen calli formed around the main and lateral roots (Figure 9). Exogenously BAP supplementation under *in vitro* culture causes callus formation not only in turnip culture but in many

different cultural systems (Tang *et al.*, 2003; Zhang *et al.*, 2005). This study clearly had shown that increasing of BAP, the turnip tuber diameter, length and weight would be decreased as well as inhibited turnip tuber formation (<5mm) in six turnip accessions. Similarly, under inducing condition (short day) cytokinins are functioning as a part of the potato tuberization stimulus present in the leaves but under long photoperiods it has been alternately an unidentified inhibitor could be formed (Kefi, 1995; Pelacho and Mingo-Castel, 1991). Storage organ didn't stimulate by application of IAA and BAP into inorganic culture in radish (Ting and Wren, 1980b).

Morphologically, the turnip plants which treated with BAP have been shown dwarf in growth (Appendix 8). This might be BAP application interrupt the GAs or sugar or sucrose pathways or sucrose transporter. GAs is a class of plant hormones which are important for elongation and growth of the cells. In tomato study, GA and BAP have been shown as an antagonistic which GA might inhibit early steps in the cytokinin response pathway via a DELLA-independent pathway and cytokinin also affect the downstream branches of the GA signaling pathway in tomato (Fleishon *et al.*, 2011). In addition, in *Arabidopsis* Gibberellins (GA) and cytokinins act antagonistically in leaf formation and meristem maintenance (Gan *et al.*, 2007). Sucrose is also the main source of carbon for plant growth under *in vitro* condition. The *Brassica rapa subspp. rapa* plants have been shown non-turnip thickening which grown without or less sucrose under *in vitro* condition.

4.2.3. Potato and Turnip tuber formation

Sucrose, medium, photoperiod and GA_3 hormone results were different from potato studies. The Maximum turnip formation was 6% of sucrose concentration under long day conditions. In contrast, for tuberization the maximum concentration is 8% sucrose concentration under short day conditions. The standard MS-medium was the earliest and suitable medium for the turnip tuber formation but for potato tuber there is specially medium is called a tuber inducing medium which has 10% of nitrogen content than standard MS-medium. However, there is one thing similarity to potato and turnip tuber formation which both are decreased when sucrose concentration is decreased (Guan and Zhang, 2009; Xu *et al.*, 1998). Application of exogenous GA_3 inhibits potato tuber formation but non-inhibitor for turnip tuber formation. Application of exogenous sucrose has a negative effect for endogenous GA concentration in potato. Exogenous sucrose application causes a decreasing of bioactive free gibberellin levels with an increase in levels of inactive conjugated gibberellins in potato (Gibson, 2004).

The combined action of cytokinin and higher sucrose (8%) concentration has shortened the potato microtuber induction time from 21 & 18 days (8% sucrose alone) to 10 days and stimulated tuber

initiation when MS medium supplemented with BA (5mg/l) and 8% of sucrose (ASLAM and IQBAL, 2010). In turnip the combination of BAP (5mg/l) and 6% sucrose was shown a formation callus than turnip tuber formation.

4.3. Gene expression

VTuni_02, VTuni_09, VTuni_14 and BraARF3_2 genes were more expressed up to 15 fold compared to reference gene in all treatments in turnip tuber and shoot tissues. VTuni_02 and VTuni_14 are identified as for water transport. Indeed, in tissue culture (*in vitro*) has high amount of water in the medium. The expressions of those two genes (VTuni_02 and VTuni_14) were decreased in BAP treated samples in turnip tuber tissues. Exogenous application of BAP was inhibited turnip tuber formation *in vitro* conditions. Turnip contains 91-97% water in fresh weight. This gene expression may be decreasing in water stress condition.

VTuni_09 and VTuni_30 was 2 to 8 fold more expressed in 6 % sucrose with BAP compared to 6% sucrose without BAP in shoot tissues. Interestingly, the biological ontologies of these two genes are ion transport. VTuni_09 is helps to diffusion of small hydrophilic molecules thought the mitochondrial outer membrane such as anions. VTuni_30 gene helps the plant to transporter ZIP family metal. Manganese is one of ZIP family which is high in callus tissues of *Nicotiana tabacum* (Petolino and Collins, 1985). This might indicate those two genes helps as a signal for callus induction in the leaf or shoot tissues. Specially, VTuni_09 was more expressed in BAP contain medium until time point 5 and decreased at time point 6 in shoot tissues. At time point 6, the plant already formed big callus (Appendix 15). In addition to callus induction signaling, VTuni_09 might also be tuber formation signaling. The expression of this gene was increased until time week 5 then decreased at week 6 in shoot tissues. Turnip thickness was really visibility (≥5mm) of formation on Week 6 (Appendix 15).

VTuni_05 gene was less expressed up to 23 fold compared to reference gene. However, in shoot tissues it was more expressed (up to 8 fold) than turnip tuber tissues in all time points. The role of this gene is ATP synthases in the thylakoid membrane of the chloroplast. It is also highly expressed in leaf tissues in *A. thaliana* compare to other tissues (Arabidopsis eFP Browser). This gene also might be involved in turnip tuber formation because it was less expressed in BAP and 2% sucrose treated samples in the shoot as well as in turnip tuber tissues. This enzyme is also a key for starch synthesis and important for storage organ development in sweet potato. Application exogenous sucrose enhanced

Adenosine 5'-diphosphate pyrophosphorylase (AGPase) activity and storage root production in sweet potato (Tsubone *et al.*, 2001). VTuni_23 was lower expressed up to 52 fold compared to the reference gene. It was also up to 9 fold lower expressed in 6% sucrose with BAP contain medium compared to 6% sucrose without BAP contain medium in tuber as well as leaf tissues.

VTuni_26 had lower expression and higher Ct values (more than 36). This indicated that this gene was not involved or very low expression in turnip tuber formation in *vitro* condition. Researchers are found turnip formations differences between *in vivo* and *in vitro* condition. The biological annotation of this gene is cyclic nucleotide gated channel (non-selective cation) which involve for Ca^{2+} conductance across the plasma membrane (PM) and facilitate cytosolic Ca^{2+} elevation in response to pathogen signals (Ma and Berkowitz, 2011). Turnip tuber formation is early *in vivo* condition compared to in *vitro* culture condition (Fangwen presentation). This gene might involve in early turnip thickness *in vivo* condition or it might in involve in disease protection signalling action compared to other turnip morphotype (Chiifu).

Interestingly, SUS3 was lower expressed up to 6 fold compared to the reference gene. It also was lower expressed in shoot tissues compared to turnip tuber tissues. Similarly, the sucrose content of different turnip accessions were lower in leaf tissues compared to turnip tuber tissues. SUT2 also has been shown a less-expression in leaf tissues compared to turnip tuber tissues. This might indicate sucrose converted to reduce sugars (glucose/fructose) directly after synthesis in leaves. So, glucose fructose synthesis and transport genes might be more expressed than sucrose synthesis genes. The expression of SUS3 and SUT2 was not clearly differentiated between sucrose with and without BAP until week 4. Sucrose synthesis and transport genes were more expressed in sucrose contain medium compared to BAP contain medium at week 5 and 6 in tuber tissues. Turnips need energy for turnip tuber formation in sucrose contain medium and BAP contain media as well for callus formation. Turnip formation as well as the callus was clearly observed (>5mm) at week 6. This indicated that week 6 was the starting point to see the differential expression of sucrose synthesis and transport genes expression in sucrose with BAP and without BAP media.

Addition of exogenous BAP decreased the expression of BraARF1 and BraARF3-2 genes up to 3 fold than without BAP contain medium in turnip tuber tissues. BraARF3-2 gene was up to 3 fold more expressed compared to the reference gene except turnip grown in BAP contain medium. In BAP contain medium turnip growth was affected and BraARFs genes involve in Auxin mediated response. This might be indicating the antagonistic of cytokinins hormones on auxins pathways as well as involvement in

turnip tuber formation. In *A. thaliana* root meristem, cytokinin promotes cell differentiation by repressing both auxins signaling and transport (Moubayidin *et al.*, 2009).

Turnip grew on 6% sucrose with BAP contain medium has been shown that lower BraDDF1 genes expression compared to 6% sucrose without BAP in all treatments in all time points in the shoot and turnip tuber tissues. I expected more expression in BAP contain medium than sucrose contain medium. As result of exogenous BAP application, it was inhibited turnip growth (dwarf) than sucrose contain medium. Overexpression of the *DWARF AND DELAYED FLOWERING 1* (*DDF1*) gene was caused dwarfism mainly by reducing the levels of bioactive gibberellin (GA) in transgenic Arabidopsis (Magome *et al.*, 2008, 2004). But I don't know the clear reasons but might be dwarfism control by another mechanism in turnip plant. Those genes (BraDDF1) might be involved in turnip tuber formation rather than effecting GA pathways.

BraFT gene was more expressed in BAP contain medium than sucrose contain medium in shoot tissues. This gene also interested, it was more expressed in BAP contain medium than without BAP at time point 2 till 4 then decreased at time point 5 to 6. Based on this result, this gene (BrFT) served as a signal for callus formation in tuber tissues.

5. Conclusion and recommendation

The difference of glucose, fructose, sucrose and starch were observed in greenhouse and field condition from different turnip accessions. Some of the accessions had higher glucose, fructose, sucrose and starch in field than greenhouse condition and vise verse. It concluded that the sucrose, glucose, fructose and starch content depends on the condition which the turnip grows and as well as the accessions. The samples were harvested in different growth stages in greenhouse and field conditions. I would recommend to do further research on sugar and starch content which the samples have similar growth stage. Sucrose content was lower in the leaf tissues than turnip tuber tissues at all turnip growth stages. Similarly, Sucrose synthesis and transport genes were lower expressed in shoot tissues compared to turnip tuber tissues. The expression of sucrose synthesis and transport gene confirmed that sucrose synthesis in the leaf and directly converted to reduce sugar such as glucose and fructose. In addition, most of turnip accessions had high glucose and fructose content.

The exogenous sucrose application and photoperiod had a significant effect on the turnip formation under *in vitro* condition. Standard MS-medium with 6% sucrose concentration gives better turnip diameter and weight. This study would recommend 6 % sucrose and standard MS-medium in long day (Turnip tuber inducing) condition for early and better turnip tuber formation. Sucrose might produce a strong turnip tuber formation signal that might either change endogenous hormone levels affecting turnip tuber formation or activate several genes coding proteins for turnip formation and enzymes related to reduce sugar synthesis.

GA_3 hormone has been shown a non-significant effect on turnip tuber formation almost in all accessions. It was clear to conclude GA_3 has no effect on turnip tuber formation. BAP was inhibitor of turnip growth. This study would recommend to do another experiment which the concentration less than 5mg/l because, 5mg/l or more of BAP had an inhibitor effect of thickening as well as the growth of turnip. GA and BAP might have an antagonistic effect which means the application of exogenous BAP might be interrupted GAs pathways and vise verse. Turnip grew on BAP medium has been shown dwarf growth. In contrast, GAs can promote growth and elongation of cells. Since, due to the antagonistic effect of BAP to GAs, exogenous application of BAP might inhibit turnip grown as well as the plant could be dwarfed. BraARF3_2 gene expression confirmed that the antagonistic effect of cytokinins (BAP) on auxin pathway. VT-117 and VT-115 accessions were the earliest accession to respond for thickening of

turnip. So, if you want to *in vitro* culture study on turnip formation, this study recommends using those two (VT-117 and VT-115) accessions.

The result of gene expression indicated that VTuni_02, VTuni_05, VTuni_09, VTuni_14, VTuni_30, BraDDF1, BraARF1 and BraARF3_2 genes were involved on turnip tuber formation *in vitro* condition. VTuni_09, VTuni_30 and BraFT were served as callus formation signaling. The effect of sucrose synthesis and transport genes were not clearly known on turnip tuber formation *in vitro* grew turnip. However, the result helps for indication of involvement of sucrose synthesis and transport genes for turnip formation. So, this study would recommend gene further study such as knockout or silence or overexpression those genes which showed turnip tuber formation to make concrete conclusion. These studies also recommend developing markers and a genetic map on those genes in RILs population to see real involvement in turnip tuber formation.

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Appendixes

Appendix 1. Pipette scheme for glucose, fructose and sucrose test into microtiter plate

Pipette into microtiterplate	Blank Glu/Fru	Sample for Glu/Fru	Blank sucrose	Sample Suc	Blank air
Solution 1*	-	-	20µl	20µl	-
Sample solution	-	10µl	-	10µl	-
Shake for 15 seconds in reader and incubate 15 minutes at room temperature					
Solution 2*	100µl	100µl	100µl	100µl	-
H_2O	200µl	190µl	180µl	170µl	-
Shake for 5 seconds in reader and measure after 3 minutes at 340nm (=A1)					
Solution 3*	2µl	2µl	2µl	2µl	-
Shake for 5 seconds in reader and measure after 15 minutes at 340nm (A2)					
Solution 4*	2µl	2µl	-	-	-
Shake for 5 seconds in reader and measure after 15 minutes at 340nm (A3)					

*see appendix 3

Appendix 2. Pipette scheme into microtiter plate for starch test

	Reagent blank	sample
Solution 1*	20µl	20µl
Sample solution	-	20µl
H_2O	20µl	-
Close the tube, mix and incubate at 57^{O}C for 15 minutes		
Spin down and transfer total solution in microtitrplate		
Solution 2*	100 µl	100 µl
H_2O	100 µl	100 µl
Mixed for 5 seconds in reader and measure after 3 minutes at 340nm(A1)		
Solution 3*	2 µl	2 µl
Mixed for 5 seconds in reader and measure after 15 minutes at 340nm(A2)		

* See table 4

Appendix 3. Sugars(glucose, fructose and sucrose) combination test with the Boehringer cat. No. 716260 contains the following solutions

Bottle 1	Bottle 2	Bottle 3	Bottle 4
0.5g lyophilisate: • citrate buffer, pH 4.6 • β-fructosidase= 720U • stabilizers	7.2g powder mixture of: • Triethanolamine buffer pH 7.6 • NADP 110mg • ATP 260mg • Magnesium sulphate • Stabilizers	1.1ml enzyme suspension of: • Hexokinase=320U • Glucose-6-phosphate dehydrogenase= 160U	• 0.6ml phosphoglucose isomerise = 420U
Solution 1: bottle 1 diluted with 10ml of demi water	Solution 2: bottle 2 diluted with 45ml of demi water	Solution 3: bottle 3 (undiluted)	Solution 4: bottle 4 (undiluted)

Appendix 4. Starch test with the Boehringer cat. No. 10 207 748 035 contains the following solutions

Bottle 1	Bottle 2	Bottle 3	Bottle 4
100mg lyophilizate: • citrate buffer = pH 4.6 • amyloglucosidase 84U	5g powder mixture of: • Triethanolamine buffer= pH 7.6 • NADP 75mg • ATP 190mg • Magnesium sulphate	0.7ml enzyme suspension of: • Hexokinase=200U • Glucose-6-phosphate dehydrogenase=160U	• Starch assay control
Solution 1: bottle 1 diluted with 6ml of demi-water	Solution 2: bottle 2 diluted with 27ml of demi-water	Solution 3= bottle 3 (undiluted)	

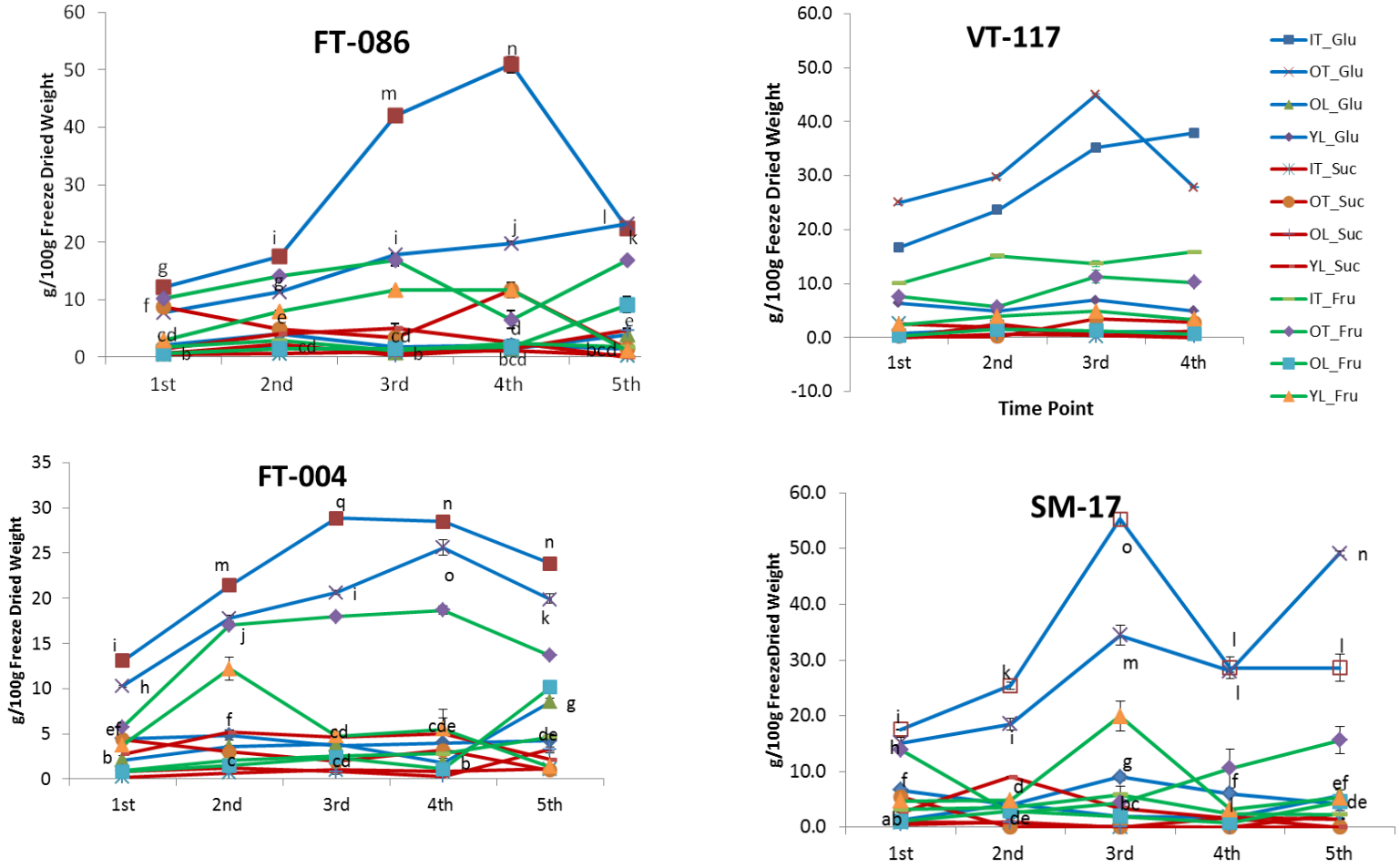
Appendix 5. Glucose, fructose, Sucrose and starch content from field and greenhouse grown turnip accessions

	Accessions	Glucose	Fructose	Sucrose	Starch	Total
		g/100g freeze dried weight				
Green-house	FT-002	20.02±1.10fg	14.99±1.44ghi	4.53±0.41b	16.98±0.36e	56.53±0.44f
	FT-004	**27.01±0.12kl**	13.94±0.23fgh	4.57±0.58b	5.13±0.48c	50.67±0.17c
	FT-047	14.74±0.50d	17.48±1.17l	15.36±0.83e	18.87±0.58gh	66.47±0.22i
	FT-051	19.97±0.27fg	9.29±0.56d	4.99±0.48b	**24.06±0.24j**	58.32±0.89g
	SM-15	6.84±0.04b	5.03±0.34b	23.50±0.65g	3.58±0.18a	38.96±0.89a
	T-738V	19.25±0.32f	12.14±0.35e	1.92±0.76a	4.47±0.44bc	37.79±0.69a
	VT-007	11.87±0.61c	4.82±0.31b	19.16±1.27f	**25.37±0.28k**	61.24±0.46h
	VT-008	14.94±1.2d	**31.46±1.09m**	**32.24±4.43i**	19.94±0.63h	98.61±1.13j
	VT-009	18.11±0.64e	6.40±1.07c	8.58±1.23c	14.87±1.02d	47.97±1.90c
	VT-010	**28.09±0.81m**	16.84±0.74ik	3.29±1.64ab	4.10±0.01ab	52.33±0.91d
	VT-012	25.30±0.18i	15.10±0.93hi	3.58±1.03ab	17.40±0.64ef	61.39±0.76h
	VT-013	26.02±0.14ijk	15.82±0.03ij	5.02±0.30b	19.25±0.55h	66.11±0.60i
	VT-044	20.46±0.27g	12.90±0.38ef	11.96±0.58d	19.91±0.28h	65.22±0.24i
	VT-052	26.8±0.14jk	13.69±0.04fg	1.68±0.29a	18.69±0.67fg	60.86±0.61h
	VT-053	2.09±0.15a	0.82±0.06a	**29.90±0.91h**	21.96±0.17i	54.78±1.17e
	VT-089	21.10±0.23g	9.28±0.44d	4.21±1.05ab	18.09±0.21fg	52.68±0.42d
	VT-115	23.04±0.15h	14.98±0.51ghj	3.57±0.23ab	16.84±0.29e	58.43±0.52g
	VT-117	25.65±0.83ij	14.08±1.19fgh	3.17±0.86ab	XXX	42.90±1.2b

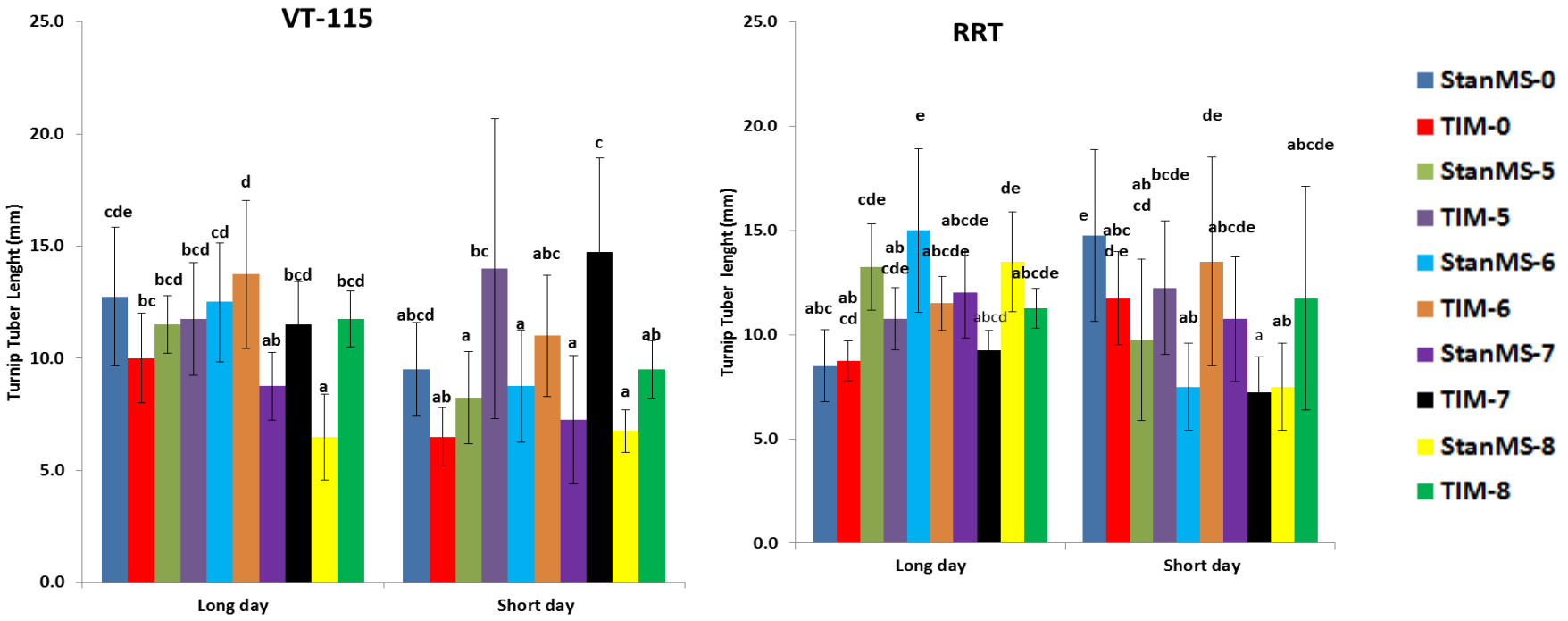
Field	FT-004	10.22±0.79d	**17.84±0.95cd**	8.44±3.79c	12.17±0.09e	48.66±3.63i
	FT-047	18.23±0.02g	6.70±0.94f	0.04±0.03d	14.51±0.19h	39.49±0.74f
	FT-051	17.07±0.52f	8.38±1.23d	0.74±1.09ac	19.91±0.37g	46.10±2.03gh
	VT-007	6.66±0.09c	6.57±0.68c	0.58±0.13ac	4.92±0.21c	18.73±0.42a
	VT-008	9.42±0.56d	4.62±0.67cd	7.11±1.49ac	13.39±0.83d	34.54±1.69e
	VT-009	17.42±0.89fg	5.85±0.76d	1.52±0.06e	15.10±0.02h	37.90±0.87f
	VT-010	**33.51±1.00i**	11.22±0.2g	0.88±1.43f	**20.85±0.69j**	66.46±1.2j
	VT-012	17.58±0.29fg	11.99±0.04e	10.04±0.80c	16.59±0.47h	47.20±1.32gh
	VT-013	18.23±0.15a	14.43±2.34a	2.97±0.59a	3.63±0.09a	24.39±1.04b
	VT-044	13.36±0.46e	6.34±0.86d	0.92±0.76a	10.99±0.41fg	31.61±1.30d
	VT-053	3.08±0.18b	1.74±0.08b	**23.39±0.17a**	17.22±0.18b	45.44±0.48g
	VT-089	19.28±0.76h	5.98±0.30f	16.9±0.03b	3.72±0.02i	45.88±0.48c
	VT-120	12.95±0.57e	5.76±0.99d	2.65±0.83c	2.51±0.05f	23.87±1.51b

XXX= the sample was finished before measuring of starch and no data available for turnip tuber weight. The bold shows the maximum sugar contents(* the turnip weight was taken at harvesting time; the same letters in one column shows a non-significant differences and different letter in the one column shows a significant difference at α=0.05; the significant difference also made within greenhouse and field grown conditions).

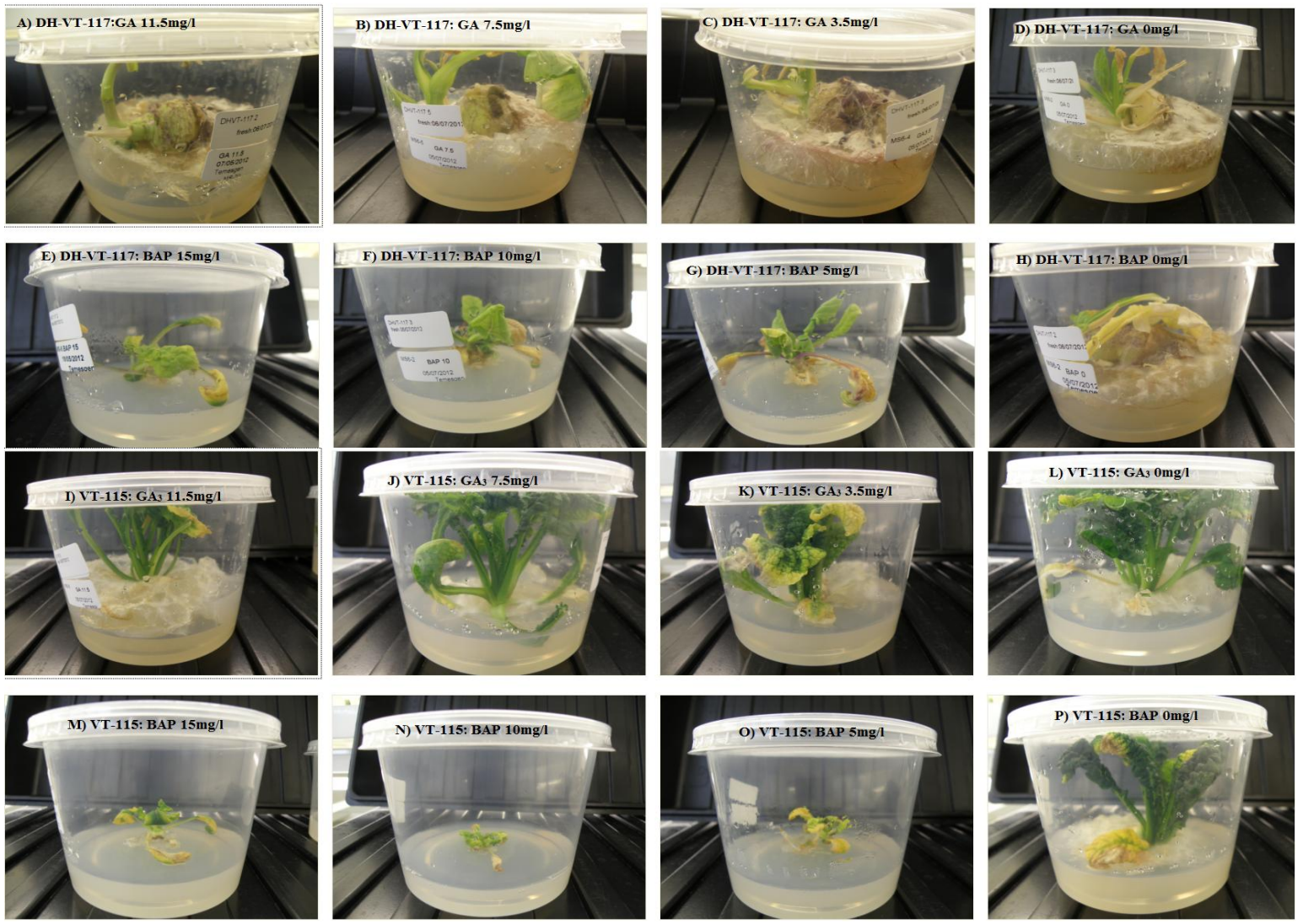
Appendix 6. Translocation of glucose, fructose and sucrose during turnip formation in four turnip accessions in different time points (1st 1 month after sowing, 2nd – 4th time points had 15 days interval and 5th time point was 9 months after sowing except for accession SM-17 which 5th time point for SM-17 was 10 months after sowing; IT=inner tuber; OT=outer tuber; OL=old leaf; YL=young leaf; Glu=Glucose; Fru= fructose; Suc=sucrose).

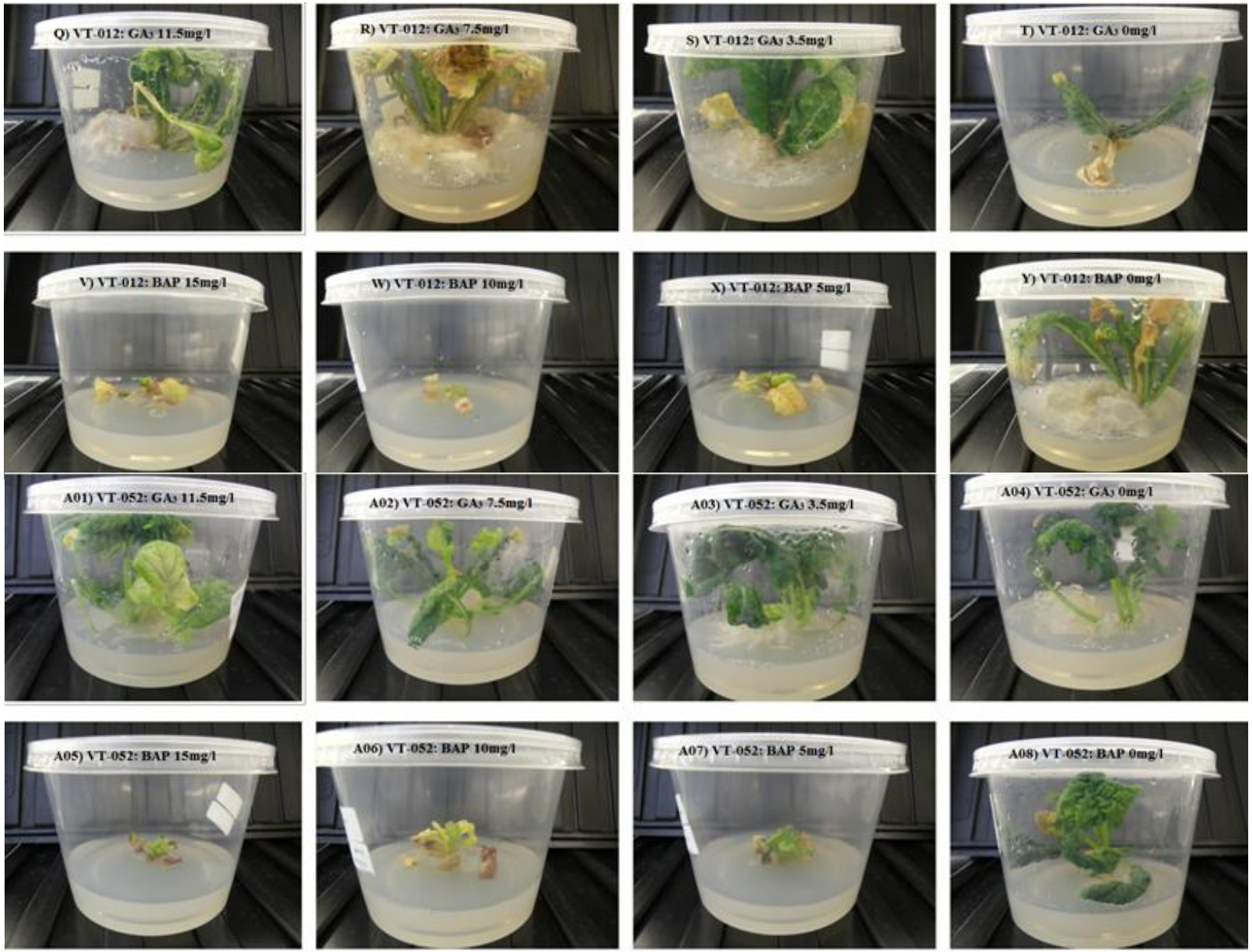


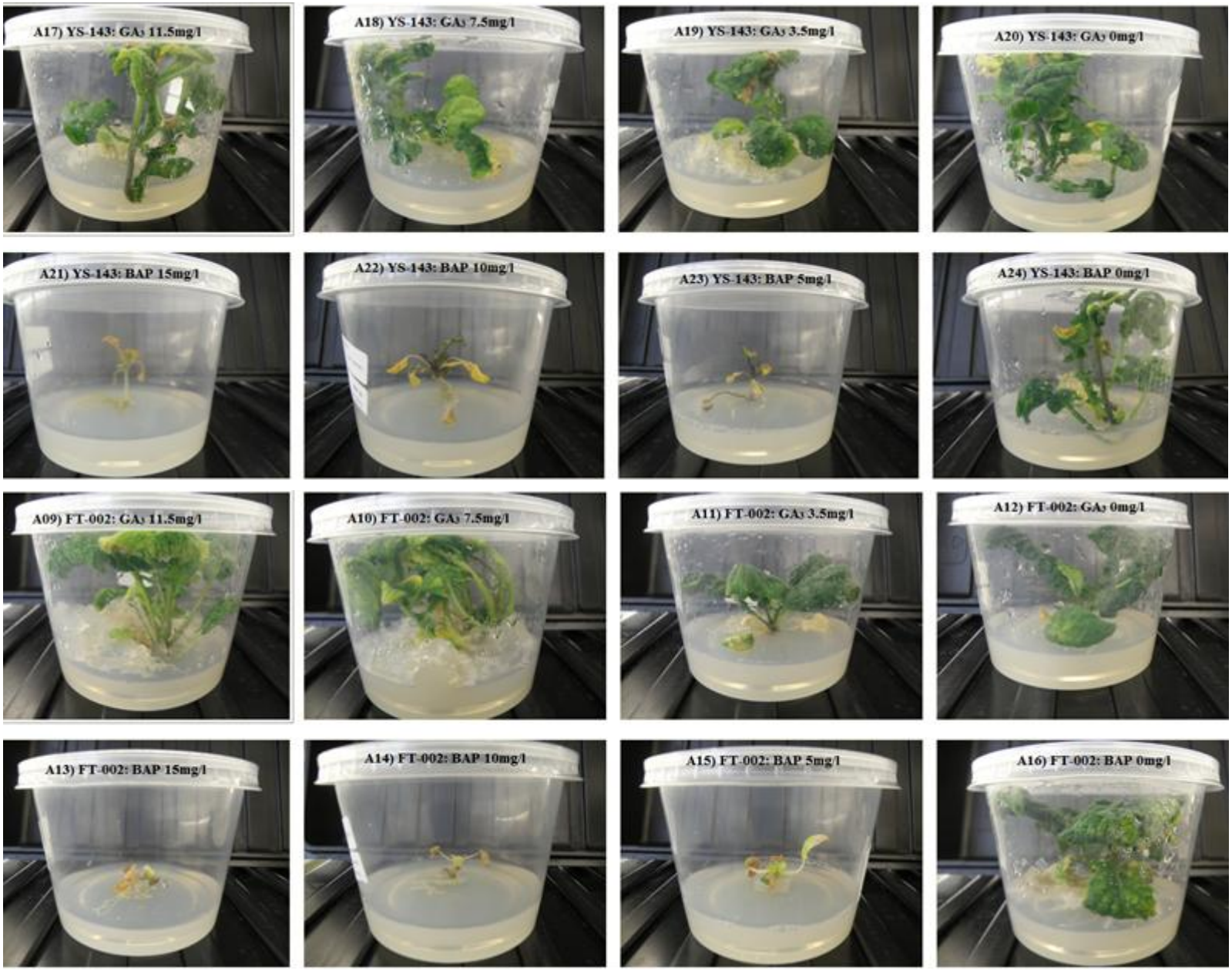
Appendix 7. Effect of medium, sucrose and photoperiod in turnip tuber length *in vitro* grown VT-115 and RRT accessions at 55 days after transplanting (the bar shows the standard deviation; the letters above the standard deviation is significant (all over the treatments) difference at α=0. 05; similar letter shows non-significant; different letter shows a significant difference; TIM= tuber inducing medium; StanMS= standard MS medium; the number shows the sucrose concentration percentage per liter).



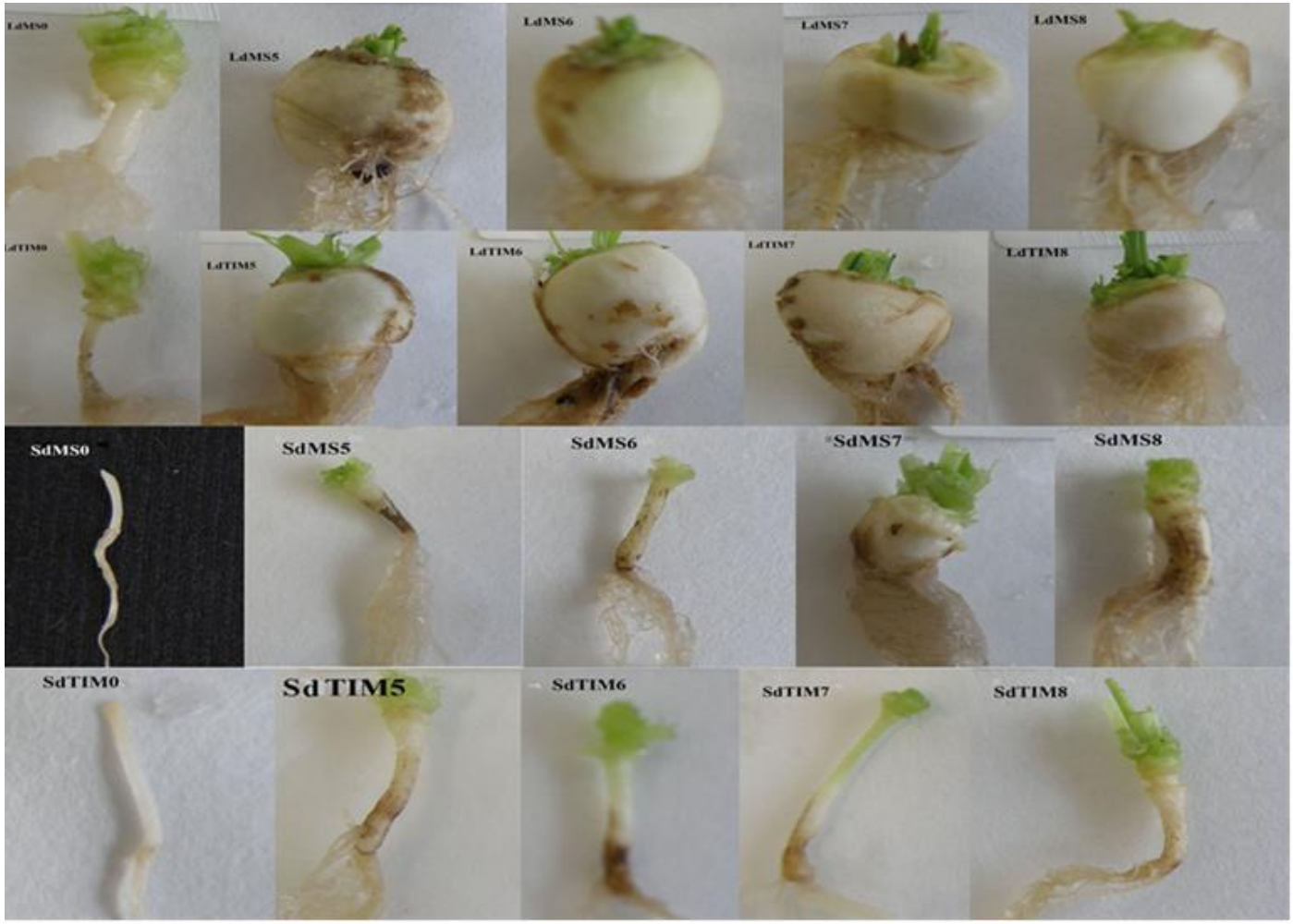
Appendix 8. Effect of GA3 and BAP hormones on morphology of turnip genotypes grown *in vitro* condition 69 days after sowing.




Q) VT-012: GA3 11.5mg/l
R) VT-012: GA3 7.5mg/l
S) VT-012: GA3 3.5mg/l
T) VT-012: GA3 0mg/l
V) VT-012: BAP 15mg/l
W) VT-012: BAP 10mg/l
X) VT-012: BAP 5mg/l
Y) VT-012: BAP 0mg/l
A01) VT-052: GA3 11.5mg/l
A02) VT-052: GA3 7.5mg/l
A03) VT-052: GA3 3.5mg/l
A04) VT-052: GA3 0mg/l
A05) VT-052: BAP 15mg/l
A06) VT-052: BAP 10mg/l
A07) VT-052: BAP 5mg/l
A08) VT-052: BAP 0mg/l


A17) YS-143: GA3 11.5mg/l
A18) YS-143: GA3 7.5mg/l
A19) YS-143: GA3 3.5mg/l
A20) YS-143: GA3 0mg/l
A21) YS-143: BAP 15mg/l
A22) YS-143: BAP 10mg/l
A23) YS-143: BAP 5mg/l
A24) YS-143: BAP 0mg/l
A09) FT-002: GA3 11.5mg/l
A10) FT-002: GA3 7.5mg/l
A11) FT-002: GA3 3.5mg/l
A12) FT-002: GA3 0mg/l
A13) FT-002: BAP 15mg/l
A14) FT-002: BAP 10mg/l
A15) FT-002: BAP 5mg/l
A16) FT-002: BAP 0mg/l

Appendix 9. Effect of photoperiod in VT-115 accession which grown in tuber induced medium and standard MS-medium with different concentration of sucrose at 55 days after transplanting (Ld=long day; MS= standard MS-medium; Sd= short day; TIM= tuber induced medium the number indicate percentage of sucrose concentration; e.g. LdMS0= long day condition-standard MS-medium-0%sucrose; SdTIM= short day-Tuber induced medium-0%sucrose).



Appendix 10. Effect of photoperiod in Red Round Turnip (RRT) which grown in tuber induced medium and standard MS-medium with different concentration of sucrose at 55 days after transplanting (Ld=long day; MS= standard MS-medium; Sd= short day; TIM= tuber induced medium the number indicate percentage of sucrose concentration; e.g. LdMS0= long day condition-standard MS-medium-0%sucrose; SdTIM= short day-Tuber induced medium-0%sucrose).

Appendix 11. effect of GA (1-4) and BAP(5-8) hormones in six turnip genotypes under *in vitro* condition(Row 1 =VT-117, row 2 =VT-115, row 3= VT-012, row 4= VT-052, row 5= YS-143, row 6= FT-002; 1=0mg/l GA3, 2=3.5mg/lGA3, 3=7.5mg/lGA3, 4=11.5mg/lGA3, 5=0mg/lBAP, 6=5mg/lBAP, 7=10mg/lBAP, 8=15mg/lBAP)

Appendix 12. Protocol for RNA extraction, purification and cDNA synthases

Total RNA extraction

The 1st step was cleaning of each material with SDS buffer and preparation of RLC buffer with β-Mercaptoethanol (β-ME) with the proportional of 1ml RLC:10µlβ-ME. The centrifuge tube (1.5ml) and spoon were deep in liquid nitrogen before taking of the samples which helps to reduce RNA degradation. The samples were taken in 1.5ml centrifuge tubes and immediately added 450µl of RLT buffer with vortex vigorously. The whole lysate (sample + RLT) was transferred to a QIAsheredder spin column (lilac) placed in a 2ml of collection tube and centrifuge for 2 minutes at full speed. The supernatants of the flow-through were collected into a new centrifuge tube without disturbing the cell-debris pellet. The ethanol was added with the amounts of half of the total volume of lysate, mixed and immediately transfer to RNeasy spin column (pink) placed in a 2ml collection tube. Centrifuge for 15sec at full speed and discard the flow-through. The RNeasy spin column membrane washed with 700µl of buffer RW1 and centrifuge for 15 seconds at full speed. The flow-through was discarded and added 500µl buffer RPE to wash the spin column membrane with centrifugation for 15sec at full speed (2 times but at the 2^{nd} centrifuge was 2 minutes at 10,000rpm). The old collection tubes were discarded and replace with new, centrifuge for 1 minute to be sure no any possible carryover buffer RPE as well as other residue remains on the outside the RNeasy spin column. The RNeasy spin column was placed in new 1.5ml collection tube. RNase-free water (30µl) was added directly to the spin column membrane. Centrifuge for 1 minute at 10,000rpm to elute the RNA. Take the eluted solution putted back to the RNeasy spin column and centrifuge for 1 minute at 10,000rpm again to get a high concentration of RNA. The quality (integrity) and quantity were check by electrophoresis gel and absorbance ratio 260/280 is around 2.0.

RNA cleanup

RNA was cleaned by using off-column DNase I, AMP Grade which digests single and double stranded DNA to oligodeoxy-ribonucleotides containing a 5'-phosphate. The cleanup step was started from addition of 3µl of 10xDNase I reaction buffer and DNase I Amp Grade I enzyme to 25µl of RNA sample. It was incubated for 15 minutes at room temperature. DNase enzyme was inactivated by the addition of 3µl of 25mM EDTA solution to the reaction mixture by heating for 10 minutes at 65^{O}C. The sample volume was adjusted to 100µl with RNase-free water. Buffer RLT (350µl) was added to the reaction mixture. Ethanol was added to the diluted RNA with mixing by pipetting. The sample was transferred immediately to an RNeasy Mini spin column paced in a 2ml collection tube and centrifuge for 15 seconds at full speed. The flow-through was discarded. The RNeasy spin column was washed by buffer RPE (500µl) at twice and

centrifuge 15 seconds for 1st wash and 2 minutes for a 2nd wash. To eliminate any possible carryover of buffer RPE or residua flow-through left on the outside of the RNeasy spin column, the old collection tube was discarded and replace with a new one with centrifuge for 1 minutes at full speed. Elution of RNA was done by adding of 30µl RNase-free water with incubation for 5 minutes at room temperature and centrifugation for 1 minute at full speed. Elution has also done again by back up the eluted solution to spin column which helps increase of the concentration of RNA.

cDNA Synthesis

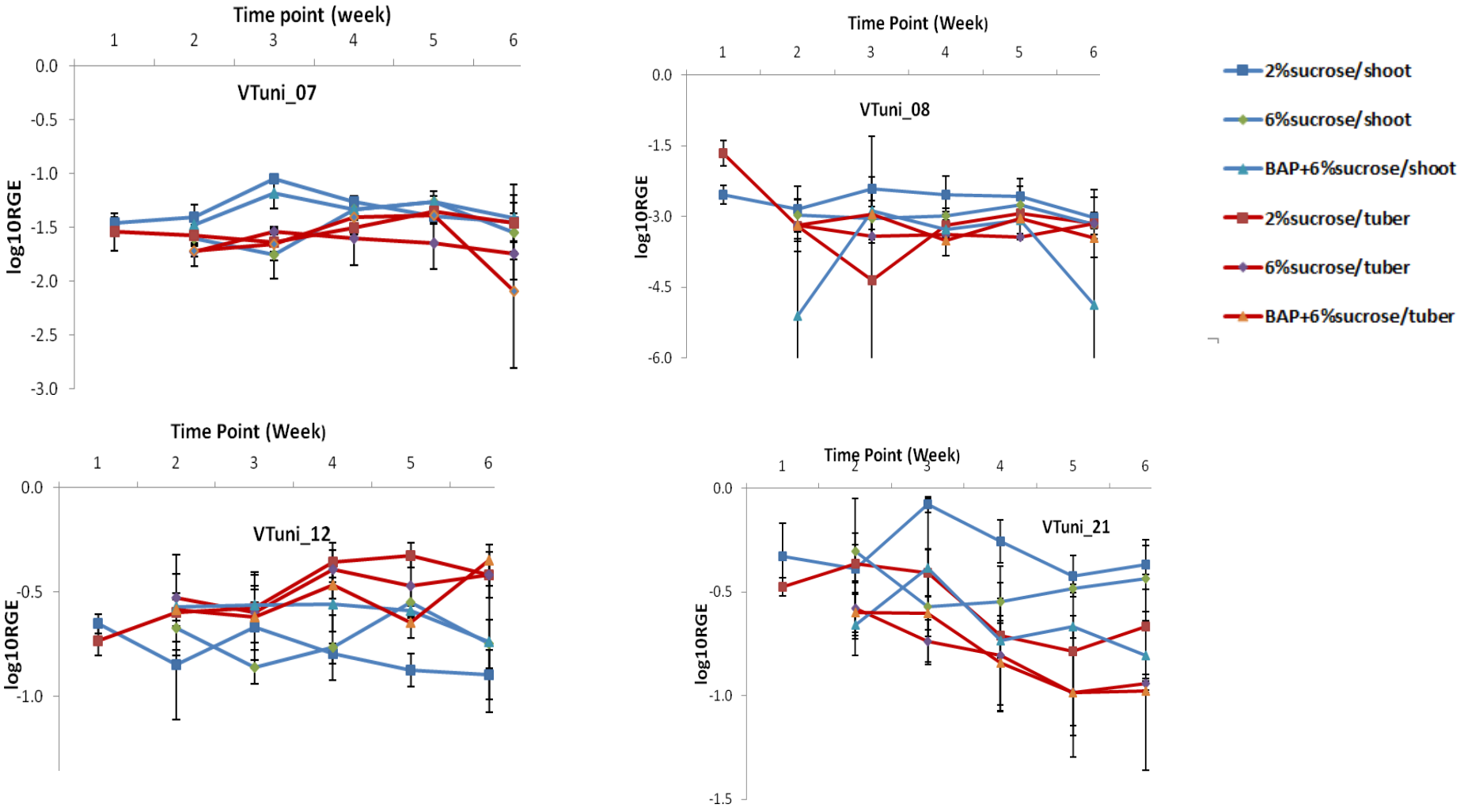
cDNA was synthesized by iScript CDNA synthesis kit using of reverse transcriptase. The total RNA was diluted to 100ng/l±2 before cDNA synthesis. The reaction step was done by mixing of 5µl nuclease-free water, 4 µl 5x reaction mix, 1 µl reverse transcriptase and 10 µl RNA template. The reaction mix was incubated for 5 minutes at 25^{O}C, 30 minutes at 42^{O}C, 5 minutes at 85^{O}C and forever at 4^{O}C. The cDNA was diluted 40x for qRT-PCR. The iScript reverse transcriptase is RNase H+ and preblended with RNase inhibitor to prevent indiscriminate degradation of RNA template. To enhance first-strand priming no need to add enhancer solution because the iScript select cDNA synthesis kit incorporates a proprietary which blended with oligo (dT) and random hexamer primers.

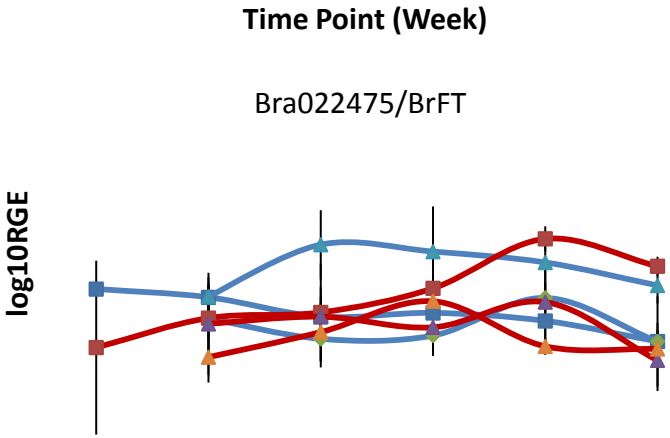
Appendix 13. Primer combination list for *in vitro* gene expression study

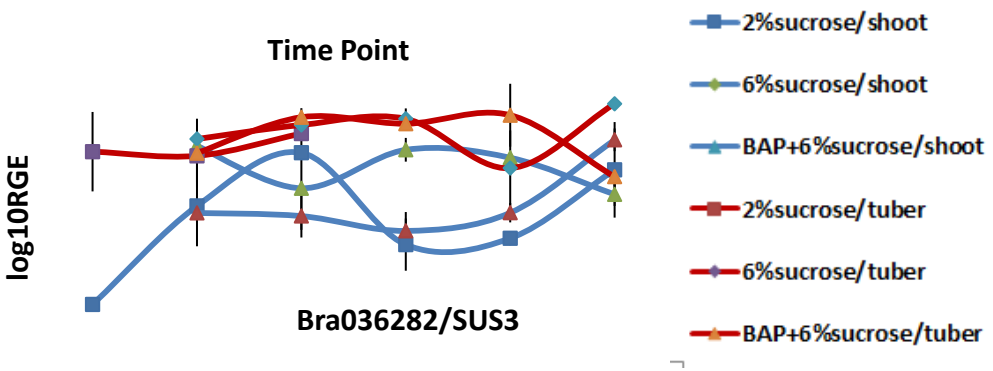
Name	Blast name	sequences	function
BrARF1-2F	Bra035427_1_F	AAGAAGCTCCCGAGAAAAGG	Auxin mediated response
BrARF1-2R	Bra035427_1_R	TTGAACCTTCAGCGATCTCC	
BrARF3-2-1F	Bra021885_1_F	GAGTTTTGTGGGATGGTTCG	
BrARF3-2-1R	Bra021885_1_R	AGGATTCGATGCAGACTTGG	
BrDDF1-1-1F	Bra026963_F	ATTATGCTGGCGGAGATGAG	Dwarf and delay flowering gene
BrDDF1-1-1R	Bra026963_R	ATTTGTCACCGTTCCTACGC	
BrDDF1-2-3F	Bra019777_2_F	ATTATGCTGGCGGAGATGAC	
BrDDF1-2-3R	Bra019777_2_R	GCCGTATTCCTCTGTAAACTGG	
BrDDF1-3-1F	Bra016763_F	AACCACCCGGATGTCATAAG	
BrDDF1-3-1R	Bra016763_R	TACCGTAAGAGGGCAAAACC	
BrFT-3F	Bra022475_2_F	CAGCGACAACTGGAACAAAC	Flowering locus T
BrFT-3R	Bra022475_2_R	ACGATGAATACCCGAGTTGG	
BrSUS3-1F	Bra036282_F	GGTGTTTGGGAATACGTTCG	sucrose synthesise
BrSUS3-1R	Bra036282_R	GCTCGAAATCAAGCTCAAGG	
BrSUT2-1-1F	Bra026576_F	TGGTTCCCATTCCTAACCAG	sucrose transport
BrSUT2-1-1R	Bra026576_R	CTTGCCAACGGAATCTCTTC	
BrSUT2-2-1F	Bra024802_F	AACATGGCTATCGTGGTTCC	

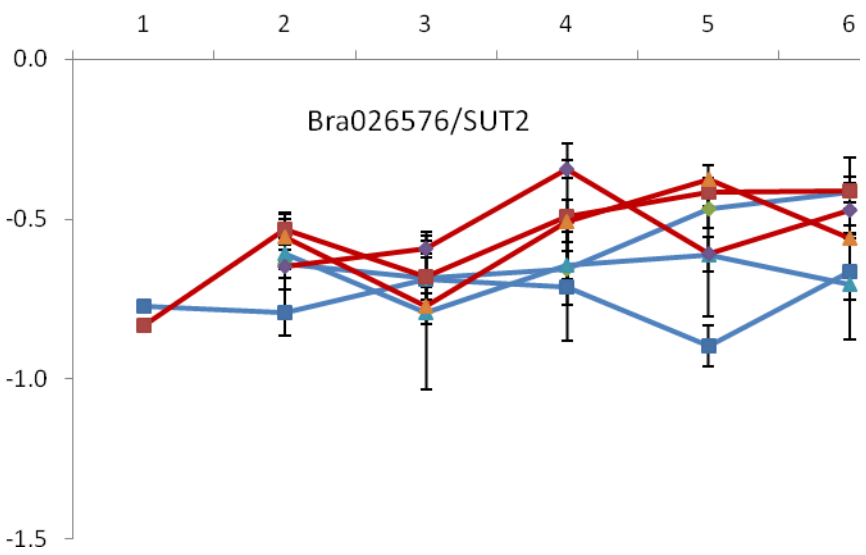
BrSUT2-2-1R	Bra024802_R	AGTTCCACGCGCAAACTATC	
VTuni_02_2F	VTuni_02_2F	ATGTCTTGGTGCCATCTGTG	Unique genes for DHVT-117 turnip
VTuni_02_2R	VTuni_02_2R	AATTTCAGCACCGAGACCAG	
VTuni_05_1F	VTuni_05_1F	GTAGCTGAAATGGCGGATTC	
VTuni_05_1R	VTuni_05_1R	AAGCTTGTGCCTGTTTGGAG	
VTuni_07_1F	VTuni_07_1F	TTCTGCGATATACGGTGTGG	
VTuni_07_1R	VTuni_07_1R	CATATCAGCAATGCCACCAG	
VTuni_08_3F	VTuni_08_3F	CTTCTTTTGTGGTGCCCTTC	
VTuni_08_3R	VTuni_08_3R	AGCATAACCGACGCCAATAC	
VTuni_09_3F	VTuni_09_3F	ACGGTGAATCCAACCACATC	
VTuni_09_3R	VTuni_09_3R	CCCAGCTTTCCCATTGTTAG	
VTuni_12_2F	VTuni_12_2F	ATTGGACCAGGAAGTGTTGG	
VTuni_12_2R	VTuni_12_2R	GCTCTCCACAAAAGGAAACG	
VTuni_14_2F	VTuni_14_2F	AGACAACCCATCGGAACATC	
VTuni_14_2R	VTuni_14_2R	AGAAACGTGGCGATGAACTC	
VTuni_21_1F	VTuni_21_1F	TGGTGCAATTGCTAGTGGTC	
VTuni_21_1R	VTuni_21_1R	GACACCAAACCAAAGCCTTC	
VTuni_23_3F	VTuni_23_3F	CATTGGTCGCAGGAGTAGATAG	
VTuni_23_3R	VTuni_23_3R	GATTCCTGCCACAAAACACC	
VTuni_26_1F	VTuni_26_1F	TCAGTGGTGTCGAAGGTGATAC	
VTuni_26_1R	VTuni_26_1R	AAGCAGTTCCAACCCACTTC	
VTuni_30_3F	VTuni_30_3F	ACGACACCAATCGGAATAGC	
VTuni_30_3R	VTuni_30_3R	GATGCAGCATTAAGCACTCC	

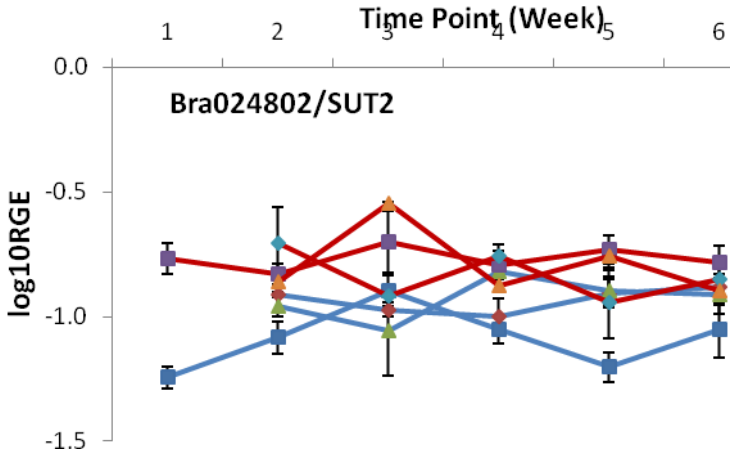
Appendix 14. Relative gene expression in VT-117 *in vitro* grown condition.




Time Point (Week)
Bra022475/BrFT
log10RGE


Time Point
log10RGE
Bra036282/SUS3
2%sucrose/shoot
6%sucrose/shoot
BAP+6%sucrose/shoot
2%sucrose/tuber
6%sucrose/tuber
BAP+6%sucrose/tuber


1
2
3
4
5
6
0.0
-0.5
-1.0
-1.5
Bra026576/SUT2


Time Point (Week)
1
2
3
4
5
6
0.0
-0.5
-1.0
-1.5
Bra024802/SUT2
log10RGE

Appendix 15. VT-117 turnip growth stage in 2% sucrose, 6% sucrose with BAP and without BAP contain medium *in vitro* condition at different time point (a week interval).