EFFECTS OF GENETICS AND PLANT NUTRIENTS ON ANTIOXIDANTS IN TOMATO (*Solanum lycopersicum* L.)

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ผลของพันธุกรรมและธาตุอาหารพืชต่อสารต้านอนุมูลอิสระในมะเขือเทศ (*Solanum lycopersicum* L.)

นายนิรุจน์ คำจุมพล

มหาวิทยาลัยเทคโนโลยีสุรนารี

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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คำสำคัญ: มะเขือเทศ cherry/มะเขือเทศ non–cherry/เครื่องหมาย DNA/ไลโคปีน/โพแทสเซียม/นิกเกิล

มะเขือเทศเป็นแหล่งของสารต้านอนุมูลอิสระหลายชนิด เช่น วิตามินเอ วิตามินซี แคโรทีนอยด์ และสารประกอบฟีนอล ซึ่งปริมาณสารต้านอนุมูลอิสระในมะเขือเทศขึ้นอยู่กับหลายปัจจัย ได้แก่ พันธุกรรม และสภาพแวดล้อม (อุณหภูมิ ความเข้มแสง และความชื้นสัมพัทธ์ เป็นต้น) ซึ่งการทดลองในครั้งนี้มีวัตถุประสงค์ คือ 1) ประเมินความหลากหลายทางพันธุกรรมของมะเขือเทศ 15 จีโนไทป์ 2) เพื่อคัดเลือกพันธุกรรมของมะเขือเทศที่มีปริมาณไลโคปีนสูงโดยใช้เครื่องหมาย DNA และ 3) เพื่อศึกษาการจัดการธาตุอาหารพืชที่เหมาะสมสำหรับเพิ่มปริมาณสารต้านอนุมูลอิสระในมะเขือเทศ โดยการทดลองที่ 1 ทำการประเมินความหลากหลายทางพันธุกรรมของมะเขือเทศ 15 จีโนไทป์ ได้แก่ มะเขือเทศ cherry 3 พันธุ์ และมะเขือเทศ non-cherry 12 พันธุ์ โดยใช้การประเมินลักษณะทางการเกษตรร่วมกับการใช้เครื่องหมาย ISSR จำนวน 32 ไพรเมอร์ ผลการทดลองพบว่าเครื่องหมาย ISSR ทั้ง 32 ไพรเมอร์ ให้จำนวนแถบ DNA ทั้งหมด 214 แถบ ซึ่งในจำนวนนี้มี 111 แถบ ที่แสดงความแตกต่างทางพันธุกรรมของมะเขือเทศ โดยแต่ละไพรเมอร์ให้ความแตกต่างของแถบ DNA ที่ 52 เปอร์เซ็นต์ ผลการวิเคราะห์แยกกลุ่มทางพันธุกรรมด้วยวิธี UPGMA ของมะเขือเทศ 15 จีโนไทป์ พบว่าสามารถจัดกลุ่มความแตกต่างทางพันธุกรรมของมะเขือเทศออกเป็น 3 กลุ่ม ที่ระดับความเหมือนทางพันธุกรรม 53 เปอร์เซ็นต์ โดยจากการจัดแบ่งมะเขือเทศกลุ่มที่ 1 เป็นมะเขือเทศเชอรี่ กลุ่มที่ 2 ได้แก่ มะเขือเทศ non–cherry พันธุ์การค้าที่ปลูกในประเทศไทย และกลุ่มที่ 3 เป็นมะเขือเทศ non–cherry จากศูนย์วิจัยและพัฒนาพืชผักแห่งเอเชีย (AVRDC) ประเทศไต้หวัน จากการจำแนกลักษณะความแตกต่างทางการเกษตร พบว่าลักษณะการเจริญเติบโต สีผล ลักษณะผล จำนวนช่อต่อต้น จำนวนผลต่อช่อ และน้ำหนักผล ในกลุ่มของมะเขือเทศเชอรี่มีความแตกต่างจาก non-cherry ทั้ง 2 กลุ่มอย่างชัดเจน นอกจากนี้ยังพบว่าลักษณะความแตกต่างของสีผลสอดคล้องกับความแตกต่างทางพันธุกรรมของมะเขือเทศแต่ละกลุ่ม ซึ่งผลการทดลองนี้บ่งชี้ว่าเครื่องหมาย DNA มีประสิทธิภาพในการจำแนกความแตกต่างทางพันธุกรรมของมะเขือเทศสูงกว่าการจำแนกโดยใช้ลักษณะทางการเกษตร สำหรับการคัดเลือกพันธุกรรมมะเขือเทศที่มีปริมาณไลโคปีนสูงในมะเขือเทศ 15 จีโนไทป์โดยใช้เครื่องหมายที่มีรายงานว่าเชื่อมโยงกับปริมาณไลโคปีน 6 เครื่องหมายคือSSR 4 เครื่องหมาย และ SCAR 2 เครื่องหมาย ที่มีรายงานว่าเชื่อมโยงกับปริมาณไลโคปีน ผลการทดลอง

พบว่าไพรเมอร์ Og^c ของเครื่องหมาย SCAR สามารถเพิ่มปริมาณ DNA และแสดงความแตกต่างของแถบ DNA ในมะเขือเทศทั้ง 15 จีโนไทป์ จากการวิเคราะห์รีเกรชชันระหว่างแถบ DNA ที่ได้จากไพรเมอร์ Og^c กับปริมาณไลโคปีนที่วิเคราะห์โดยวิธีทางเคมี พบว่ามีความสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติ โดยมีค่า R^2 36.80% ซึ่งบ่งชี้ว่าไพรเมอร์ Og^c สามารถนำมาใช้ประโยชน์ในการคัดเลือกมะเขือเทศที่มีปริมาณไลโคปีนสูงได้ การทดลองที่ 2 ใช้มะเขือเทศ 2 พันธุ์ (Ranger และ Sweet Girl) ปลูกในโรงเรือน 2 สภาพแวดล้อม เพื่อทดสอบการจัดการธาตุอาหารที่เหมาะสมในการเพิ่มปริมาณสารต้านอนุมูลอิสระในมะเขือเทศ การปลูกในแต่ละสภาพแวดล้อมใช้สารละลายธาตุอาหารพืช 6 สูตร ประกอบด้วย T1 (control)–Hoagland solution (H), T2– H + K400, T3–H + K300, T4–H + Ni20, T5–H + Ni10, and T6–H + K300 + Ni10 บันทึกข้อมูลผลผลิตต่อต้น ดัชนีสีผล TSS ปริมาณไลโคปีน และปริมาณเบต้าแคโรทีน จากผลการทดลองพบว่าความแตกต่างของสูตรธาตุอาหารไม่มีผลต่อผลผลิตของมะเขือเทศทั้งสองพันธุ์ อย่างไรก็ตามธาตุอาหารสูตร H+K400 (T2) ส่งผลให้ปริมาณไลโคปีนในมะเขือเทศทั้งสองพันธุ์สูงขึ้นเมื่อปลูกในทั้งสองสภาพแวดล้อม นอกจากนี้ธาตุอาหารสูตรนี้ยังส่งผลให้ดัชนีสีผล (ค่าอัตราส่วนสีแดง a*/b*) ค่า TSS และเบต้าแคโรทีนในผลมะเขือเทศโดยเฉพาะในมะเขือเทศ Sweet Girl สูงขึ้นได้เมื่อเปรียบเทียบกับสูตรสารละลายธาตุอาหารสูตรอื่น นอกจากนี้ในสภาพแวดล้อมที่ 2 (31.78–36.45 °C และความชื้นสัมพัทธ์ 75.26–79.64%) เป็นสภาพแวดล้อมที่เหมาะสมส่งผลให้มะเขือเทศทั้งสองพันธุ์มีคุณภาพผลผลิต และปริมาณสารต้านอนุมูลอิสระสูง

มหาวิทยาลัยเทคโนโลยีสุรนารี

สาขาวิชาเทคโนโลยีการผลิตพืช ลายมือชื่อนักศึกษา ปิยอน คำมงคล

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NIRUT KHAMCHUMPHOL : EFFECTS OF GENETICS AND PLANT NUTRIENTS ON ANTIOXIDANTS IN TOMATO (*Solanum lycopersicum* L.). THESIS ADVISOR : ASST. PROF. THITIPORN MACHIKOWA, Ph.D., 88 PP.

Keyword: Cherry tomato/Non-cherry tomato/DNA marker/Lycopene content/ Potassium/Nickel

Tomatoes are an abundant source of bioactive compounds including vitamin A, vitamin C, carotenoids, and phenolic compounds. The antioxidant content in tomatoes is dependent on several factors including genetic and environments (temperature, light intensity and relative humidity). The aims of this study were to i) evaluate the genetic diversity of 15 tomato genotypes (ii) screen tomato genotypes for high antioxidant content using DNA markers, and (iii) identify the suitable plant nutrient for high antioxidant in tomato. Experiment 1 was conducted to evaluate genetic diversity and morphological diversity of 15 tomato genotypes comprised of three cherry tomatoes and twelve non-cherry tomatoes using 32 ISSR markers and agronomic traits. The results showed that 32 ISSR markers generated 214 DNA bands, 111 being polymorphic, with an average of percentage polymorphic band 52%. Clustering analysis based on the Unweighted Pair Group Method with the arithmetic mean (UPGMA) method was able to divide 15 tomato genotypes into three groups with the similarity coefficient of 53%. Group 1 was cherry tomato cultivars, group 2 was non-cherry cultivated cultivars in Thailand, and group 3 was non-cherry tomato from *AVRDC Taiwan*. Agronomic traits (growth habit, fruit color, fruit shape, cluster per plant, number of fruits per cluster, fruit weight) were used to classify cherry tomato from non-cherry tomato. In addition, it was found that the difference in fruit color was related to the genetic distance in various tomatoes groups. The result indicated that DNA markers was more efficient for classifying the genetic diversity than using the agronomic characteristics to screen lycopene content in 15 tomato genotypes using 6 primers including 4 SSR and 2 SCAR markers that were reported to be linked to lycopene contents. The results indicated that the Og^c primer of SCAR markers was able to amplify reproducibly of clear DNA bands and show polymorphic of 15 tomato genotypes. The regression analysis between Og^c primer results and lycopene contents

by the chemical analysis was significant with a coefficient of determination (R^2) of 36.80%. The result indicated that the Og^c marker can be used for screening of high lycopene content in tomatoes. Experiment 2, two tomato cultivars (Ranger, Sweet Girl) were sown under two greenhouse conditions to identify the suitable plant nutrient management for high antioxidant tomatoes. In each condition, six plant nutrient formulas including T1 (control)–Hoagland solution (H), T2–H + K400 (400 ppm K), T3–H + K300 (300 ppm K), T4–H + Ni20 (20 ppm Ni), T5–H + Ni10 (10 ppm Ni), and T6–H + K300 + Ni10 were applied. Yield per plant, fruit color index, TSS, lycopene, and β–carotene were recorded. The results showed that different plant nutrient formulas did not affect tomato yield but the H+K400 formula resulted to the highest lycopene content across 2 environments in both cultivars. In addition, this formula could promote fruit color (the redness ratio a^*/b^*), TSS, and β–carotene, especially in the Sweet Girl cultivar compared to other nutrient formulas. Furthermore, the E2 condition (31.78–36.45°C with 75.26–79.64% RH) was a more suitable environment for the high yield quality and antioxidant tomato production on both cultivars.

มหาวิทยาลัยเทคโนโลยีสุรนารี

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Student's Signature .....
Advisor's Signature .....

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NIRUT KHAMCHUMPHOL

มหาวิทยาลัยเทคโนโลยีสุรนารี

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

มหาวิทยาลัยเทคโนโลยีสุรนารี

LIST OF TABLES

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

มหาวิทยาลัยเทคโนโลยีสุรนารี

LIST OF FIGURES

Figure **Page**

มหาวิทยาลัยเทคโนโลยีสุรนารี

LIST OF FIGURES (Continued)

Figures **Page**

มหาวิทยาลัยเทคโนโลยีสุรนารี

LIST OF ABBREVIATIONS

AVRDC	=	Asian Vegetable Research and Development Center
DNA	=	Deoxyribonucleic acid
DPPH	=	2,2-diphenyl-1-picrylhydrazyl
ISSR	=	Inter-simple sequence repeat
PCoA	=	Principle coordinate analysis
PIC	=	Polymorphism information content
RH	=	Relative humidity
SCAR	=	Sequence characterized amplified regions
SSR	=	Simple sequence repeat
UPGMA	=	Unweighted pair group method with arithmetic mean

มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER 1
INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important commercial fruit vegetable and is widely cultivated in Thailand where the area and total production of tomato is about 6,028 ha and 122,593 tons, respectively. Sixty seven percent of tomatoes are used as raw material in tomato processing industries and 33% are shipped to fresh market (Office of Agricultural Economics, 2020). Tomato is a source of minerals, organic acids, and antioxidants such as vitamin C, vitamin E, and phenolic compounds (Mattila and Hellström, 2007). Lycopene, which is one of the common carotenoids, is the main pigment that is widely present in mature tomatoes (Nguyen and Schwartz, 1999) and is fairly stable for storage and cooking. It is one of the antioxidants protecting humans against cancers. Thus, tomato fruit enriched with these compounds is desirable. However, the antioxidants content in tomato fruit is usually at a moderate to low level compared to other plants (Wang et al., 1996). Improving fruit quality has become an important goal in tomato breeding programs. The genetic diversity in the tomato population was evaluated in the first step of breeding programs. Ketsakul et al. (2015) reported the variation of tomatoes in Thailand including cherry type, sida type, and table type. The various types of tomatoes showed differences in agronomic traits and fruit quality within the same and different groups. In addition, exploring biodiversity as a source to improve the quality and nutritional value of fruit is an important target. The antioxidant variation was evaluated in plant breeding programs. Previous studies reported that the antioxidant content of interspecific tomato plants had variability where *L. pimpinellifolium* was significantly higher than *L. esculentum* (Hanson et al., 2004). The antioxidant content of intraspecific tomatoes also had variability between cherry and non–cherry tomatoes (*L. esculentum*). It was reported that 76 varieties of non–cherry tomatoes were derived from commercial varieties and their germplasm lines produced β–carotene ranging from 2.9–17.1 mg/100g FW and lycopene from 3.8–20.1 mg/100g FW, which were higher than cherry tomatoes. Bhandari et al. (2016) also

reported that the germplasm lines of cherry tomatoes have higher vitamin C and total phenolic compounds than non–cherry tomatoes. In addition, the measurement of these morphological and agronomic characteristics provides criteria for genetic variation in plant breeding programs. The plant habit, day to flowering, day to ripening, cluster per plant, number of fruits per cluster, fruit shape, fruit color, average fruit weight, and yield per plant were evaluated for each variation in tomato populations (Henareh et al., 2015; Ketsakul et al., 2015; Bhattarai et al., 2016). However, the morphological diversity evaluation was limited by influences from environmental factors and the developmental stage of the plant. Thus, the molecular markers were used to evaluate the genetic variation as well. Inter simple sequence repeats (ISSR) and simple sequence repeats (SSR) were used to study genetic diversity for tomato plant breeding (Hassan et al.,2013; Henareh et al., 2016; Sharifova et al., 2017). Marker–assisted selection (MAS) is an indirect selection process where a trait of interest is selected based on a marker (morphological, biochemical, or DNA/RNA) linked to the trait of interest. Singh et al. (2018) compared SSR markers and morphological markers to evaluate the genetic diversity of 24 tomato varieties. Lycopene specific primers phytoene synthase (Psy–1), phytoene desaturase (Pds–1), zeta carotene desaturase (Zds–1), and lycopene cyclase (Lcy–1) were used. The results indicated that the morphological markers could classify the genetic diversity of 24 varieties at a 90% similarity coefficient. Four lycopene–specific primers could classify the genetic diversity of 24 varieties at a 65% similarity coefficient. However, gene actions of fruit quality characters were governed by non–additive gene effects and revealed moderate narrow–sense heritability (Akhtar and Hazra, 2012). Therefore, fast and accurate techniques are required for screening the biodiversity of tomatoes such as marker–assisted selection. Babak et al. (2018) studied the development of tomato hybrids by combining two pigment content genes including β–carotene (B), old–gold crimson (Og^c), high pigment–2–dark green ($hp–2^{dg}$) and long shelf life (gf–3), non–ripening (nor), ripening inhibitor (rin), and alcobaca (norA), which develops from F_1 hybrids using the method of successive crosses and selected in F_2 and F_3 progenies. Antioxidant analysis in tomato fruit found that characteristics of long shelf life gene in nor, rin, and norA are affected to decrease the lycopene content in tomato fruit. The pigment content

gene B, Og^c, $hp–2^{dg}$, and gf–3 had an influence on the accumulation of lycopene content in tomato fruit, especially $hp–2^{dg}$ and Og^c.

Several studies have reported that environmental conditions such as temperature, plant nutrients, relative humidity (RH), and light intensity can affect plant growth and antioxidant content in tomatoes (Kim et al., 2004; Xu et al., 2007; Taber et al., 2008). Plant nutrients are considered limiting factors for growth and development, as well as, yield and quality. They also greatly affect the content of secondary metabolites including those with antioxidant properties. Macronutrients are irreplaceable and required for normal crop growth and reproduction (Sainju and Singh, 2003). There were positive relationships between the level of macronutrients (N, P, K) and the level of phenolics, lycopene, β–carotene, flavonoids, tomato fruit firmness, and sweetness in tomato fruits (Rebouças et al., 2017; Aina et al., 2019). Excessive N application can result in tomato yield reduction caused by abnormal vegetative growth and lodging which makes the harvesting more difficult. Optimum P can regulate root initiation and stimulate early flowering and fruit set in tomatoes. It can also improve the skin color and pulp, vitamin C content, taste, and hardiness. The increasing K concentration can increase the levels of citric and malic acids, TSS, sugars, and carotene in tomato fruits, thus improving the tomato's storage quality. In the case of micronutrients, Ni plays an essential role in several enzyme activities which directly affect plant growth and development. However, too much Ni can be toxic to the tomato plant and can inhibit growth and yields (Rehman et al., 2016; Kamboj et al., 2018). To achieve optimal yield with high nutritional quality, growers must provide plant nutrients fitting for their tomatoes, however, the nutrient management may need to be specific to the tomato cultivars. In addition, ethylene production during the fruit ripening stage can be accelerated by increasing temperature which in turn rises carotenoid accumulation in tomato fruit (Shivashankara et al., 2015), however, too high a temperature may cause stress on tomato and decrease yield quality (Abdelmageed et al., 2003). The natural light condition produces higher yield and TSS in tomato fruit compared to shading condition but shading condition can induce lycopene content (Zoran et al., 2012). The suitable RH can promote antioxidant and fruit quality in tomatoes. Different RH affects tomato fruit quality under greenhouse conditions, 72%

RH at 32.4°C can produce higher lycopene content and TSS than 62% RH at the same temperature (Leyva et al., 2013).

Therefore, the aims of this study were to (i) evaluate the genetic diversity of 15 tomato genotypes, (ii) screen tomato genotypes for high antioxidant content using DNA markers and (iii) identify the suitable plant nutrient for high antioxidant in tomato.

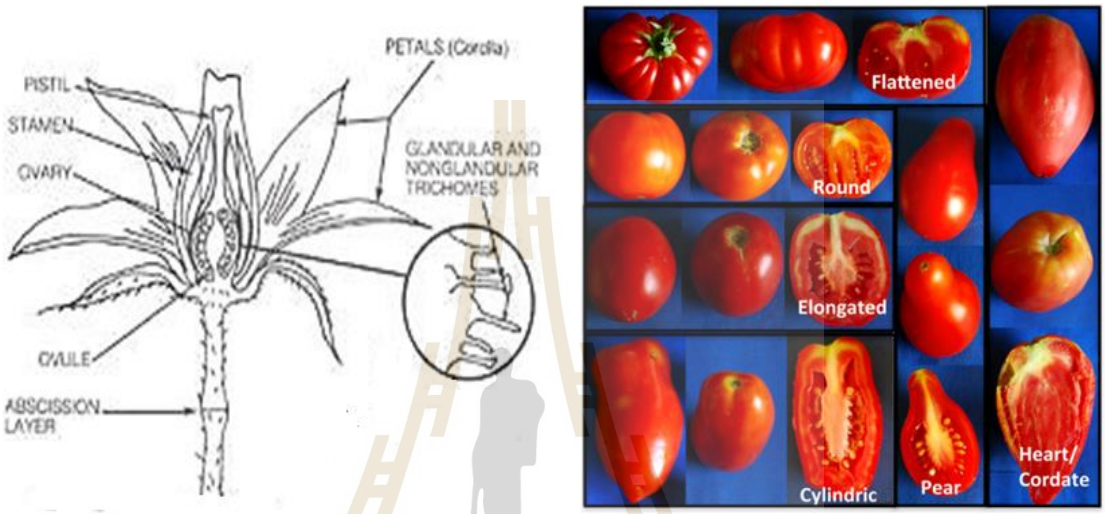
CHAPTER 2
LITERATURE REVIEWS

2.1 The importance and characteristics of tomato

2.1.1 The importance of tomato, tomato is an important commercial and dietary vegetable crop and it is widely cultivated in all regions of Thailand. During 2019–2020, the area and total production of tomatoes in the country were about 39,555 rais and 132,625 tonnes, respectively and its productivity was about 3.5 tonnes/rai. Sixty–seven percent of tomatoes are used as raw material in processing tomato industries and 33% are in the fresh market (Office of Agricultural Economics, 2020). Tomato is a good source of minerals and organic acids. In addition, it is a source of other groups of antioxidants, vitamin C, vitamin E, and phenolic compounds (Mattila and Hellstom, 2007). Previous studies have shown that high consumption of tomatoes is correlated with a reduced risk of some types of cancer (Chan et al., 2005; Rao and Ali, 2007) and heart disease (Rao and Agarwal, 1999).

2.1.2 Characteristics of tomato, tomato (*Solanum lycopersicum* L.) belongs to the diverse family Solanaceae, such as chili, potato, and tobacco. It is one of the most important vegetables in tropical and subtropical areas. Tomato has a large main root, which is known as taproot, which consists of small lateral roots. It has a straight stem with a semi–tall shrub height, which is about 50–200 cm. Tomato is classified into indeterminate, determinate, and semi–determinate categories. The indeterminate tomato will bloom, set new fruit and ripen fruit all at the same time throughout the season. While the determinate tomato will grow to a compact height (generally 90–120 cm) and stop growing when the fruit sets on the top bud. Semi–determinate tomatoes will grow through the lateral bud, set new fruit, and ripen fruit all at the same time throughout the season, but generally, remain smaller than indeterminate plants. All fruits from the plant ripen at approximately the same time (Fridman et al., 2002). Tomato flowers develop on the apical meristem with 5–10 petals and 5 stamens, which are surrounding the pistil in the center of the flower (Figure 2.1A) (Zitter, 1985).

Tomato has single fruit with different colors and shapes. The shape of fruit can be round, oblate, pear–shaped, or torpedo–shaped. Fruit size range from 3–10 cm. The colors of fruit depend on two pigments including lycopene (red color) and carotene (yellow, yellow–red, orange, and brown) (Figure 2.1B) (Sacco et al., 2015). The seed size of a tomato is about 0.2–0.5 cm.



(A) (B)

Figure 2.1 Flower structures and various types of tomato fruits (Zitter, 1985: Sacco et al., 2015).

2.2 Genetic diversity and breeding in tomato

Tomato plant breeding is critical for improving tomato quality. The general goals of tomato breeding are fruit yield, fruit quality, and resistance to diseases and pests. The desirable traits should have variations in populations during the plant breeding program. Generally, genetic diversity evaluation is the first step of plant breeding, it helps evaluate gene pools for the breeding programs.

2.2.1 Morphological diversity, the morphology diversity evaluation is a general technique for classifying the different genotypes. Morphological traits such as growth habit, hypocotyl color, leaf type, petal color, fruit shape, fruit size, and fruit color were used to determine the genetic diversity. Henareh et al. (2015) used 19 traits including morphological and fruit quality (seedling size, plant size, leaf type, fruit size, color of mature fruit, fruit firmness and fruit, blossom–end rot of fruit, and fruit cracking) of 97

tomato genotypes. The cluster analysis was classified into 6 groups. The highest group was found in cluster 1, including 27 genotypes that had seedling size, plant size, foliage density, fruit cracking, the ratio of the amount of seed/fruit size less than and fruit firmness, sunscald more than other genotypes. The 22–tomato inbred line indicated morphology characteristics for 6 quantitative traits and 21 qualitative traits (Salim et al., 2020). The predominant traits in this study included hypocotyl color, hypocotyl pubescence, leaf type, green shoulder trips in the fruit, and fruit cross–sectional shape can be used to differentiate the genotypes. The difference inbred line parents was used to generate the heterosis in progenies.

2.2.2 Genetic diversity based on molecular markers, Marker–assisted selection is an indirect selection process where a trait of interest is selected based on a marker linked to the trait of interest. The morphological marker is used to classify plant species by the expression of the phenotype. However, plant species classify based on only morphological criteria, especially quantitative traits, which may give incorrect information, because some morphological traits may affect both environment and genotype. The molecular marker can identify genetic diversity by using molecules contained in a sample, which are taken from an organism such as protein, DNA, and RNA. The effect of the environment is low and high performance for genetic diversity in plants. The study of genetic diversity in DNA markers is one of the techniques that can shorten the selection time and efficient technique of genetic materials. The DNA marker can indicate the specificity of living things from the polymorphism of nucleotides in DNA molecules. There are two techniques to differentiate the sequence of the DNA molecule or DNA fingerprinting i. e. , hybridization–based DNA fingerprint and PCR–based DNA fingerprint.

— DNA fingerprinting by the technique of hybridization– based. The DNA fingerprint is based on DNA hybridization of restriction fragment length polymorphism (RFLP) to identify DNA fragments that are derived from restriction enzymes.

— DNA fingerprinting using polymerase chain reaction (PCR) is based on the technique of increasing DNA in vitro using DNA polymerase enzymes and primer for increasing target DNA by PCR reaction.

— Simple sequence repeat (SSR) or microsatellite is base on the repetitive sequence and tandem repeats for distinguishing DNA. This technique is used to study genomics and genome maps. SSR is generally distributed in the genome, and the number of SSR has a specific plant or animal in each species. Therefore, this marker is suitable for differentiating of genetic diversity of various organisms. SSR is a specific primer that has high specificity with target DNA. SSR–DNA band derives from PCR reaction, which shows the differentiation among heterozygous, homozygous, and reproducibility (Jongkolphan, 2005).

— Inter simple sequence repeats (ISSR) or microsatellite primed PCR (MP–PCR) is a marker developed from a microsatellite, which combines the advantage of a microsatellite marker, amplified fragment length polymorphism (AFLP), and random amplified polymorphic DNA (RAPD). The sequence base on ISSR is microsatellite 16–25 nucleotides. This method is a technique that is similar to the RAPD technique, but it uses a longer primer than RAPD (Fang and Roose, 1997).

There were several studies evaluated the genetic diversity of tomato genotypes. Evaluation of the genetic variation is necessary for a preliminary test of the appropriate selection lines (Todorovska et al., 2014). ISSR markers were highly effective in detecting polymorphism in tomato accessions because of targeting multiple microsatellite loci distributed throughout the genome. The high efficiency of ISSR markers was reported by Hassan et al. (2013). Eighteen primers were used of inter–simple sequence repeat (ISSR) to identify 11 genotypes including 9 tomatoes (*Lycopersicon esculentum* L.), 1 Mexican husk tomato (*Physalis philadelphica*), and 1 cherry tomato (*Solanum lycopersicum* var. cerasiforme) accessions. the results showed a high polymorphism in this population. The 18 ISSR primers includes polymorphism ranging from 69% to 100% and averaged 83.44%. The cluster analysis was classified into 2 major groups. The 1 tomatillo (*Physalis philadelphica*) was classified in the first major group and 1 cherry tomato (*Solanum lycopersicum* var. cerasiforme) was classified in subgroup 1 of the second major group. The 9 tomatoes (*Lycopersicon esculentum* L.) were classified in subgroups 2 and 3 of the second major groups. The high polymorphic bands in 100% polymorphism of 14 ISSR primers were found in the experiment of Henareh et al. (2016). They found the variation of 93 tomato landraces from the East Anatolian region of Turkey and North–West of Iran and 3 commercial

varieties. The cluster analysis was generated in 9 groups. The genotypes from the same regions were often classified into the same groups. This heterogeneous population variation was helpful for hybrid variety productions.

2.3 Tomato breeding for high antioxidants

2.3.1 Antioxidants in tomato, antioxidants are the secondary metabolite that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. There are various types of antioxidants in plants and differences in quality depending on the species of plants. Tomato is a rich source of antioxidants, especially the carotenoid group. Lycopene is the major carotenoid present in tomatoes, which has red pigment, accounting for 80% of the total tomato carotenoids in fully red–ripe fruits (Nguyen et al., 1999) and it is fairly stable for storage and cooking. In addition, tomato is a source of other groups of antioxidants in tomato such as vitamin C, vitamin E, and phenolic compounds (Giovanelli et al., 1999).

— Flavonoids are mostly found in the peel of tomatoes (Taiz and Zeiger, 2006). Flavonoids can generate by 2 pathways, which are the shikimic acid pathway and the malonic acid pathway (Figure 2.2) (Verhoeyen et al., 2002). The maturation of tissue affected phenolic content. The tomato peel contains the highest contents of flavonoids 45 days after fertilization (Krause and Galensa, 1992; Stewart et al., 2000).

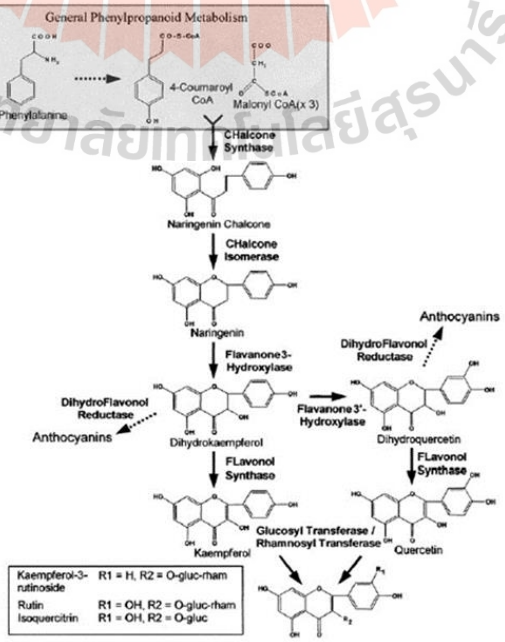


Figure 2.2 Flavonoid synthesis pathway (Verhoeyen et al., 2002).

— Carotenoid is one group of antioxidants that are mostly found in tomatoes, such as β–carotene, lutein, cryptoxanthin, and lycopene. Lycopene is the major carotenoid present in tomatoes, which has red pigment, accounting for 80% of the total tomato carotenoids in fully red–ripe fruits (Nguyen et al., 1999). Lycopene plays an important role in preventing destruction from exposure to photosynthesis in plants. Ruiz-Sola and Rodriguez-Concepcion (2012) reported that carotenoids can generate 2 pathways including a mevalonate (MAV) pathway that occur in the cytosol and methylerythritol 4–phosphate (MEP) pathway (Figure 2.3). Two groups of carotenoids are the carotene and xanthophyll in tomatoes that can be generated in the maturation stage and observed by the change of green color from chlorophyll to yellow–orange or red color from β–carotene and lycopene (Sousa et al., 2014)

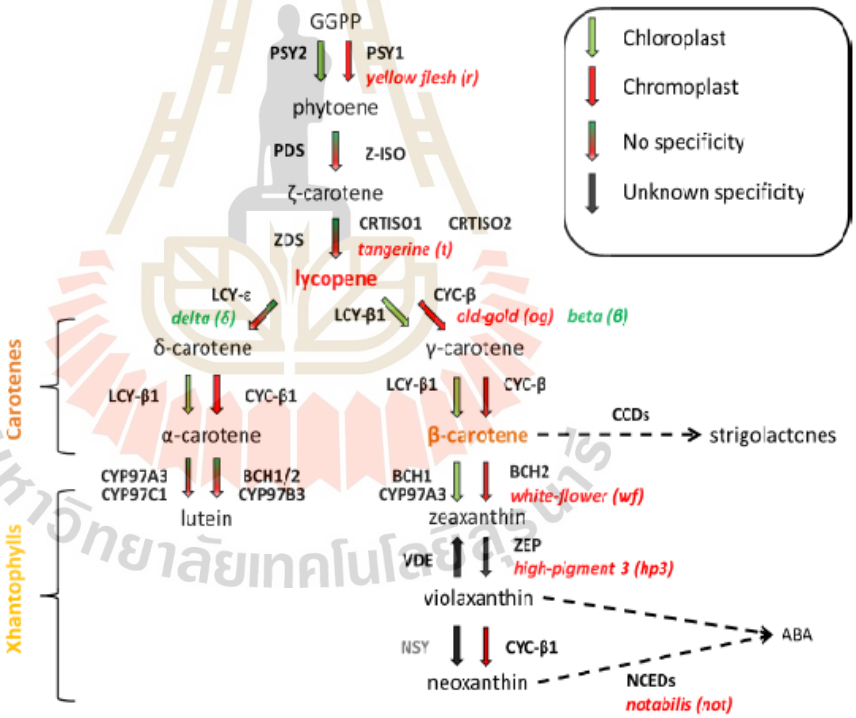


Figure 2.3 Carotenoid synthesis pathway (Ruiz-Sola and Rodriguez-Concepcion, 2012).

— Vitamin C is one group of antioxidants that is mostly found in tomatoes (Serio et al., 2007). Vitamin C or ascorbate is a lactone compound with 6 carbon atoms. The structure of vitamin C is a hydroxyl group, which has water–solubility properties (Smirnoff et al., 2001). The accumulation of ascorbate is high in the mature tomato

fruit. Previous research reported that the ascorbate content was low in dark green fruits and it increased when the fruits turn red by more than 80 % (Duma et al., 2015).

2.3.2 Genetic variation of antioxidants in tomato, there are hundreds of tomato varieties, from marble–sized grape or cherry tomatoes. The classification of tomato species in Thailand can be classified according to the application into two groups.

1) Fresh consuming tomato is direct consumption without processing such as cherry tomato, sida tomato, and salad tomato.

1.1) Cherry tomato is a popular variety, which is a type of small round tomato, the fruit size of 2.5 cm, fruits weight is 12–15 g and total soluble solids (TSS) > 8 °Brix. The shapes of fruit can range from spherical to slightly oblong fruit. Most of the fruit colors are red, thinner skin, and the taste is sweeter than in other varieties.

1.2) Sida tomato is a specie used for food components, such as an ingredient in papaya salad. The size of the fruit is about 4–5 cm and the weight of the fruit is about 20 g with a sour taste. TSS is less than 4 °Brix and the popular color of the fruit is pink.

1.3) Salad tomato (Loog Too variety) is often used for slicing on sandwiches or chopping into salads. These tomatoes have large fruit (5–7.5 cm diameter) and the weight of the fruits is> 40 g.There are several shapes of fruit including round, oblate, and pear–shaped. The colors of fruit are red, pink, orange, yellow, purple, and green. TSS is < 4 °Brix. They are usually a little tarter and juicier than cherry tomatoes.

2) Industrial tomatoes are used as raw materials for tomato sauce production and cosmetics. These tomatoes have a round shape and red color with high firmness and high total soluble solid (> 4 °Brix) and fruit weight is less than 40 g.

Types and varieties of tomato influence antioxidant contents (Hanson et al., 2004). Previous studies reported the difference in antioxidant content between a cherry tomato and a non–cherry tomato. It was reported that 76 varieties of non–cherry tomatoes derived from commercial varieties and germplasm lines produced β–carotene ranging from 29.7–173.1 mg/kg dry weight and lycopene ranging from 386.8–2,067.1 mg/kg dry weight, both traits were higher than a cherry tomato. However, cherry tomato of germplasm lines has higher vitamin C and total phenolic compounds than non–cherry tomatoes (Bhandari et al., 2016). In addition, there are highly correlated

coefficients of fruit colors and fruit sizes with antioxidant content in tomatoes.Red fruit has lycopene, ascorbic acid, and phenolic compound more than high beta fruit. Moreover, small red–fruit (11–30 g) has lycopene, β–carotene, and ascorbic acid more than large red–fruit (51–90 g). However, some studies reported that dark–green fruit had higher lycopene and β–carotene than large red–fruited. Therefore, the preliminary assessment of the genetic characteristics is necessary for the selection of high antioxidant genotypes.

2.3.3 Environmental effect on antioxidants

2.3.3.1 Temperature and relative humidity, temperature and relative humidity (RH) are factors affecting transpiration and translocation of water nutrient uptake in the plant. The temperature affects enzyme activity in plant metabolism. A temperature higher than 35°C decreases photosynthesis, transpiration rate in the reproductive state, leaf area, plant growth, dry weight, fruit set, and delays the development of normal fruit colors (Abdelmageeda et al., 2003; Sato et al., 2000). In addition, high temperature (>38°C) conditions could inhibit lycopene production, TSS, carotenoid accumulation, and antioxidant activity in tomatoes (Islam, 2005; Lokesha et al., 2019). Shivashankara et al. (2015). Lycopene and β–carotene accumulation in tomato ripening depend on temperature. Balcerowicz et al. (2020) reported that temperatures lower than 24°C and higher than 30°C on reproductive growth caused the reduction in lycopene and β–carotene accumulation in tomatoes. This is because the elevated temperature shifts the balance from the active Pfr form to the inactive Pr form of phytochromes. Reduced phytochrome activity causes decreased accumulation of carotenoid in the ripening stage (Figure 2.4). Generally, the optimum temperature for tomato production is an average temperature >18–27°C (Araki et al., 2000).

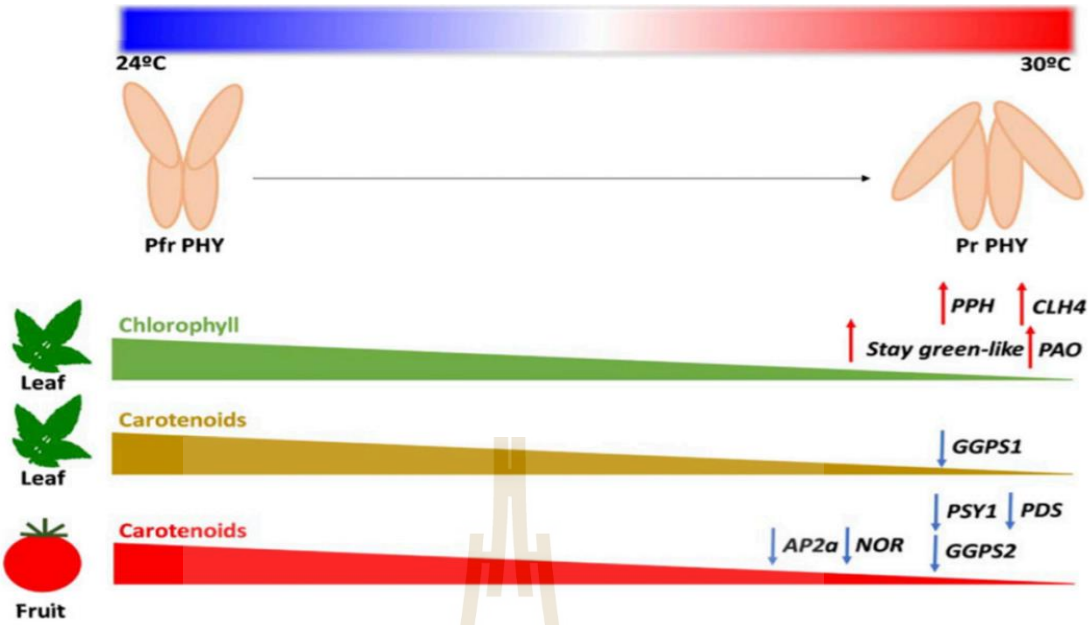


Figure 2.4 Effects of the temperature–controlled activity of phytochromes on pigment metabolism in tomatoes (Balcerowicz, 2020).

2.3.3.2 Effect of plant nutrients on plant growth and the accumulation of antioxidants. Plant nutrients are essential for plant growth and development. They can stimulate the metabolizing process in plants and are a precursor for photosynthesis in plants. Plant nutrients can be divided into 2 groups 1) macronutrients are the nutrient that plants require for growth, normally highly concentrate in plants, including C, H, O, N, P, K, Ca, Mg, and S, 2) micronutrients or trace elements are plant nutrients that plants require for growth and development less than macronutrients, including B, Fe, Cu, Zn, Mn, Mo, Cl, and Ni (Table 2.1). They are irreplaceable and required for normal crop growth and reproduction (Sainju, 2003). Plant nutrients are considered limiting factors for growth and development, as well as, yield and quality. They also greatly affect the content of secondary metabolites including those with antioxidant properties. This is not an exception for tomatoes, the results of Aina et al. (2019) suggested a positive relationship between the level of macronutrients (N, P, K) and the level of phenolics, lycopene, β–carotene, and flavonoids in tomato fruits. Rebouças et al. (2017) have also shown a positive relationship between N level and tomato fruit firmness and sweetness but a negative relationship was found with the ascorbic acid and titratable acidity. Excessive N

application can result in tomato yield reduction caused by abnormal vegetative growth and lodging which makes the harvesting more difficult. Similarly, excess availability of some other essential plant nutrients, such as B and Mn, can cause toxic effects on tomatoes (Sainju et al., 1999). Optimum P can regulate root initiation and stimulate early flowering and fruit set in tomatoes. It can also improve skin color and pulp, vitamin C content, taste, and hardiness. In the case of K, it activates ATP production enzymes which are important in regulating plant growth and stimulating the early flowering and setting of tomato fruits. This plant nutrient also affects the quality of tomato fruit such as the titratable acidity of tomato juice since it could activate translocation of glucose from source to sink in plant phloem (Hasanuzzaman et al., 2018). The increasing K concentration can increase the levels of citric and malic acids, TSS, sugars, and carotene in tomato fruits, thus improving the tomato's storage quality. A study by Woldemariam et al. (2018) reported that K can improve the sweetness of tomato fruit. It plays an essential role in carotenoid production by activating the enzyme phytoene synthase which changes pigment in the carotenoid group during the ripening stage (Rodriguez, 2001). A high level of K can increase the antioxidant activity in tomatoes (Serio et al., 2007), similar to a study by Taber et al. (2008) which found that 350–450 $mg.L^{-1}$ of K in a soilless culture can promote lycopene accumulation in tomato fruits. Furthermore, at the fruit setting stage, the K:N ratio is crucial for tomato yield and quality. Fallah et al. (2021) reported that the nutrient solution with the K:N ratio of 2:1 increased yield per plant, fruit firmness, and TSS. However, the highest ascorbic acid was found in K:N ratio of 4:1, while this ratio of K:N decreased vegetative growth and the translocation ratio from source to sink (Scanlan and Morgan, 1982). In the case of micronutrients, Ni plays an essential role in several enzyme activities which directly affect plant growth and development. However, too much Ni can be toxic to the tomato plant and can inhibit growth and yields (Rehman et al., 2016; Kamboj et al., 2018).

Table 2.1 Relative of nutrients to biomass in tomato plants.

Element	Chemical Symbol	Form Available to Plant	Concentration in dry tissue	
			$mg.L^{-1}$	%
Carbon	C	CO_2	450,000	45
Oxygen	O	O_2, H_2O	450,000	45
Hydrogen	H	H_2O	60,000	6
Nitrogen	N	NO_3^-, NH_4^+	15,000	1.5
Potassium	K	K^+	10,000	1.0
Calcium	Ca	Ca^{++}	5,000	0.5
Magnesium	Mg	Mg^{++}	2,000	0.2
Phosphorous	P	$H_2PO^-_4$, HPO_4^{-2}	2,000	0.2
Sulfer	S	SO^-_4, SO_2	1,000	0.1
Chloride	Cl	CL^-	100	0.01
Iron	Fe	Fe^{++}, Fe^{+++}	100	0.01
Manganese	Mn	Mn^{++}	50	0.005
Boron	B	H_3BO_3	20	0.002
Zinc	Zn	Zn^{++}	20	0.002
Copper	Cu	Cu^+, Cu^{++}	6	0.0006
Molybdenum	Mo	MoO_4^-	0.1	0.00001
Nickel	Ni	Ni^{++}	0.1	0.00001

Kumar et al. (2015) reported that 50 *μM* of Ni can induce stress and also activate antioxidant enzymes i.e. guaiacol peroxidase (GPX) and ascorbate peroxidase (APX). In addition, 15–50 $mg.L^{-1}$ Ni can increase the TSS, vitamin C, and N, P, and K accumulation in tomato fruits (Palacios et al., 1998; Gad et al., 2007; Kumar et al., 2015). To achieve optimal yield with high nutritional quality, growers must provide plant nutrients fitting

for their tomatoes, however, the nutrient management may need to be specific to the tomato varieties.

2.3.4 Tomato breeding using molecular markers for high antioxidant contents, Antioxidants in tomatoes are quantitative traits controlled by multiple genes, which is located on different chromosomes (Okmen et al., 2011). Heritability testing of antioxidants in 35 varieties/ species of tomato, broad–sense heritability of vitamin A, lycopene, and vitamin C were 99.7, 95.1 and 87.4 %, respectively (Hedau et al., 2008). However, it found moderate to low narrow–sense heritability (16–50.13%) and low value of genetic advance of lycopene content in tomatoes (Kumar et al., 2016). Kaushik and Dhaliwal (2018) reported that the inheritance of lycopene is very low additive gene action. In addition, there are possible genes that control this characteristic, which is the dominance of the gene action.

Molecular markers are widely used to classify genetic characteristics in quantitative traits. Generally, high antioxidant contents improvement requires high genetic diversity germplasm (Kavitha et al., 2014). Singh et al. (2018) compared SSR markers and morphological markers to evaluate the genetic diversity of 24 tomato varieties. Lycopene specific primers (phytoene synthase (Psy–1), phytoene desaturase (Pds–1), zeta carotene desaturase (Zds–1), and lycopene cyclase (Lcy–1)) were used. The results indicated that the morphological markers could classify the genetic diversity of 24 varieties at a 65% similarity coefficient. Four lycopene–specific primers could classify the genetic diversity of 24 varieties at a 90% similarity coefficient. Quantitative trait loci QTL for increasing carotenoid contents in tomatoes was generated by Kinkade abd Foolad (2003). The recombinant inbred line (RIL) populations generated from *S. lycopersicum* × *S. pimpinellifolium* (high lycopene species) were used to detect QTLs for high lycopene contents. There were two QTLs include *lyc7.1* (chromosomes 7) and *lcy12.1* (chromosomes 12) were linked to lycopene traits in RIL populations. To generate marker–assisted selection, two QTLs were validated the effect of phenotype on lycopene content of BC_2 population that generated from selected RIL × *S. lycopersicum* (recurrent parent). The result showed *lyc7.1* was not a significant difference in lycopene content in BC_2 populations while lcy12.1 significantly

increased lycopene higher than recurrent parent by 70.3%. Therefore, the *lcy12.1* had potential targets for marker–assisted selection of tomato lycopene contents.

Based on the data obtained, molecular markers can be applied to the selection of inbreeding lines that contain genes that are specific to lycopene in the population with differences in morphology characteristics, to generate tomato varieties that have high antioxidant content. In addition, Babak et al. (2018) studied the development of tomato hybrids by combining two pigment content genes including β–carotene (B), old–gold crimson (ogc), high pigment–2–dark green (hp2dg), and long shelf life (gf–3), are non–ripening (nor), ripening inhibitor (rin), and Alcobaca (norA), which develop of F_1 hybrids using the method of successive crosses and selected in F_2 and F_3 progenies. Populations that combined pigment content genes and long shelf life gene, are B/rin/gf, B//rin/hp, B/nor/gf, B/nor/hp, ogc/rin/gf, ogc/rin/hp, ogc/nor/gf, ogc/nor/hp. Designed co–dominant sequence characterized amplified region (SCAR)and using gene–specific primers. Antioxidant analysis in tomato fruit found that characteristics of long shelf life gene in nor, rin, and norA are affected to decrease the lycopene content in tomato fruit. The pigment content gene B, ogc, hp2dg, and gf–3 had an influence on the accumulation of lycopene content in tomato fruit, especially hp2dg and ogc. When thein expression causes high accumulation of lycopene at 19.9 mg/100g fresh weight, even though there was expression of other genes that can inhibit the generation of lycopene content in the species. Therefore, molecular marker utilization for the identification of target gene and genetic diversity of plants is the most important in the field of plant breeding.

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CHAPTER 3
MATERIALS AND METHODS

Experiment 1. Genetic Diversity Evaluation and Selection of Tomato Genotypes for High Antioxidants

3.1 Genetic diversity evaluation

3.1.1 Genetic diversity evaluation using ISSR markers

- Plant materials

Fifteen (15) tomato genotypes from Thailand and the Asian Vegetable Research and Development Center (AVRDC, Taiwan) including seven hybrid cultivars (Phet Chompoo, Petch Rung, Sweet Boy, Sida, Loog Too, Ranger), two open –pollinated cultivars (Sida Namkhem and Sweet Cherry 154) and six inbred lines of high carotenoid tomato from AVRDC (AVTO0102 (CLN2366B), AVTO1422 (CLN3670F), AVTO1424 (CLN3682C), AVTO1420 (CLN3670B), AVTO1418 (CLN3669A), AVTO1008 (CLN3078C) and AVTO1008 (CLN3078C) were used in this study (Table 3.1). The experiment was conducted at the Suranaree University of Technology. The seeds of all tomato genotypes were germinated and grown under greenhouse conditions with an average temperature of 27 °C and average relative humidity of 70%.

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Table 3.1 Fifteen tomato genotypes used in the experiment.

Genotypes	Cultivars/lines	Type of Cultivars/lines	Resources
Cherry	Sweet Cherry 154	OP	Tropical Vegetable Plant Research Center
Cherry	Sweet Girl	Hybrid	Chia Tai Company, TH
Cherry	Sweet Boy	Hybrid	Chia Tai Company, TH
Non–cherry	Phet Chompoo	Hybrid	East–West Seed Thailand
Non–cherry	Sida Namkhem	OP	Siam Agrichem Company, TH
Non–cherry	Petch Rung	Hybrid	Chia Tai Company, TH
Non–cherry	Sida	Hybrid	Chia Tai Company, TH
Non–cherry	Loog Too	Hybrid	Chia Tai Company, TH
Non–cherry	Ranger	Hybrid	Chia Tai Company, TH
Non–cherry	AVTO0102(CLN2366B)	Inbred line	AVRDC, Taiwan
Non–cherry	AVTO1422 (CLN3670F)	Inbred line	AVRDC, Taiwan
Non–cherry	AVTO1424 (CLN3682C)	Inbred line	AVRDC, Taiwan
Non–cherry	AVTO1420 (CLN3670B)	Inbred line	AVRDC, Taiwan
Non–cherry	AVTO1418 (CLN3669A)	Inbred line	AVRDC, Taiwan
Non–cherry	AVTO1008 (CLN3078C)	Inbred line	AVRDC, Taiwan

- Extraction and quantification of DNA

Fifteen days after sowing, five seedlings of each genotype were randomized for DNA extraction. From each tomato genotype, samples of young leaves (5 plants per genotype) of approximately 2–3 g were crushed into liquid nitrogen using a mortar and pestle. Then, genomic DNA was isolated by using the modified CTAB method of

(Doyle and Doyle, 1990). Transfer the powder sample to a 1,500 µl microcentrifuge tube and keep it in liquid nitrogen.

- Add 600 µl of preheating (65 °C) extraction buffer 600 µl to microcentrifuge tube that contained powder sample and swirl to mix using the vortex mixer
- The sample was incubated in an incubator at 65 °C and 30 minutes.
- Add 24 chloroform: 1 isoamyl alcohol (v/v) 600 µl and mixed by slight inversion
- Centrifuge at 13,000 rpm for 20 minutes at room temperature in a microcentrifuge
- Transfer the supernatant in the upper aqueous phase, which contains the DNA of approximated 500 µl, to a new 1,500 µl microcentrifuge tube.
- Repeat extraction method 3.1.4–3.1.6, finally to get 400 µl of supernatants
- Add 0.5 volume (200 µl) of 5 M NaCl to the sample and swirl gently to mix
- Add 1 volume (600 µl) of 100% isopropanol (cold) to the sample and swirl gently to mix
- Centrifuge at 13,000 rpm for 15 minutes at room temperature
- Wash the DNA pellet with 500 µl of 70% ethanol (cold) and centrifuge at 13,000 rpm for 5 minutes at room temperature, discard 70% ethanol from the tube
- Wash the DNA pellet with 500 µl of 100% ethanol (cold) and centrifuge at 13,000 rpm for 5 minutes at room temperature, discard 100% ethanol from the tube
- Invert the centrifuge tube on filter paper and dry the DNA pellet at 37°C in a heated vacuum desiccator until the DNA pellet dried
- Dissolve the DNA pellet in 100 µl deionized water (DI)
- Add 40 µl RNAase A (1 mg/ml) and incubated for 30 minutes at 37°C

Store the DNA at –20°C

**Extraction buffer reagent, the extraction buffer contained 3% cetyltrimethylammonium bromide (CTAB) (1v), 1.4 M NaCl, 0.1 M Tris–HCl pH 8.0 (1v), 20 mM ethylenediaminetetraacetic acid (EDTA) pH 8.0 (1v), 2% polyvinylpyrrolidone (PVP)

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(1v) and 0.2% β–mercaptoethanol (1v). Tris/Borate/EDTA buffer (TBE) was prepared in stock concentration 10x TBE. The buffer contained 108 g Tris, 55 g boric acid, and 7.5 g Na_2EDTA. Dissolve the reagents and adjust the volume to 1,000 by DI water and store at room temperature.

- DNA concentration analysis; DNA concentration was determined by ND–1,000 (Nanodrop Technologies, Inc., Wilmington, DE, USA). The concentration and purity of DNA should be determined by measuring the absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) in a spectrophotometer.

$$\textbf{Concentration of genomic DNA (ng/µl)} = (A_{260}) / (A_{280})$$

where A_{260} is DNA absorption, A_{280} is protein absorption.

DNA solutions with different concentrations were prepared to 150 ng/µl by serial dilutions using DI water. Stock DNA samples of each genotype were combined in a new microcentrifuge tube of about 100 µl.

- Amplification conditions; Thirty–two (32) ISSR markers were used for PCR amplification of genomic DNA (Table 3.2). PCR was carried out in 20 µl of reaction mixtures containing 150 ng of DNA, 1x buffer (500 mM KCL, 100 mM Tris–HCL (PH 9.1 at 20 ℃ and 0.1% Triton X–100), 1.5 mM $MgCl_2$, 0.2 mM of dATP, dCTP, dGTP and dTTP, primer 0.5 µM and 1 unit of *Taq* DNA polymerase. T100™ Thermal cycle (Bio–rad Laboratory, Inc., California, USA) was used for DNA amplification. PCR cycles were started with initial (1) denature at 95 ℃ for 5 minutes, (2) denaturation at 95 ℃ for 30 seconds, annealing at 54–56 ℃ for 45 seconds, extension at 72 ℃ for 2 minutes for 37 cycles, and (3) extension at 72 ℃ for 7 minutes. The PCR sample was inspected for DNA amplification in gel electrophoresis.

Table 3.2 Thirty–two ISSR primers sequence and annealing temperature.

Primer code	Nucleotide sequence 5'–3'	Annealing temperature	Reference
ISSR1	$CAC(TCC)_5$	61.0 °C	Hassan et al. (2013)
ISSR2	$AGA(TCC)_5$	45.0 °C	
ISSR890	$ACG(TG)_7$	49.0 °C	
ISSR891	$TCT(TG)_7$	47.0 °C	
ISSR811	$(GA)_8C$	47.0 °C	
ISSR807	$(AG)_8T$	47.0 °C	
AW–3	$(GT)_7AG$	47.0 °C	
ISSR3	$(CA)_8AT$	47.0 °C	
ISSR16	$CGTC(AC)_7$	47.0 °C	
ISSR17	$CAGC(AC)_7$	58.0 °C	
DAT	$(GA)_7AC$	47.0 °C	
ISSR–34	$(AG)_8CT$	47.0 °C	
ISSR842	$(GA)_8TG$	50.0 °C	

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Table 3.2 (Continued).

Primer code	Nucleotide sequence 5'–3'	Annealing temperature	Reference
ISSR 814	$(CT)_8TG$	47.6 °C	Sharifova et al. (2017)
UBC 825	$(ACACAC)_2ACA\ CT$	51.4 °C	
ISSR 857	$(AC)_8YT$	53.1 °C	
UBC 860	$(TGTGTG)_2TGT\ GRA$	53.1 °C	
ISSR HB8	$(GA)_6GG$	41.9 °C	
ISSR HB9	$(GT)_6GG$	46.6 °C	
A7	$(AG)_{10}T$	54.0 °C	
UBC867	$(GGC)_8$	54.0 °C	Henareh et al. (2016)
UBC820	$(GT)_8C$	55.0 °C	
A12	$(GA)_6CC$	53.0 °C	
UBC848	$(CA)_8R*G$	54.0 °C	
UBC855	$(AC)_8Y*T$	56.0 °C	
UBC818	$(CA)_8G$	54.0 °C	
UBC849	$(GT_)_8CG$	54.0 °C	
UBC808	$(AG)_8C$	54.0 °C	
UBC840	$(GA)_8Y*T$	54.0 °C	
UBC815	$(CT)_8T$	54.0 °C	
UBC880	$(GGAGA)_3$	53.0 °C	
ISSR430	$(TGG)_7A$	56.0 °C	

R= A or G, Y= C or T.

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- Agarose gel electrophoresis
  - Agarose was prepared in 2% (w/v) for gel electrophoresis. Agarose was weighed at 2 g in 100 ml of 0.5X TBE buffer. Heating agarose solution in the microwave until completely dissolved. Let the agarose solution cool down for about 5 minutes and pour the agarose solution into a gel cast that wared well comb in place.
  - Place the sample gel at room temperature until completely solidified about 10 minutes. Well comb was pulled out of the sample gel and put gel in the horizontal electrophoresis tank.
  - Add 0.5x TBE buffer until the gel is flooded.
  - Loading dye that contained 6x loading dye: SYBR loading dye (2:1) was added to 2 µl in a 4 µl DNA sample, that received PCR reaction.
  - DNA in each sample was mixed with loading dye and load the mixed DNA sample well.
  - Run gel electrophoresis for 10 minutes at 120 V until the dye lines approximately 50% of the gel.
  - Remove the gel from the electrophoresis tank and placed the gel on the UV transilluminator. DNA fragments were visualized the band appearance on the gel.
- Acrylamide gel electrophoresis
  - Preparation of grass plates; DI water was used for washing the grass plates, spacer, and combs for the first time, a 95% ethyl alcohol was repeated washing. Grass plates were assembled in the gel casting mold and placed on the rubber of the mounting bracket.
  - Acrylamide gel preparation 6% acrylamide (20 ml)

Urea	8.4 g	Vortex to dissolved
10x TBE	2.0 ml	
40% Acrylamide/Bis (19:1)	3.0 ml	
DI water	(Adjust volume in 20 ml)	
10% APS	133.3 µl	Added before use
TEMED	13.3 µl	

- Pure the gel solution; The gel solution was placed on an ice pod. Micropipette 1,000 µl was loaded gel solution into gel casting mold until almost full. Put the comb in the gel being careful not to bubble and let the gel at room temperature until completely solidified about 60 minutes.
- Pre –run; The gel was removed from the gel casting mold and the comb was pulled out of the gel. Tap water was washed the excess gel outside grass plates and bubbles above the well. The gel was assembled in a support gel plate and put in a vertical electrophoresis tank (1 tank per 4 gels). 1x TBE buffer was added in space between gel plates until the top of the plate to check for possible leaks. The buffer was added to empty channels of the electrophoresis tank to electric current complete the circuit.
- DNA sample preparation; DNA sample from PCR product was staked at the bottom PCR tube by vortex mix. PCR product 5 µl of each sample was sucked in a new PCR tube that contained 3x SSR loading dye 2.5 µl. they were mixed for about 1 minute in a vortex mixer and preheat at 94 °C placed sample on icebox after being heated for 5 minutes. 100 bp ladder (vivantis DNA ladder) was used for an estimate by comparison with a molecular weight of DNA bands.
- DNA sample loading; Syringe was used to expel urea out well above the gel. DNA in each sample was loaded in the well. The first well was loaded 100 bp ladder for standard molecular weight DNA and DNA sample respectively. The free well was loaded with 3x SSR loading dye to maintain the DNA bands straight.
- Gel running; Close the cover tank and turn on the device for running, using 80 minutes at 200 V 400 A.

- Gel Stained

  The gel stain was accorded to the method of Sambrook and Russel (2001);

  - Remove the gel from the grass plate and place the gel in the gel box. The gel was soaked in 10% ethyl alcohol and placed on an orbital shaker for 10 minutes at 80 rpm.

- The gel was washed with DI water one time and soaked in 0.7% HNO_3 solutions and placed on an orbital shaker for 6 minutes at 80 rpm.
- HNO_3 solution was poured out of the gel box and then the gel was washed with DI two times. The gel was soaked in 0.2% $AgNO_3$ solutions and closed the cover box. Placed gel box on orbital for 30 minutes at 80 rpm.
- $AgNO_3$ solutions were poured out of the gel box and washed DNA with DI two times for about 2 minutes. The gel was soaked in the developer and placed on an orbital shaker until the DNA band was appearances.
- The developer was poured out of the gel box and washed DNA with DI one time. The gel was soaked in 3% acetic acid and placed on an orbital shaker for 5 minutes.
- Acetic acid was purred out of the gel box and washed DNA with DI two times. Finally, the gel was soaked in 10% ethyl alcohol for 10 minutes.

- Gel dried; Cellophane sheet and glass plate were soaked in water to prepare the gel dried. The first cellophane was placed on the glass plate and placed gel on the cellophane sheet. Wet the area of the sheet where the gel was placed with water. The second cellophane sheet was slowly placed over the gel. Remove water and bubbles trapped between two sheets. Clamp the cellophane sheet on the glass plate using the clamps. Place the grass plate in the vertical position and allow it to dry at room temperature.

- Data analysis; Total of 32 ISSR primers (A7, A12, AW–3, DAT, ISSR HB8, ISSR HB9, ISSR1, ISSR2, ISSR3, ISSR16, ISSR17, ISSR–34, ISSR430, ISSR807, ISSR811, ISSR 814, ISSR842, ISSR857, ISSR890, ISSR891, UBC808, UBC815, UBC818, UBC820, UBC825, UBC840, UBC848, UBC849, UBC855, UBC860, UBC867 and UBC880) were used to evaluate polymorphism of 15 tomato genotypes as shown in Table 3.2 (Hassan et al., 2013). For each ISSR marker, PCR–amplified bands were identified and scored visually on 15 genotypes of tomatoes. Final scoring and data analysis for all the markers were done with only distinguishable and reproducible bands. The ISSR amplified band was coded as absence (0) and presence (1). A cluster analysis was made with the unweighted paired grouped mean arithmetic average (UPGMA), generating the corresponding dendrogram using the statistical package NTSYSpc version 2.2 (Rohlf,

2000). With the information of the bands analyzed for each primer, a matrix of binary data was used to calculate the Jaccard similarity coefficient. Polymorphism information content (PIC) of each primer was calculated according to the equation described by Botstein et al. (1980) was using the following equation:

$$PIC = 1 - \Sigma Pi^2$$

where i is the total number of alleles detected for the ISSR marker, and Pi is the frequency of the i^{th} allele in the genotypes studied.

XLSTAT software V. 2015 (Addinsoft, Inc., Paris, France) (Mantel, 1967) was used again to construct principal coordinate analysis (PCoA) to assess the partitioning of the genotypic variability at the population level. Population structure was estimated based on a grouping algorithm.

3.1.2 Agronomic traits evaluation for genetic diversity

The greenhouse experiments were conducted in an RCBD with three replications and each treatment included 30 plants at the Suranaree University of Technology Farm, Nakhon Ratchasima, Thailand. The first condition was performed in an evaporation cooling greenhouse with an average relative humidity of 78% and an average temperature of 28–35 °C (December 2018–March 2019). The second condition was an uncontrollable environment greenhouse with a relative humidity of 62 % and an average temperature between 35–40 °C (June–September 2019). The relative humidity and temperature were collected daily after the experiment began. Fifteen genotypes of tomato were used in this study including seven hybrid cultivars (Phet Chompoo, Petch Rung, Sweet Boy, Sida, Loog Too, and Ranger), two open –pollinated cultivars (Sida Namkhem and Sweet Cherry 154), and six inbred lines of high carotenoid tomato from AVRDC (AVTO0102 (CLN2366B), AVTO1422 (CLN3670F), AVTO1424 (CLN3682C), AVTO1420 (CLN3670B), AVTO1418 (CLN3669A) and AVTO1008 (CLN3078C)) (Table 3.1) were germinated in peat moss. One–month–old tomato seedlings were transplanted in a growing bag (8×16 inch) which contain coconut coir and coconut bark. The inter–row and within–row spacing is 50×50 cm. Standard agronomical techniques were used for plant nutrition and pest management. Briefly, all plants were irrigated using drip irrigation with nutrient solutions (Hoagland and Arnon, 1950) once a day (EC 2.5 dS/m and pH 6.5). The solution A consisted of $Ca(NO_3)_2$ (354 $mg.L^{-1}$), Fe–EDDHA (0.12 $mg.L^{-1}$), and

Fe–EDTA (5.3 mg.L^{-1}), and the solution B consisted of KNO_3 (302 mg.L^{-1}), KH_2PO_4 (135 mg.L^{-1}), $MgSO_4$ (135 mg.L^{-1}) and completed micronutrients (Nickspray, 4.3 mg.L^{-1}). Pest management was done according to the pest outbreak. The experiment was laid out in a randomized complete block design (RCBD) with three replications (30 plants per replication). The biometrical observations were recorded on five randomly taken plants from each cultivar in each replication. The morphology characters, yield, yield components, fruit quality, antioxidant contents, and antioxidant activity were estimated in the experiment.

- Data collection
  - Morphological traits; Morphological traits of 15 tomato genotypes including growth habit, plant height, days to flowering, days to ripening, fruit color, and fruit shape were recorded by repeating three measurements in each treatment, randomly.
    - Growth habit, the traits including indeterminate, determinate, and semi–determinate plants were classified in among tomato genotypes.
    - Plant height was measured from the ground to the tip of the plant using a tape measure and reported as a centimeter (cm).
    - Days to flowering were counted from the day of transplanting until the day of 50% flowering in the first cluster.
    - Days to ripening were counted from the day of transplanting until the day to 50% of fruit ripening in the first cluster.
    - Fruit color was estimated in the fruit ripening period (at 45–50 days after anthesis). The fruit colors of various genotypes were measured by visual assessment.
    - Fruit shapes were classified according to Rodriguez et al. (2011). There are eight fruit shape categories, flat, round, rectangular, ellipsoid, heart, long, obovoid, and oxheart shape (Figure 3.1). Three replicates were repeated randomly in each treatment.

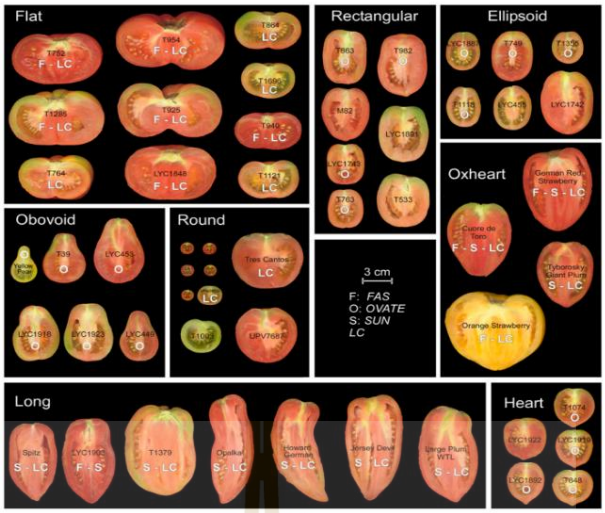

Figure 3.1 Tomato fruit shape classified from Rodriguez et al. (2011).

— Yield and yield components; Yield and yield components of 15 tomato genotypes included cluster per plant, fruit per cluster, fruit weight and yield per plant. Three replicates were repeated randomly in each treatment.

  — Cluster per plant was measured in periods of the days of ripening in the first cluster of various genotypes. All of the fruit clusters were tagged and counted from upper to lower plant.

  — Fruit per cluster, the total fruits per cluster were measured in each pre – tagged cluster between fruit ripening periods. The fruit cluster in the bottom, middle and above plants were randomized for counted total fruit per cluster.

  — Fruit weight was measured in the ripening stage about 45 days after anthesis. The 10 fruits of various genotypes were randomized to weighing using a Sartorius TE1502S balance. Average fruit weight was recorded in each genotype as gram (g).

  — Yield per plant, the ripened tomato fruit of various genotypes was used for determining the yield per plant. The yield of tomatoes was measured on the pre –tagged number of the cluster on the day of ripening. The total fruit weight per plant was weighed using a Sartorius TE1502S balance. Yield per plant was recorded in gram (g).

— Fruit quality; After 45 days of anthesis, tomato fruits at a ripening stage were randomly collected from each plant. Two fruits were randomly sampled on a second cluster from the trunk of each plant.

— Fruit color index

Fruit color index, the fruit samples were used to measure color values *L**, *a**, and *b** using CR–400 Chroma Meter, expressed in numerical terms of *a**/*b** (redness/yellowness). The *L** value indicated the lightness reflected. The *a** value is a measurement of the intensity of the red (+values) or green (–values) coloration, and the *b** value exhibited the intensity of the yellow (+ values) or blue (–values) coloration (Figure 3.2).

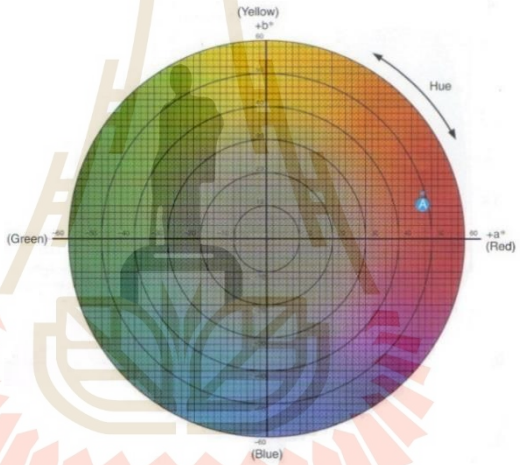

Figure 3.2 Chromaticity diagram (CIE, 1976).

— Total soluble solid (TSS) was measured using the refractometer MASTER–100H/ ATAGO. Sample fruits were homogenized in a blender and filtered in Whatman filter paper. The tomato juice was dropped in between the prisms of the hand refractometer and note the reading at the demarcation line. The result was reported as °Brix (Joomwong and Sornsrivichai, 2005).

— Fruit firmness was measured by the TA–XT2i texture analyzer (Texture Technologies, United States). The firmness tester was equipped with an 0.5 mm probe and compressed prob on the center fruit sample deep 0.5

— Antioxidant contents and antioxidant activity; Antioxidant contents and antioxidant activity including lycopene, β–carotene contents, and DPPH° scavenging assay were measured.

— Lycopene and β–carotene contents

Lycopene content was estimated following the method of Sadler et al. (1990). Fruits were homogenized in a blender and a gram of freshly homogenized was placed in a 25 ml Erlenmeyer flask wrapped with aluminum foil. Lycopene extraction was done by adding 20 ml of hexane: acetone: ethanol (2:1:1, v:v:v) in darkness. The flasks were placed on a shaker to mix the content at 180 rpm, after 15 minutes of shaking, 10 ml of cold deionized water was added and the shaking was continued for 5 minutes. The absorbance of the non–polar (upper) layer was measured in a 1 cm path length glass cuvette at 503 nm and compared with a blank of hexane solvent using UV–Visible Spectrophotometers–UH5300. The lycopene content (mg/100 g fresh weight, FW) was estimated using the following equations:

$$\text{Lycopene (mg/100g FW)} = (X/Y) \times A_{503} \times 3.12$$

where X was hexane volume (ml) and Y was the sampling fresh weight (g)

The β–carotene content was determined according to Thomnuad (2008). A similar extraction procedure like lycopene extraction was carried out, the absorbance of the non–polar (upper) layer was measured in a 1 cm path length glass cuvette at 663, 645, 505, and 453 nm (UV–Visible Spectrophotometers–UH5300) and compared with a blank of hexane solvent. The β–carotene content (mg/100 g FW) was estimated according to Nagata and Yamashita (1992) using the following equations:

$$\beta\text{–carotene (mg/100g FW)} = 0.216A_{663} - 1.22A_{545} - 0.304A_{505} + 0.452A_{453}$$

where, A_{663}, A_{545}, A_{505}, and A_{453} are absorbance at 663nm, 545 nm, 505nm, and 453 nm.

— Antioxidant activities (DPPH° scavenging assay); Antioxidant activities were analyzed according to the method of Erge and Karadeniz (2011). Fifteen grams of tomato puree was blended with acetone (1:2 w/v) for 12 hours in darkness. The extraction was filtered through Whatman no 1 paper. The filtrates were collected and acetone was evaporated at 45 °C. Tomato extracts were made to the volume of 10 ml and stored at –20 °C.ใ.The stock solution of DPPH was prepared by dissolving 25 mg DPPH with 100 ml methanol. The 50 µl of tomato extract was added to 2,450 µl of DPPH solution. The absorbance was measured at 517 nm using a UV–VIS spectrophotometer. A control treatment was treated with 50 µl of methanol. The antioxidant activity was determined as the basis of percent quenching of DPPH radical and was expressed as the decreasing percent absorbance of the DPPH radical solution by using the following equation.

$$\mathbf{A.A.}\,(\%) = \frac{(\mathbf{Control\ absorbance} - \mathbf{Sample\ absorbance})}{\mathbf{Control\ absorbance}} \times \mathbf{100}$$

Results were analyzed by linear regression of the standard curves at 500, 1,000, 1,500, and 2,000 µM of Trolox. Preparing the solution for antioxidant activities and antioxidant activities of tomato cultivars were expressed as µM Trolox equivalent per 100 g fresh weight (µM TE 100 g).

- Data analysis

Means of growth, yield, and quality of yield including plant height, days to flowering, days to ripening, cluster per plant, fruit per cluster, fruit weight, yield per plant, fruit color index, fruit firmness, total soluble solid, lycopene content, β–carotene content and antioxidant activity are assessed by analysis of variance (ANOVA). When a significant difference was detected, means were compared using multiple comparisons by DMRT (Duncan' New Multiple Range Test). All statistical comparisons will be performed using SPSS v.14 for the window. A cluster analysis of morphological characters was made with the unweighted paired grouped mean arithmetic average (UPGMA), generating the corresponding dendrogram using the statistical package NTSYSpc version 2.2 (Rohlf, 2000).

3.2 Marker–assisted selection in tomato for high lycopene content

Total of four SSR primers (Psy–1, Pds–1, Zds–1 and Lcy–1) and two SCAR primers (Hp–2^{dg} and Og^{c}) as shown in Table 3.3 were used to screen for high lycopene content of 15 tomato genotypes.

Table 3.3 Four SSR and two SCAR primers sequence and annealing temperature.

Marker	Primers Code	Primer sequence (5'–3')	Annealing temperature	Reference
SSR	Psy–1	TTGCGCTTGTTGAGTGAACG (F)	55.7 °C	Singh et al. (2018)
		TGTCGTTGCCTTTGATTCAGG (R)	56.0 °C	
	Pds–1	TTGTGTTTGCCGCGCCAGTGGAT (F)	60.9 °C	
		GCGCCTTCCATTGAAGCCAAGTAT (R)	60.0 °C	
	Zds–1	ATTATTACATTGAGGGACAAG (F)	54.2 °C	
		TCATCAGACAAGACTCAACTCATC (R)	54.3 °C	
	Lcy–1	CAGAGAGCTGTTGGAATCGGTGG (F)	59.4 °C	
		CATTCTTATCCTGTAACAAATTGT (R)	53.6 °C	
SCAR	Hp–2^{dg}	TTCTTGGATTGTATGGT (F)	55.0 °C	Babak et al. (2018)
		CACCAATGCTATGTGAAA (R)		
	Og^{c}	TAGGTCTATTTTCCAACAA (F)	55.0 °C	
		AAGACTCTGGCTTTGGATG (R)		

- Data scoring and statistical analysis

For each SSR marker and SCAR, PCR–amplified bands were identified and scored visually on 15 tomato genotypes. Final scoring and data analysis for all the markers were done with only distinguishable and reproducible bands. The SSR and SCAR amplified bands were presence target band (1) presence wild type band (3). Multiple linear regression (MR): MR model was conducted using the stepwise method for the selection of variables and the markers were modeled as fixed effects. Forward and backward selection was employed for the identification of significant markers (α= 0.1). Regression coefficients of markers included in the final model were used as markers effects to predict the genetic value of the individuals belonging to the validation population, being the model:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots \beta_k X_k + e_k,$$

where β_0= mean, β_k= fixed effect of marker k, X_k= genotype matrix of marker k and e_k= residual with N (0, σ^2_e).

Experiment 2. Plant Nutrients Management for High Antioxidant Tomato Production.

- Experimental design and plant materials

The greenhouse experiment was conducted in a randomized complete block design (RCBD) with three replications (20 plants/replication) at the Suranaree University of Technology Farm, Nakhon Ratchasima, Thailand. The experiment was divided into two environments: environment 1 was conducted between January–April 2020 and environment 2 was conducted between June–September 2020. Two tomato cultivars (Ranger and Sweet Girl) were used in this study, they were germinated in germination trays filled with peat moss. One–month–old tomato seedlings were transplanted into 2.10-liter growing bags containing growing media (coconut coir and coconut bark (4:1)). The bags were placed inside the greenhouse with the inter–row and within–row spacing of 50×50 cm.

Drip irrigation with nutrient solution (Hoagland and Arnon, 1950) was applied once a day (EC 1.5 dS/m and pH 6.5). Fifty days before flowering, tomato plants received the following nutrients formula: T1 (control)–Hoagland solution (H), T2–H + K400 (400 mg.L^{-1} K), T3–H + K300 (300 mg.L^{-1} K), T4–H + Ni20 (20 mg.L^{-1} Ni), T5–H + Ni10 (10 mg.L^{-1} Ni), and T6–H + K300 + Ni10 (Table 1). After flowering until the harvesting period, the nutrient formulas were maintained for each treatment but the solutions' EC were modified to 2.5 dS/m with a pH of 6.5.

Table 3.4 Detail of plant nutrient formulas used in this study.

Nutrients	Concentration ($mg.L^{-1}$)					
	T1 (Hoagland)	T2	T3	T4	T5	T6
N	242.65	242.65	242.65	242.65	242.65	242.65
P	30.86	30.86	30.86	30.86	30.86	30.86
K	231.22	400.00	300.00	231.22	231.22	300.00
Ca	202.37	202.37	202.37	202.37	202.37	202.37
Mg	48.22	48.22	48.22	48.22	48.22	48.22
S	63.69	63.69	63.69	63.69	63.69	63.69
Fe	4.99	4.99	4.99	4.99	4.99	4.99
Zn	0.05	0.05	0.05	0.05	0.05	0.05
Cu	0.02	0.02	0.02	0.02	0.02	0.02
Mn	0.50	0.50	0.50	0.50	0.50	0.50
B	0.51	0.51	0.51	0.51	0.51	0.51
Mo	0.01	0.01	0.01	0.01	0.01	0.01
Ni	–	–	–	20.00	10.00	10.00

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- Data collection

— Environmental conditions

During the experiment, temperature and RH were daily recorded as environmental condition traits using a thermo-hygrometer (TFA Accuracy). The monthly averages were later calculated.

— Yield and yield quality

After 45 days of anthesis, tomato fruits at a ripening stage were randomly collected from each plant. They were weighed and yield/ plant was estimated. Afterward, the collected tomato fruits were measured for fruit quality. Then, two fruits were randomly sampled on a second bunch from the trunk of each plant.

— Fruit color index, the fruit samples were used to measure color values *L**, *a**, and *b** using CR–400 Chroma Meter, expressed in numerical terms of *a**/b* (redness/yellowness). The *L** value indicated the lightness reflected. The *a** value is a measurement of the intensity of the red (+values) or green (–values) coloration, and the *b** value exhibited the intensity of the yellow (+ values) or blue (–values) coloration (Figure 3.2).

— Fruit firmness was measured on the TA–XT2i texture analyzer (Texture Technologies, United States). The firmness tester was equipped with an 0.5 mm probe and compressed prob on the center fruit sample deep 0.5 mm. The compression force was calculated and reported as the newton (N) force (Joomwong and Sornsrivichai, 2005).

— Total soluble solid (TSS) were measured on the refractometer MASTER-100H / ATAGO. The tomato fruits were homeginized in blender and filtered in Whatman filter paper. The tomato juice was droped in between the prisms of hand refractometer and note the reading at the demarcation line. Result were reported as (°Brix) (Joomwong and Sornsrivichai, 2005).

— Antioxidant contents

Antioxidant contents were measured in this study including lycopene and β–carotene contents. The lycopene content was estimated following the method of Sadler et al. (1990). Fruits were homogenized in a blender and a gram of freshly homogenized was placed in a 25 ml Erlenmeyer flask wrapped with aluminum foil. Lycopene extraction was done by adding 20 ml of hexane: acetone: ethanol (2:1:1, v:v:v) in darkness. The flasks were placed on shaker to mix the content at 180 rpm, after 15 minutes of shaking, 10 ml of cold deionized water was added and the shaking was continued for 5 minutes. The absorbance of the non–polar (upper) layer was measured in a 1 cm path length glass cuvette at 503 nm and compared with a blank of hexane solvent using UV–Visible Spectrophotometers–UH5300. The lycopene content (mg/100 g fresh weight, FW) was estimated using the following equations:

$$\text{Lycopene (mg/100g FW)} = (X/Y) \times A_{503} \times 3.12$$

when X was hexane volume (ml) and Y was the sampling fresh weight (g)

The β–carotene content was determined according to Thomnuad (2008). A similar extraction procedure like lycopene extraction was carried out, the absorbance of the non–polar (upper) layer was measured in a 1 cm path length glass cuvette at 663, 645, 505 and 453 nm (UV–Visible Spectrophotometers–UH5300) and compared with a blank of hexane solvent. The β–carotene content (mg/100 g FW) was estimated according to Nagata and Yamashita (1992) using the following equations:

$$\beta\text{–carotene (mg/100g FW)} = 0.216A_{663} - 1.22A_{545} - 0.304A_{505} + 0.452A_{453}$$

where, A_{663}, A_{545}, A_{505} and A_{453} are absorbance at 663nm, 545 nm, 505nm and 453 nm.

- Data analysis

Analysis of variances (ANOVA) of all traits was performed using SPSS v. 14 for window (Norman et al., 1970). When a significant difference was detected, means were compared using multiple comparisons by DMRT (Duncan's New Multiple Range Test). The correlation coefficients were used to determine the relationship between yield, yield quality, and antioxidant contents.

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CHAPTER 4

Results and Discussion

Experiment 1. Genetic Diversity Evaluation and Selection of Tomato Genotypes for High Antioxidants

This study was conducted on 15 genotypes of tomatoes. To estimate the genetic diversity of these tomato genotypes, 32 ISSR primers and morphological traits evaluation were used, whereas 4 SSR and 2 SCAR markers were used to identify the variation of lycopene content.

4.1 Genetic diversity evaluation in tomato genotypes

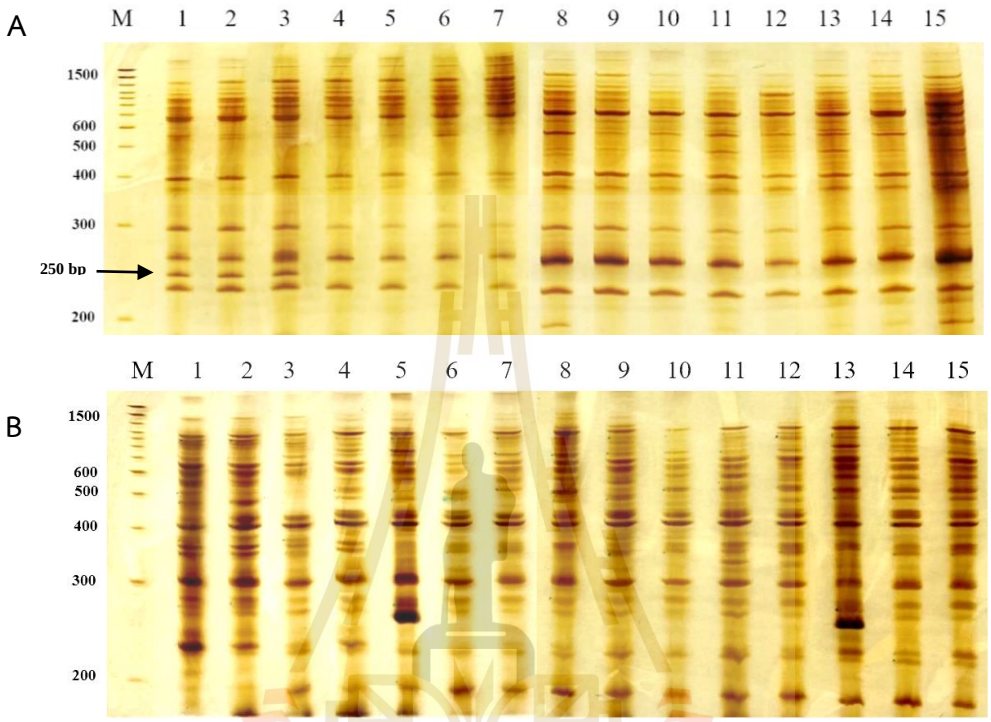
The different areas of collection and origin of these tomato genotypes used in this experiment were shown in Table 4.1. The nine genotypes (Sweet Cherry 154, Sweet Girl, Sweet Boy, Phet Chompoo, Sida Namkhem, Petch Rung, Sida, Loog Too, and Ranger) representing Thai cultivars and six genotypes (CLN3078C, CLN3670F, CLN3670B, CLN3669A, CLN3682C, and CLN2366B) located in Taiwan.

4.1.1 Genetic diversity evaluation using ISSR markers

Thirty–two ISSR primers were used to characterize the genetic diversity of the 15 tomato genotypes. The 32 ISSR primers generated a total of 214 amplified bands for all populations, of which 111 amplified bands were polymorphic (52%) (Table 4.1). Thirteen primer out of 32 primers (808, 812, 816, 825, 829, 835, 841, 846, 848, 855, 856, 857 and 880) could amplify and exhibit scorable bands. Similarity, when 10 ISSR primers were used for genetic diversity evaluation of 96 tomato accessions a total of 144 amplified bands and 53 polymorphic bands (37%) were found (Elsayed et al., 2011). Todorovska et al. (2014) showed that a total of 160 amplified bands and 81 polymorphic bands (51%) were detected by 5 SSR markers in 8 accessions of Bulgarian tomato. Therefore, these polymorphic 13 ISSR primers were used for the molecular characterization of 15 tomato genotypes. The size of amplified bands ranged from 200 to 1,500 bp. The primers amplified average total fragments of 16 bands, ranging from 8 to 26 bands. The percentage of polymorphic fragments varied between 35–67% of the total amplified bands with an average of 52%. The average polymorphic fragment

level in this study was high when compared with other previous studies. While, Figueiredo et al. (2016) reported the genetic diversity of 10 tomato lines using 12 ISSR markers, there were 27.62% of average percentage polymorphism and ranged from 11–55%. Polymorphism of primers depends on the number of samples and genotype variation in plant species. Vargas et al. (2020) studied the genetic diversity in different tomato species (*S. pimpinellifolium, S. lycopersicum* var. *cerasiforme, S. lycopersicum and S. peruvianum*) with 7 ISSR primers. They reported that the variation of tomato various species showed high polymorphism over 90% of 7 primers. The vast origins and geographical distribution of the genotypes showed high polymorphism in primers detection. Henareh et al. (2016) evaluated 31 tomato genotypes in Iran and the East Anatolian region of Turkey by 14 ISSR primers, which showed 100% polymorphism. For the results of this experiment, 15 tomato genotypes were restricted to the polymorphism value of the primers. However, primer ISSR 808, 841, 846, and 848 exhibited high polymorphisms of over 60%. The profiles generated by primer 841 ($(GA)_8YC$) contained the highest number of the polymorphic bands (16 bands) (Figure 4.1 (B)), primer 848 ($(CA)_8RG$) exhibited the highest polymorphism of 67% and. While primer 856 ($(AC)_8YA$) identified the lowest polymorphic bands (35%). Tikunov et al. (2003) reported that high polymorphic bands were found in primer CA, GA, and AG repeat identified in five tomato species. In addition, Reddy et al. (2002) reported the di–nucleotide repeats primers including (AG), (GA), (CT), (TC), (AC), and (CA) generated higher polymorphism than other di–nucleotide, tri–nucleotide and tetra–nucleotide repeats of ISSR primers in tomato. However, di–nucleotide repeat in ISSR–841 and 846 primers produced the highest number of scorable bands, polymorphism, and numbers of polymorphic bands in this study. Polymorphism information content (PIC) ranged from 0.25 to 0.47 with an average of 0.33. The highest value of PIC (0.47) was found in primer 812, while the lowest was observed in primer 835 (0.25). The PIC value indicated the ratio of polymorphic allele on the whole alleles in each primer and show the highest PIC at 0.5 for dominant markers (Nagl et al., 2011). Previous studies reported PIC values between 0.0–0.54 with an average of 0.27 of 27 tomato genotypes using 20 microsatellite markers (Kaushal et al., 2017), and PIC values ranging from 0.26 to 0.46 with an average of 0.36 of 31 landrace tomato genotypes in different regions of Turkey by 14 ISSR primers (Henareh et al., 2016). Therefore, PIC values obtained in this study,

111 polymorphic bands from 13 ISSR primers (808, 812, 816, 825, 829, 835, 841, 846, 848, 855, 856, 857, and 880) were useful for studies of genetic diversity in tomato genotypes.



M is marker of 100 bp, tomato genotypes ; 1=Sweet Cherry 154, 2=Sweet Girl, 3=Sweet Boy, 4= Loog Too, 5=Ranger, 6=Phet Chompoo, 7=Sida, 8=Petch Rung, 9=Sida Namkhem, 10= CLN3670B, 11=CLN3670F, 12=CLN3078C, 13=CLN3682C, 14=CLN2366B, 15= CLN3669A.

Figure 4.1 Profiles of DNA amplification of tomato genotypes, (A) profiles of the primer ISSR–856 marker, and (B) profiles of the primer ISSR–846 marker.

Table 4.1 Sequence and polymorphism of 32 ISSR primers used to evaluate genetic diversity in tomato genotypes.

ISSR primers	Primer sequence (5'– 3')	No. of scorable DNA bands	Number of polymorphic bands	Size of amplified fragments (bp)	PIC
ISSR808	$(AG)_8C$	8	5 (63)[1]	400–1,400	0.39
ISSR812	$(GA)_8A$	11	5 (45)	200–1,200	0.47
ISSR816	$(CA)_8T$	23	11 (48)	200–1500	0.31
ISSR825	$(AC)_8T$	16	7 (44)	200–1,500	0.28
ISSR829	$(TG)_8T$	10	4 (40)	300–1500	0.28
ISSR835	$(AG)_8YC$	16	9 (56)	200–1,200	0.25
ISSR841	$(GA)_8YC$	26	16 (62)	200–1500	0.29
ISSR846	$(CA)_8RT$	23	15 (65)	200–1,400	0.28
ISSR848	$(CA)_8RG$	15	10 (67)	200–1,200	0.33
ISSR855	$(AC)_8YT$	18	8 (44)	200–1,500	0.35
ISSR856	$(AC)_8YA$	17	6 (35)	200–1,500	0.28
ISSR857	$(AC)_8RG$	14	6 (43)	200–1,200	0.39
ISSR880	$(GGAGA)_3$	17	9 (53)	200–1,400	0.34
Total		214	111		
Average		16	(52)		0.33

[1]percentage of polymorphic fragments, R= A or G, Y= C or T.

- Cluster Analysis

To estimate the genetic diversity and relationships among 15 tomato genotypes, Jaccard's similarity coefficient was used. Using 13 ISSR primers, the similarity coefficient ranged from 0.751 to 0.953 (Table 4.2). Genetic clustering analysis by the UPGMA method grouped the 15 tomato genotypes into three groups (Figure 4.2) at a similarity coefficient of 83%. Elsayed et al. (2011) classified the 96 tomato accessions into two groups including, accession of Brazil origin and United States origin accession by 54% similarity coefficients. In addition, Kochieva et al. (2002) indicated the relationship of 54 wild tomatoes in different species with 14 ISSR primers and classified these tomatoes into two groups with a 65% similarity coefficient. Therefore, the degree of genetic diversity of 15 tomato genotypes in this study detected 83% similarity was sufficient to classify tomato distance of this population. For Group 1, three cherry tomato genotypes (Sweet cherry 154, Sweet Girl, and Sweet Boy) were grouped representing 20% of the total population. Group 2 was commercial cultivars of non–cherry in Thailand (Ranger, Loog Too, Phet Chompoo, Sida Namkhem, Sida, and Petch Rung) which corresponds to 40% of the population. Group 3, six genotypes of non–cherry tomato from AVRDC included CLN2366B, CLN3078C, CLN3669A, CN3670B, CLN3670F and CLN3682C were grouped, representing 40% of the population. The average genetic similarity coefficient between Group 1 and group 2 was 45%, between Group 1 and Group 3 was 43%, and between Group 2 and Group 3 was 51%. The average genetic similarity coefficient of Group 1 and other groups showed low average values, because of Cherry tomato in Group 1 *(S. lycopersicum* var. *cerasiforme)* was classified in sub–varieties of *S. lycopersicum* L. (Kumar et al., 2016), which was generated in the same group of tomato species *S. pimpinellifolium* by 80% similarity coefficient (Kochieva et al., 2002). In this study, three cherry tomato genotypes (Sweet Cherry 154, Sweet Girl, and Sweet Boy) possessed an allele size of 250 bp of primer 856 ($(AC)_8YA$) (Figure 4.1 (A)), which was classified cherry tomato out of non–cherry tomato groups. The average genetic distance between groups 2 and 3 were 49% and the difference in genotypes based on their growth habitat and agro–ecological regions. The genetic distance between groups may derive fromm the different origins (Keneni et al., 2005; Gashaw et al., 2007; Henareh et al. 2016).

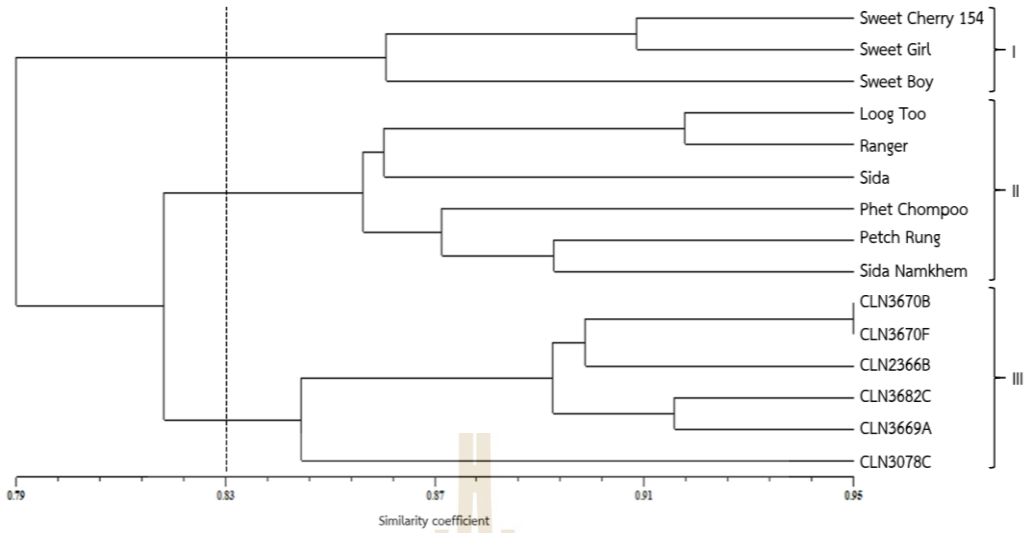


Figure 4.2. Dendrogram of 15 tomato genotypes based on the Jaccard similarity coefficient and the UPGMA.

The similarity coefficient ranged from 0.860 to 0.911 with an average of 0.878 in Group 1 (Table 4.2). The minimum similarity coefficient between Sweet Boy and Sweet Girl was 0.860 and the maximum similarity between Sweet Girl and Sweet Cherry 154 was 0.911. In group 2, the similarity coefficient ranged from 0.821 to 0.920 with an average of 0.867. The minimum similarity coefficient between Ranger and Sida Nankhem was 0.821 and the maximum similarity between Loog Too and Ranger was 0.920. The similarity coefficient ranged from 0.837 to 0.953 with an average of 0.885 in Group 3. The minimum similarity coefficient between CLN3670F and CLN3078C was 0.837. The similarity coefficient in Group 3 exhibited maximum similarity between CLN3670B and CLN3670F (0.953). A similar result to Hanson and Yang (2016), which reported that the relationship between CLN3670B and CLN3670F were generated from the same parents' lines.

The principal coordinate analysis (PCoA) was performed with 15 tomato genotypes based on ISSR data (Figure 4.3). The 3 groups of tomato population were separated by the variation of three dimensions. The smallest groups (group 1) contained three cherry tomato genotypes and non-cherry tomatoes were mainly clustered into two groups (group 2, group 3). The results of PCoA confirmed the separation of tomato populations as grouped by the UPGMA. A similar result by Choudhary et al. (2018) reported that the cluster analysis on both UPGMA and PCoA grouped tomato populations into three clusters with different species.

Table 4.2 Similarity matrix for 15 tomato genotypes based on 13 ISSR markers.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.911	0.865	0.793	0.788	0.775	0.760	0.793	0.759	0.772	0.754	0.751	0.762	0.795	0.802
2		0.860	0.823	0.807	0.793	0.767	0.811	0.766	0.791	0.774	0.770	0.757	0.767	0.777
3			0.881	0.844	0.830	0.828	0.859	0.840	0.816	0.788	0.761	0.760	0.793	0.777
4				0.920	0.872	0.881	0.878	0.872	0.859	0.832	0.782	0.782	0.814	0.809
5					0.869	0.844	0.841	0.821	0.821	0.793	0.811	0.800	0.821	0.816
6						0.830	0.894	0.854	0.875	0.849	0.854	0.809	0.864	0.847
7							0.859	0.888	0.851	0.824	0.773	0.784	0.782	0.800
8								0.895	0.870	0.855	0.849	0.816	0.859	0.820
9									0.852	0.812	0.784	0.784	0.805	0.766
10										0.953	0.841	0.889	0.885	0.903
11											0.837	0.886	0.918	0.901
12												0.855	0.841	0.859
13													0.889	0.919
14														0.903

1=Sweet Cherry 154, 2=Sweet Girl, 3=Sweet Boy, 4= Loog Too, 5=Ranger, 6=Phet Chompoo, 7=Sida, 8=Petch Rung, 9=Sida Namkhem, 10= CLN3670B, 11=CLN3670F, 12=CLN3078C, 13=CLN3682C, 14=CLN2366B, 15= CLN3669A.

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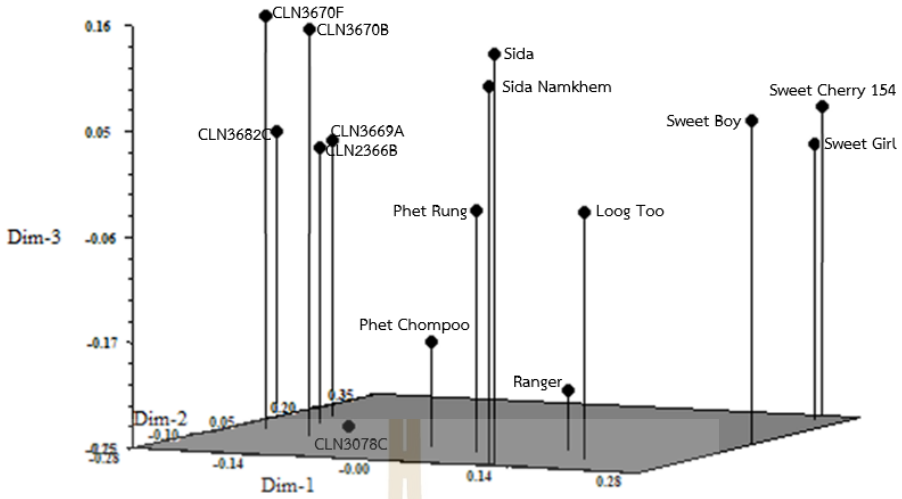

Figure 4.3 Principle coordinate analysis (PCoA) of 15 tomato genotypes.

ISSR molecular markers were used to determine the genetic diversity of 15 tomato genotypes from different sites. The results of this experiment, ISSR markers were a useful tool for determining the genetic diversity and establishing the relationship of tomato genotypes from different areas. The high genetic diversity of tomato genotypes could be used for future breeding programs for the economically important traits in tomatoes.

4.1.2 Agronomic trait evaluation for genetic diversity in tomato genotypes

Cultivated tomatoes exhibited a wide range of morphological diversity i.e. fruit size, fruit shape, fruit colors, quality, etc.

- Morphological traits

— Growth habit, 15 tomato genotype growth habit traits (indeterminate, determinate, and semi–determinate plant) were classified among tomato genotypes. Sweet Girl, Sweet Boy (Cherry tomato), and Sida Namkhem (non–cherry) had indeterminate growth (Table 4.3). Sweet Cherry 154 (cherry tomato), Phet Chompoo, Petch Rung, Ranger, CLN3670F, CLN3682C, CLN3670B, and CLN3669A (non–cherry tomato) had semi–determinate growth. While, Sida, Loog Too, CLN2366B, and CLN3078C (non–cherry) showed determinate growth habits. Bhattarai et al. (2016) reported variation in growth habit traits of 71 tomato genotypes including advanced breeding lines and wild genotypes. Bhattarai et al. (2018) also identified 91 tomato

genotypes including breeding lines and hybrid genotypes, which were classified into determinate (71%), indeterminate (17.6%), dwarf (2.2%), and semi–determinate (2.2%).

— Days to flowering was measured from the day after transplanting until 50% flowering of the first cluster. The result showed that the effect of genetics on days to flowering of 15 genotypes was significantly different. The period between transplanting to flowering among tomato cultivars ranged from 39.20 to 52.30 days. Phet Chompoo, Ranger, CLN3078C, Petch Rung, and Loog Too (non–cherry) were the earliest flowering (39.20–40.80 days), while CLN3682C, CLN3670F, and CLN3670B were late flowering (50.60–52.30 days). Days to the flowering of cherry tomatoes were moderate flowring ranging from 41.70 to 42.90 days. Previous studies evaluated the diversity of days to flowering on both cherry tomato and non–cherry tomato genotypes which ranged from 40.68 to 56.67 days after transplanting (Shah et al., 2011; Sindhu et al., 2020).

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Table 4.3 Agronomic characters of 15 tomato genotypes.

Genotype	Special features							Description
	Type	Growth habit	Days to flowering[1] (days)	Days to ripening (days)	Fruit color	Fruit shape	Plant height (cm)	
Sweet Cherry 154	Cherry	Semi–determinate	42.60^{cd}	84.70^{e}	Red	Ellipsoid	140.00^{d}	Tropical Vegetable Plant Research Center
Sweet Girl	Cherry	Indeterminate	42.90^{cd}	86.80^{de}	Red	Ellipsoid	221.60^{a}	*Chia Tai Company*
Sweet Boy	Cherry	Indeterminate	41.70^{cde}	86.40^{de}	Yellow	Ellipsoid	198.00^{b}	*Chia Tai Company*
Phet Chompoo	Non–cherry	Semi–determinate	39.20^{f}	85.20^{e}	Pink	Rectangular	114.50^{ef}	East–West Seed Thailand
Sida Namkhem	Non–cherry	Indeterminate	42.65^{cd}	89.00^{cd}	Pink	Rectangular	155.50^{c}	Siam Agrichem Company
Petch Rung	Non–cherry	Semi–determinate	40.55^{def}	86.30^{de}	Pink	Rectangular	168.80^{c}	*Chia Tai Company*
Sida	Non–cherry	Determinate	41.90^{cde}	88.00^{de}	Pink	Rectangular	158.20^{e}	*Chia Tai Company*
Loog Too	Non–cherry	Determinate	40.80^{def}	86.10^{de}	Red	Rectangular	119.70^{ef}	*Chia Tai Company*
Ranger	Non–cherry	Semi–determinate	40.10^{ef}	85.15^{e}	Red	Rectangular	109.20^{f}	*Chia Tai Company*

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Table 4.3 Agronomic characters of 15 tomato genotypes (continued).

Genotype	Type	Special features						Description
		Growth habit	Days to flowering[1] (days)	Days to ripening (days)	Fruit color	Fruit shape	Plant height (cm)	
AVTO1008 (CLN3078C)	Non–cherry	Determinate	40.20^{def}	85.20^{e}	Red	Rectangular	118.50^{ef}	AVRDC, Taiwan
AVTO1422 (CLN3670F)	Non–cherry	Semi–determinate	52.20^{a1}	102.30^{a}	Red	Rectangular	122.50^{ef}	AVRDC, Taiwan
AVTO1420 (CLN3670B)	Non–cherry	Semi–determinate	50.80^{a}	103.00^{a}	Red	Rectangular	120.70^{ef}	AVRDC, Taiwan
AVTO1418 (CLN3669A)	Non–cherry	Semi–determinate	52.30^{a}	97.10^{b}	Red	Rectangular	125.50^{e}	AVRDC, Taiwan
AVTO1424 (CLN3682C)	Non–cherry	Semi–determinate	44.90^{bc}	91.75^{c}	Red	Rectangular	124.30^{e}	AVRDC, Taiwan
AVTO0102 (CLN2366B)	Non–cherry	Determinate	46.50^{b}	96.40^{b}	Orange	Round	127.90^{de}	AVRDC, Taiwan
F–test			**	**			**	
C.V. (%)			2.88	1.62			4.18	

** highly significant difference at P=0.01.

[1]Values in column followed by the same letter are not significantly different at P=0.05.

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— Days to ripening was measured from the day after transplanting until 50% fruit ripening in the first cluster. The trait values ranged from 84.70 to 103.00 days. Sweet Cherry 154, Ranger, Phet Chompoo, CLN3078C, Loog Too, Petch Rung, Sweet Boy, Sweet Girl, and Sida were the earliest day of fruit ripening (84.70–88.00 days), while the late–ripening was found in CLN3670F and CLN3670B (102.30 and 103 days, respectively). Tomato diversity evaluation in a previous study reported that days to ripening ranged from 57.27 to 98.00 days after transplanting (Naz et al., 2011; Shan et al., 2011; Sindhu et al., 2020).

— Fruit colors including orange, pink, red, and yellow color were classified. For cherry tomatoes, Sweet Cherry 154 and Sweet Girl expressed red color fruit, while Sweet Boy showed yellow fruit. For non–cherry tomatoes, Loog Too, Ranger, CLN3670F, CLN3682C, CLN3670B, CLN3669A, and CLN3078C were red color fruit while CLN2366B was orange color fruit. However, Phet Chompoo, Sida Namkhem, Petch Rung, and Sida showed pink fruit color. This result agrees with the report of Nochai and Pongjanta (2013) which reported that normally sida tomato (Phet Chompoo, Sida Namkhem, Petch Rung, and Sida genotypes) in Thai commercials expressed pink fruit color.

— Fruit shapes, there were three fruit shapes (ellipsoid, rectangular, and round shapes) classified in this experiment. All genotypes of cherry tomato were ellipsoid shape fruit, while non–cherry genotypes expressed both rectangular shapes (Phet Chompoo, Sida Namkhem, Petch Rung, Sida, Loog Too, Ranger, CLN3670F, CLN3682C, CLN3670B, CLN3669A, and CLN3078C) and round fruit shape (CLN2366B). Tomato fruit shape was defined by these mutations' genes (Rodriguez et al., 2011) and variation of tomato fruit shapes depends on four genes–controlled fruit shapes. The elongated shape was controlled by SUN and OVATE genes, whereas fruit locule numbers and flat shape were controlled by fascinated (FAS) and locule number (LC) genes.

— Plant height, the highest plant height was found in Sweet Boy (198.00 cm) and Sweet Girl (221.60 cm), which were indeterminate growth tomatoes. Plant height of non–cherry genotypes (determinate and semi–determinate types) ranged from 109.20–168.80 cm and the lowest plant height was found in Ranger (109.20 cm). The plant height of cherry tomato ranged from 143.44 to 210.77 cm at first harvesting

(Kamnoo et al., 2020), while the plant height of non–cherry ranged from 47.79–58.30 cm at 120 days after sowing (Sindhu et al., 2020). However, plant height depended on the type of growth habits indeterminate growth habits had higher plant height than determinate and semi–determinate tomatoes (Kathimba et al., 2021).

- Yield and yield component

— Clusters per plant**,** the cluster per plant was measured in the fruit ripening stage and presented in Table 4.4 The result showed a significant difference in cluster per plant of 15 tomato genotypes. The highest clusters per plant was found in Sweet Cherry 154 (8.15 clusters/plant), while the lowest was found in Loog Too (5.40 clusters/plant). The result was higher than the previous reported 3.38–5.44 clusters/plant (Sindhu et al., 2020), but lower than the previous study by Sureshkumara et al. (2017) which reported 6.22–13.63 clusters/plant.

— Fruits per cluster of 15 tomato genotypes were shown in Table 4.4. Sweet Cherry 154 had the highest fruits per cluster (18.30 fruits/cluster), while the lowest was found in CLN3669A, CLN3078C, and Loog Too (4.50, 4.70, and 4.90 fruits/cluster, respectively). The number of fruits per cluster of cherry tomato varied from 13.70 to 18.30 fruits/cluster which was higher than non–cherry tomato (4.50–9.30 fruits/cluster). Previous studies indicated fruit per cluster of non–cherry tomatoes ranged from 2.27 to 22.0 (Shah et al., 2011; Regassa et al., 2012), whereas cherry tomatoes ranged from 11.22 to 22.00 fruit per cluster (Kamnoo et al., 2020).

— Fruit weight, there were highly significant differences in fruit weight of tomato genotypes (Table 4.4). The highest fruit weight was found in CLN3078C (80.51 g/fruit) while the lowest fruit weight was found in Sweet Boy, Sweet Cherry 154, and Sweet Girl (9.90, 10.90, and 13.75 g/fruit, respectively). The result indicates that the fruit per plant of non–cherry (21.90–80.51 g/fruit) was higher than that of cherry tomato (9.90–13.75 g/fruit). These results agree with Luitel et al. (2012) who reported that the fruit weight of tomatoes depended on fruit size. Fruit weight of non–cherry tomatoes ranged between 36.40–97.40 g (Luitel et al., 2012; Sindhu et al., 2020), while cherry tomato fruit weight ranged from 4.13–16.03 g (Najeema et al., 2018; Yimchunger et al., 2018; Kamnoo et al., 2020).

Table 4.4 The yield and yield components of tomato 15 genotypes.

Cultivars	Clusters per plant[1] (No.)	Fruits per cluster (No.)	Fruit weight (g/fruit)	Yield/plant (g)
Cherry tomato				
Sweet Cherry 154	8.15^{a}	18.30^{a}	10.90^{g}	710.00^{b}
Sweet Girl	6.40^{cdef}	14.40^{b}	13.75^{g}	695.00^{bc}
Sweet Boy	6.60^{bcde}	13.70^{b}	9.90^{g}	560.00^{bcde}
Non–Cherry tomato				
Phet Chompoo	6.40^{cdef}	9.30^{c}	34.43^{e}	595.00^{bcde}
Sida Namkhem	5.75^{fg}	7.30^{de}	21.90^{f}	465.00^{e}
Petch Rung	6.55^{cde}	7.90^{de}	26.87^{f}	600.00^{bcde}
Sida	6.90^{bc}	8.40^{d}	24.38^{f}	505.00^{de}
Loog Too	5.40^{g}	4.90^{f}	45.43^{cd}	705.00^{b}
Ranger	6.05^{defg}	6.50^{def}	62.76^{b}	1,065.00^{a}
CLN2366B	5.90^{efg}	5.90^{ef}	39.95^{cd}	545.00^{cde}
CLN3670F	6.05^{defg}	6.00^{ef}	61.06^{b}	610.00^{bcde}
CLN3682C	6.70^{bcd}	6.00^{ef}	39.23^{de}	520.00^{cde}
CLN3670B	6.40^{cdef}	6.30^{ef}	58.71^{b}	660.00^{bcd}
CLN3669A	6.00d^{efg}	4.50^{f}	48.61^{c}	540.00^{bcde}
CLN3078C	7.30^{b}	4.70^{f}	80.51^{a}	705.00^{b}
F–test	**	**	**	**
C.V. (%)	4.68	10.58	8.89	12.29

** highly significant differences at P=0.01.

[1]Values in column followed by the same letter are not significantly different at P=0.05.

— Yield per plant, there were significant differences in yield per plant among 15 tomato genotypes. The results showed yield per plant varied from 465 to 1,065 g/plant (Table 4.4). Cherry tomatoes were yield per plant ranged from 560–710 g/plant and non–cherry tomato was 465–1,065 g/plant. Yield per plant had a high correlation with the number of fruits per cluster and fruit weight (Sindhu et al., 2020). Ranger had the highest yield per plant (1,065 g/plant), while Sida Namkhem had low yield components and had the lowest yield per plant (465 g/plant). The yield per plant of

cherry tomato was limited by low fruit weight, which had yield per plant ranging from 367 to 1,779 g/plant (Najeema et al., 2018; Yimchanger et al., 2018).

- Fruit quality

— Fruit color index can be expressed in numerical terms of *L** (lightness), *a** (redness), and *b** (yellowness), which were presented in Table 4.5 There were significant differences in fruit color index among genotypes. There were significant differences in *a** value among genotypes, which ranged from 12.47 to 32.46. The results indicated that CLN3669A and Ranger had the highest *a** value (32.33 and 32.46, respectively) while Sweet Boy and CLN2366B had the lowest *a** value (12.47 and 16.52, respectively). For *b** value was found significant difference among cultivars, the highest *b** value was found in CLN2366B and Sweet Boy (36.59 and 36.93, respectively), while the lowest was observed in Sida (17.57). However, *L** value varied from 33.26 to 48.12, which was not a significant difference among genotypes.

— Total soluble solid (TSS), there were significant differences in TSS among genotypes. TSS of tomato genotypes varied from 3.55–7.38 °brix. The highest TSS was observed in Sweet Girl and Sweet Boy (7.37 and 7.38 °brix, respectively). The result indicates that the TSS cherry tomato (varied from 6.16–7.38 °brix) was higher than that of non–cherry tomato (varied from 3.55–4.41 °brix).

— Fruit firmness, there were significant differences in fruit firmness among tomato genotypes. The highest average fruit firmness was found in Ranger (15.21 N), CLN3669A (15.37 N), CLN3670B (17.60 N), and CLN3670F (18.41 N). Sida, CLN3682C, Sida Namkhem, Petch Rung, and Phet Chompoo had the lowest fruit firmness (9.00, 9.04, 9.26, 9.41, and 9.99 N, respectively). The fruit firmness of cherry tomatoes ranged from 13.16–13.38 N, which was moderate when compared with non–cherry tomatoes.

- Antioxidant contents and antioxidant activity

— Lycopene content, the result showed significant differences in lycopene among tomato genotypes. The highest lycopene content was found in Sweet Cherry 154, Ranger, CLN3078C, Sweet Girl, CLN3669A, Loog Too, and CLN3682C (9.20, 9.68, 9.71, 11.30, 11.43, 11.48 and 12.19 mg/100 g FW, respectively), while the lowest was found in Sweet Boy and CLN2366B (0.56 and 2.57 mg/100 g FW, respectively).

— β–carotene content of all tomato genotypes was shown in Table 4.5. There was no significant difference in β–carotene contents among genotypes which ranged from 1.30–2.47 mg/100 g FW.

— DPPH activity, the DPPH activity was measured as μM trolox equivalent. Antioxidant activities varied from 63.28–72.03 μM TE/100g FW but were no significant differences between cultivar.

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Table 4.5 Fruit quality, antioxidants, and antioxidant activity of 15 tomato genotypes.

Cultivars	Fruit color index			TSS	Fruit firmness	Lycopene	β–carotene	DPPH
	*L**	*a**[1]	*b**	(°brix)	(N)	(mg/100 g FW)	(mg/100 g FW)	(μM TE/100g FW)
Cherry tomato								
Sweet Cherry 154	43.93	29.59^{abc}	27.08^{abcde}	6.16^{b}	13.26^{bc}	9.20^{abcde}	2.11	70.14
Sweet Girl	33.26	27.67^{abc}	23.21^{abcde}	7.37^{a}	13.38^{bc}	11.30^{abc}	2.47	69.45
Sweet Boy	43.72	12.47^{e}	36.93^{a}	7.38^{a}	13.16^{bc}	0.56^{f}	1.31	69.99
Non–Cherry tomato								
Phet Chompoo	42.66	22.25^{bcde}	18.58^{de}	4.05^{c}	9.99^{d}	8.30^{cde}	2.15	66.45
Sida Namkhem	45.23	21.66^{bcde}	18.07^{e}	3.94^{c}	9.26^{d}	7.84^{de}	1.57	72.03
Petch Rung	41.88	22.39^{bcd}	20.84^{bcde}	4.28^{c}	9.41^{d}	8.45^{bcde}	1.66	64.72
Sida	43.20	21.39^{bcde}	17.57^{f}	3.66^{c}	9.00^{d}	6.50^{e}	1.30	63.83
Loog Too	37.09	31.11^{ab}	24.75^{abcde}	4.41^{c}	12.58^{bc}	11.48^{ab}	2.40	63.28
Ranger	38.77	32.46^{a}	28.69^{abcde}	3.99^{c}	15.21^{ab}	9.68^{abcd}	2.20	66.15
CLN2366B	45.32	16.52^{de}	36.59^{a}	4.31^{c}	10.44^{cd}	2.57^{f}	2.12	63.68
CLN3670F	48.12	20.65^{cde}	34.50^{abc}	3.90^{c}	18.41^{a}	9.35^{abcde}	1.87	68.97
CLN3682C	33.68	24.94^{abcd}	19.05^{cde}	4.16^{c}	9.04^{d}	12.19^{a}	2.38	66.26
CLN3670B	47.94	19.32^{cde}	34.00^{abcd}	3.91^{c}	17.60^{a}	9.15^{abcde}	1.95	67.24
CLN3669A	42.89	32.33^{a}	31.83^{abcde}	3.55^{c}	15.37^{ab}	11.43^{ab}	2.34	67.31
CLN3078C	46.35	28.57^{abc}	33.71^{ab}	4.33^{c}	13.34^{bc}	9.71^{abcd}	2.24	71.17
F–test	ns	**	**	**	**	**	ns	ns
C.V. (%)	15.8	17.36	23.93	9.13	12.20	14.88	27.34	6.37

ns, ** = non–significant, highly significant differences at P=0.01, respectively.

[1]Values in column followed by the same letter are not significantly different at P=0.05.

*L** = lightness, *a** = redness, *b** = yellowness, TSS = Total soluble solids, DPPH = radical scavenging assay diphenyl–picrylhydrazyl.

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- Correlation between yield, fruit quality, and antioxidants

The correlation coefficients of fruit yield, fruit quality, and antioxidants were estimated for non–cherry tomato and cherry tomato (Tables 4.6 and 4.7).

Table 4.6 Correlation coefficients between yield, fruit quality, antioxidants, and antioxidant activity of non–cherry tomato.

Traits	L^*	a^*	b^*	Fruit firmness	TSS	Lycopene	β–carotene	DPPH
Yield/plant	0.025	0.532**	0.356	0.397	0.196	0.217	0.331	–0.150
*L**		–0.172	0.556**	0.279	0.153	–0.440*	–0.277	0.063
*a**			0.023	0.166	0.119	0.579**	0.486*	–0.122
*b**				0.629**	0.188	–0.133	0.269	–0.024
Fruit firmness					–0.197	0.157	0.136	0.123
TSS						0.024	0.075	–0.202
Lycopene							0.401	0.023
β–carotene								–0.199

*, ** significant differences at p = 0.05 and 0.01, respectively.

*L** = lightness, *a** = redness, b* = yellowness, TSS = Total soluble solids, DPPH = radical scavenging assay diphenyl–picrylhydrazyl.

The results indicated that fruit yield had positively correlated with the *a** value in non–cherry tomatoes, but it had a negative correlation with the *b** value in cherry tomatoes. *L** value was positively correlated with the *b** value in non–cherry tomatoes, but it had a negative correlation with lycopene content. The *a** value was positively correlated with lycopene and β–carotene contents in both cherry and non–cherry tomatoes. The *b** value was positively correlated with fruit firmness in cherry tomatoes. Lycopene content was positively correlated with β–carotene content in cherry tomatoes but did not correlate with non–cherry tomatoes. Furthermore, lycopene and β–carotene contents had no correlation with fruit yield, TSS, fruit firmness, and DPPH in both types.

Table 4.7 Correlation coefficients between yield, fruit quality, antioxidant and antioxidants activity of cherry tomato.

Traits	L^*	a^*	b^*	Fruit firmness	TSS	Lycopene	β–carotene	DPPH
Yield/plant	–0.448	0.641	–0.823*	0.350	–0.645	0.596	0.595	–0.414
*L**		–3.338	0.547	–0.382	–0.313	–0.432	–0.628	–0.026
*a**			–0.580	0.035	–0.345	0.844**	0.766*	0.125
*b**				0.130	0.537	–0.673	–0.640	0.631
Fruit firmness					0.134	–0.128	–0.133	0.393
TSS						–0.235	–0.121	0.580
Lycopene							0.843**	–0.042
β–carotene								–0.029

*, ** = significant differences at p=0.05 and 0.01, respectively.

*L** = lightness, *a** = redness, *b** = yellowness, TSS = Total soluble solids, DPPH = radical scavenging assay diphenyl–picrylhydrazyl.

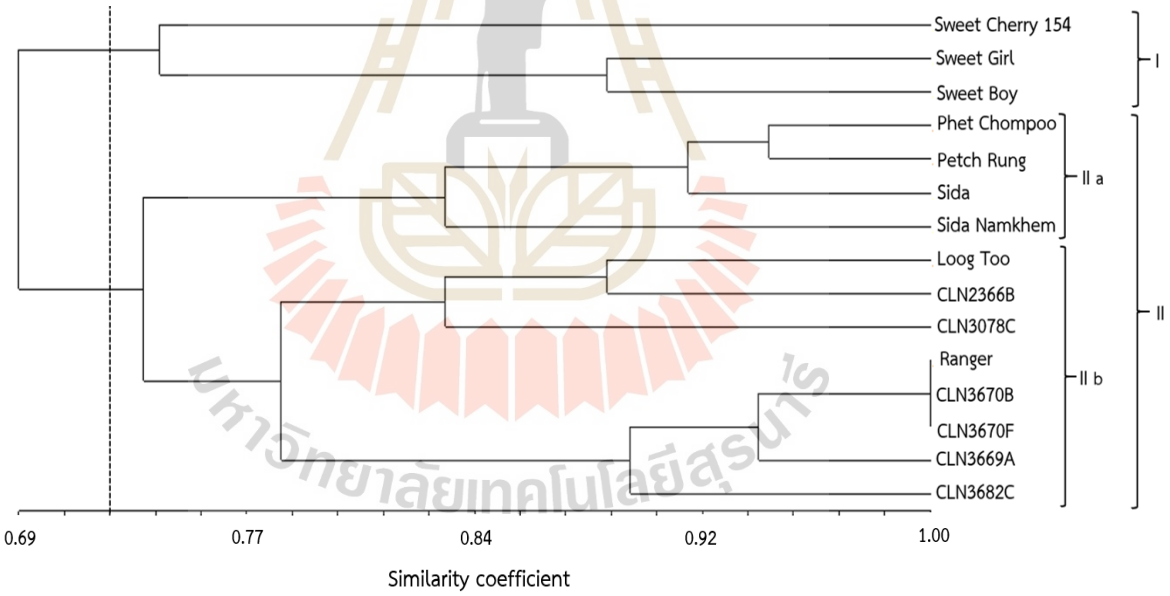

Figure 4.4 Dendrogram of morphological characteristic of 15 tomato genotypes based on the Jaccard similarity coefficient and the UPGMA.

- Cluster Analysis

To estimate the morphological diversity and relationships among 15 tomato genotypes, Jaccard's similarity coefficient was used. Using six morphological traits (growth habit, fruit color, fruit shape, cluster per plant, number of fruits per cluster, fruit weight), the similarity coefficient ranged from 0.69 to 1.00 (Figure 4.4). The degree of morphological diversity of 15 tomato genotypes in this study detected 71% similarity was sufficient to classify tomato distance of this population. For Group 1, three cherry tomato genotypes (Sweet cherry 154, Sweet Girl, and Sweet Boy) were grouped representing 20% of the total population. Group 2 was devided into 2 sub-group i.e. group 2a consisted of 4 non–cherry cultivars in Thailand (Phet Chompoo, Petch Rung, Sida, and Sida Namkhem) representing 27% of the total population and group 2b contained of 2 cultivars from Thailand (Loog Too and Ranger) and all genotypes from AVRDC (CLN2366B, CLN3078C, CLN3670B, CLN3670F, CLN3669A, and CLN3682C) representing 53% of the population.

- The relationship between DNA markers and morphological traits

Fifteen tomato genotypes in this experiment were classified into three groups by 13 ISSR primers. Group 1 were cherry tomato which had a low genetic similarity coefficient with group 2 and 3 (81% and 77%, respectively) (Table 4.2). The cherry tomato (*S. lycopersicum* L. var. *cerasiforme*) was classified into sub–varieties of *S. lycopersicum* L. (Kumar et al., 2016). For the morphological traits of cherry tomato, it expressed low fruit weight (9.90–13.75 g) but had the highest TSS (6.16–7.38 °brix) when compared with other groups (Table 4.4, 4.5). A similar result was reported by Garden et al. (2014), which found that cherry tomatoes had a small fruit weight (6.6–8.2 g) and higher sweetness (6.8–7.4 °brix) than non–cherry tomatoes. In this experiment, the fruits per cluster of cherry tomato ranged from 13.70 to 18.30 and was higher than non–cherry tomato (4.50–9.30 fruits/cluster). In addition, the fruit shape of cherry tomato was classified as ellipsoid fruit shape, which was different from non–cherry tomato that showed rectangular and round shapes (Table 4.3). Rodriguez et al. (2011) reported that four genes controlling tomato fruit shapes (SUN and OVATE controlled elongated shape, FASCIATED (FAS), and LOCULE NUMBER (LC) control fruit

locule number and flat shapes). Therefore, agronomic traits including fruit weight, TSS, number of fruits per cluster, and fruit shape were controlled by genes that might be detected by 13 ISSR primers and classified the cherry tomato group from non–cherry tomato. Similar results were grouped by morphological traits (Figure 4.4), which found that six morphological traits (growth habit, fruit color, fruit shape, cluster per plant, number of fruits per cluster, and fruit weight) were classified cherry tomato (group 1) from non–cherry tomato (group 2). Group 2 and 3 had a high average similarity coefficient (82%) between–group (Table 4.2). However, tomato genotypes in groups 2 and 3 showed a grouping of genotypes based on different growth habitats and agro–ecological regions and show genetic dissimilarity of 18% between groups. Group 2 (non–cherry) tomatoes were commercial cultivars from Thailand, and group 3 were non–cherry tomatoes from AVRDC, Taiwan. Non–cherry tomatoes could be classified into sub-group (2a and 2b) according to morphological traits (Figure 4.4). The morphological distance was found in the tomato population within groups. The CLN3078C in group 3 was early flowering and fruit ripening (40.20 and 85.20 days) than other genotypes, it was the lowest average similarity within group 3 (85%) (Table 4.2). CLN2366B had different fruit colors (orange color) and fruit shapes (round shape) in group 3 were a low average similarity within group 89%. In addition, the yellow fruit color of Sweet Boy in group 1 was affected by the lowest similarity coefficient between groups (86%). Tomato fruit color was dependent on the pigments in carotenoid groups. The result of Manoharan et al. (2017) studied the effect of β–lycopene cyclase (CYC–B) carotenoid accumulation gene and STAY–GREEN (SGR) chlorophyll accumulation gene on tomato fruit colors. they reported that the mutation on base sequences of the genes affected the gene expression deficiency and tomato fruit showed differences in fruit colors. Therefore, the mutation of pigment genes determined the genetic distance of Sweet Boy in group 1 and CLN2366B in Group 2 (Figure 4.2). Ranger and Loog Too genotypes were the highest similarity coefficient (92%) in group 2. The red fruit color of Ranger and Loog Too were different pink fruit colors of other genotypes in group 2. Similarly, morphological traits could be use to classify the tomato into 2 groups (Figure 4.4). Phet Chompoo, Sida Namkhem, Petch Rung, and Sida cultivars (Sida type) were classified as group 2a which had pink fruit color (Nochai and Pongjanta

et al., 2013). Ranger and Loog Too were classified as group 2b which had higher *a** value, fruit firmness and lycopene content than group 2a.

The DNA marker is more efficient for classifying the genetic diversity compared with the morphological characteristic. A high percentage of polymorphism and allele diversity were found in primers ISSR 808, 841, 846, and 848. They were highly efficient for classifying the genetic variation in tomatoes.

4.2 Marker–assisted selection for lycopene contents using SSR and SCAR markers

Four SSR markers and two SCAR markers which were reported to be linked to lycopene contents were used to DNA amplify 15 tomato genotypes. The results indicated that four SSR markers failed to produce a clear DNA band, which was not sufficient to identify the differences in genotypes. However, two SCAR markers ($Hp–2^{dg}$ and Og^{c}) amplified reproducibly clear DNA bands, and Og^{c} marker was found polymorphic between tomato genotypes (Figure 4.6), while $Hp–2^{dg}$ marker showed monomorphism (Figure 4.5). These SCAR markers produced a similar allele size (697 bp for $Hp–2^{dg}$ and 139 bp for Og^{c}) which were designed using lycopene–specific primers (Babak et al., 2018). The $Hp–2^{dg}$ band was found at 697 bp and the wild type band at 578 bp. The Og^{c} band was found at 139 bp and wild type band at 140 bp. The marker $HP–2^{dg}$ was designed from the mutation of *DEETIOLATED1* (*DET1*) gene which involved the signal transduction of light perception in phytochrome. The *DET1* mutation controlled the dark mature–green and high red fruit color of ripe fruit (Levin et al., 2003). The old–gold–crimson marker (Og^{c}) was generated by the mutation of the Beta (B) gene on chromosome 6 which involved the lycopene β–cyclase enzyme to convert lycopene to β–carotene contents. The B gene mutation showed high lycopene contents and low β–carotene contents in ripening fruit (Ronen et al., 2000). The study of Foolad (2007) reported that $HP–2^{dg}$ and Og^{c} genes influenced lycopene accumulation in tomatoes, which showed that the tomato genotypes containing Og^{c} gene had 25% higher lycopene content than the normal genotype.

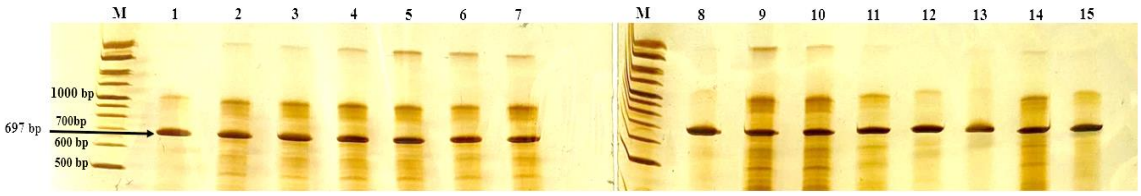

Figure 4.5 DNA amplification of tomato genotypes with the Hp–2^{dg} marker. Genotype (1) Sweet Cherry 154, (2) Sweet Girl, (3) Sweet Boy, (4) Loog Too, (5) Ranger, (6) Phet Chompoo, (7) Sida, (8) Petch Rung, (9) Sida Namkhem, (10) CLN3670B, (11) CLN3670F, (12) CLN3078C, (13) CLN3682C, (14) CLN2366B, (15) CLN3669A.

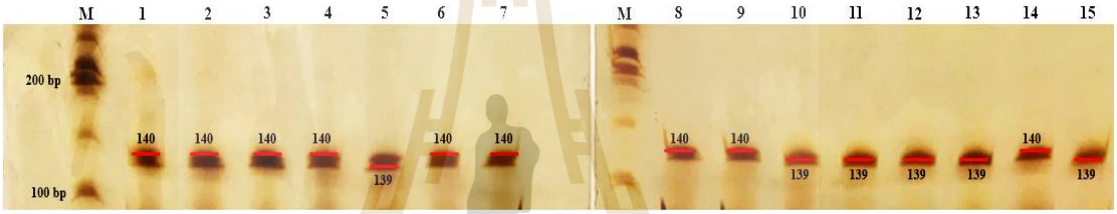

Figure 4.6 DNA amplification of tomato genotypes with the Og^c marker. Genotype (1) Sweet Cherry 154, (2) Sweet Girl, (3) Sweet Boy, (4) Loog Too, (5) Ranger, (6) Phet Chompoo, (7) Sida, (8) Petch Rung, (9) Sida Namkhem, (10) CLN3670B, (11) CLN3670F, (12) CLN3078C, (13) CLN3682C, (14) CLN2366B, (15) CLN3669A.

In this study, six tomato genotypes (Ranger, CLN3670B, CLN3670F, CLN3078C, CLN3682C, and CLN3669A) possessed an allele size of 139 bp of Og^c allele, which was accorded with lycopene content of tomato. Sweet Cherry 154, Sweet Girl, and Loog Too genotypes had high lycopene content, but they did not possess Og^c allele. However, the lycopene contents of 6 genotypes ranged from 9.15–12.19 mg/100 g FW and were classified as high lycopene groups (Table 4.8). This result agrees with the previous study which reported the Og^c gene could produce higher lycopene content of 25 to 29.6% than the normal genotype (Foolad, 2007; Hazra et al., 2012).

Table 4.8 Tomato genotypes and lycopene content analysis using Hp–2^{dg} and Og^{c} marker.

cultivars	Lycopene contents[1] (mg/100 g FW)	Lycopene levels[2]	Markers scores[3]	
			Hp–2^{dg}	Og^{c}
Cherry tomato				
Sweet Cherry 154	9.20^{abcde}	3	1	3
Sweet Girl	11.30^{abc}	3	1	3
Sweet Boy	0.56^{f}	1	1	3
Non–Cherry tomato				
Phet Chompoo	8.30^{cde}	2	1	3
Sida Namkhem	7.84^{de}	2	1	3
Petch Rung	8.45^{bcde}	2	1	3
Sida	6.50^{e}	2	1	3
Loog Too	11.48^{ab}	3	1	3
Ranger	9.68^{abcd}	3	1	1
CLN2366B	2.57^{f}	1	1	3
CLN3670F	9.35^{abcde}	3	1	1
CLN3682C	12.19^{a}	3	1	1
CLN3670B	9.15^{abcde}	3	1	1
CLN3669A	11.43^{ab}	3	1	1
CLN3078C	9.71^{abcd}	3	1	1
F–test	**			
C.V. (%)	14.88			

** highly significant differences at P=0.01.

[1]Values in column followed by the same letter are not significantly different at P=0.05, [2]lycopene level was classified into three classes: Low (1), medium (2), High (3), [3]Marker scores were presence target band (1), and presence wild type band (3).

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In addition, the highest lycopene contents 19.9 mg/100 g FW was found in F_3 progenies (combination of Hp–2^{dg} and Og^c alleles) compared with the parents (Babak et al., 2018). In this experiment, the lycopene contents of 15 tomato genotypes were classified into three levels low (0.56–2.57 mg/100 g FW) (1), medium (6.50–8.45 mg/100 g FW) (2), and high (9.15–12.19 mg/100 g FW) (3). DNA marker scores 1 and 3 representatives in the target band and wild type band were used to assess simple linear regression in conjunction with lycopene levels. The correlation between Og^c marker and lycopene levels was evaluated by simple linear regression. This marker was significantly associated with lycopene level and had a coefficient of determination (R^2) of 36.80%. Salari et al. (2010) reported a high correlation between RAPD markers ($OPC4_{950}$ and $OPC4_{300}$ markers) and lycopene content in tomatoes, especially $OPC4_{300}$ had a high coefficient of determination (R^2) of 31.75%.

The marker–assisted selection (MAS) was more efficient for the selection of the quantitative traits in the breeding program. The Og^c alleles had a high association with the lycopene contents gene in tomatoes and were also useful for selecting high lycopene genotypes in the population.

Experiment 2. Plant nutrients Management for High Antioxidant Tomato Production

4.3 Environmental conditions

The temperature and RH of the two environments were slightly different (Figure 4.7). For E1 which the experiment was conducted during January–April 2020, the temperature ranged from 29.04–37.56 °C and RH was from 71.00–73.00% while in E2 (June–September 2020), the temperature was 31.78–36.45 °C and RH ranged from 75.26–79.64%.

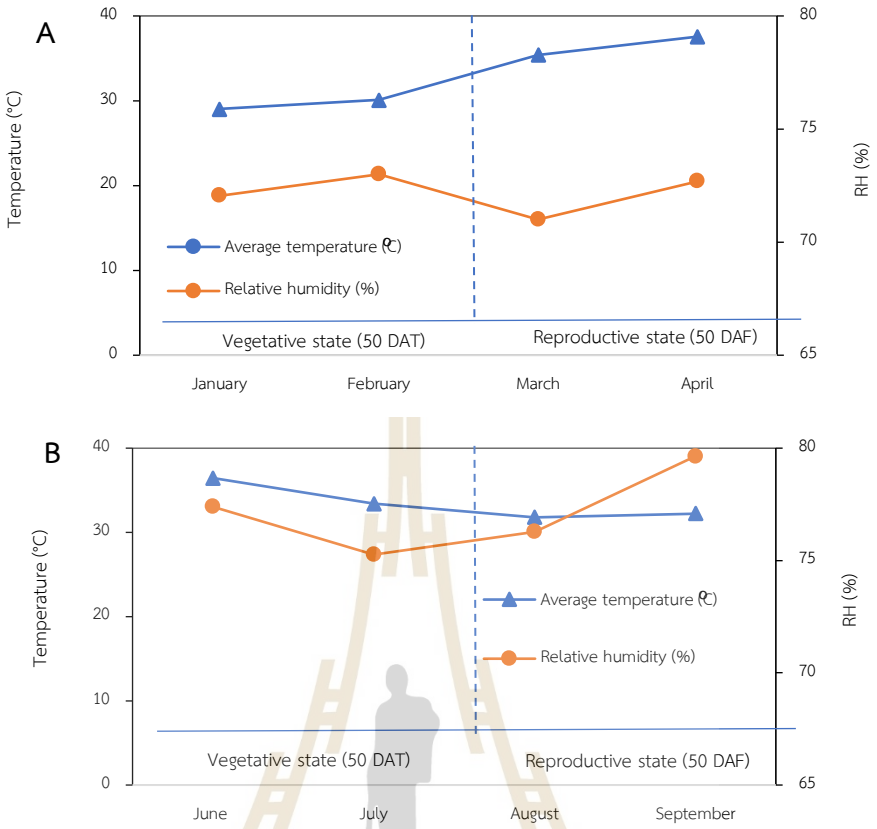

Figure 4.7 The relative humidity (RH) and temperature of E1 (A) and E2 (B); DAT (Days after transplanting) and DAF (Days after flowering).

4.4 Yield and fruit quality of Ranger cultivar

The results of yield, fruit quality, and antioxidant contents of Ranger tomato were presented in Table 4.9. Yield per plant of tested environmental conditions and plant nutrient formula were similar and without a combined effect of the two factors. Different environmental conditions yielded significantly different results on tomato fruit quality and antioxidant contents. Increasing monthly temperature during the reproduction period with lower RH in E1 appeared to significantly reduce fruit quality and antioxidant contents of Ranger tomato. The studied nutrient formulas also significantly affected a*/b*, fruit firmness, TSS, and lycopene content but the β–carotene content was unaffected. Extra application of K did not significantly improve *a*/b**, fruit firmness, TSS, and lycopene content compared to the use of the Hoagland solution. While the addition of Ni to nutrient formula appeared to have a negative effect on *a*/b**, fruit firmness, TSS, and lycopene content. This negative effect was

mitigated when 300 mg.L^{-1} K was added in combination with 10 mg.L^{-1} Ni for *a*/b**, fruit firmness, and TSS. Furthermore, the combined effects of environmental conditions and nutrient formulas were found on fruit firmness and lycopene content (Figure 4.8). Ranger tomato grown in E2 with H + K400 and Hoagland solution gave the highest lycopene content whereas the fruit firmness was maximized under E2 condition with Hoagland solution and H + K300 + Ni10 formula.

Table 4.9 Effects of plant nutrition on yield, fruit quality and antioxidants of Ranger cultivar.

	Yield (g/plant)	Fruit color index (*a*/b**)[1]	Fruit Firmness	TSS (°Brix)	Lycopene (mg/100 g FW)	β-carotene (mg/100 g FW)
Environment						
E1	963	0.94^{b}	14.06^{b}	4.62^{b}	5.22^{b}	0.93^{b}
E2	918	1.04^{a}	15.97^{a}	5.75^{a}	10.32^{a}	5.28^{a}
p-value	ns[3]	**	**	**	**	**
Nutrient formula						
Hoagland solution (H)	1020	1.03^{ab}	16.41^{a}	5.73^{ab}	9.35^{ab}	3.31
H+K400	954	1.05^{a}	15.27^{abc}	5.84^{a}	11.14^{a}	3.34
H+K300	934	1.04^{a}	15.22^{abc}	5.33^{abc}	8.23^{ab}	2.79
H+Ni20	924	0.93^{c}	15.00^{abc}	4.93^{bcd}	5.86^{c}	3.22
H+Ni10	946	0.97^{b}	13.65^{c}	4.72^{d}	6.11^{c}	2.83
H+K300+Ni10	913	0.96^{b}	15.51^{ab}	5.34^{abc}	5.92^{c}	3.16
p-value	ns	**	*	**	*	ns
E x T	ns	ns	*	ns	**	ns

ns, *, ** = non-significant, significant, and highly significant differences, respectively.

a/b** = fruit color index as a ratio between redness and yellowness, TSS = total soluble solid.

[1]Values in column followed by the same letter are not significantly different at P=0.05.

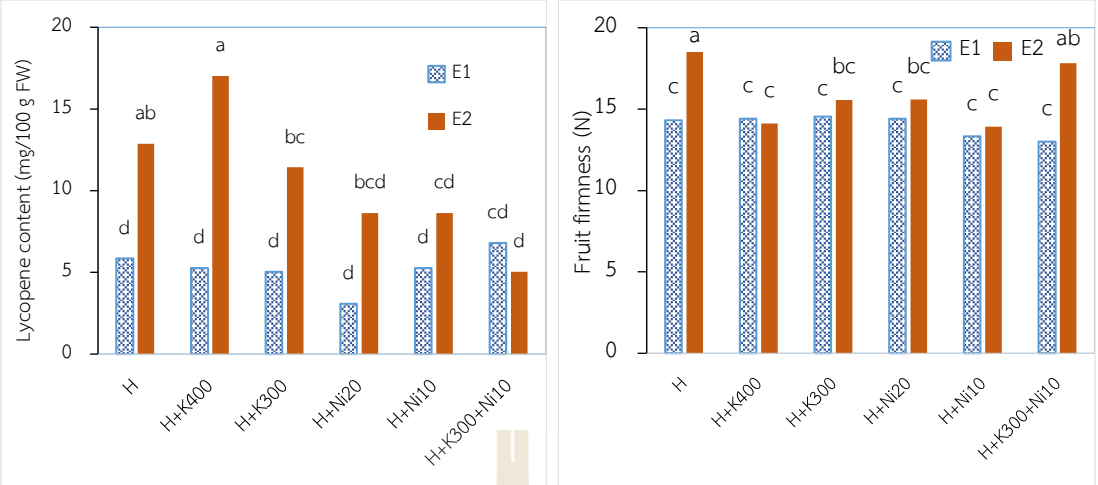

Figure 4.8 Combined effects of environmental conditions and plant nutrient formulas on lycopene content (left) and fruit firmness (right) of Ranger cultivar.

Table 4.10 The correlation coefficients between yield, yield quality, and antioxidants of Ranger cultivar.

Traits	*a*/b**	Fruit firmness	TSS	Lycopene content	β–carotene content
Yield/plant	–0.36*	–0.29	–0.41*	–0.11	–0.35*
a/b**		0.22	0.65**	0.57**	0.55**
Fruit firmness			0.57**	–0.06	0.48**
TSS				0.51**	0.72**
Lycopene content					0.46**

*, ** significant, and highly significant differences, respectively.

a/b** = fruit color index as a ratio between redness and yellowness, TSS = total soluble solid.

The correlation coefficients of yield, yield quality, and antioxidant contents of the Ranger cultivar were shown in Table 4.10. The results indicated that tomato yield was negatively correlated with *a*/b**, TSS and, β–carotene, whereas the *a*/b** was positively correlated with TSS, and lycopene and β–carotene contents. Positive

correlations were also found between fruit firmness with TSS and β–carotene content, TSS with lycopene and β– carotene contents, as well as between lycopene and β–carotene contents.

4.5 Yield and fruit quality of Sweet Girl cultivar

For Sweet Girl tomato, significant differences were found when compared to the studied environmental conditions. Yield/plant was significantly greater under the E1 condition, however, the E2 condition yielded significantly higher results for yield quality and antioxidant traits (Table 4.11). The nutrient formulas did not cause a significant difference in yield/plant, however, they affected yield quality and antioxidant traits of Sweet Girl tomato. The addition of K in nutrient formulas did not obviously improve Sweet Girl tomato fruit quality and antioxidant contents compared with the H formula, except for TSS and lycopene content in which the H +K400 gave significantly higher results. Whereas the additional Ni in plant nutrient formula did not show any obvious benefit for the quality of tomato fruit or antioxidant contents and some traits such as *a*/b**, fruit firmness, lycopene, and β–carotene contents, the presence of Ni in nutrient formulas worsen the values when compared with the H. The use of 300 mg.L^{-1} K can not mitigate negative effects from Ni as previously observed in the Ranger cultivar. The combined effects of the two factors studied were found in fruit firmness and lycopene content. Sweet Girl tomato grown in E2 with H and H + Ni10 had the highest fruit firmness and were not significantly different from H + K400 and H + K300. Lycopene content of tomatoes grown under E2 condition, H + K400 was the highest and not significantly different from the following treatments: H, H + K300, and H + Ni20 (Figure 4.9).

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Table 4.11 Effects of plant nutrient management on yield, fruit quality, and antioxidants of Sweet Girl cultivar.

	Yield (g/plant)[1]	Fruit color index (*a*/b**)	Fruit Firmness	TSS (°Brix)	Lycopene (mg/100 g FW)	β–carotene (mg/100 g FW)
Environment						
E1	615.69^{a}	0.95^{b}	14.41^{b}	7.09^{b}	4.24^{b}	0.86^{b}
E2	514.29^{b}	1.12^{a}	16.31^{a}	7.77^{a}	10.37^{a}	2.83^{a}
p–value	**	**	*	**	**	**
Nutrient formula						
Hoagland solution (H)	589.45	1.06^{ab}	16.15^{a}	7.31^{b}	7.66^{bc}	1.98^{ab}
H+K400	523.12	1.11^{a}	15.37^{ab}	7.99^{a}	9.53^{a}	2.18^{a}
H+K300	561.32	1.09^{a}	15.20^{ab}	7.57^{ab}	8.08^{ab}	1.85^{abc}
H+Ni20	614.63	0.98^{c}	14.40^{b}	7.59^{ab}	8.10^{ab}	1.48^{c}
H+Ni10	547.43	1.00^{bc}	15.87^{a}	7.11^{b}	4.46^{d}	1.94^{ab}
H+K300+Ni10	553.99	0.97^{c}	15.17^{ab}	7.31^{b}	5.99^{cd}	1.64^{bc}
p–value	ns	**	*	*	**	**
E x T	ns	ns	*	ns	**	ns

ns, *, ** = non–significant, significant, and highly significant differences, respectively.

a/b** = fruit color index as a ratio between redness and yellowness, TSS = total soluble solid, FW = sample fresh weight.

[1]Values in a column followed by the same letter are not significantly different at P=0.05.

The correlation coefficients of yield, yield quality, and antioxidant contents of the Sweet Girl cultivar were shown in Table 4.12. The results indicated that tomato yield was negatively correlated with *a*/b**, fruit firmness, and β–carotene. The *a*/b** was positively correlated with all other fruit quality and antioxidant contents while fruit firmness was positively correlated with lycopene and β–carotene contents. In addition, the TSS was positively correlated with lycopene and β– carotene contents and lycopene content also had a positive relationship with β–carotene content.

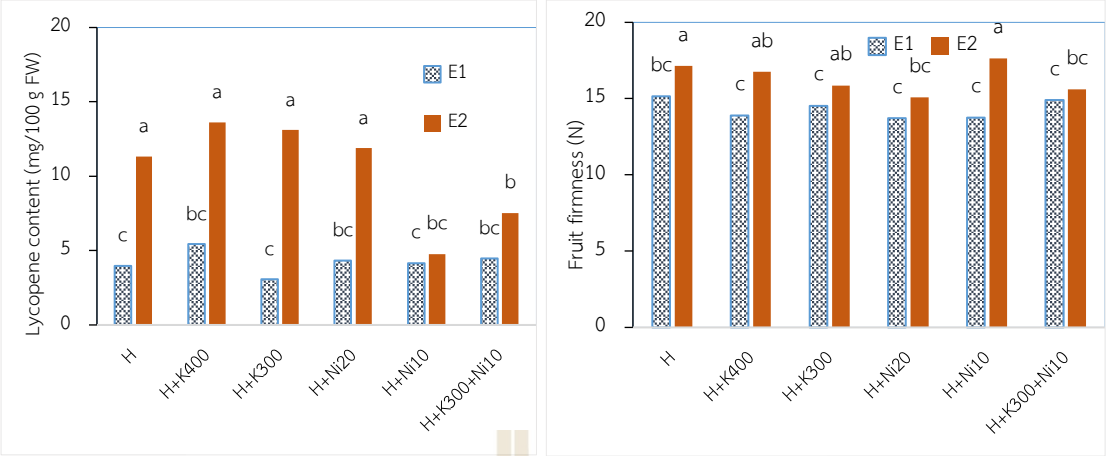

Figure 4.9 Combined effects of environmental conditions and plant nutrient formulas on lycopene content (left) and fruit firmness (right) of Sweet Girl cultivar.

Table 4.12 The correlation coefficients between yield, yield quality, and antioxidants of Sweet Girl cultivar.

Traits	*a*/b**	Fruit firmness	TSS	Lycopene content	β–carotene content
Yield/plant	–0.41*	–0.37*	–0.25	–0.07	–0.43**
a/b**		0.53**	0.42*	0.52**	0.67**
Fruit firmness			0.23	0.38*	0.75**
TSS				0.46**	0.49**
Lycopene contents					0.68**

*, ** significant, and highly significant differences, respectively.

a/b** = fruit color index as a ratio between redness and yellowness, TSS = total soluble solid.

4.6 Discussion

In terms of plant nutrient formula, the finding indicates that the yield of Ranger and Sweet Girl did not affect by plant nutrient formulas. However, they affected fruit quality and antioxidant contents of both tested tomatoes cultivars. The Hoagland solution is normally can induce high production yield since it contains a high

concentration of N and K which essential for enzyme cofactor biosynthesis. Furthermore, the supplement of N and K can increase the soluble sugar that leads to increasing starch accumulation (Osotspa, 2015). The study of Rebouças et al. (2017) has found that fruit firmness and sweetness were increased while the titratable acids were decreased. In this study, the nutrient formula with a high K concentration (H + K400) resulted in the highest TSS in tomato fruit, especially in the Sweet Girl cultivar. This formula also stimulated the highest lycopene content in both cultivars and the highest β–carotene in the Sweet Girl cultivar. This finding may relate to the K function in phytoene synthase activation which leads to increasing lycopene and β–carotene production in tomatoes**.** A similar result was obtained by Serio et al. (2007) and Taber et al. (2008) who reported that the application of 195–450 mg.L^{-1} K can enhance lycopene biosynthesis in tomatoes.

In addition, the K:N ratio is also an important factor affecting tomato production and yield quality. In this current study, the 1:1 of K:N ratio in the Hoagland solution gave a slightly higher yield compared with other plant nutrient formulas. Leal et al. (2015) reported that fruit production was maximized when the ratio of K:N was 1:1 (188.7 and 177.2 mg.L^{-1}, respectively). Fallah et al. (2021) also found that the 2:1 ratio of K:N can increase fruit firmness and TSS of tomatoes while the highest ascorbic acid was found in the 4:1 of K:N ratio. However, the nutrient solution with a high K:N ratio can result in a reduction of vegetative growth and thus, reduction of the source to sink ratio in tomatoes (Scanlan and Morgan, 1982). Furthermore, a high concentration of K may disturb the cation (K, Ca, Mg, and Na) balance in the plant and may cause a reduction of calcium ion (Ca^{+}) and magnesium ion (Mg^{+}) uptake (Kasinath et al., 2017; Xie et al., 2021) which can affect tomato growth and yields. Fanasca et al. (2005) have shown that a 1:1 of K:Ca ratio, which is rather high, can increase plant growth and yield of tomatoes while the Hoagland solution with a low K concentration (242.65 mg.L^{-1}) may induce more Ca+ uptake than the high K concentration formulas (H + K400 and H + K300). However, a low K concentration may result in the uneven ripening fruit, while a low Ca concentration may cause the blossom end rot (Nzanza et al., 2009) and the loss of fruit firmness in tomatoes (Fagaria, 2001).

Ni was an essential micronutrient for the growth and development of plants because it involves nitrogen metabolism (Marschner, 2011). The Ni concentration in plants is normally very low, in leaf tissue, it may range from 0.05–5 mg/kg dry weight (Liu et al., 2011). The Ni concentration higher than 10 $mg.L^{-1}$ is considered to be toxic to sensitive species or cultivars and the toxicity symptoms are likely to occur (Yusuf et al., 2011). These toxicity symptoms usually happened as a result of high Ni concentration inhibiting the absorption of other cations including Cu, Mn, Fe, and Zn which are also essential elements for plant growth and development (Clarkson and Lüttge, 1989; Pandolfini et al., 1996). In this study, the lycopene content and a^*/b^* were reduced in H + Ni10 and H + Ni20. These high concentrations of Ni (10–20 $mg.L^{-1}$) may be toxic to tomatoes and could inhibit lycopene synthesis. A similar result was obtained by Palacios et al. (1998) who reported that 5–30 $mg.L^{-1}$ Ni increased N accumulation in tomato fruit and a high concentration of nitrogen then reduced lycopene contents.

The optimal temperature for the growth and reproduction of tomatoes is considered to range between 18.0–32.0 °C (Panthee et al., 2018; Angmo et al., 2021), in this study, the E1 condition (29.04–37.56 °C with 71.00–73.00% RH) is appeared to be a favorable growing condition of two studied tomatoes as it significantly increased the yield/plant of Sweet Girl tomato with a slightly increasing trend in Ranger tomato. The average air temperature during the first two months of the E1 condition being in a suitable range for the tomatoes' growth and development might be a reason for the finding. In the E2 condition, the temperature during the first two months (33.40–36.45 °C) was greater than optimal for tomatoes, it induced stress and resulted in a reduction of yield. This result agreed with previous studies of Sato et al. (2000) Zhu et al. (2017), and Shamshiri et al., 2018 that showed effects of high temperature on flower and fruit abscission, as well as abnormal fruit development. Despite an increasing yield, the fruit quality and antioxidant contents of the two varieties were reduced under the E1 condition. This was also an effect of temperature higher than the optimal (35.40–37.56 °C) which occurred in the E1 during fruit development and ripening stages while in the E2, the average air temperature was lower to optimal range. Shamshiri et al., 2018 reported that a temperature greater than 35 °C could result in a reduction of fruit

redness. Shivashankara et al. (2015) also suggested that fruit quality and antioxidant contents of tomatoes could be enhanced when growing at 33.40 °C but could be dropped when growing at an air temperature of 35.40 °C because of the limiting lycopene biosynthesis rate (Helyes et al, 2007) and degradation (Demiray et al, 2013). While Balcerowicz (2020) found that a temperature higher than 30 °C could cause a reduction in lycopene and β–carotene accumulation in tomatoes. This phenomenon occurred because the rising temperature can alter the phytochrome Pfr which is an active form to the inactive form, Pr. The reduction of phytochrome activity then causes a lower accumulation of the carotenoids during the ripening stage of tomato fruit. Thus, the optimal temperature affecting this biosynthesis was varied with the tomato cultivar (Garcia and Barrett, 2006). In addition, the RH also greatly contributed to the fruit quality and antioxidant contents of tomatoes. A study by Liu et al. (2006) had suggested that the RH inside a greenhouse should range between 55–90% for optimal photosynthesis of tomatoes. In this study, the RH in both E1 and E2 conditions was in this optimal range. Leyva et al. (2013) have also reported that the 72% RH allows tomatoes to produce higher lycopene content when compared with the 62% RH at a similar air temperature. Considering both environmental traits studied, the E2 condition should be considered to produce tomatoes with high antioxidant contents.

The negative correlation found between yield and other studied traits indicates the trade-off between yield and yield quality or secondary metabolites in tomatoes. Therefore, optimum production management is vital to produce high antioxidants tomatoes. For the mentioned purpose, the temperature in the production greenhouse should be maintained at the optimum level (not greater than 35 °C), especially during the fruit development and ripening stages with RH higher than 70%. The H + K400 is recommended for tomato production as it can promote fruit quality such as the a^*/b^* and TSS, as well as the antioxidant content.

CHAPTER 5
CONCLUSION

This study was conducted on 15 genotypes of tomatoes from different areas of collection and origin. To estimate the genetic diversity of these tomato genotypes, 32 ISSR primers and morphological traits evaluation were used. Thirteen out of 32 primers (808, 812, 816, 825, 829, 835, 841, 846, 848, 855, 856, 857 and 880) could amplify and exhibit evaluable bands. The 15 tomato genotypes were grouped into three groups at a similarity coefficient of 53%. Group 1 was cherry tomato; group 2 was non–cherry cultivated cultivars in Thailand, and group 3 was non–cherry tomato from AVRDC Taiwan. In addition, agronomic traits including fruit weight, TSS, number of fruits per cluster, and fruit shape were controlled by genes that might be detected by 13 ISSR primers and classified the cherry tomato group from non–cherry tomato. The genetic distance between groups may derive form the different tomato type and origin. Similarly, morphological traits including growth habit, fruit color, fruit shape, cluster per plant, number of fruits per cluster, and fruit weight, were used to differentiate cherry from non–cherry tomatoes. Moreover, using these morphological traits, non–cherry tomatoes could be classified into sub-group 2a and 2b.

For high antioxidant genotypes selection, this study exposes the effectiveness of high lycopene contents marker including four SSR and two SCAR markers for screen tomato genotypes for high lycopene content. The 15 tomato genotypes were significant difference in lycopene content. The Og^c primers had a high association with the lycopene contents gene in tomatoes. Therefore, genetic diversity and lycopene markers obtained from DNA markers and morphological traits are important for germplasm management and were also useful for selected high lycopene genotype in the population.

The environment condition and plant nutrient management influenced on lycopene content in tomato. The nutrient formula with a high K concentration (H + K400) resulted in the highest *a*/b** and TSS in tomato fruit, especially in the Sweet

Girl cultivar. This formula also stimulated the highest lycopene content in both Sweet Girl and Ranger cultivars. The Ni concentration higher than 10 mg.L^{-1} is considered to be toxic on tomato. In addition, the E2 condition (31.78–36.45°C with 75.26–79.64% RH) was a more suitable environment for the high antioxidant tomato production. The H + K400 is recommended for tomato production as it can promote fruit quality such as the redness ratio *a*/b** and TSS, as well as the lycopene contents and β–carotene.

Therefore, genetics and environment can affect lycopene and other antioxidant content in tomatoes. The plant nutrient solution is another factor affecting enzyme activities which directly involves the antioxidant accumulation. Therefore, appropriate plant nutrients management is able to increase antioxidant contents and productivity in tomato.

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