GENETICS AND HERITABILITY OF CHILI (*Capsicum annuum* L.) GERMPLASM ON CUCUMBER MOSAIC VIRUS AND AGRO-MORPHOLOGICAL TRAITS

By

ABDUL AZIM BIN ADNAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

October 2019

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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October 2019

Chairman : Professor Mohd Rafii bin Yusop, PhD
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Chili (*Capsicum annuum* L.), one of the most widely and frequently consumed vegetables worldwide, is susceptible to cucumber mosaic virus (CMV). High yield and highly resistant chili to CMV is vital to farmers. Genetic based knowledge play important role for the development of a new variety in chili. In this study, two experiments were carried out. First experiment is to study the basic genetic components among 21 *Capsicum annuum* L. genotypes in two different planting seasons which the first season took place from February until June 2015 while the second season was conducted from July until December 2015. Second experiment is to assess and pre-determine the unknown viruses that infected chili. The assessment of viral diseases was conducted using disease severity index (DSI) and further verified using reverse transcriptase polymerase chain reaction (RT-PCR). In the first planting season, the highest yield per plant was recorded from genotype PP0337-7554 (447.71 g). The most resistant genotype to CMV was 2331 HJR. High heritability was observed in days to flowering, fruit length, yield per plant and total leaf area indicated high genetic advance. The highest genotypic coefficient of variation (GCV) was shown by the total leaf area (66.00%) while the highest phenotypic coefficient of variation (PCV) was found in number of fruits per plant (95.18%). In the second planting season, the highest yield per plant was recorded from genotype Cili Bangi 3 (409.63 g). The most resistant genotype to CMV was PP0537-7539. High heritability was observed in stem diameter, fruit length, fruit diameter, yield per plant, plant canopy diameter and days to flowering. The highest GCV was shown by the number of fruit per plant (78.02%) while the highest PCV was found in number of fruit per plant (78.18%). From both planting season analysis, 21 chili genotypes were clustered into four major groups based on their quantitative traits. Out of the 21 chili genotypes, six genotypes were selected as the best genotypes which have high fruit yields and mild resistant to CMV over two planting seasons. The three chili genotypes shown highest yield per plant over

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two planting seasons were Cili Bangi 3, AVPP 0902 and PP0337-7554 with 420.29 g, 340.36 g and 325.71 g per plant, respectively. While the three chili genotypes shown to have mild resistant to CMV over two planting seasons were PP0042-31, PP0537-7539 and 2331 HJR with DSI of 39.10, 45.20 and 52.10, respectively. In the second experiment, all of the six best chili genotypes were selected for preliminary identification of the specific groups of viruses. Total RNA of infected chili leaves showing mosaic, mottling, chlorosis and leaf deformation symptoms was extracted using CTAB method. Three fragments of 800, 500, 255 bp, specific to CMV subgroup II, PepLCV and 18S rRNA, respectively, were successfully amplified. This confirms the occurrence of CMV and mix infection in chili.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

KAJIAN GENETIK DAN KETERWARISAN PLASMA GERMA POKOK CILI (*Capsicum annuum* L.) TERHADAP VIRUS MOSAIK TIMUN DAN CIRI-CIRI AGRO-MORFOLOGINYA

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Cili (*Capsicum annuum* L.), yang merupakan salah satu sayuran yang paling tersebar dan kerap dimakan oleh manusia di seluruh dunia adalah terdedah kepada virus mosaik timun (CMV). Hasil yang tinggi dan cili yang rintang kepada CMV adalah sangat penting bagi para petani. Pengetahuan berasaskan genetik memainkan peranan penting untuk penghasilan varieti cili yang baharu. Dalam kajian ini, dua eksperimen telah dijalankan. Eksperimen pertama adalah untuk mengkaji secara asas komponen genetik antara 21 genotip *Capsicum annuum* L. dalam dua musim penanaman yang berbeza iaitu musim pertama ditanam pada bulan Februari hingga Jun 2015 manakala musim kedua pula ditanam pada Julai hingga Disember 2015. Eksperimen kedua adalah bertujuan untuk mengenalpasti virus yang tidak diketahui yang telah menjangkiti pokok cili. Penilaian tentang penyakit virus telah dijalankan dengan menggunakan Indeks Keseriusan Penyakit (DSI) dan pengenalpastian lanjutan dilakukan dengan menggunakan Tindak balas Berantai - Polimerase Reverse Transcriptase (RT-PCR). Dalam penanaman musim yang pertama, penghasilan buah sepokok paling tinggi direkodkan daripada genotip PP0337-7554 (447.71 g). Genotip paling rintang kepada CMV adalah 2331 HJR. Keterwarisan tertinggi telah didapati pada tempoh berbunga, panjang buah, penghasilan buah sepokok dan jumlah luas daun yang menunjukkan kemajuan genetik yang tinggi. Pekali variasi genotip (GCV) yang tertinggi ditunjukkan oleh jumlah luas daun (66.00%) sedangkan pekali variasi fenotip (PCV) yang tertinggi telah ditemui pada jumlah buah dalam satu-satu tumbuhan (95.18%). Dalam penanaman musim kedua, penghasilan buah sepokok paling tinggi direkodkan daripada genotip Cili Bangi 3 (409.63 g). Genotip paling rintang kepada CMV adalah PP0537-7539. Keterwarisan tertinggi telah didapati pada diameter batang, panjang buah, diameter buah, penghasilan buah sepokok, diameter kanopi pokok dan tempoh berbunga. Pekali variasi genotip (GCV) yang tertinggi ditunjukkan oleh jumlah buah dalam satu-satu tumbuhan (78.02%) sedangkan pekali variasi fenotip (PCV) yang tertinggi telah ditemui pada jumlah buah dalam satu-satu tumbuhan

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(78.18%). Daripada analisis kedua-dua musim penanaman, 21 genotip cili telah diklusterkan ke dalam empat kumpulan besar berdasarkan kepada ciri kuantitatif masing-masing. Daripada 21 genotip cili, enam genotip telah terpilih sebagai genotip terbaik dalam penghasilan buah sepokok dan kesederhanaan rintang kepada CMV antara dua musim penanaman. Tiga genotip cili terbaik dalam penghasilan buah sepokok antara dua musim penanaman adalah Cili Bangi 3, AVPP 0902 dan PP0337-7554 dengan masing-masing 420.29 g, 340.36 g dan 325.71 g untuk setiap pokok. Manakala genotip terbaik dalam kesederhanaan rintang kepada CMV antara dua musim penanaman adalah genotip PP0042-31, PP0537-7539 dan 2331 HJR dengan DSI masing-masing sebanyak 39.10, 45.20 dan 52.10. Dalam eksperimen kedua, keenam-enam genotip cili terbaik telah dipilih untuk pengenalpastian awal bagi kumpulan virus tertentu. Sejumlah RNA daripada daun yang dijangkiti yang menunjukkan tanda mosaik, berbintik-bintik, klorosis, dan kecacatan daun telah diekstrak menggunakan kaedah CTAB. Tiga bahagian daripada 800, 500, 255 bp yang spesifik kepada sub-kumpulan II CMV, PepLCV dan 18S rRNA, masing-masing telah berjaya diamplifikasikan. Ini membuktikan bahawa kehadiran CMV dan jangkitan secara bercampur berlaku dalam pokok cili.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of Variance
AVRDC	World Vegetable Centre
Ca	Calcium
C	Carbon
cDNA	Complimentary deoxyribonucleic acid
cm	Centimetre
CVMV	Chili Veinal Mottle Virus
CMV	Cucumber Mosaic Virus
^{o}C	Degree Celcius
ddH2O	Double distilled water
DEPC	Diethyl pyrocarbonate
DI	Disease Incidence
DNA	Deoxyribonucleic acid
et al	et alia
g	Gram
g	Gravitational Force
HCl	Hydrochloric Acid
Fe	Iron
kg	Kilogram
LAI	Leaf Area Index
LSD	Least Significant Difference
L	Litre
MARDI	Malaysian Agriculture Research and Development Institute

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Mg	Magnesium
Mn	Manganese
m	Metre
µ	Micro
µg	Microgram
µl	Microlitre
µm	Micrometer
µmol	Micromole
mg	Milligram
ml	Millilitre
mm	Millimetre
mM	Millimolar
N	Nitrogen
PepLCV	Pepper Leaf Curl Virus
%	Percentage
P	Phosphorus
PCR	Polymerase Chain Reaction
RT-PCR	Reverse transcriptase Polymerase Chain Reaction
K	Potassium
K_2SiO_3	Potassium silicate
RNA	Ribonucleic Acid
RCBD	Randomized completely blocked design
SEM	Scanning electron microscopy
Si	Silicon
NaOH	Sodium hydroxide
SEA	South East Asia

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spp	Species
SE	Standard Error
SAS	Statistical analysis software
UKM	Universiti Kebangsaan Malaysia
UPM	Universiti Putra Malaysia
σ^2	Variance

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The most common *Capsicum* species is known as red chile, chili pepper, hot red pepper, tabasco, paprika, and cayenne that comes from Solanaceae family. There are about 25 species of *Capsicum* and might be more worldwide. Among 25 species of capsicum, there are five major species being cultivated which includes *Capsicum annuum* (*C. annuum*), *Capsicum frutescens (C. frutescens), Capsicum chinense (C. chinense), Capsicum pendulum (C. pendulum)* and *Capsicum pubescens (C. pubescens)* (De, 2003). However, in Malaysia, only *C. annuum* (*Cili Besar*) and *C. frutescens* (*Cili Padi*) is widely cultivated due to economically importance while *C. chinense* is getting place. Among all, *C. annuum* is indisputably one of the most important fruit vegetable crops.

In Malaysia, Chili is a lowland crop. From 2018 report, chili was cultivated in a wide area of around three thousand hectares (JPSM, 2019). However, current chili production did not satisfy the current demand of around 50 thousand tons per year and thus, Malaysia had to import chili from neighboring countries. Besides importing chili, there are other ways such as increasing the cultivation area as well as crop productivity as suggested by Awang *et al*. (2013).

To date, there are four major vegetable family grown in Malaysia which include Cucurbitaceae, Solanaceae, Criciferae and Leguminosae (Fujisawa *et al.,* 1990). These vegetable especially Solanaceae and Cucurbitaceae have been severely affected by virus diseases and in recent years the problem has been amplified with the new emerging diseases. Chili crops is also highly susceptible to many diseases despite high economic importance. One of the major production constrain is viral diseases, which result in great yield loss and force the farmers to apply a huge amount of pesticide to maintain high yield production (Awang *et al.,* 2013; Lob *et al.,* 2017). The infected chili plants are generally suffered from mosaic, yellowing and curling leaves, flowers fall, growth retardation and stunted plants (Solahudin *et al.*, 2015).

1.2 Problem Statement

More than 45 viruses have been reported to be infecting chili pepper worldwide. In Malaysia, some of the major virus are cucumber mosaic virus (CMV), chili veinal mottle virus (CVMV), tobacco leaf curl virus (TLCV), tobacco mosaic virus (TMV), tomato spotted wilt virus (TSWV) and potato virus y (PVY). CMV and CVMV were found to be the most infectious virus damaging chili crops which results in 11 to 50% of yield loss when infected at fruiting stage, and up-to 100%

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at vegetative stage (Rahman *et. al.,* 2016). Controlling virus infection is difficult due to non-availability of effective chemicals. However, instead of focusing on chemical control, growing resistant chili cultivar is the most preferable option to control these aphid-transmitted viruses (Berke *et al.,* 2005).

Therefore, the development of resistant chili towards CMV is highly important. This study was carried out to evaluate numbers of chili genotypes from different countries which have high resistant to CMV virus. With this findings, the development of new variety of high yield and CMV resistant chili varieties can be established in a future breeding programs.

1.3 Objectives of the Study

i. To determine the genetic heritability values, phenotypic, correlation, divergence and combined analysis of vegetative and yield and yield components traits among 21 chili genotypes over two different planting seasons.
ii. To assess the damage of chili plant based on the viral symptom observation using disease severity index.
iii. To preliminary determine unknown viruses that infected chili through molecular screening especially by using reverse transcriptase polymerase chain reaction (RT-PCR)

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CHAPTER 2

LITERATURE REVIEW

2.1 The origin of *C. annuum* L.

The discovery of red chili by Columbus in 1492 made this fiery vegetable well-known across the globe. The highly prized of black pepper at that time started facing stiff challenges from the new advent species of red chili which becoming an excellent substitute to the black pepper. More than 450 years ago, Portuguese and Spanish traders introduced this fruit to the Asian continent. Historically, it is known to be used to impart flavour and hotness in food. Its popularity as a condiment, spice and vegetable is remarkably pivotal part of the diet in Asia, from the curries of India to the sambals of Indonesia, from the spicy soups of Thailand to the hot dishes of China. Nowadays, chili is widely planted in South Asia, China, Korea and South-East Asia. About one billion people consume chili in daily basis in one form to another, making it one of the largely consumed vegetables on the earth (Tan, 2012). The introduction of this newly spice variety are becoming inevitable part of indigenous diets.

2.2 Worldwide Production of Chili

Nowadays, Asia is the most significant player in chili industry that primarily traded by Portuguese and Arabs in the past. Asian community embraced it very well and in fact it has become the world largest producer in 2017 with 64.9% production followed by Americas, Europe, Africa and Oceania with 13.4%, 11.5%, 10% and 0.2% respectively (FAOSTAT, 2019). One of the reason is chili usage in food has becoming popular “ethnic food”. As per 2017 world production statistics, the total global production of chili was 40.7 million tonnes (FAOSTAT, 2019). This figure is inclusive of both dried and green chili. China tops the list with 17.8 million tonnes accounting for 44% of global production, tailed by Mexico 3.3 million tonnes, Turkey 2.6 million tonnes, Indonesia 2.4 million tonnes, Spain 1.3 million tonnes and other countries combine to produce 37 million tonnes (Figure 2.1). However, in India, 97% of 2.2 million tonnes produced is composed of dried chilies. This has placed India as the top listed for dried chili producer worldwide in 2017 (FAOSTAT, 2019).

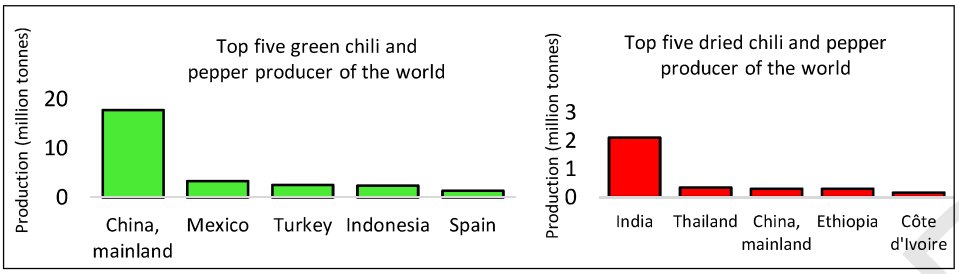

Figure 2.1: Production of chili (green and dried) in top five chili producing countries in 2017 (FAOSTAT, 2019)

2.3 Production of Chili in Malaysia

In Malaysia, *Capsicum* is locally known as "cili merah", "lada besar", "cabai" or "cili". Two species are widely cultivated, namely *C. annuum* and *C. frutescens.* The latter is of greater economic value (JPSM, 2019). Pungent chili is likely cultivated extensively on the lowland area and the non-pungent bell pepper (*Capsicum annuum* var grossum), is cultivated in the higher land due to cooler temperature. The bell pepper in the lowlands often grown with modified environment (netted houses and rain shelters) and equipped with fertigation systems (Rejab, 2008).

In 2018, total chili cultivated area are around 4052 hectares of crop equivalent per year. On this cultivation area, about 78.8% is planted with Cili Besar (*C. annuum*) and 21.2% is cultivated with Cili Padi (*C. frustescens*) (JPSM, 2019). The latter, however, is gaining popularity. *C. chinense* is one of the hottest chili species planted locally as an ornamental. *C. annuum*, includes both pungent and non-pungent types which originated from Mexico or South America and adopted to Malaysian Tropical Climate hundred years ago. It is among the most cultivated vegetable in Malaysia due to high demand and stable price (Rejab, 2008). The most popular chili variety in Malaysia is Cili Kulai while other varieties also can be found in the local market such as MC 11, MC 12, MC 4, MC 5, Cili Ipoh, Cili Bangi and Cili Minyak Beku. This herbaceous and short-lived perennial is cultivated as an annual crop and grow up to 1 m in height. It is cultivated throughout Malaysian peninsular from sea level up to an altitude 1600 m like in Cameron Highland, Pahang (Rejab, 2008).

Popularly, Cili Besar is cultivated on 3195 hectares of land area in Malaysia in 2018. This chili is grown in several major producing state such as Johor, Pahang, and Kelantan in a commercial scale with a total production of 33,755 tonnes (JPSM, 2019). The total hectareage of chili plantation in Malaysia from 2014 until 2018 is shown in the Figure 2.2. Chili is suitable in temperature from 20 - 30 °C with rainfall between 1,500 mm - 2,000 mm monthly and it can adapt to pH between 5.5 - 6.8 on mineral soil, peat or Bris Soil (with proper irrigation

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system) (Berke et al. 2005). Red chili or green chili are the product depend on harvesting period and different market needs. Malaysian took about 50,000 tonnes a year of fresh chili annually whereby the local farmer only can produce about 30,000 tonnes of chili yearly (JPSM, 2019). Thus, Malaysian government had to import chili from neighboring countries like Thailand, Vietnam and China and spend a considerable amount of currency due to low production in local chili (Awang *et al.,* 2013).

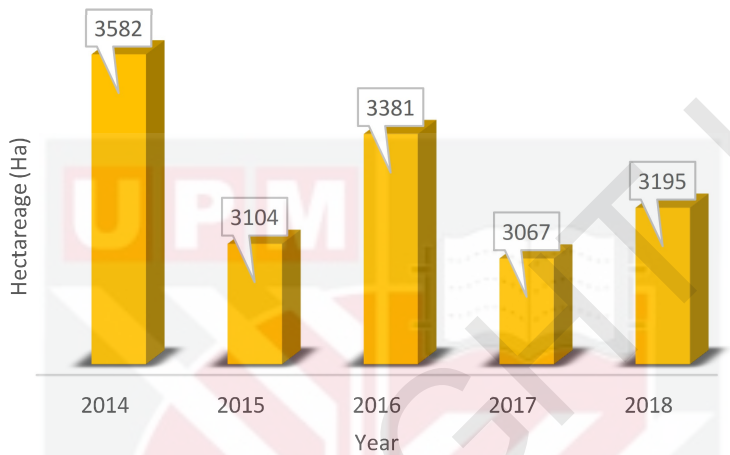

Figure 2.2: Hectareage of *C. annuum* L. in Malaysia from 2014-2018 (JPSM, 2019)

2.4 Nutritive Properties of Chili

Chili possess many important beneficial molecules such as capsaicinoids, oxidative compounds, carotenoids, vitamin C and A (Lim, 2012). It is also able to help the uptake of other food constituents. Chili provides Capsaicin ($C_{18}H_{27}O_3N$) which is the main alkaloid of capsaicinoid. The highest concentration of Capsaicin can be found in the inner wall where the seed attached and also can be found in every part of chili except in the seeds. Capsaicin is known to be a strong substance for anti-cancer as always been used in the pharmaceutical industry. The importance of Capsaicin as antioxidant is vital for defense mechanism of living cells to prevent degenerative diseases, including cancer, diabetes and cardiovascular conditions. Other than that, capsaicin also has great implication for analgesic affect, gastric, airways diseases and urological disorders (Zhuang *et al.,* 2012; Lanteri and Rotino, 2013; Cao *et al.,* 2015; Fattori *et al.,* 2016). Carotenoid pigments especially capsanthin and capsorubin are exclusively responsible for the red colour of chili while the green colour of immature fruit predominantly due to the presence of chlorophyll, and in minor extent to xantophylls as well as carotenoids (De Masi *et al.,* 2007).

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2.5 Viral diseases

2.5.1 Infection on chili in Malaysia

Chili is infested by more than 21 insects and non-insect pests. It is one of the limiting factor for the production of chili in Malaysia (Awang *et al.,* 2013) and at least nine different viruses have been reported to infect a number of chili plants in Malaysia (Table 2.1). Since 1984, Fujisawa had leaded the investigation on the occurrence and distribution of viral infection from several places in Peninsular Malaysia. Samples of infected chili leaves were collected from several states in west Malaysia including Pahang, Selangor and Johor. Plant samples showing mosaic, yellow mosaic, top necrosis or mixture of these symptoms were screened using serological tests. The test result revealed that chili veinal mottle virus (CVMV) and tomato spotted wilt virus (TSWV) were detected causing severe loss of chili yield (Fujisawa *et al.,* 1986). Since that, five main group of viruses have been commonly reported infecting chili in Malaysia. These includes cucumovirus, begomovirus, tobamovirus, potyvirus, and tospovirus. Among all, cucumovirus and potyvirus are the most damaging groups in term of incidence and lost in chili production (Mohamad Roff et al., 1994). However latest information associated to viral infection of chili in Malaysia are still limited.

Table 2.1: Five main group of viruses that reported infect chili in Malaysia

Main Group	Subgroup	Type	Reference
Cucumovirus	CMV subgroup II, CMV subgroup I	RNA RNA	Mohammad Roff and Anang (1989), Mohamad Roff and Ong (1992), Mohamad Roff *et al.* (2006), Norsazilawati (2012), Saad *et al.* (2017), Azizan *et al.* (2017)
Begomovirus	PepLCV	DNA	Shih *et al.* (1998)
Tobamovirus	TMV, ToMV	RNA	Sulaiman and Gim (1981), Mohamad Roff and Ong (1992), Lob *et al.* (2017)
Potyvirus	ChiVMV, PVY, PVMV, TEV	RNA	Soh *et al.* (1977), Ong *et al.* (1979), Sulaiman and Gim (1981), Fujisawa *et al.* (1986), Mohamad Roff and Ong (1992)
Tospovirus	TSWV	RNA	Fujisawa *et al.* (1986), Mohamad Roff and Ong (1992)

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2.5.2 Cucumber Mosaic Virus (CMV)

CMV is one of the most important virus causing severe diseases on the chili plant showing chlorotic leaves and stunted plant (Figure 2.3). In the worst cases, CMV together with other viruses, can cause massive damage to the chili plants. It was first reported in Peninsular Malaysia by Burnett (1947) as mosaic disease of chili. However, the report was based on the symptoms only. To date, efforts had been made by many researchers in Malaysia and continuously reporting the presence of CMV infecting chili. Yet, CMV is still prevalent in chili farms throughout the country. CMV is aphid-transmitted virus from genus *Cucumovirus* (family *Bromoviridae*). In nature, plant viruses are transmitted from one plant to another through vectors which can be either insects, fungi, mites, and nematodes (Zhang, 2005). According to Edwardson *et al.* (1991), CMV can be transmitted into the plant by more than 85 species of aphids in a non-persistent manner. Hull (2013) stated that CMV has been developed successfully with a broad host range of more than 1200 plant species in 100 botanical families, in both monocots and dicots plants whereby the hosts are reported in Cruciferae, Solanaceaea, Cucurbitaceae, Leguminosae and Compositae.

In Malaysia, since 1984, more than 80% of the cultivated crops were infected with mosaic disease and become a major constrain on the production of chili locally (Fujisawa *et al.,* 1986). CMV exhibit complex symptoms like mosaic, mottling, leaf distortion, fruit lesions, vein chlorosis and plant stunting which greatly affected the chili yield on the market (Ben Chaim *et al.,* 2001). Disease interference is one of the major reason for lower production of chili in Malaysia (Awang *et al.,* 2013). Many strategies have been practised to eradicate the source of infection from reaching the chili plants by controlling the vector using chemical application, virus free planting material, and breeding for disease resistance. The identification and understanding the genetic behaviour of virus-infecting chili is essential for resistance breeding programs (Naresh and Reddy, 2016). Accurate, quick, and early detection of plant viruses is very important as well as a rapid assay for the diagnosis of CMV in both field and laboratory is essential. This is possible with modern serological assays and molecular techniques (Shetti *et al.,* 2013).

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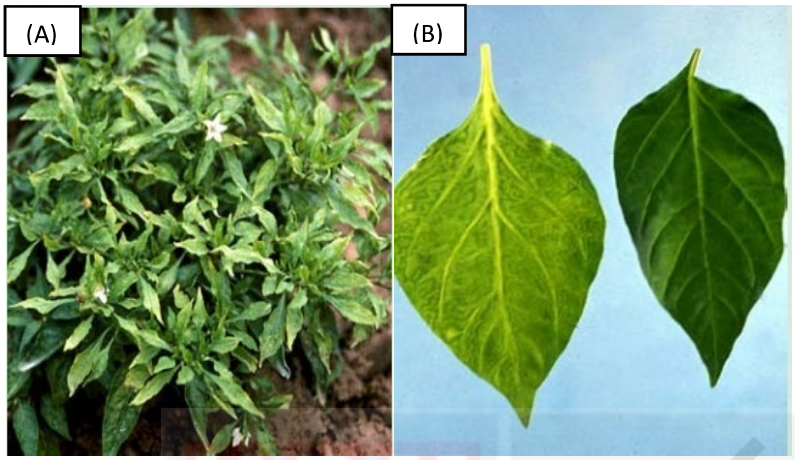

Figure 2.3: (A) Chlorotic leaves. (B) Stunted, pale plant with no distinctive leaf markings (Cerkauskas, 2004)

2.5.3 Emerging virus

Begomovirus is another important virus that infect dicotyledonous plants. This emerging virus in Malaysia is the largest genus in family of *Geminiviridae*. Begomovirus is transmitted by whiteflies (*Bemisia tabaci* Genn.) and have a broad host range to infect plant which resulted in substantial economic damage to agricultural revenue. Severe crop losses from many parts of the world due to begomovirus are continuously been reported (Oliveira *et al.,* 2001). Begomovirus can be differentiated through host range, genome organization and insect vector specificity (Saunders *et al.,* 1991).

Lazarowitz (1992) revealed that most of the begomoviruses have a bipartite genome; DNA-A and DNA-B components whereby DNA-A encodes for genes responsible for gene silencing, gene expression, and viral replication while DNA-B encodes genes for viral movement, host range determination and symptom development. Begomovirus has been seriously affected economically important food and fibre crops include chilies, tomatoes, cucurbits, cassava, beans and cotton worldwide (Varma and Malathi, 2003).

Begomovirus incidence in Malaysia was first reported in 1990 by Fujisawa *et al.* (1990) and in 1998 by Shih *et al*. (1998). Less report about this kind of viruses has been studied in Malaysia. This is due to the fact that begomovirus are under control of the usage of pesticide application. Some farmers used a huge amount of pesticides to control the whitefly infestation by neglecting the negative consequences on biodiversity and nature. It is vital to now clear the overall application of pesticides by searching for possible alternatives of agrochemicals and pesticides. To do that aggressive effort must been made in characterize the incidence of begomovirus in Malaysia.

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2.5.4 Mixed viral infection

Viral diseases on chili in Asia are found at least 35 viruses (Damiri, 2014) and throughout the world about 68 viruses (Pernezny *et al.,* 2003), consistently acknowledged and considered as the main limiting factor in chili production (Damiri, 2014). Biologically, some viruses are co-infect the chili crops because those viruses have large host range and are transmitted by many species of aphids. Complete control of viral diseases is consequently extremely difficult hence genetic resistant cultivar in host plant would be the best solution to counter those viruses (Chaim *et al.,* 2008). Mixed infection of the viruses can occur and cause more severe diseases on the chili plant (Arogundale *et al.,* 2012; Soleimani *et al.,* 2014). Many research had proved the substantial effect of this mixed infection to the chili cultivation like in Indonesia (Damiri, 2014; Subiastuti *et al.,* 2019), Iran (Soleimani *et al.,* 2014), Mexico (Rentería-Canett *et al.,* 2011), China (Chen *et al.,* 2011), Korea (Kim and Yun, 2013), India (Kumar *et al.,* 2011) and Malaysia (Fujisawa *et al.,* 1986; Fujisawa *et al.,* 1990; Mohamad Roff and Ong, 1992).

Mixed infection led to severe symptoms that reflected the interaction among virus occurs within the plant tissues. This interaction can occur either in antagonism, synergistic or additive behaviour (Oku, 1994). Soleimani *et al.,* (2014) established that the mixed infection can occur not only in single infection but also in double, triple and multiple infection. The aggregation dynamic of this interaction often changes greatly. Notwithstanding chemical agents are not effective in controlling virus diseases, breeding the virus resistant varieties is the best practise.

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CHAPTER 3

MATERIALS AND METHODS

3.1 Genotypes

Twenty-one chili genotypes were selected in this study. The chili genotypes were obtained from three sources; fourteen genotypes from the World Vegetable Center, formerly known as Asian Vegetable Research and Development Centre (AVRDC) in Taiwan, six from local institutes in Malaysia and one from Sriwijaya University (SU), Indonesia. All genotypes code, name, and origin were displayed in Table 3.1.

3.2 Experimental Site Preparation

The field experiment was laid under the rain shelter to minimize rain drop impact to the chili plants at Agrotechnology Unit, Institute of Tropical Agriculture and Food Security, (ITAFoS), Universiti Putra Malaysia (UPM). The rain shelter was located at 31 m above the sea level, between 2°98'N and 101°71'E. The climate for that location was hot humid tropic. The areas also fell under the rain forest. The experiment was executed in two different planting seasons. First planting season was carried out on February until June 2015, the mean daily ranges from 25-35 °C while in the second planting season was carried out from July until December 2015, the mean daily range from 22-30 °C.

3.3 Experimental Design

The experimental plots were set up randomly in randomized complete block design (RCBD) with four replications. Each of the replication consist of two polybags per genotypes and was randomly located under the rain shelter. Chili plants were been spaced 45 cm between plants in a row, 75 cm between rows, 80 cm between adjacent rows and 100 cm between each block. The experimental design was illustrated in Figure 3.1. Total of the experimental unit in this study was 168 chili plants.

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Table 3.1: 21 chili genotypes of local and foreign varieties

Code of Accessions	Name of Genotype	Origin of Genotype
CS 1	Kulai Mardi	MARDI, Malaysia
CS 2	Kulai Jernih	MARDI, Malaysia
CS 3	PP0537-7539	AVRDC, Taiwan
CS 4	PP0042-31	AVRDC, Taiwan
CS 5	Cili Bangi 5	UKM, Malaysia
CS 6	2331 HJR	SU, Indonesia
CS 7	MC11	MARDI, Malaysia
CS 8	PP0337-7554	AVRDC, Taiwan
CS 9	Cili Bangi 2	UKM, Malaysia
CS 10	Cili Bangi 3	UKM, Malaysia
CS 11	AVPP 0002	AVRDC, Taiwan
CS 12	AVPP 0116	AVRDC, Taiwan
CS 13	AVPP 0306	AVRDC, Taiwan
CS 14	AVPP 0512	AVRDC, Taiwan
CS 15	AVPP 0513	AVRDC, Taiwan
CS 16	AVPP 0514	AVRDC, Taiwan
CS 17	AVPP 0705	AVRDC, Taiwan
CS 18	AVPP 0902	AVRDC, Taiwan
CS 19	AVPP 9703	AVRDC, Taiwan
CS 20	AVPP 9813	AVRDC, Taiwan
CS 21	AVPP 9905	AVRDC, Taiwan

Note: MARDI = Malaysian Agricultural Research & Development Institute; UKM = Universiti Kebangsaan Malaysia; SU = Sriwijaya Universiti, AVRD = World Vegetable Center.

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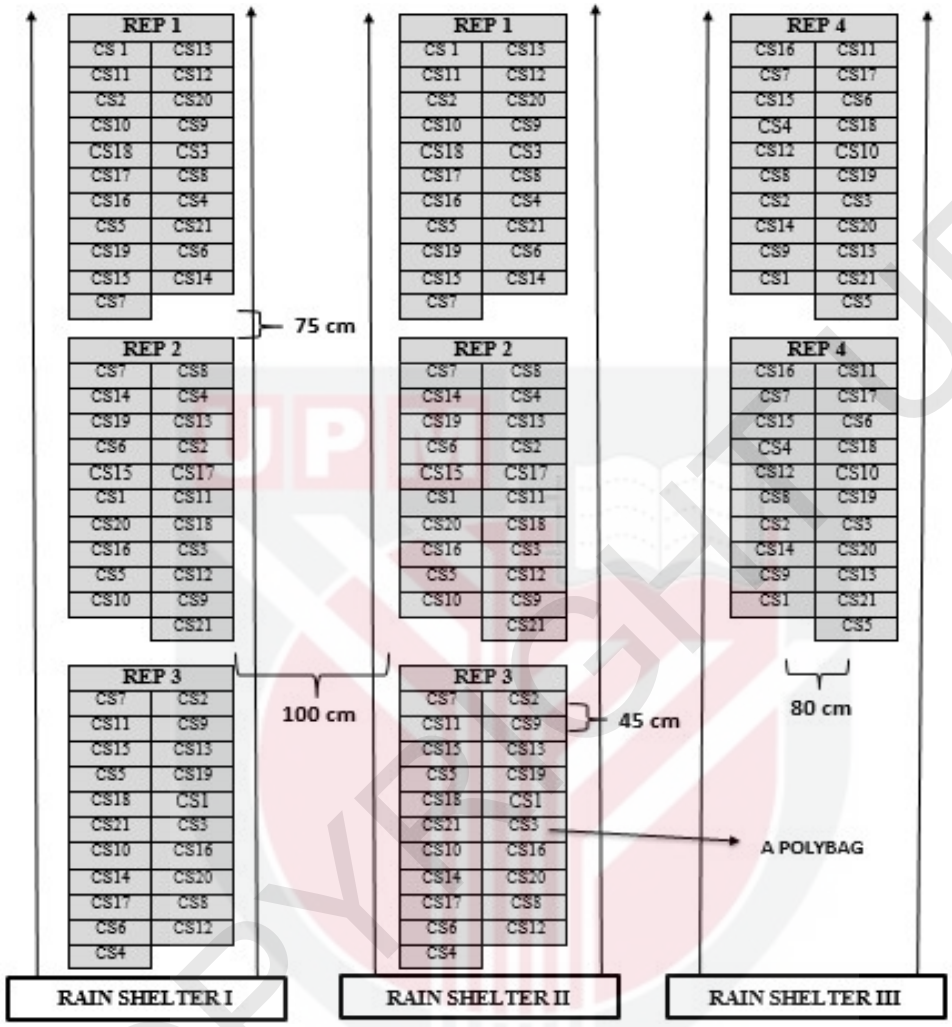

Figure 3.1: Experimental design layout of total 168 chili plants in white polybags within four replications in three rain shelters; designed for collection of data for vegetative, yield and yield's components of 21 chili genotypes

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3.4 Crop Husbandry

All of 21 chili genotypes were sown in 104-holes seed trays containing peat moss as germinating media. The seed trays were placed inside an insect-proof greenhouse at MARDI. Watering were done twice a day. Four weeks old chili seedlings with four true leaves were transplanted into white polybag with size 30 cm x 30 cm filled with coconut dust as the planting media. For transplanting, only healthy and uniform seedlings were chosen. To maintain plant health, the fertigation system were set up according to the 'Fertigation System Manual' First Edition from MARDI (Figure 3.2). The chemical fertilizer were applied from seedling stage until harvesting period within 90 days. There were two set of solutions which are solution A and solution B that been used in this study. Both solutions are described in Table 3.2. To protect plant from insects and fungi, insecticide and fungicide such as Decis®, Confidor® (Bayer, UK) and Mapa malathion® (Hextar, China) were applied twice per month throughout the cropping period.

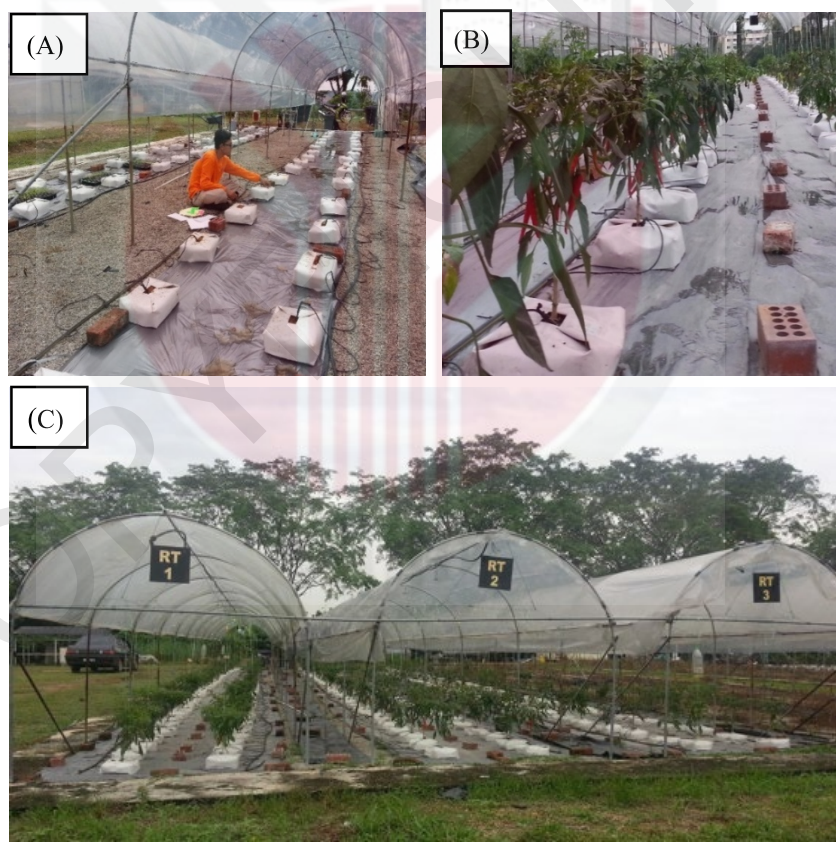

Figure 3.2: (A) 168 white polybags were prepared under the rain shelter with fertigation system from MARDI. (B) Plants were maintained with chemical fertilizer and pesticides until fruiting stage. (C) Front view of three rain shelters

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Table 3.2: Chemical fertilizer consists of two type of solutions

Solution	Nutrients	Amount in g
A	Calcium Nitrate	7132.50
	Ferum EDTA	237.50
B	Potassium Nitrate	1327.50
	Potassium Sulphate	4365.00
	Magnesium Sulphate	4012.50
	Manganese Sulphate / EDTA	4012.50
	Potassium Dehydrogen Phosphate	641.25
	Boric Acid	12.75
	Cooper Sulphate / EDTA	2.93
	Zinc Sulphate / EDTA	3.30
	Ammonium Molybdate	2.78

Solution A for macronutrients and solution B for micronutrients. The solution was from Cooper standard formulation. (A'fifah *et al.,* 2015)

3.5 Data Collection

3.5.1 Vegetative Traits

Data were collected from two consecutive planting seasons from February until June 2015 (first planting season) and from July until December 2015 (second planting season). The data was divided into two types of data; vegetative traits and yield traits data. Firstly, the data of vegetative traits such as plant height (PH), stem diameter (SD), plant canopy diameter (PCD), total leaf area, (TLA), days to flowering (DF) and days to maturity (DM) were measured using specific measuring tools and further described in the Table 3.3.

3.5.2 Yield and Yield Components

Secondly, six yield traits such as total seed (TS), fruit number (NFPP), fruit weight (FWPF), fruit length (FL), fruit diameter (FD) and yield per plant (YPP) were included as data of collection and further described in the Table 3.4.

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Table 3.3: Way of collecting data from six vegetative traits

Traits	Description
PH (cm)	Plant height is measured at every growth stage with two weeks interval from week 0 until week 12. It was measured using measuring tape from medium surface to the tip of the plant
SD (cm)	Main stem diameter was measured at the level of the first internode using Vernier caliper. It was determined at mid-vegetative stage (27 DAT), flowering or fruit setting stage (40 DAT), early fruiting stage (60 DAT) and maturation stage (80 DAT)
PCD (cm)	Plant canopy diameter was recorded using a tape. It was determined at mid-vegetative stage (27 DAT), flowering or fruit setting stage (40 DAT), early fruiting stage (60 DAT) and maturation stage (80 DAT)
TLA (cm^2)	The area of each leaf was measured using leaf area meter for total leaf area determination of each plant
DF	The days from transplanting until first fully open flower
DM	The days of first fruit ripening on the plant

The data was taken at interval time. Four chili genotypes from the experimental unit were randomly selected. DAT- day after transplant (Awang, 2013).

Table 3.4: Way of collecting data from six yield and yield components traits

Traits	Description
TS	Total number of chili seeds for each fruits was calculated
NFPP	Total number of fruits from the first harvest to 13 weeks of age. The number of days taken from transplant to the first fruit setting was observed until fruit successfully developed
FWPF (g/plant)	Fruit fresh weight was recorded from first harvest until 13 weeks of age using digital balance
FL (cm)	From the base to the tips of the fruit
FD (cm)	The average of longest chord of chili fruit was recorded using Vernier caliper
YPP (g)	Chili fruits were harvested every five days when the colour turn from green to intermediate reddish colour until

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	13 weeks of age. Data from fruit weight was added to get a cumulative yield per plant using digital balance

All yield traits data were taken at 90 days after transplanting period. Four plants from each of chili genotypes were randomly selected (Awang, 2013).

3.5.3 Analysis of cucumber mosaic virus (CMV)

Data were collected based on the appearance of the chili plant. These data were first analysed from the physical appearance using method in 3.5.3.1 and further grouped into several resistant level. Then six of selected chili genotypes were further analysed from RNA using methods in 3.5.3.2 to verify group of viruses.

3.5.3.1 Disease Severity Index of CMV

Disease severity index (DSI) was mean to compute systematically the damage index that caused by chili virus, the viral symptom that observed on the plant which affected the yield traits were calculated according to Galanihe *et al.* (2004). From DSI, each of the chili genotypes were categorized into several resistant levels. CMV that had many reviews was symptomatically studied using DSI. The symptom of viral diseases were rated using visual rating method on the exposed leaves. Several signs of damage plants such as chlorosis, defoliation, necrosis, mosaic and under developed were observed. The assessment of plant health can be referred as relative area of the plant tissue damage which often represented as a proportion. The observation was taken place for every two weeks starting from 15 days after transplanting for expression of viral symptoms. The symptomatic plant caused by virus were described in Table 3.5.

Table 3.5: Rating scale assessment of CMV for viral disease evaluation among chili genotypes based on symptomatic appearance

Virus	Scale	Symptomatic plant description
CMV	1	No visible symptoms.
	2	Mild chlorosis, necrosis.
	3	Moderate mosaic, chlorosis, and necrosis with some plant stunting.
	4	Severe mosaic, chlorosis, necrosis, pale leaf and some plant stunting.
	5	Stunted, systemic mosaic, severe chlorosis, necrotic ring spots and lines and pale plant with no distinctive leaf markings.

(Awang, 2013)

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The formula to calculate a disease incidence in chili was calculated as in the following equation:

$$\text{Disease incidence (DI \%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Results from above DI were used as reference in Table 3.6. This reference was then incorporated into the formula given in the equation 3.2 to define the DSI of each chili genotypes. Using the DSI results, chili genotypes were classified into several resistant levels such as immune, resistant and susceptible based on the description in Table 3.7.

Table 3.6: Severity score for DSI

Damage rate	Description of damage
0	No disease symptoms
1	10% of the canopy showing disease symptom
3	10-25% of the canopy showing disease symptom
5	25-50% of the canopy showing disease symptom
7	50-75% of the canopy showing disease symptom
9	> 75% of the canopy showing disease symptom

(Awang, 2013).

$$\text{Disease Severity Index (\%)} = \frac{\sum(V \times n) \times 100}{N \times Z}$$

V - Severity score
n - Number of affected plants having same score
N - Total number of plants observed
Z – Maximum scale number (9)

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Table 3.7: Resistant levels of chili genotypes for viral disease assessment

Resistant level	Scale	Description
Immune, I	0-20	No symptoms, plant grow healthy at optimum condition. 0-20% of canopy showing disease symptom.
Highly Resistant, HR	21-40	Mild symptoms. 21-40% of canopy showing disease symptom.
Mild Resistant, MR	41-60	Mild or delayed mild symptoms. 41-60% of the canopy showing disease symptom.
Susceptible, S	61-80	Symptoms, positive, 61-80% of the canopy showing disease symptom.
Highly Susceptible, HS	81-100	Symptoms, positive and severe. > 80% of the canopy showing disease symptom.

(Awang, 2013)

3.5.3.2 Isolation of viral RNA

Among 21 chili genotypes, only six of chili genotypes were selected for viral verification. The selection was based on quality in yield performance and resistance level. Viral RNA was isolated from healthy leaf and infected leaf from selected genotypes (Table 3.8). In this study, the verification of selected genotypes were carried out to preliminary identifying the virus presence through molecular technique specifically using reverse transcriptase polymerase chain reaction (RT-PCR).

Viral RNA was extracted from leaf tissues by Trizol reagent following method (Chomczynski and Sacchi, 1987) and (Vitti *et al.,* 2015). Leaf tissues (100 mg) of each selected genotypes were randomly chosen from 168 chili plants. Samples were replicated into four samples. Leaf samples were grounded with mortar and pestle with 1 ml of sterile water RNase-free water. A 1 ml (1000 µl) of Trizol reagent was added and incubated in room temperature for 5 mins. 300 µl of chloroform was added and shaken for 15 sec. Then, the samples were incubated at room temperature about 2 to 5 min. After that, all samples were centrifuged at 12,000 G about 5 to 15 min at 2 to 8ºC to attain phase separation.

The upper phase was transferred and added with 0.75 ml isopropanol into a new tube. Then, all tubes were incubated about 10 min at room temperature. Maximum centrifugation was carried out at 18000 G about 10 to 15 min at 4ºC. The supernatants were discarded. Pellets were collected and washed with 70%

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ethanol prepared using DEPC water. Next, the pellets were vortexes briefly. Again, the pellets were centrifuged at 13000 G about 2 to 10 min at 4ºC and supernatants were discarded. Pellets were air dried about 5 to 10 min then were dissolved in appropriate amount of RNase-free water by pipetting up and down. Total of RNA were checked with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) for the concentration and purity. RNAs were stored in RNase-free water at -80ºC until next analysis.

Table 3.8: Selected genotypes for verification of viruses

Design	Genotypes
A	Healthy Chili
B	Inoculated Chili with CMV
C	Infected Chili PP0337-7554
D	Infected Chili 2331 HJR
E	Infected Chili AVPP 0902
F	Infected Chili Bangi 3
G	Infected Chili PP0042-31
H	Infected Chili PP0537-7539

3.5.3.3 Determination of RNA concentration and its quality

RNA concentration were determined photometrically. The RNA sample were diluted 1:100 with double distilled water, ddH_2O as in the following equation. The absorbance of solution were measured at 260 and 280 nm, using water as blank. An OD 260 of 1 corresponds to a RNA concentration of 40 (RNA) µg/ml were carried out. The ratio 260/280 provides an indication of the purity of the RNA. The value should be between 2.0 and 2.2 (Usman *et al.,* 2014).

$$\text{Dilution of RNA stock Volume (µl)} = \frac{\text{Required final concentration of RNA (ng/µl)}}{\text{Actual concentration of RNA stock (ng/µl)}} \times \text{Final}$$

$$= \text{Volume of stock to add (µl)}$$

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3.5.3.4 Reverse Transcriptase (RT)-Polymerase Chain Reaction (PCR)

Twelve set of degenerate primers as in Table 3.9 capable of amplifying part of the viral gene in some viruses were used to amplify virus DNA in the samples. 50 µl of reaction mixture containing 25 µl of master mixture (Promega), 5 µl of each primer (10mM), 4 µl of diluted (1:100) infected or healthy RNA and sterile water was added to make up the volume. 18S rRNA primer was used as internal control and CMV inoculated sample as the positive sample. The PCR condition used was one cycle of initial denaturation at 94°C for 4 min, melting at 54°C for 1 min. Then, 72°C for 1 min followed by 39 cycles of denaturation at 94°C for 1 min. The annealing temperature was 55°C for 1 min, extension at 72°C for 1 min. Lastly, a final extension was set at 72°C for 10 min.

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Table 3.9: List of twelve degenerate primers used for RT-PCR amplification of viral RNA in *C. annuum* L

Virus	Primer Designation	Primer Sequence (5' -----3`)	Position	Product Size (bp)	References
PepLCV	5´-CP (Forward) 3´-CP (Reverse)	F: AGAATTATGTCCAAGCGACCA R: AAGCGTTGGGGATACACAAA	295-316 1282-1262	750	Sinha *et al.* (2011)
Potato Virus Y	5´-CP (Forward) 3´-CP (Reverse)	F: CAACTCCAGATGGAACAATTG R: CCATTCATCACAGTTGGC	7861-7882 8858-8875	1000	Fakhrabad *et al.* (2012)
ChiVMV	5´-Pol (Forward) 3´-Oligo(dT) (Reverse)	F: AGCATGGAGAGAGCGACATTAGTC R:GCGGGATCCTTTTTTTTTTTTTTTTT	-	1200	Tsai *et al.* (2008)
Potyviruses	5´-CP (Forward) 3´- Oligo(dT) (Reverse)	F:TGAGGATCCTGGTGYATHGARAAYGG R: GCGGGATCCTTTTTTTTTTTTTTTTT	-	800	Tsai *et al.* (2008)
CMV subgroup II	5´-CP (Forward) 3´-CP (Reverse)	F: CTACGTTTATCTTCC R: AACCGGTGATTTACCATCGC	970–984 1655–1674	704	Chen *et al.* (2011)
CMV subgroup I	5´-CP (Forward) 3´-CP (Reverse)	F: GCCACCAAAAATAGACCG R: ATCTGCTGGCGTGGATTTCT	1484–1502 2057–2076	593	Chen *et al.* (2011)
TMV	5´-CP (Forward) 3´-CP (Reverse)	F: CGATGATGATTCGGAGGC R: GAGGTCCARACCAAMCCAG	5664–5681 6157–6175	512	Chen *et al.* (2011)
ToMV	5´-CP (Forward) 3´-CP (Reverse)	F: CATCTGTATGGGCTGAC R: GAGGTCCARACCAAMCCAG	5746–5762 6148–6166	421	Chen *et al.* (2011)

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Table 3.9: Continued.

18S rRNA (internal control)	5´-18S (Forward) 3´-18S (Reverse)	F: GAGAAACGGCTACCACATCCA R: CGTGCCATCCCAAAGTCCAAC	399–419 633–653	255	Chen *et al.* (2011)
Tospovirus	5´-Pol (Forward) 3´-Pol (Reverse)	F: AATTGCCTTGCAACCAATTC R: ATCAGTCGAAATGGTCGGCA	-	276	Perez-Colmenares *et al.* (2015)
TSWV	5´-CP (Forward) 3´-CP (Reverse)	F: TTAACTTACAGCTGCTTT R: CAAAGCATATAAGAACT	-	823	Perez-Colmenares *et al.* (2015)
ToLCV	5´-CP (Forward) 3´-CP (Reverse)	F: TAAGAAATGACCAGTCCGAGG R: AATTTCTGGCCCTGATAAC	1200 300	1800	Maruthi *et al.* (2007)

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3.5.3.5 Agarose gel electrophoresis

The PCR fragments were electrophoresed by 1% metaphor agarose gels in TAE buffer for 30 min at 70 V and visualized by staining with Ultra Power® Nucleic Acid Stain (BioTeke Corp.) under UV light at 254 nm using a Trans illuminator. Gels were photographed to record the results.

3.6 Statistical Analysis

3.6.1 Keys-out of Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) was done with 0.05 and 0.01 probability level to calculate all the vegetative and yield and yield components attributes to determine the variability among the 21 chili genotypes. This calculation included mean, range, coefficient of variation (CV) and standard deviation of each trait while mean comparison were separated with least significant different (LSD) using statistical analysis software (SAS) version 9.4. The ANOVA analysis was divided into two parts; individual season analysis and combined season analysis. The keys-out of ANOVA was presented in the Table 3.10 for individual season and Table 3.11 for combined season.

Table 3.10: Keys-out of ANOVA table for individual season analysis

SOV	df	SS	MS	EMS
Replications (*R*)	*r*-1	SS *R*	MS *R*	$\sigma^2_e + a\sigma^2_r$
Genotypes (*G*)	*g*-1	SS *G*	MS *G*	$\sigma^2_e + r\sigma^2_g$
Error (*E*)	(*r*-1)(*g*-1)	SS *E*	MS *E*	σ^2_e

SOV = Source of variation; df = Degree of freedom; SS = Sum of squares MS = mean squares; EMS = Expected mean squares.

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Table 3.11: Keys-out of ANOVA table for combined season analysis

SOV	df	SS	MS	EMS
Replications (*R*)	*s*(*r*-1)	SS *R*	MS *R*	$\sigma^2_e + a\sigma^2_r$
Genotypes (*G*)	*g*-1	SS *G*	MS *G*	$\sigma^2_e + r\sigma^2_g$
Seasons (*S*)	*s*-1	SS *S*	MS *S*	$\sigma^2_e + r\sigma^2_s$
G × *S*	(*s*-1) (*g*-1)	SS *G* × *S*	MS *G* × *S*	$\sigma^2_e + r\sigma^2_g + a\sigma^2_r + r\sigma^2_s$
Error (*E*)	*s*(*r*-1)(*g*-1)	SS *E*	MS *E*	σ^2_e

SOV = Source of variation; df = Degree of freedom; SS = Sum of squares MS = mean squares; EMS = Expected mean squares.

3.6.2 Variance Components and Heritability Estimates

Variance components were estimated to determine the genetic variation among 21 chili genotypes. The genetic and heritability component were calculated according to Oladosu *et al.* (2014) as in the Table 3.12.

3.6.3 Multivariate Analysis

Cluster and principal component analysis (PCA) were carried out with Numerical Taxonomy Multivariate Analysis System Software (NTSYS) version 2.1. All chili genotypes were classified into a group that were similar together. These analysis were useful to mathematically cluster each genotypes into a group such that each group was as homogenous as possible with respect to certain characteristics. The relationships among the traits were also determined using correlation analysis. The methods used were based on Euclidian distance method, Dice's and Jaccard's similarity coefficient. While genetic relationships among the chili genotypes were determine using unweighted pair group method using arithmetic average (UPGMA) algorithm and SAHN clustering method. The STAND program in this software was used first to standardize the yield data prior to analysis and PCA analysis was performed using EIGEN value which explains the variance in each of the principle component.

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Table 3.12: Variance and variance components calculation

Variance Components	*Consider;*
Error Variance (σ^2_e)	$\sigma^2_e = MSE$
Genotypic Variance (σ^2_g)	$\sigma^2_g = \frac{(MSG - MSE)}{r}$
Phenotypic Variance (σ^2_p)	$\sigma^2_p = \sigma^2_g + \sigma^2_e$
Phenotypic and Genotypic Coefficient of Variation (PCV and GCV)	$PCV = \frac{\sqrt{\sigma^2_p}}{X} \times 100$
	$GCV = \frac{\sqrt{\sigma^2_g}}{X} \times 100$
Heritability Estimate	$h^2_B = \frac{\sigma^2_g}{\sigma^2_p}$
Estimated and Expected Genetic Advance (GA)	$GA = K \times \sqrt{\sigma^2_p} \times h^2_B$
	$GA\ (\%) = K \times \frac{\sqrt{\sigma^2_p} \times h^2_B}{X} \times 100$

MSG: Mean square of genotype, MSE: Mean square of Error, r: number of replication, σ^2_g: genotypic variance, σ^2_p: phenotypic variance, σ^2_e: error variance, X: mean of the trait, $\sqrt{\sigma^2_p}$: phenotypic standard deviation, GCV and PCV values were categorized as low (0-10%), moderate (10-20%), and high (20% and above), h^2_B: broad sense heritability was categorized as low (0-30%), moderate (10-30-60%), and high (≥60% and above), Genetic advance was categorized as low (0-10%), moderate (10-20%), and high (>20% and above), K is a constant which represents the selection intensity, when K is 5%, the value is 2.06 . (Oladosu *et al.,* 2014).

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Genetic variation study over season by vegetative and yield component characters

A total of 12 traits (vegetative and yield components) were studied over two seasons of planting, containing 21 chili genotypes. Vegetative traits studied were plant height, stem diameter, plant canopy diameter, total leaf area, days to flowering and days to maturity. Next, yield components studied were total seed, number of fruit per plant, fruit weight per plant, fruit length, fruit diameter and yield per plant. All of these traits were tested in two different planting seasons which taken place in February until June 2015 (first season) and from July until November 2015 (second season). By combining both seasons in combined analysis, the most stable trait over seasons were determined.

4.1.1 Vegetative traits

In term of growth performance, all vegetative traits were normally developed in a sigmoidal curve growth pattern which rose steadily from the first day of transplant until week 12. Rapid growth taken place from week 4 until week 10 (Appendix C1). Table 4.1 showed highly significant differences (P<0.01) for all vegetative traits computed among the 21 chili genotypes tested except in days to flowering trait which was found not significant within the replication during the first season. Table 4.2 was combined analysis between both seasons showing significant differences among all data parameters tested excluding days to flowering which was not significant.

Table 4.3 presented means for the plant height in both season and combined season. Plant height was taken in two weeks intervals from the week 0 (PH 0) until the weeks 12 (PH 12). The range of the plant height in week 12 for the first planting season was between 40.67 cm and 99.00 cm and the second planting season was between 45.33 cm and 80.67 cm. Kulai Jernih and Cili Bangi 2 from the both planting seasons recorded the highest plant height with respect to 99.00 cm and 80.67 cm. While AVPP 9905 and AVPP 9703 recorded the lowest value of the plant height with respect to 40.67 cm and 45.33 cm. It appears that genotype MC 11 was the most stable genotypes over seasons with the mean value of 76.67 cm followed closely by Cili Bangi 2 and Kulai Jernih with the mean value 76.17 cm and 74.33 cm respectively.

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Table 4.4 displayed means for stem diameter and plant canopy diameter in both season and combined season. The mean values for the stem diameter ranged from 1.10 to 1.57 cm in the first planting season while 0.90 to 1.30 cm in the second planting season. Kulai Jernih had the highest value, whereas AVPP 0512 had the lowest value in the first planting season. In the second planting season Cili Bangi 2 and AVPP 0116 shared the highest value while Kulai Jernih, Cili Bangi 5 and AVPP 0512 shared the lowest value. Seemingly, genotypes AVPP 0116 was the most stable genotypes over seasons with the mean value of 1.42 cm followed by Cili Bangi 2 and PP0337-7554 with the mean value of 1.37 cm and 1.28 cm respectively. In the case of plant canopy diameter, the mean values were ranged between 30.33 to 84.67 cm in the first planting season and 29.33 to 60.00 cm in the second planting season. The highest plant canopy diameter were 2331 HJR and Cili Bangi 2 with respect to 84.67 cm and 60.00 cm. While the lowest plant canopy diameter were AVPP 0512 in both planting season with respect to 30.33 cm and 29.33 cm. Apparently, genotypes 2331 HJR was the most stable genotypes over seasons with the mean value of 66.33 cm.

Table 4.5 displayed means for total leaf area, days to flowering and days to maturity in both season and combined season. Total leaf area ranged from 1185.71 cm^2 to 9288.54 cm^2 and 1029.76 cm^2 to 9101.93 cm^2 for respective seasons. 2331 HJR had the highest of total leaf area for both planting seasons while AVPP 0513 had the lowest mean value of total leaf area for the couple season. However, 2331 HJR was the most stable total leaf area across the seasons with the mean value of 9195.24 cm^2.

For days to flowering in the first planting season, the mean values were ranged between 23 and 34 days as observed in PP0537-7539, 2331 HJR and PP0337-7554, respectively while in the second planting season the mean value was between 23 and 35 days as observed in PP0537-7539 and 2331 HJR, respectively. Over the seasons, the most stable days to flowering was PP0537-7539 with the mean value of 23 days. For days to maturity, the earliest days to maturity was belonged to Cili Bangi 5 in the first planting season and PP0537-7539 in the second planting season with respect to day 66 and day 63. The longest days to maturity was belonged to Cili Bangi 3 in the first planting season and Kulai Jernih, PP0337-7554 and Cili Bangi 3 in the next planting season with the same mean value of 75 days. Yet, the most stable days to maturity between seasons with the same mean value of 65.90 days were Cili Bangi 5 and PP0537-7539.

Table 4.1: Analysis of variance for six vegetative characters of *C. annuum* L. in different planting seasons

Trait\SOV	Replications (df = 3)		Genotypes (df = 20)		Error (df = 60)	
	S1	S2	S1	S2	S1	S2
Plant height (cm)	34.33**	34.73**	654.45**	245.95**	2.45	0.31
Stem diameter (cm)	0.03*	0.21**	0.06**	0.03**	0.01	0.00
Plant canopy diameter (cm)	270.33**	23.05**	515.74**	150.58**	2.50	0.03
Total leaf area (cm^2)	9664167.50**	1452716.10**	19642555.30**	19563391.90**	37523.70	10424.00
Days to flowering (days)	1.18*ns*	173.54**	85.38**	89.83**	0.52	0.02
Days to maturity (days)	55.08**	64.84**	23.43**	58.61**	6.69	0.16

*Significant at 0.05 probability level; **highly significant at 0.01 probability level; *ns* = non-significant; SOV = source of variation; df = degree of freedom, S = season.

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Table 4.2: Combined analysis of variance for vegetative and yield and yield components traits of *C. annuum* L

Traits\SOV	Replications (df = 3)	Genotypes (*G*) (df = 20)	Seasons (*S*) (df = 1)	*G* × *S* (df = 20)	Error (df = 120)
Plant height (cm)	44.18**	382.02**	196.27**	234.81**	293.52**
Stem diameter (cm)	7.38**	7.42**	235.52**	4.88**	11.54**
Plant canopy diameter (cm)	77.26**	158.20**	502.14**	70.86**	121.81**
Total leaf area (cm^2)	923167.50**	1082555.30**	1952716.10**	10163391.90**	2356.30**
Days to flowering (days)	29.30**	66.30**	0.52*ns*	0.14*ns*	32.15**
Days to maturity (days)	5.34**	12.39**	4.21*	2.41**	7.15**
Total seed (no)	18.84**	200.38**	14.71**	12.36**	96.55**
Number of fruit per plant (no)	1.16*ns*	12.49**	6.49*	1.88*	6.64**
Fruit weight per plant (g)	6.51**	47.69**	4.91*	3.76**	23.55**
Fruit lenght (cm)	33.08**	269.69**	39.58**	8.17**	127.31**
Fruit diameter (mm)	41.00**	196.77**	14.26**	0.00*ns*	91.42**
Yield per plant (g)	605.36**	13713.80**	13665.50**	3477.63**	7998.11**

*Significant at 0.05 probability level; **highly significant at 0.01 probability level; *ns* = non-significant; SOV = source of variation; df = degree of freedom.

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Table 4.3: Means for plant height (PH) studied in 21 genotypes of *C. annuum* L. from different planting seasons

Genotype\Trait	PH 0 (cm)			PH 2 (cm)			PH 4 (cm)			PH 6 (cm)			PH 8 (cm)			PH 10 (cm)			PH 12 (cm)		
	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS
Kulai mardi	7.17	11.33	9.25	13.67	15.00	14.33	33.67	38.33	36.00	63.33	44.67	54.00	71.00	51.00	61.00	81.00	51.00	66.00	81.67	51.67	66.67
Kulai Jernih	7.33	13.00	10.17	11.67	17.00	14.33	32.83	39.00	35.92	62.67	44.00	53.33	78.67	45.00	61.83	97.00	46.33	71.67	99.00	49.67	74.33
PP0537-7539	8.00	12.33	10.17	12.00	16.67	14.33	25.67	35.00	30.33	47.33	49.33	48.33	56.67	55.00	55.83	58.67	53.00	55.83	56.67	59.67	58.17
PP0042-31	7.83	13.67	10.75	11.50	18.00	14.75	27.67	34.67	31.17	48.33	39.00	43.67	48.33	40.00	44.17	51.33	44.00	47.67	51.67	47.00	49.33
Cili Bangi 5	9.50	10.00	9.75	13.00	15.00	14.00	37.33	35.33	36.33	60.00	39.67	49.83	69.00	44.00	56.50	69.33	44.00	56.67	67.67	51.00	59.33
2331 HJR	7.83	11.33	9.58	11.67	16.67	14.17	33.33	31.00	32.17	67.00	39.67	53.33	74.83	53.67	64.25	75.33	55.00	65.17	75.00	57.00	66.00
MC11	6.00	13.00	9.50	9.67	17.00	13.33	25.83	44.00	34.92	55.67	56.00	55.83	69.50	67.33	68.42	81.83	71.00	76.42	80.33	73.00	76.67
PP0337-7554	5.33	14.00	9.67	9.00	19.67	14.33	21.00	45.33	33.17	48.17	47.00	47.58	63.67	49.00	56.33	62.67	50.33	56.50	62.67	56.33	59.50
Cili Bangi 2	6.83	15.00	10.92	10.33	20.67	15.50	50.33	56.00	53.17	59.83	60.67	60.25	71.33	69.67	70.50	71.67	70.67	71.17	71.67	80.67	76.17
Cili Bangi 3	8.00	14.67	11.33	13.33	20.00	16.67	48.00	43.00	45.50	54.00	45.33	49.67	59.00	48.33	53.67	60.00	50.67	55.33	58.00	55.67	56.83
AVPP 0002	6.50	12.67	9.58	11.67	17.00	14.33	42.33	44.33	43.33	51.67	50.00	50.83	60.00	59.67	59.83	62.67	63.00	62.83	62.33	68.33	65.33
AVPP 0116	7.00	13.67	10.33	12.33	18.67	15.50	43.00	45.67	44.33	52.00	54.33	53.17	60.17	60.00	60.08	61.00	60.33	60.67	60.67	63.33	62.00
AVPP 0306	7.00	14.00	10.50	12.00	17.67	14.83	37.33	40.33	38.83	47.00	48.67	47.83	53.00	53.00	53.00	54.67	57.33	56.00	54.33	64.00	59.17
AVPP 0512	8.00	11.00	9.50	12.00	16.17	14.08	38.33	41.00	39.67	47.00	49.00	48.00	51.67	52.33	52.00	53.67	53.33	53.50	54.00	54.00	54.00
AVPP 0513	8.00	11.00	9.50	12.00	15.33	13.67	34.67	35.33	35.00	41.33	40.00	40.67	41.67	41.67	41.67	46.33	46.00	46.17	45.67	51.00	48.33
AVPP 0514	9.00	12.00	10.50	12.00	15.67	13.83	42.33	44.67	43.50	48.00	50.33	49.17	56.33	55.67	56.00	55.67	55.33	55.50	54.33	55.00	54.67
AVPP 0705	9.00	11.67	10.33	12.00	16.67	14.33	33.00	36.00	34.50	38.00	40.00	39.00	42.00	41.67	41.83	42.67	43.00	42.83	42.67	46.00	44.33
AVPP 0902	6.50	12.67	9.58	10.33	19.00	14.67	42.00	44.67	43.33	49.00	50.00	49.50	52.67	53.00	52.83	52.00	53.00	52.50	51.67	63.00	57.33
AVPP 9703	6.00	9.67	7.83	9.83	12.17	11.00	27.67	30.33	29.00	33.00	35.33	34.17	38.33	38.00	38.17	42.33	44.33	43.33	42.33	45.33	43.83
AVPP 9813	6.00	9.67	7.83	11.17	13.33	12.25	40.67	43.00	41.83	47.67	49.00	48.33	50.67	51.00	50.83	51.33	51.00	51.17	51.00	51.00	51.00
AVPP 9905	6.67	11.00	8.83	12.67	15.33	14.00	33.67	36.67	35.17	39.00	39.67	39.33	42.67	43.00	42.83	42.67	44.67	43.67	40.67	58.00	49.33
Mean	7.31	12.25	9.78	11.61	16.79	14.20	35.75	40.17	37.96	50.48	46.27	48.37	57.67	51.05	54.36	60.66	52.73	56.69	60.19	57.17	58.68
Stdev	1.10	1.58	1.34	1.20	2.12	1.66	7.61	5.98	6.79	8.79	6.45	7.62	11.70	8.52	10.11	14.41	8.18	11.29	14.77	9.05	11.91
CV	15.04	12.93	13.99	10.35	12.64	11.49	21.28	14.89	18.08	17.41	13.94	15.68	20.29	16.68	18.49	23.76	15.51	19.63	24.54	15.84	20.19
Stderr	0.24	0.35	0.29	0.26	0.46	0.36	1.66	1.31	1.48	1.92	1.41	1.66	2.55	1.86	2.21	3.15	1.78	2.46	3.22	1.98	2.60
LSD (P≤0.05)	0.86	0.65	0.76	1.75	0.83	1.29	3.43	0.89	2.16	3.14	0.89	2.01	1.64	0.90	1.27	3.53	1.23	2.38	2.58	0.92	1.75

PH 0 = Plant height at week 0; PH 2 = Plant height at week 2; PH 4 = Plant height at week 4; PH 6 = Plant height at week 6; PH 8 = Plant height at week 8; PH 10 = Plant height at week 10; PH 12 = Plant height at week 12; S = Season; CS = Combined seasons; CV = Coefficient of variation; Stderr = Standard error of mean; LSD = Least significant difference.

* ☐ Main result

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Table 4.4: Means for stem diameter (SD) and plant canopy diameter (PCD) studied in 21 genotypes of *C. annuum* L. from different planting seasons

Genotype\Trait	SD 27 (cm)			SD 40 (cm)			SD 60 (cm)			SD 80 (cm)			PCD 27 (cm)			PCD 40 (cm)			PCD 60 (cm)			PCD 80 (cm)		
	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS
Kulai mardi	0.47	0.50	0.48	0.83	0.70	0.77	1.37	1.00	1.18	1.53	1.00	1.27	23.50	21.33	22.42	35.00	35.00	35.00	61.33	36.00	48.67	56.00	36.00	46.00
Kulai Jernih	0.53	0.40	0.47	1.07	0.60	0.83	1.43	0.90	1.17	1.57	0.90	1.23	23.50	22.67	23.08	39.00	36.00	37.50	63.33	44.33	53.83	71.00	45.67	58.33
PP0537-7539	0.50	0.40	0.45	0.93	0.60	0.77	1.33	1.00	1.17	1.43	1.00	1.22	22.83	26.00	24.42	51.00	39.00	45.00	55.33	53.33	54.33	57.00	51.00	54.00
PP0042-31	0.43	0.40	0.42	0.80	0.60	0.70	1.23	1.00	1.12	1.13	1.00	1.07	22.83	22.33	22.58	40.67	39.00	39.83	56.00	46.00	51.00	52.67	47.00	49.83
Cili Bangi 5	0.47	0.50	0.48	0.80	0.70	0.75	1.27	0.90	1.08	1.23	0.90	1.07	27.17	23.33	25.25	49.00	38.00	43.50	73.67	42.00	57.83	70.00	38.00	54.00
2331 HJR	0.40	0.40	0.40	0.80	0.60	0.70	1.20	1.00	1.10	1.33	1.00	1.17	23.00	26.33	24.67	45.00	41.00	43.00	77.00	51.33	64.17	84.67	48.00	66.33
MC11	0.47	0.50	0.48	0.87	0.70	0.78	1.13	1.00	1.07	1.30	1.00	1.15	21.33	22.33	21.83	36.00	37.00	36.50	49.00	50.33	49.67	57.00	49.00	53.00
PP0337-7554	0.47	0.40	0.43	1.17	0.60	0.88	1.40	1.10	1.25	1.47	1.10	1.28	24.83	24.33	24.58	41.67	39.33	40.50	67.33	51.33	59.33	63.67	51.00	57.33
Cili Bangi 2	0.43	0.40	0.42	0.87	0.60	0.73	1.33	1.30	1.32	1.43	1.30	1.37	22.33	36.00	29.17	45.33	51.00	48.17	62.67	62.00	62.33	59.00	60.00	59.50
Cili Bangi 3	0.43	0.40	0.42	0.83	0.60	0.72	1.13	1.10	1.12	1.27	1.10	1.18	22.33	25.00	23.67	33.00	40.33	36.67	53.67	54.33	54.00	49.67	47.00	48.33
AVPP 0002	0.43	0.40	0.42	0.83	0.60	0.72	1.17	1.10	1.13	1.27	1.10	1.18	21.33	23.33	22.33	39.67	38.00	38.83	46.00	46.33	46.17	48.00	50.00	49.00
AVPP 0116	0.43	0.40	0.42	0.83	0.60	0.72	1.33	1.30	1.32	1.53	1.30	1.42	20.33	21.67	21.00	35.00	36.00	35.50	43.33	43.33	43.33	50.00	51.00	50.50
AVPP 0306	0.43	0.40	0.42	0.83	0.60	0.72	1.13	1.10	1.12	1.40	1.10	1.25	22.33	22.33	22.33	35.33	37.00	36.17	39.33	40.33	39.83	39.33	40.00	39.67
AVPP 0512	0.43	0.40	0.42	0.83	0.60	0.72	1.03	0.90	0.97	1.10	0.90	1.00	16.17	20.67	18.42	28.33	31.00	29.67	34.67	35.33	35.00	30.33	29.33	29.83
AVPP 0513	0.43	0.40	0.42	0.83	0.60	0.72	1.17	1.10	1.13	1.17	1.10	1.13	20.67	20.33	20.50	32.00	25.33	28.67	30.00	31.33	30.67	33.00	34.00	33.50
AVPP 0514	0.43	0.40	0.42	0.83	0.60	0.72	1.13	1.10	1.12	1.20	1.10	1.15	24.00	22.00	23.00	34.67	37.00	35.83	42.67	43.33	43.00	46.00	47.00	46.50
AVPP 0705	0.43	0.40	0.42	0.87	0.60	0.73	1.17	1.10	1.13	1.20	1.10	1.15	23.50	21.33	22.42	33.67	36.33	35.00	42.67	43.33	43.00	43.33	44.00	43.67
AVPP 0902	0.43	0.40	0.42	0.87	0.60	0.73	1.13	1.10	1.12	1.23	1.10	1.17	29.17	24.00	26.58	37.17	39.00	38.08	49.00	50.33	49.67	47.67	48.00	47.83
AVPP 9703	0.43	0.40	0.42	0.87	0.60	0.73	1.07	1.00	1.03	1.17	1.00	1.08	21.33	23.00	22.17	37.67	38.00	37.83	36.00	37.00	36.50	38.33	37.00	37.67
AVPP 9813	0.47	0.50	0.48	0.87	0.70	0.78	1.10	1.10	1.10	1.20	1.10	1.15	20.67	25.00	22.83	31.33	36.00	33.67	40.67	41.67	41.17	43.00	43.00	43.00
AVPP 9905	0.47	0.50	0.48	0.83	0.70	0.77	1.10	1.10	1.10	1.20	1.10	1.15	20.67	25.00	22.83	34.83	35.33	35.08	42.33	43.33	42.83	47.33	48.00	47.67
Mean	0.45	0.42	0.44	0.87	0.62	0.75	1.21	1.06	1.13	1.30	1.06	1.18	22.56	23.73	23.15	37.87	37.37	37.62	50.76	45.08	47.92	51.76	44.95	48.36
Stdev	0.03	0.04	0.04	0.09	0.04	0.07	0.12	0.11	0.11	0.14	0.11	0.13	2.61	3.28	2.95	5.85	4.60	5.23	13.01	7.29	10.15	13.11	7.08	10.10
CV	6.48	10.30	8.39	10.20	7.00	8.60	9.63	10.09	9.86	10.97	10.09	10.53	11.57	13.82	12.70	15.46	12.31	13.88	25.62	16.18	20.90	25.33	15.76	20.55
Stderr	0.01	0.01	0.01	0.02	0.01	0.01	0.03	0.02	0.02	0.03	0.02	0.03	0.57	0.72	0.64	1.28	1.00	1.14	2.84	1.59	2.21	2.86	1.55	2.20
LSD (P≤0.05)	0.09	0.00	0.04	0.13	0.00	0.07	0.11	0.00	0.05	0.14	0.00	0.07	2.07	0.64	1.35	1.96	0.42	1.19	1.80	1.97	1.88	2.61	0.29	1.45

SD 27 = Stem diameter at 27 DAT; SD 40 = Stem diameter at 40 DAT; SD 60 = Stem diameter at 60 DAT; SD 80 = Stem diameter at 80 DAT; PCD 27 = Plant canopy diameter at 27 DAT; PCD 60 = Plant canopy diameter at 60 DAT; PCD 40 = Plant canopy diameter at 40 DAT; PCD 80 = Plant canopy diameter at 80 DAT; S = Season; C = Combined seasons; CV = Coefficient of variation; Stderr = Standard error of mean; LSD = Least significant difference

* ☐ Main result

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Table 4.5: Means for total leaf area (TLA), days to flowering (DF) and days to maturity (DM) studied in 21 genotypes of *C. annuum* L. from different planting seasons

Genotype\Trait	TLA (cm2)			DF (days)			DM (days)		
Season	S1	S2	CS	S1	S2	CS	S1	S2	CS
Kulai mardi	1279.63	1232.07	1255.85	31.60	32.00	31.80	70.40	72.00	71.20
Kulai Jernih	1657.89	1629.15	1643.52	28.80	30.00	29.40	69.00	75.00	72.00
PP0537-7539	1830.72	1815.47	1823.10	23.40	23.00	23.20	68.80	63.00	65.90
PP0042-31	1483.76	1444.90	1464.33	25.00	25.00	25.00	67.80	65.00	66.40
Cili Bangi 5	6940.39	6987.38	6963.89	25.00	25.00	25.00	66.80	65.00	65.90
2331 HJR	9288.54	9101.93	9195.24	34.40	35.00	34.70	67.40	70.00	68.70
MC11	2073.13	2033.51	2053.32	33.80	34.00	33.90	71.60	74.00	72.80
PP0337-7554	1837.11	1698.76	1767.94	34.40	34.80	34.60	72.80	75.00	73.90
Cili Bangi 2	4751.98	4716.21	4734.10	32.40	32.00	32.20	72.60	72.40	72.50
Cili Bangi 3	3643.19	3636.90	3640.05	34.00	34.00	34.00	73.80	75.00	74.40
AVPP 0002	3747.07	3731.61	3739.34	24.40	25.00	24.70	68.60	70.00	69.30
AVPP 0116	2841.53	2823.72	2832.63	24.40	24.00	24.20	67.20	68.00	67.60
AVPP 0306	2430.81	2432.88	2431.85	24.40	25.00	24.70	70.00	71.00	70.50
AVPP 0512	2097.04	2004.29	2050.67	24.80	25.00	24.90	72.00	71.00	71.50
AVPP 0513	1185.71	1029.76	1107.74	24.80	25.00	24.90	68.40	70.00	69.20
AVPP 0514	2119.67	2084.11	2101.89	24.00	24.00	24.00	67.20	67.00	67.10
AVPP 0705	2113.67	1993.92	2053.80	24.60	24.80	24.70	68.20	70.00	69.10
AVPP 0902	2377.55	2360.46	2369.01	26.00	26.00	26.00	71.80	73.40	72.60
AVPP 9703	1853.11	1825.71	1839.41	26.00	26.00	26.00	72.20	73.00	72.60
AVPP 9813	3558.90	3522.17	3540.54	24.00	24.00	24.00	68.80	69.00	68.90
AVPP 9905	3886.70	3834.77	3860.74	24.00	24.00	24.00	68.40	69.00	68.70
Mean	2999.91	2949.51	2974.71	27.34	27.50	27.42	69.70	70.37	70.04
Stdev	1982.05	1978.05	1980.05	4.13	4.24	4.19	2.16	3.42	2.79
CV	66.07	67.06	66.57	15.11	15.41	15.26	3.11	4.87	3.99
Stderr	432.52	431.65	432.08	0.90	0.92	0.91	0.47	0.75	0.61
LSD (P≤0.05)	243.81	128.50	186.16	0.90	0.90	0.90	3.25	3.25	3.25

TLA= Total leaf area; DF= Days to flowering; DM= Days to maturity; S = Season; CS = Combined seasons; CV = Coefficient of variation; Stderr = Standard error of mean; LSD = Least significant difference mean; LSD = Least significant difference

* ☐ Main result

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4.1.2 Yield and Its Components

Mean comparison for all yield characters were illustrated in respective graph in the Appendix C2. Table 4.6 showed highly significant differences (P<0.01) for all yield characters calculated among the 21 chili genotypes tested except for the total seed number and number of fruit per plant which was found not significant within the replication throughout the first season. Six traits on chili yield comprising both consecutive planting seasons were analysed for genetic variability. All of the mean values for all traits had been presented in the Table 4.7. The mean value of total seed was varied from 53.40 to 166.00 in the first planting season and 50.00 to 150.00 in the next planting season. The highest mean value of total seed was calculated in Cili Bangi 5 and Cili Bangi 3 with 166.00 and 150.00, respectively, while the lowest mean value of total seed was computed in AVPP 0514 and 2331 HJR with mean value of 53.40 and 50.00, respectively. Nevertheless, combined analysis exposed that Cili Bangi 5 was the most stable genotypes in total seed over the seasons with the mean value of 157.50.

The highest mean value of number of fruit per plant was recorded from Cili Bangi 3 in the first season and AVPP 0902 in the second season with the mean value of 97.40 and 129.00, respectively. While the lowest mean value for number of fruit per plant was recorded from AVPP 9905 in the first season and Kulai Mardi in the second season with the mean value of 11.60 and 10.00, respectively. Meanwhile, the most stable genotypes in number of fruit per plant across the seasons was recorded from AVPP 0902 with the mean value of 124.60. In fresh weight per fruit, the highest mean value was observed in AVPP 9905 for both seasons with the value of 21.78 g and 23.87 g, respectively. While the lowest mean value of fresh weight per fruit was observed in AVPP 0902 for both seasons with the value of 2.66 g and 2.80 g, respectively. To be noted, the most stable genotypes in fresh weight per fruit across the seasons was logged by AVPP 9905 with the mean value of 22.83 g. The highest mean value of fruit length was detected in AVPP 9905 for both planting seasons with the mean value of 20.00 cm in the first season and 20.20 cm in the second season. While the lowest mean value of fruit length was indicated by AVPP 0513 and AVPP 9813 in both planting seasons with the value of 11.00 cm and 11.20 cm, respectively. Though, the most stable genotypes in fruit length between different seasons was recorded from AVPP 9905 with the mean value of 20.10 cm.

The highest mean value of fruit diameter was discovered in Kulai Mardi, Cili Bangi 3 and AVPP 9905 in the first and second planting season with the value of 2.04 mm and 2.00 mm, respectively. While the lowest mean value of fruit diameter was identified in AVPP 0902 and AVPP 9703 with the value of 0.94 mm and 0.90 mm, respectively. Without regard to, the most stable genotypes in fruit diameter between different seasons was recorded from three genotypes, Kulai Mardi, Cili Bangi 3 and AVPP 9905 which had a similar mean value of 2.02 mm. Mean value of yield per plant varied from 89.55 to 447.71 g in the first planting season and 91.61 to 409.63 g in the next planting season. The highest mean value of yield per plant was determined from PP0337-7554 and Cili Bangi 3 with

the mean value of 447.71 g and 409.63 g, respectively, while the lowest mean value of yield per plant was detected from AVPP 0705 with the mean value of 89.55 g and 91.61 g in each of respected seasons. For all that, the most stable genotypes in yield per plant over the seasons was obtained from Cili Bangi 3 with the mean value of 420.29 g.

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Table 4.6: Analysis of variance for six yield traits of *C. annuum* L. in different planting seasons

Trait\SOV	Replications (df = 3)		Genotypes (df = 20)		Error (df = 60)	
	S1	S2	S1	S2	S1	S2
Total seed (no)	35.97*ns*	1518.91**	4719.91**	4558.23**	49.17	3.58
Number of fruits per plant (no)	1370.99*ns*	436.60**	3474.67**	3045.06**	863.21	2.59
Fruit weight per plant (g)	3.53**	72.54**	81.307**	109.26**	0.32	4.68
Fruit length (cm)	1.41**	12.08**	24.89**	25.70**	0.00	0.00
Fruit diameter (mm)	0.27**	0.21**	0.58**	0.58**	0.00	0.00
Yield per plant (g)	1520.90**	1258.53**	44657.34**	34078.45**	5.35	3.69

*Significant at 0.05 probability level; **Highly significant at 0.01 probability level; *ns* = Non-significant; SOV = Source of variation; df= Degree of freedom; S = Season

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Table 4.7: Means for yield and yield traits in 21 genotypes of *C. annuum* L. from different planting seasons

Genotype\Trait	TS (no)			NFPP (no)			FWPF (g)			FL (cm)			FD (mm)			YPP (g)		
	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS	S1	S2	CS
Kulai mardi	110.40	132.40	121.40	38.20	10.00	24.10	7.32	16.04	11.68	18.50	17.70	18.10	2.04	2.00	2.02	278.89	137.61	208.25
Kulai Jernih	123.60	146.00	134.80	28.20	12.00	20.10	7.54	10.35	8.95	16.70	17.20	16.95	1.44	1.40	1.42	211.93	112.46	162.19
PP0537-7539	114.80	112.00	113.40	41.20	21.00	31.10	5.99	8.32	7.16	14.80	18.20	16.50	1.34	1.30	1.32	246.70	169.38	208.04
PP0042-31	142.80	136.00	139.40	29.20	26.00	27.60	8.38	8.30	8.34	16.70	17.70	17.20	1.54	1.50	1.52	243.93	211.66	227.79
Cili Bangi 5	166.00	149.00	157.50	43.20	20.00	31.60	6.42	7.33	6.88	15.30	15.70	15.50	1.44	1.40	1.42	276.97	141.73	209.35
2331 HJR	90.20	50.00	70.10	62.20	25.00	43.60	5.06	5.70	5.38	16.50	16.70	16.60	1.34	1.30	1.32	314.24	139.50	226.87
MC11	147.20	125.00	136.10	22.20	26.00	24.10	10.82	9.88	10.35	18.00	17.70	17.85	1.64	1.60	1.62	238.86	251.82	245.34
PP0337-7554	132.40	126.00	129.20	38.20	28.80	33.50	11.74	7.18	9.46	15.50	16.20	15.85	1.54	1.50	1.52	447.71	203.70	325.71
Cili Bangi 2	127.00	113.00	120.00	45.20	50.00	47.60	4.81	5.49	5.15	14.00	14.20	14.10	1.44	1.40	1.42	217.20	273.24	245.22
Cili Bangi 3	149.60	150.00	149.80	97.40	32.20	64.80	12.25	12.87	12.56	15.50	15.70	15.60	2.04	2.00	2.02	430.95	409.63	420.29
AVPP 0002	82.00	100.00	91.00	25.40	29.00	27.20	4.30	4.25	4.28	15.50	15.70	15.60	1.34	1.30	1.32	109.55	121.45	115.50
AVPP 0116	130.60	116.00	123.30	25.20	25.00	25.10	9.15	9.44	9.29	13.50	13.70	13.60	1.74	1.70	1.72	229.86	220.80	225.33
AVPP 0306	86.00	94.80	90.40	30.20	37.00	33.60	5.65	5.17	5.41	12.50	12.70	12.60	1.34	1.30	1.32	170.33	185.27	177.80
AVPP 0512	89.00	84.00	86.50	35.40	41.00	38.20	5.67	5.27	5.47	16.00	16.20	16.10	1.04	1.00	1.02	200.65	214.32	207.49
AVPP 0513	90.40	81.00	85.70	17.20	22.00	19.60	7.11	6.44	6.78	11.00	11.20	11.10	1.24	1.20	1.22	121.22	130.15	125.68
AVPP 0514	53.40	58.00	55.70	46.20	44.00	45.10	6.09	6.32	6.21	13.48	13.70	13.59	1.04	1.00	1.02	280.92	276.48	278.70
AVPP 0705	81.20	86.00	83.60	16.20	17.00	16.60	5.57	5.64	5.61	15.48	15.70	15.59	1.04	1.00	1.02	89.55	91.61	90.58
AVPP 0902	81.60	80.00	80.80	120.20	129.00	124.60	2.66	2.80	2.73	15.48	15.70	15.59	0.94	0.90	0.92	320.21	360.52	340.36
AVPP 9703	113.00	103.00	108.00	17.20	21.00	19.10	5.88	6.15	6.01	14.48	14.70	14.59	0.94	0.90	0.92	100.34	125.20	112.77
AVPP 9813	56.20	56.00	56.10	31.20	36.00	33.60	6.49	6.23	6.36	11.00	11.20	11.10	1.24	1.20	1.22	202.03	222.03	212.03
AVPP 9905	103.20	99.00	101.10	11.60	12.00	11.80	21.78	23.87	22.83	20.00	20.20	20.10	2.04	2.00	2.02	250.11	258.53	254.32
Mean	108.12	104.63	106.38	39.10	31.62	35.36	7.65	8.24	7.95	15.23	15.60	15.42	1.42	1.38	1.40	237.25	202.72	219.98
Stdev	30.72	30.19	30.46	26.36	24.68	25.52	4.03	4.67	4.35	2.23	2.27	2.25	0.34	0.34	0.34	94.51	82.56	88.53
CV	28.42	28.86	28.64	67.41	78.05	72.73	52.70	56.73	54.71	14.64	14.53	14.59	24.04	24.74	24.39	39.83	40.73	40.28
Stderr	6.70	6.59	6.65	5.75	5.39	5.57	0.88	1.02	0.95	0.49	0.49	0.49	0.07	0.07	0.07	20.62	18.02	19.32
LSD (P≤0.05)	8.83	8.83	8.83	36.98	36.98	36.98	0.71	0.71	0.71	0.02	0.02	0.02	0.00	0.00	0.00	2.91	2.91	2.91

TS = Total seed; NFPP = No. of fruit per plant; FWPF = Fresh weight per fruit; FL = Fruit length; FD = Fruit diameter; YPP = Yield per plant; S = Season;

* ☐ Main result

According to Usman *et al.* (2014) and Ridzuan *et al.* (2018), genetic constitution might be a possible reason of variation among the growth characters studied. In general, high performance of chili genotypes in term of vegetative growth were seen in MC 11, Cili Bangi 2, Kulai Jernih, AVPP 0116, 2331 HJR and PP0537-7539 while in term of yield components were seen in Cili Bangi 5, Cili Bangi 3, PP0337-7554, AVPP 0902 and AVPP 9905 (Table 4.8). Most of the Malaysian genotypes such as Cili Bangi 3, Cili Bangi 5, and Kulai Jernih were highly performed compare to the other foreign genotypes (Taiwan, AVRDC and Indonesia). This finding may due to the adaptability of local genotypes to the local environment. However, certain foreign genotypes like AVPP 0116, 2331 HJR, PP0337-7554, AVPP 0902 and AVPP 9905 were becoming locally adapted.

The interaction of foreign genotypes to the local environment also affected the days to flowering. As cited by Mends-Cole *et al.* (2019), flower formation are influenced by genetic and environment factors which involve many complex processes. In this study, most of the foreign genotypes had the earliest days to flowering (23 to 24 days) and produced earlier day of maturity (65-72 days) in fruit when grown in local environment. Earliness or lateness in the days of flowering could be due to the early adaptation to the growing environment inherited characters (Delelegn *et al.,* 2014), biotic and abiotic stress (Faoro and Gozzo, 2015) to enhance growth and development. DeWitt and Bosland (2009) and Mend-Cole *et al.* (2019) also observed the fewer number of days to flowering.

Analysis of vegetative and yield traits data displayed in the Table 4.3, 4.4, 4.5 and 4.7 disclosed that different planting seasons elucidated significant effect on the performance of chili genotypes. Obviously, local and foreign genotypes showed a contradicted result. Local cultivar such as Kulai Mardi, Kulai Jernih, Cili Bangi 2, Cili Bangi 3 and Cili Bangi 5 had decreasing in term of yield per plant from first the planting season to the second planting season. The results were associated with decreasing in plant height, plant canopy diameter and number of fruit per plant. Decreasing in yield for local chili genotypes were due to the pathogenic attack for the plant growth and subsequent retardation to fruits for producing optimum yield. This finding was also in line with Srivastava *et al.* (2017) , Kavyashri and Nagaraju (2019) and Katediya *et al.* (2019) where most of the local chili genotypes were susceptible to natural disease incidence. In contrast to foreign genotypes, most of the genotypes indicated a significant increment in yield per plant even though it is not at the optimum yield. The increment is unknown but for some reason the plants might be challenged due to the pathogens which inducing the expression of defense gene to improve the resistance mechanism against the infection (Faoro and Gozzo, 2015). For all that, the genetic variation observed among the 21 chili genotypes provide room for potential improvement and selection (Usman *et al.,* 2014).

Table 4.8: The most stable chili genotypes with respected traits over two planting seasons

Traits	Genotypes
Plant height (cm)	MC 11, Cili Bangi 2, and Kulai Jernih
Stem diameter (cm)	AVPP 0116, Cili Bangi 2, and PP0337-7554
Plant canopy diameter (cm)	2331 HJR
Days to flowering	PP0537-7539
Days to maturity	Cili Bangi 5
Total leaf area (cm^2)	2331 HJR
Total seed	Cili Bangi 5
Number of fruits per plant (g)	AVPP 0902
Fruit weight per plant (g)	AVPP 9905
Fruit length (cm)	AVPP 9905
Fruit diameter (mm)	Kulai Mardi, Cili Bangi 3 and AVPP 9905
Yield per plant (g)	Cili Bangi 3

4.3 Heritability, Selection Gain, and Coefficient of Variation

The estimation of genotypic variance (GV), phenotypic variance (PV), phenotypic coefficient variance (PCV), genotypic coefficient variance (GCV), broad sense heritability (h^2_B), and genetic advance (GA) of vegetative traits, yield and yield components for consecutive planting seasons are presented in Table 4.9.

In the first planting season, h^2_B was high for all traits except in number of fruit per plant (37.70%) and days to maturity (33.37%). The highest heritability value (100%) was found in days to flowering, whereas fruit length, yield per plant, and total leaf area had 99.99%, 99.94%, and 99.05% values, respectively. In addition, high level of variability was observed in genotypic coefficient variation and phenotypic coefficient variation. The highest value of GCV were recorded from total leaf area (66.00%) while the lowest value was found in days to maturity (2.63%). The highest PCV values was found in number of fruit per plant (95.18%) but the lowest value was found in days to maturity (4.54%). Furthermore, the GA for 12 traits had its peak with the total leaf area and fresh weight per plant with value more than 100% followed by the yield per plant (82.03%), whereby the lowest value was recorded from days to maturity (3.12%). Further, the GA calculated had shown the high values (≥20%) for all yield components except for stem diameter and days to maturity.

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In the second planting season, all traits have high h^2_B value. The highest heritability value (100%) was found in stem diameter, fruit length, and fruit diameter followed by yield per plant, plant canopy diameter, and days to flowering with 99.95%, 99.94%, and 99.90% values, respectively. Moreover, high level of variability was monitored in GCV and PCV. The highest value of GCV were recorded from number of fruit per plant (78.02%) whereas the lowest value was found in days to maturity (4.86%). The highest PCV values was found in number of fruit per plant (78.18%) whereas the lowest values were found in days to maturity (4.89%) and stem diameter (9.42%). Furthermore, the genetic advance for 12 traits had its peak with number of fruit per plant, total leaf area, and fresh weight per plant with value more than 100% followed by yield per plant (83.87%), whilst the lowest value was recorded from days to maturity (9.94%). Besides, the GA calculated had shown the high values (≥20%) for all yield components except for stem diameter and days to maturity.

Genetic variation can be divided into genotypic and environmental components. Genotypic variation however is higher magnitude than the corresponding environmental variances for all characters, implying that environment had little influence on genetic variability (Singh *et al.,* 2009). High or low in GCV is highlighting the range of the variability in the studied characters. With the high GCV in the genotypes, it shows high variation of genetic due to additive gene effect subsequently further selection can be conducted to improve the characters in those chili genotypes whereas low GCV shows restricted improvement for the traits through selection (Singh *et al.,* 2009). A big differences of mean value between GCV and PCV were observed for the number of fruit per plant indicates the significant impact of environmental influences on the specific characters (Ridzuan *et al.,* 2018). In contrast, a small difference in value between GCV and PCV were observed in all traits except number of fruit per plant indicates a small effect of environment and mostly due to genetic factors (Oladosu *et al.,* 2014).

Heritability is the proportion of total phenotypic (observable) variation between individuals due to genetic differences in a given population. High heritability in the trait studied reveals that the magnitude of heritability is less influenced by environment, however effectiveness of selection depends not only on heritability but also on GA (Oladosu *et al.,* 2014). High heritability together with high GA, was recorded for all traits except days to maturity. This results specify that inheritance of these traits in under control of additive gene action (Singh *et al.,* 2009; Oladosu *et al.,* 2014). Therefore, selection will be more effective strategy to improve these traits. In different circumstances, days to maturity had high heritability but with low genetic advance indicate that inheritance of this trait is under the control of non-additive gene effects. Hybridization is the best way in improving the quality of this trait compare to the selection (Singh *et al.,* 2009; Oladosu *et al.,* 2014)

Table 4.9: Estimate of heritability and genetic advanced of 21 *C. annuum* L. in different seasons

Trait	GV			PV			GCV (%)				PCV (%)			h^2_B (%)			GA (%)	
	S1	S2	CS	S1	S2	CS	S1	S2	CS	S	S2	CS	S1	S2	CS	S1	S2	CS
PH (cm)	217.33	81.88	149.61	219.78	82.19	150.99	24.49	15.83	20.16	24.63	15.86	20.24	98.89	99.62	99.25	50.17	32.54	41.36
SD (cm)	0.02	0.01	0.01	0.02	0.01	0.02	10.19	9.42	9.80	12.07	9.42	10.74	71.34	100.00	85.67	17.73	19.40	18.57
PCD (cm)	171.08	50.18	110.63	173.58	50.21	111.90	25.27	15.76	20.51	25.45	15.76	20.61	98.56	99.94	99.25	51.68	32.45	42.07
TLA (cm^2)	3921006.32	3910593.58	3915799.95	3958530.02	3921017.58	3939773.80	66.01	67.05	66.53	66.32	67.14	66.73	99.05	99.73	99.39	135.33	137.93	136.63
DF (days)	16.97	17.96	17.47	17.49	17.98	17.73	15.07	15.41	15.24	15.29	15.42	15.36	97.05	99.90	98.47	30.58	31.73	31.15
DM (days)	3.35	11.69	7.52	10.03	11.85	10.94	2.63	4.86	3.74	4.54	4.89	4.72	33.37	98.63	66.00	3.12	9.94	6.53
TS (no)	934.15	910.93	922.54	983.32	914.51	948.91	28.27	28.85	28.56	29.00	28.90	28.95	95.00	99.61	97.30	56.76	59.31	58.03
NFPP (no)	522.29	608.49	565.39	1385.50	611.08	998.29	58.44	78.02	68.23	95.19	78.18	86.68	37.70	99.58	68.64	73.92	160.37	117.14
FWPF (g)	16.20	20.92	18.56	16.52	25.59	21.05	52.60	55.50	54.05	53.11	61.39	57.25	98.08	81.72	89.90	107.31	103.35	105.33
FL (cm)	4.98	5.14	5.06	4.98	5.14	5.06	14.65	14.53	14.59	14.65	14.53	14.59	99.99	100.00	100.00	30.17	29.93	30.05
FD (mm)	0.12	0.12	0.12	0.12	0.12	0.12	24.05	24.74	24.39	24.05	24.74	24.39	100.00	100.00	100.00	49.54	50.96	50.25
YPP (g)	8930.40	6814.86	7872.63	8935.75	6818.55	7877.15	39.83	40.72	40.28	39.84	40.73	40.29	99.94	99.95	99.94	82.03	83.87	82.95

PH = Plant height; SD = Stem diameter; PCD = Plant canopy diameter; TLA = Total leaf area; DF = Days to flowering; DM = Days to maturity; TS = Total seed; NFPP = No. of fruit per plant; FWPF = Fresh weight per fruit; FL = Fruit length,; FD = Fruit diameter; YPP = Yield per plant; DSI = Disease severity incidence; GV: Genotypic variance, PV: Phenotypic variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, h^2_B: Broad sense heritability, GA: Genetic advance (as percentage of mean); S = Season; C = Combined season

* ☐ Main result

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4.4 Correlation between Vegetative and Yield Components

Association analysis of different vegetative and yield components traits and inter-relationships were analyzed through the study of genotypic and phenotypic correlation coefficient. The relationships between thirteen traits were determined by Pearson correlation coefficients with significant at 0.05 probability level and highly significant at 0.01 probability level (Table 4.10).

Plant height had positive and highly significant correlation coefficient with all yield components traits except for total seed, and disease severity index. Among the vegetative traits, plant height had positive and highly significant correlation coefficient between the traits. Among the yield components traits, yield per plant had positive and highly significant correlation coefficient between the traits except for fruit length, suggesting that selection based on these traits would be worthwhile. These findings corroborate the earlier observations of Singh *et al.,* (2009); Oladosu *et al.,* (2014); Usman *et al.,* (2014); Ridzuan *et al.,* (2018) with different breeding material, implying advancement of yield by giving special focus to these traits. For disease severity index, it had negative and non-significant correlation coefficient, although positive, to all the other traits both in the vegetative and yield components traits except for the days to maturity. According to Chattopadhyay *et al.* (2011), this indicates its limited improvement towards yield per plant.

4.5 Genetic Divergence in Vegetative and Yield Components

4.5.1 Genotype Cluster Analysis

Based on the combined data of two consecutive planting seasons, a standardized of 13 vegetative and yield traits data collection were used to calculate the Euclidean distances among the 21 chili genotypes and a un-weighted pair group method with arithmetic mean (UPGMA) dendrogram was prepared using Numerical Taxonomy Multivariate Analysis System Software (NTSYS) version 2.1 (Figure 4.1). 21 chili genotypes were clustered into four major groups based on the 13 traits at 0.068 dissimilarity coefficients. Group IV had the largest number with 11 genotypes followed by group II with 7 genotypes, group I with two genotypes whereas group III contained only a genotype.

The mean values of each groups were demonstrated in the Table 4.11. For vegetative traits, the highest mean values for plant height, stem diameter and plant canopy diameter were recorded from group I with the value of 72.17, 1.50 and 59.83, respectively. While the lowest mean values for the similar parameters studied were recorded from group III with the value of 45.67, 1.17 and 33.00, respectively. For the yield per plant, the highest mean value was recorded from group I with the value of 363.30 while the lowest mean value was recorded from group III (121.22). Meanwhile, the disease severity index in group I showed the

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highest resistance mean value from disease with the value of 67.20 and the lowest resistance mean value from disease was recorded from group III (93.60).

Genetic diversity is one of the important tool analysis in selection of superior parent. It would be more effective in choosing superior parent with the knowledge of genetic diversity (Latif *et al.,* 2011) and can be used to classify different genotypes in a population (Franco *et al.,* 2001). Therefore, to obtain achievement in breeding for higher vigor among the genotypes, desired parent such as Cili Bangi 3, the highest yield parent from group II, could be hybridized with genotype 2331 HJR, the third most resistant to virus from group IV.

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Table 4.10: Correlation coefficient of 13 traits for 21 *C. annuum* L. planted on the different seasons

		PH	SD	PCD	TLA	DF	DM	TS	NFPP	FWPF	FL	FD	YPP	DSI
PH														
SD	S1	0.60**												
	S2	0.31**												
	C	0.50**												
PCD	S1	0.70**	0.42**											
	S2	0.49**	0.53**											
	C	0.68**	0.52**											
TLA	S1	0.21*	0.11 ns	0.14 ns										
	S2	0.28**	0.63**	0.59**										
	C	0.25*	0.37**	0.37**										
DF	S1	0.35**	0.04 ns	0.05 ns	0.26**									
	S2	0.10 ns	0.65**	0.57**	0.25**									
	C	0.23*	0.33**	0.31**	0.23*									
DM	S1	0.32**	-0.02 ns	0.14 ns	-0.23*	0.33**								
	S2	0.20*	0.67**	0.54**	-0.19*	0.33**								
	C	0.26*	0.33**	0.34**	-0.12 ns	0.41**								
TS	S1	0.13ns	0.11ns	0.22*	0.09 ns	0.41**	0.14 ns							
	S2	0.08 ns	0.56**	0.52**	0.10 ns	0.41**	0.14 ns							
	C	0.11ns	0.34**	0.37**	0.11 ns	0.30**	0.13 ns							
NFPP	S1	0.26**	0.08 ns	0.03 ns	0.14 ns	0.22*	0.14 ns	0.03 ns						
	S2	0.58**	0.45**	0.55**	0.18 ns	0.22*	0.14 ns	0.03 ns						
	C	0.42**	0.27*	0.29*	0.16 ns	0.12 ns	0.14*	-0.27**						
FWPF	S1	0.26**	0.37**	0.13 ns	-0.05 ns	0.14 ns	0.05 ns	0.32**	-0.14 ns					
	S2	0.52**	0.45**	0.52**	-0.05 ns	0.14ns	0.05ns	0.32**	-0.14 ns					
	C	0.39**	0.41**	0.33**	-0.04 ns	0.02 ns	0.01 ns	0.31**	-0.27**					
FL	S1	-0.05 ns	-0.05 ns	-0.05 ns	0.05 ns	0.33**	0.09 ns	0.37**	0.01 ns	0.49**				
	S2	0.61**	0.49**	0.63**	0.08 ns	0.33**	0.09 ns	0.01 ns	0.01 ns	0.49**				
	C	0.56**	0.22*	0.58**	0.05 ns	0.20**	0.00 ns	0.37**	-0.10 ns	0.54**				
FD	S1	0.06 ns	0.01 ns	0.14 ns	0.11 ns	0.40**	-0.02 ns	0.51**	-0.06 ns	0.66**	0.45**			
	S2	0.63**	0.46**	0.03 ns	0.07 ns	0.40**	-0.02 ns	0.51**	-0.06 ns	0.66**	0.45**			
	C	0.57**	0.24*	0.09 ns	0.09 ns	0.31**	0.00 ns	0.53**	-0.20**	0.71**	0.46**			
YPP	S1	0.45**	0.32**	0.33**	0.21*	0.58**	0.19*	0.36**	0.45**	0.32**	0.28**	0.42**		
	S2	0.45**	0.46**	0.03 ns	0.22*	0.58**	0.19*	0.36**	0.45**	0.32**	0.28**	0.42**		
	C	0.45**	0.39**	0.01 ns	0.21*	0.34**	0.17**	0.23**	0.50**	0.20**	0.13 ns	0.33**		
DSI	S1	-0.11 ns	0.02 ns	-0.01 ns	-0.39**	-0.24**	0.20*	-0.26**	-0.05 ns	0.07 ns	-0.25*	-0.13 ns	-0.29**	
	S2	-0.13 ns	-0.26**	-0.33**	-0.39**	-0.25**	0.20*	-0.27**	-0.04 ns	0.05 ns	-0.24**	-0.15 ns	-0.30**	
	C	-0.12ns	-0.12 ns	-0.17 ns	-0.31**	-0.24**	0.26**	-0.29**	-0.03 ns	0.05 ns	-0.24**	-0.12 ns	-0.19**	

*Significant at 0.05 probability level; **Highly significant at 0.01 probability level; ns= Non-significant; PH= plant height; SD= stem diameter; PCD= plant canopy diameter; TLA= total leaf area; DF= days to flowering; DM= days to maturity; TS= total seed; NFPP= no. of fruit per plant; FWPF= fresh weight per fruit; FL= fruit length,; FD= fruit diameter; YPP= yield per plant; DSI= disease severity index; S = season; C = combined season

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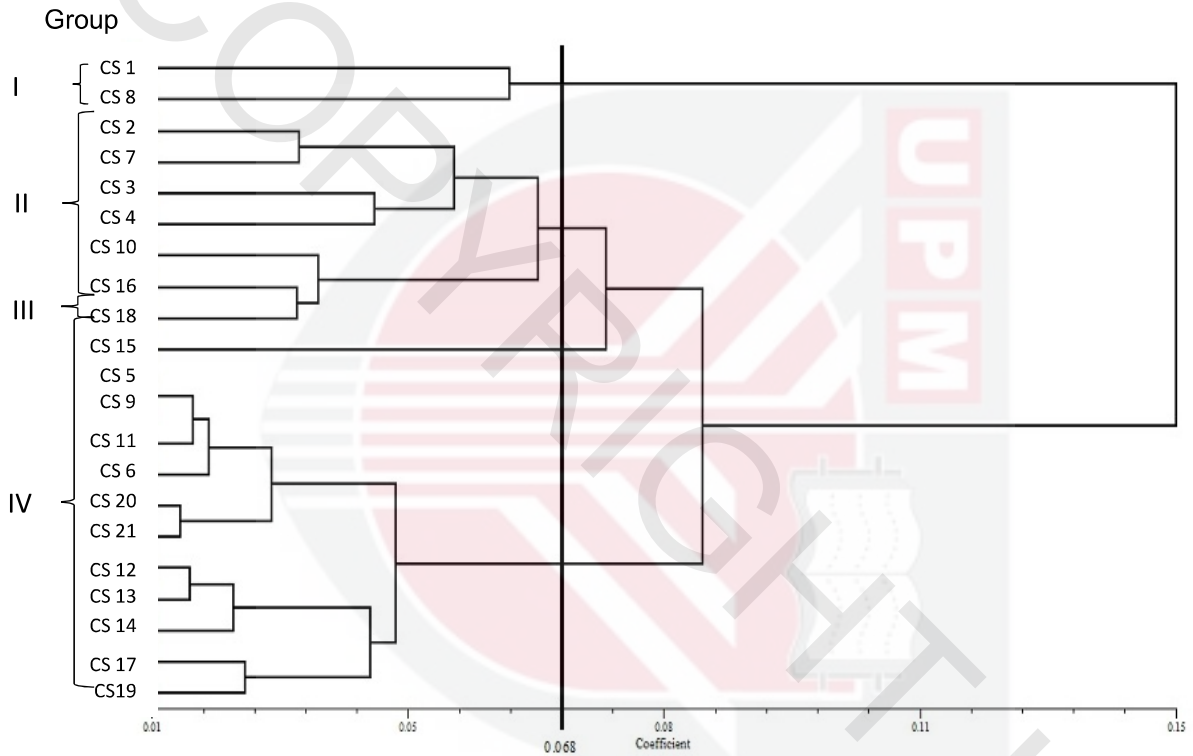
Figure 4.1: Combined dendrogram was constructed based on 13 quantitative traits grouped the 21 chili genotypes into 4 distinct groups at the coefficient of 0.068.



1 = Kulai Mardi, CS 2 = Kulai Jernih, CS 3= PP0537-7539, CS 4 = PP004231, CS 5 = Cili Bangi 5, CS 6 = 2331 HJR, CS 7 = MC11, CS 8 = PP0337-7554, CS 9 = Cili Bangi 2, CS 10 = Cili Bangi 3, CS 11 = AVPP 0002, CS 12 = AVPP 0116, CS 13 = AVPP 0306, CS 14 = AVPP 0512, CS 15 = AVPP 0513, CS 16 = AVP[illegible]514, CS 17 = AVPP 0705, CS18 = AVPP 0902, CS19 = AVPP 9703, CS20 = AVPP 9813, CS21 = AVPP 9905.

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Table 4.11: Mean values of 13 traits for four groups revealed by cluster analysis on 21 genotypes of *C. annuum* L. from combined season

Trait	Cluster				
	I	II	III	IV	Mean
Plant height (cm)	72.17	64.52	45.67	56.58	60.19
Stem diameter (cm)	1.5	1.3	1.17	1.28	1.3
Plant canopy diameter (cm)	59.83	54.43	33	50.3	51.76
Total leaf area (cm^2)	1558.37	2169.42	1185.71	3955.43	2999.91
Days to flowering (days)	33	27.86	24.8	26.22	27.34
Days to maturity (days)	71.6	70	68.4	69.29	69.7
Total seed (no)	121.4	116.14	90.4	102.22	108.12
Number of fruits per plant (no)	38.2	54.94	17.2	31.18	39.1
Fruit weight per plant (g)	9.53	7.68	7.11	7.34	7.65
Fruit lenght (cm)	17	15.81	11	14.93	15.23
Fruit diameter (mm)	1.79	1.43	1.24	1.36	1.42
Yield per plant (g)	363.3	281.93	121.22	196.44	237.25
Disease severity index (%)	67.2	69.06	93.6	78.87	75.19
Mean	111.38	131.35	78.78	196.85	161.26

PH = Plant height; SD = Stem diameter; PCD = Plant canopy diameter; TLA = Total leaf area; DF = Days to flowering; DM = Days to maturity; TS = Total seed; NFPP = No. of fruit per plant; FWPF = Fresh weight per fruit; FL = Fruit length; FD = Fruit diameter; YPP = Yield per plant; DSI = Disease severity index.

4.5.2 Principal Component Analysis

In a combined data from two consecutive planting seasons on the 13 traits, a principal component analysis was performed and portrayed in Figure 4.2. Principal component analysis was further justified the clustering analysis and grouped the similar genotypes together (Usman *et al.,* 2014). The variation percentages of the PC1 and PC2 were 43.3% and 31.4%, respectively, with PC1 showed the highest of the total variation. Most of the genotypes were dispersed at close distances and few were dispersed at great distances as revealed by the Eigenvector. The furthest chili genotypes from the centroid of principal component analysis were CS 8 (PP0337-7554) and CS 1 (Kulai Mardi). Two groups, II and IV had genotypes closely to each other in relation to the yield and yield components.

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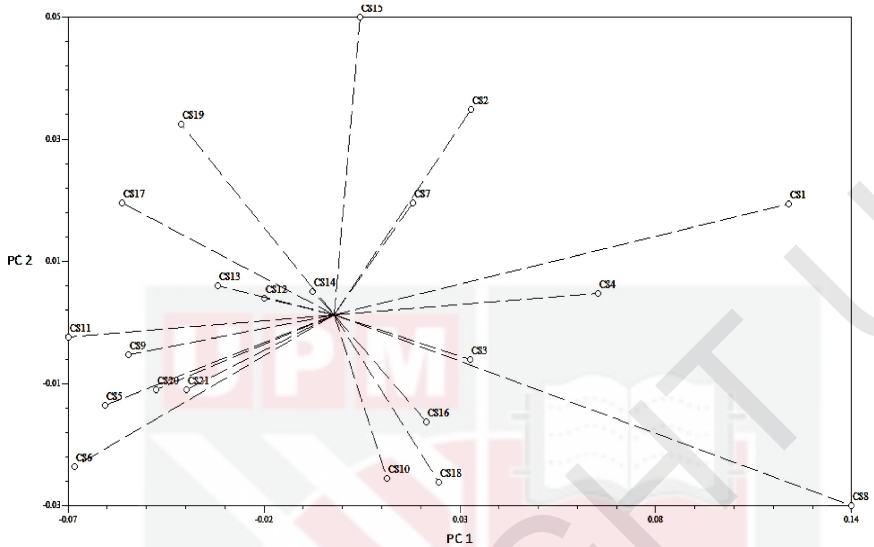
Figure 4.2: Two-dimensional graph of principal component analysis of 21 C. annuum L. genotypes from combined season showing relationship among the genotypes.



CS 1 = Kulai Mardi, CS 2 = Kulai Jernih, CS 3= PP0537-7539, CS 4 = PP004231, CS 5 = Cili Bangi 5, CS 6 = 2331 HJR, CS 7 = MC11, CS 8 = PP0337-7554, CS 9 = Cili Bangi 2, CS 10 = Cili Bangi 3, CS 11 = AVPP 0002, CS 12 = AVPP 0116, CS 13 = AVPP 0306, CS 14 = AVPP 0512, CS 15 = AVPP 0513, CS 16 = AVPP 0514, CS 17 = AVPP 0705, CS18 = AVPP 0902, CS19 = AVPP 9703, CS20 = AVPP 9813, CS21 = AVPP 9905.

4.6 Disease Severity Index

Natural infected chili plants with Cucumber Mosaic Virus (CMV) in the field were studied using general observation. Table 4.12 showed disease severity index (DSI) was highly significant among the genotypes either in individual seasons or combined season. Reading were taken in intervals between 15 days until 90 days after transplant. Symptoms were detected as early as in the 27 days after transplant. The symptoms were included mild mosaic, leaf curling, mottling, and dwarfing. These symptoms developed after 9-12 days of first infection of CMV into the plant. All of the observation data recorded were analysed using DSI. Mean values of DSI for all chili genotypes from consecutive planting seasons and combined season were presented in Table 4.13. This scoring disease was evaluated merely for viral infection particularly on CMV symptoms.

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Table 4.12: Analysis of variance for disease severity index (DSI) of *C. annuum* L. in two different planting seasons and combined season

Trait\SOV	df	Season 1	Season 2	Combined season
Replications	3	464.18*	149.68*ns*	5.34**
Genotypes (*G*)	20	3229.63**	957.76**	36.64**
Seasons (*S*)	1	-	-	54.05**
G × *S*	20	-	-	11.09**
Error Season	60	97.9**	63.83**	-
Error Combined season	120			22.89**

*Significant at 0.05 probability level; **Highly significant at 0.01 probability level; *ns* = Non-significant; SOV = Source of variation; df = Degree of freedom; G =

Table 4.13: Disease severity index (DSI) and resistant level (RL) of 21 infected *C. annuum* L. from two planting seasons and combined season

	Season 1		Season 2		Combined Season	
Genotype	DSI	RL	DSI	RL	DSI	RL
Kulai mardi	80.20	S	91.80	HS	86.00	HS
Kulai Jernih	80.40	S	77.20	S	78.80	S
PP0537-7539	33.60	HR	56.80	MR	45.20	MR
PP0042-31	27.20	HR	51.00	MR	39.10	HR
Cili Bangi 5	61.20	S	78.00	S	69.60	S
2331 HJR	12.60	I	91.60	HS	52.10	MR
MC11	77.40	S	89.20	HS	83.30	HS
PP0337-7554	54.20	MR	93.60	HS	73.90	S
Cili Bangi 2	93.60	HS	96.00	HS	94.80	HS
Cili Bangi 3	75.40	S	78.60	S	77.00	S
AVPP 0002	54.00	MR	55.00	MR	54.50	MR
AVPP 0116	91.40	HS	93.80	HS	92.60	HS
AVPP 0306	93.40	HS	89.20	HS	91.30	HS
AVPP 0512	91.20	HS	89.40	HS	90.30	HS
AVPP 0513	93.60	HS	93.80	HS	93.70	HS
AVPP 0514	95.80	HS	91.80	HS	93.80	HS
AVPP 0705	91.60	HS	93.60	HS	92.60	HS
AVPP 0902	93.60	HS	91.60	HS	92.60	HS
AVPP 9703	93.40	HS	92.80	HS	93.10	HS
AVPP 9813	91.60	HS	93.40	HS	92.50	HS
AVPP 9905	93.60	HS	91.40	HS	92.50	HS
Mean	75.19		84.7429		79.97	
Stdev	25.09		13.8403		19.47	
CV	33.37		16.3321		24.8529	
Stderr	5.48		3.02019		4.24807	
LSD (P≤0.05)	12.70		12.453		12.5775	

RL = Resistance level; I = Immune; HR = Highly resistant; MR = Mild resistant; S = Susceptible; HS = Highly susceptible; CV = Coefficient of variation; Stderr = Standard error of mean; LSD = Least significant difference

* ☐ Main result

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The highest level of DSI in first planting season was detected in AVPP 0514 with the score of 95.80, intermediate DSI were MC 11 and Cili Bangi 3 with the score of 77.40 and 75.40, respectively, while the lowest score of DSI was 2331 HJR with the score of 12.60. In the second planting season, the highest level of DSI was detected in Cili Bangi 2 with the score of 96.00, intermediate DSI were AVPP 0512, AVPP 0306 and MC 11 with the score of 89.40, 89.20 and 89.20, respectively, while the lowest score was PP0537-7539 with the score of 51.00. It was observed that the total mean of DSI in the first planting season had increased in the second planting season with the mean value of 75.19 and 84.74, respectively. Thus the infection of CMV was increased about 9% from the first planting season to the second planting season.

Using DSI score in the Table 3.6 under subsection 3.5.3.1, each of the chili genotypes were classified into several resistant levels (Table 3.7 under subsection 3.5.3.1). In the first planting season, the most resistant chili genotype or immune to the viral infection was 2331 HJR while there was no immune chili genotype in the second season. Highly resistant chili genotype was classified only from the first planting season which were PP0537-7539 and PP0042-31. Mild resistant chili genotypes were classified in both season, in first planting season were PP0337-7554 and AVPP 0002 while in the second planting season were PP0537-7539, PP0042-31 and AVPP 0002. For the susceptible group, there was an increment in number of chili genotypes from the first planting season to the second planting season with 16 chili genotypes in the first season were classified into the susceptible group while 18 chili genotypes in the second season were also classified into the same group. In the combined season, the DSI scores had categorized the genotypes into any of four groups and among the genotypes only one highly resistant, three moderately resistant, four susceptible and thirteen highly susceptible genotypes were identified against CMV.

In this study, the pesticides application at early growing stage of chili genotypes to prevent the infection viruses, perform great pertaining disease severity and yield potential. But after 30 days of transplant, it was gradually loose its effectiveness. This observation was in conformity with the work of Awang *et al.* (2013). According to Awang *et al.* (2013) and Soleimani *et al.* (2014), the chemical agents like fungicides and bactericides are not effective in controlling virus diseases. This is because viruses in chili can be transmitted in a non-persistent manner by aphids and some are transmitted by thrips, leafhopper, nematodes, beetle, fungi, through soil and even by physical contact (Soleimani *et al.,* 2014; Rahman *et al.,* 2016). From the results, none of the 21 chili genotypes were truly immune to CMV. The majority of the genotypes showed a CMV incidence of 40-90% or more in the field while only one genotype, PP0042-31 had an incidence less than 40%. The rest of the genotypes exhibited characteristic symptoms like mosaic, mottling, leaf curling, stunting, and leaf deformity. Rahman *et al.* (2016) reported that the yield loss of chili due to CMV infection ranged from 11 to 50%. The yield lost can be calculated as the reduction of yield due to CMV infection.

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Massive effort for searching resistant sources was carried out by AVRDC-The world vegetable center and members' countries. The local screening of CMV resistant genotypes are important because the screening result may varied among the member countries due to the presence of different viral strains (Roossinck, 2002; Tsai *et al.,* 2008). This study proved that CMV was still virulent in chili genotypes and development of CMV resistance should be a major part in chili breeding.

4.7 The six best selected chili genotypes

There were two interested characters to be selected using combined analysis over the both planting seasons, namely yield per plant and disease severity index. A higher yield per plant with lower disease severity index shows that genotype more prominent dependability of been selected, prompting further study for genetic hybridization. Based on the combined analysis of variance for yield per plant in Table 4.2 and combined analysis of variance for disease severity index in 4.12, planting season 1 and 2 showed highly significant values. This result suggested that the performance of the interested characters might be affected by planting at different seasons.

To decide the best genotype of the interested characters over the both planting seasons, the response of the agronomic trait together with the adaptability to viruses were compared using LSD test procedures. Consistently, this post hoc test demonstrate specific differences among 21 chili genotypes. From the Table 4.7, Cili Bangi 3 had the highest mean value of yield per plant over the two different planting seasons if compare to AVPP 0902 and PP0337-7554 with the means value of 340.36 g and 325.71 g respectively. The most bottom list of the genotypes in this test was AVPP 0705 with the mean value of 90.58 g. The best genotypes in CMV resistant was PP0042-31 with the percentage value of 39.10 whereas PP0537-7539 and 2331 HJR were the second and third in the position with the percentage values of 45.20 and 52.10 respectively.

The development of newly superior chili cultivar in a different area of cultivation need a thorough assessment. This is because chili from different parental lines demonstrated statistically different yield traits performance according to the different environment. In this study, we had successfully identified six potential chili genotypes from 21 chili genotypes to be selected for future breeding programs (Table 4.14). In Figure 4.3, three genotypes, Cili Bangi 3, AVVPP 0902 and PP0337-7554 were selected based on yield performance. All of these genotypes produced high yield and stable over the two different planting seasons. Cili Bangi 3 was from local source and the other two genotypes were from World Vegetable Center (AVRDC), Taiwan. However, these genotypes were not resistance to viruses and do not mature at early period.

On the other hand, the other three genotypes were selected based on performance on disease severity index. In Figure 4.4, genotypes PP0042-31,

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PP0537-7539 and 2331 HJR were observed to have high resistant level and stable over different planting seasons as well as mature at early stage however the yield was not very high as the former genotypes. Hybridization of high yield chili genotypes with highly resistant chili genotypes should be carried out to obtain more vigor or heterosis among the chili genotypes.

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Table 4.14: Main characteristic of the six best selected chili genotypes based on yield performance and disease scoring

Genotype	Maturity time	Plant yield	Plant size	Plant height	Days to flower	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Immature fruit colour intensity	Mature fruit colour intensity	Immature fruit colour	Mature fruit colour	CMV
Cili Bangi 3	Late	Very High	Medium	Medium	34	15.5	2.04	12.25	Medium	Medium	Green	Red	S
AVPP 0902	Medium	Very High	Medium	Low	26	15.5	0.94	2.66	Medium	Medium	Green	Red	HS
PP0337-7554	Late	Very High	Large	Medium	34	15.5	1.54	11.74	Medium	Medium	Green	Red	MR
PP0042-31	Early	High	Small	Low	25	16.7	1.54	8.38	High	High	Green	Red	HR
PP0537-7539	Early	High	Large	Low	23	14.8	1.34	5.99	Medium	Medium	Green	Red	HR
2331 HJR	Early	High	Medium	Very High	34	16.5	1.34	5.06	Medium	Medium	Green	Red	HR

All genotypes had red mature fruit colour, all had compact plant habit, chili fruit type, and elongated fruits with pointed shaped at blossom end, all had intermediate fruit position, all genotypes were susceptible to CMV except genotypes PP0042-31, PP0537-7539 and 2331 HJR. Field evaluation were conducted at TPU Field, UPM, Serdang where I = Immune (80-100% symptom free), HR = Highly resistant (60-79% symptom free), MR = Mild resistant (40-59% symptom free), S = Susceptible (20-39% symptom free) and HS = Highly susceptible (0-19% symptom free). Performance at other sites may vary.

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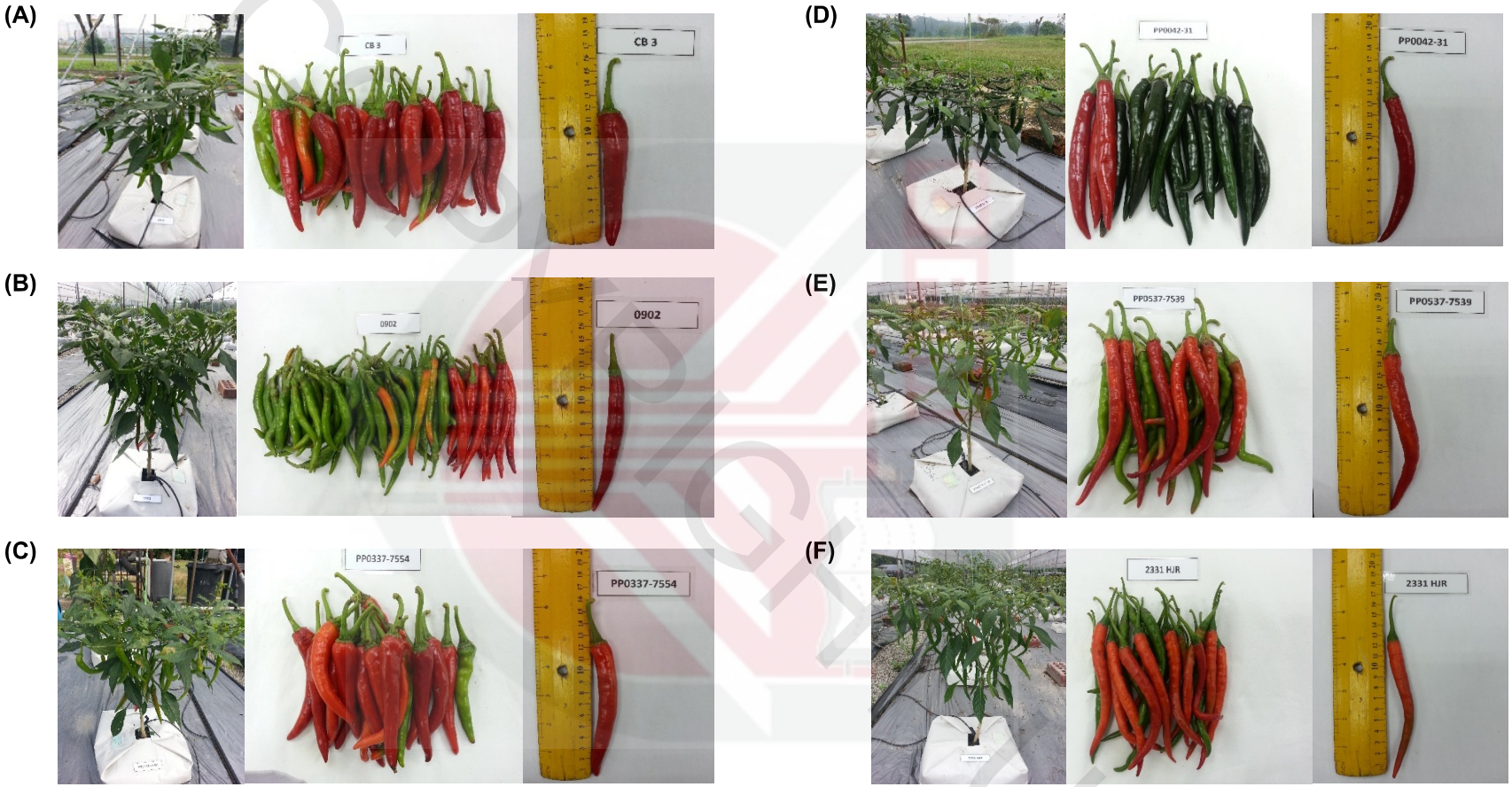

Figure 4.3: Six best selected of *C. annuum* L. genotypes based on high yield performance; (A) Cili Bangi 3, (B) AVPP 0902, (C) PP0337-7554 and highly CMV-resistant; (D) PP0042-31, (E) PP0537-7539 and (F) 2331 HJR

4.8 Molecular identification of viral diseases causing mosaic symptom in *C. annuum* L.

Figure 4.5 presented normal leaf and six infected leaf samples from selected chili genotypes (Table 4.14 in previous section). Systemic mosaic, mottling, chlorosis, and leaf deformation were found on Cili Bangi 3, mosaic and clorosis were found on genotype AVPP 0902, mosaic, chlorosis and leaf deformation were found on PP0337-7554, mosaic, mottling and leaf deformation were found on PP0042-31, mosaic and chlorosis were found on 2331 HJR, mosaic and chlorosis were found on PP0537-7539. All infected leaf samples were taken on 70 days after transplant. In general, the presence of unknown virus in diseased plant samples can be identified by the amplification of viral DNA using RT-PCR. Viral DNA was isolated from all leaf samples using CTAB method and the viral RNA was diluted from RNA stock (Table 4.15). Diluted RNA samples were amplified and the quality of cDNA samples were then checked using nanodrop (Table 4.16).

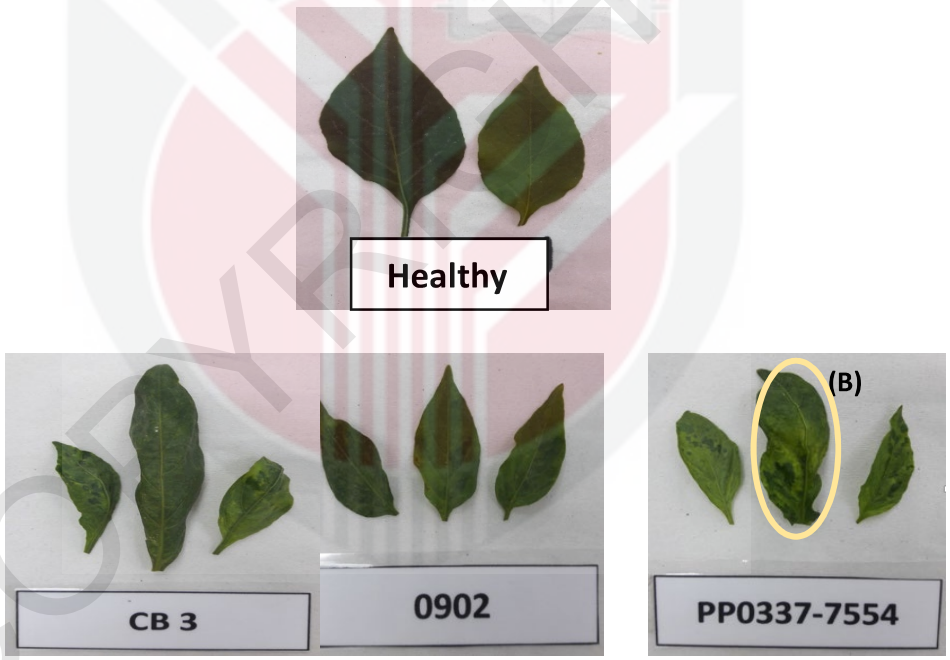

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Figure 4.4: Mosaic symptoms on several chili leaves showed symptoms like in (A) slightly colour breaking and (B) deformed shape compare to normal leaf which collected for identification of CMV infection

Table 4.15: RNA diluted from RNA stock

Sample	Conc. (ng)	260/280	260/230
A	108.5	1.75	1.49
B	76	1.73	1.20
C	85.6	1.79	1.87
D	92.2	1.78	1.87
E	101.5	1.81	1.90
F	111.9	1.78	1.81
G	144	1.80	1.75
H	109.4	1.82	1.72

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Table 4.16: Nanodrop of cDNA after been amplified (RT-PCR products) using 5 g of infected leaves samples

Sample	Conc. (ng)	260/280	260/230
A	885.6	2.01	2.91
B	716.4	2.02	2.70
C	700.6	2.02	2.49
D	871.8	1.98	2.73
E	926.9	1.98	2.97
F	926.9	2.00	2.89
G	1003.7	1.98	2.68
H	1000.3	1.97	2.75

Thirteen primers (Table 3.9 under subsection 3.5.3.4) from six main group of viruses were used in this study to detect the most highly conserved gene, CP gene. It has been accepted that CP gene sequence could be used to predict specific strains, species, as well as taxonomic lineages of different viruses and acts as desirable marker for virus identity when a full-length genomic sequence is not available (Rybicky, 1994). This feature of CP genes facilitates in detection of specific virus like CMV and PepLCV in preliminary detection and identification.

In Figure 4.6, using a diluted cDNAs from diluted RNA stock, we were able to produce an expected 800 bp fragments in all leaf samples tested from lane 3 to 7. This confirmed the presence of CMV in all chili samples tested. The results also showed that the primer pairs were target specific and RT-PCR managed to amplify all of the six fragments under the optimized conditions. Evidently, CMV was the most dominant virus-infecting chili with subgroup II detected. CMV subgroup I was not detected because it might be predominant in the tropics and subtropics area (Chen *et al.,* 2011).

In Figure 4.7, the infected leaf of lane 5 and 7 produced a typical 500 bp amplification product which confirming it to be a geminivirus. This result was similar with Sinha *et al.* (2011) which reported 550 bp DNA of specific coat protein gene region had successfully been amplified using degenerate primers. However in this study, only PP03370-7554 (lane 5) and PP0537-7539 (lane 7) were identified infected by PepLCV. The presence of PepLCV in the leaf samples along with CMV proved a mix infection occurred in chili. Obviously, the infection of PepLCV in chili are lesser than CMV and PepLCV was not virulent as CMV symptomatically. This could be explained by the dominant effect of CMV against other viruses. Saad et al. (2017) reported that the settling of whitefly, host vector of PepLCV was prominently affected by the presence of CMV in chili. CMV-

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infected chili plants released more linalool and phenylacetaldehyde than non-infected chili plant. These significantly released volatile compound which had altered the host plant attractiveness and suitability for whitefly preferences.

Figure 4.5: Gel photograph of RT-PCR amplicons of 800bp using CMV RNA and its particle as template. L= 100bp DNA ladder (GeneDirex), 1= -ve control (normal leaf), 2= +ve control (18S rRNA), 3= Infected Chili Bangi 3, 4= Infected AVPP 0902, 5= Infected PP0337-7554, 6= Infected PP004231, 7= Infected PP0537-7539, 8=Infected 2331 HJR

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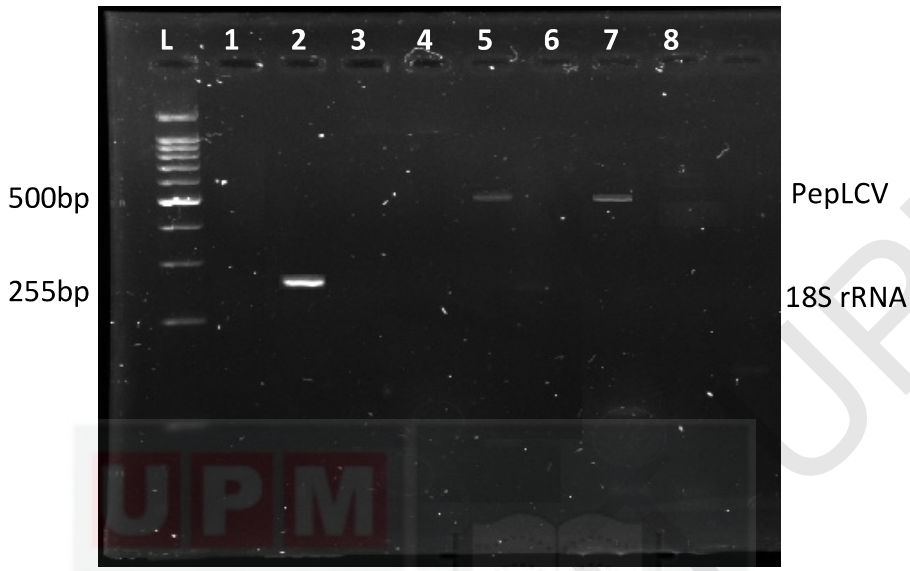

Figure 4.6: Gel photograph of RT-PCR amplicons of 500bp using PepLCV RNA and its particle as template. L=100bp DNA ladder (GeneDirex), 1= -ve control (normal leaf), 2= +ve control (18S rRNA), 3= Infected Chili Bangi 3, 4= Infected AVPP 0902, 5= Infected PP0337-7554, 6= Infected PP004231, 7= Infected PP0537-7539, 8= Infected 2331 HJR

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CHAPTER 5

GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH

5.1 General Conclusion

Twenty-one chili genotypes selected from Malaysia, Indonesia and Taiwan were evaluated for yield performance and cucumber mosaic virus (CMV)-disease severity index (DSI) assessment. Among the Malaysian genotypes, genotypes Cili Bangi 3 was selected based on its good performances in yield and yield attributing traits. In different circumstances, four genotypes namely AVPP 0902, PP0337-7554, PP0042-31 and PP0537-7539 from World Vegetable Center (AVRDC) and one genotypes from Indonesia, 2331 HJR were selected based on high yield and resistance against the CMV pathogen. From this study, two types of viruses infecting chili plants were successfully pre-identified, CMV and PepLCV were commonly found causing severe diseases on chili plants in worldwide. The findings from these identification and assessment were useful for future selection in breeding program for high yield and CMV-PepLCV resistant chili genotypes.

These studies also provided evidences that new chili genotypes contain favorable genes potential to be introgress to the local chili varieties. The use of host plant resistance is one of the most important strategies in the breeding and disease management such as the role played by AVRDC which afforded several improved resistant chili lines. The understanding on the interaction between resistant host and the plant viruses was useful in the selection of future chili parental lines. The development of resistant chili cultivar is essential as the ideal primary line to combat against the infection of viruses without application of chemical inputs or transgenic manipulation which is beneficial to maintain environmental safety and sustainability. Besides, it helps farmers to construct the organic cultivation in chili. Therefore, it can be concluded that the use of disease resistant plant is the most environmentally safe, economically efficient, and durable methods for controlling plant diseases.

5.2 Recommendation

The outcomes of this study present the strategy for the advancement of chili genotypes pertaining to CMV resistant gene using resistant host of chili plant. Looking at the agronomic performance, variability, and group distances among the studied genotypes for high yield and resistant to CMV, the crosses between Cili Bangi 3 with PP0042-31, PP0537-7539 and 2331 HJR; AVPP 0902 with PP0042-31, PP0537-7539 and 2331 HJR and PP0337-7554 with PP0042-31, PP0537-7539 and 2331 HJR could be suggested for future hybridization program for development of high resistant and high yielding chili variety.

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APPENDICES

Appendix A

A1) Stock solution for 30 litres of water

Stock A

Calcium Nitrate	7132.5 g
Ferum EDTA	237.5 g

Stock B

Potassium Nitrate	1327.5 g
Potassium Sulphate	4365.00 g
Magnesium Sulphate	4012.5 g
Manganese Sulphate / EDTA	4012.5 g
Boric Acid	12.75 g
Cooper Sulphate / EDTA	2.925 g
Potassium Dehydrogen Phosphate	641.25 g
Zinc Sulphate / EDTA	3.3 g
Ammonium Molybdate	2.775 g

Appendix B

B1) Buffer solution preparation for mechanical inoculation of virus

Material	**Preparation**
Sol. A	1.36 g KH_2PO_4 in 1 L H_2O
Sol. B	1.78 g Na_2HPO_4.12 in 1 L H_2O
0.01 M PO_4 (pH 7.0)	51 ml of sol. A + 49 ml of sol. B
Sodium diethyl-dithio carbamate (Sod. DIECA)	25% (w/v) (Mazidah et al., 2012)
Carborundum 600 mesh	5% (w/v) (Tan, 2012)
Buffer solution	100 ml of 0.01 M PO_4 + 0.25 g of Sod. DIECA + 0.5 g of carborundum

B2) Specific primer to confirm the absence of virus was confirmed by RT-PCR

Name	**Sequence from 5' to 3'**	**Genomic segment**
5' CMV-CP (Forward)	5'-atggac aaa tct gra tcw mcc-3'	RNA 3
3' CMV-CP (Reverse)	5'-ctg gat ggacaa ccc gtt c-3'	RNA 3

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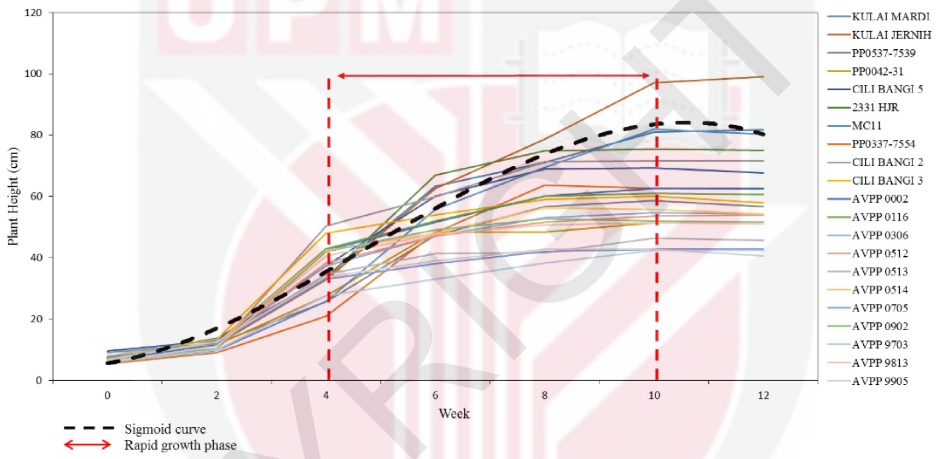
B3) Specific primer as an internal control

Name	Sequence from 5' to 3'	Genomic segment
Nad sense	5'-gatgcttcttggggcttcttgtt- 3'	-
Nad antisense	5'-ctccagtcaccaacattggcataa-3'	-

Appendix C

C1) Sigmoidal curve and mean comparison on vegetative growth on 21 *C. annuum L.* over two different planting seasons



Plant height of 21 chilli genotypes measured every two weeks interval planted on February until June 2015

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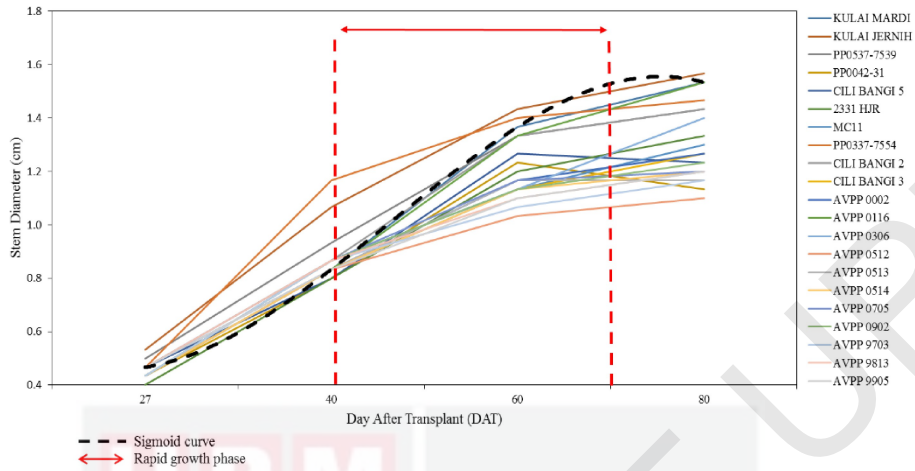
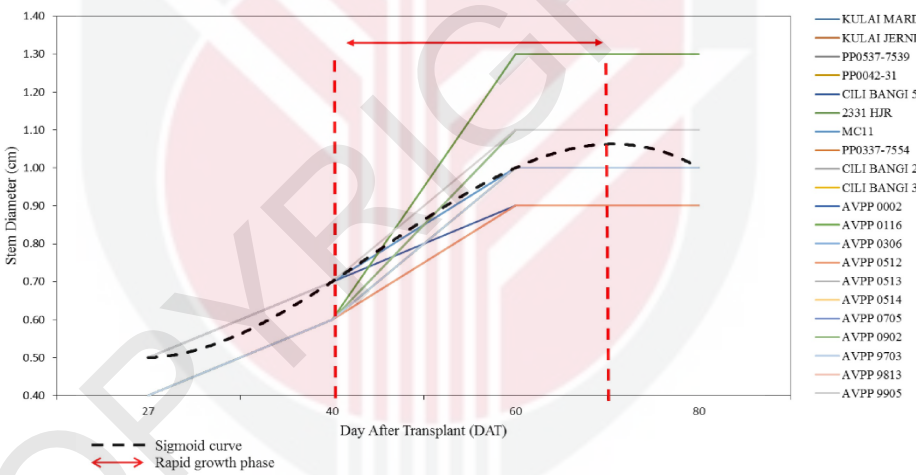


Stem diameter of 21 chilli genotypes planted on Feb until June 2015



Stem diameter of 21 chilli genotypes planted on July until December 2015

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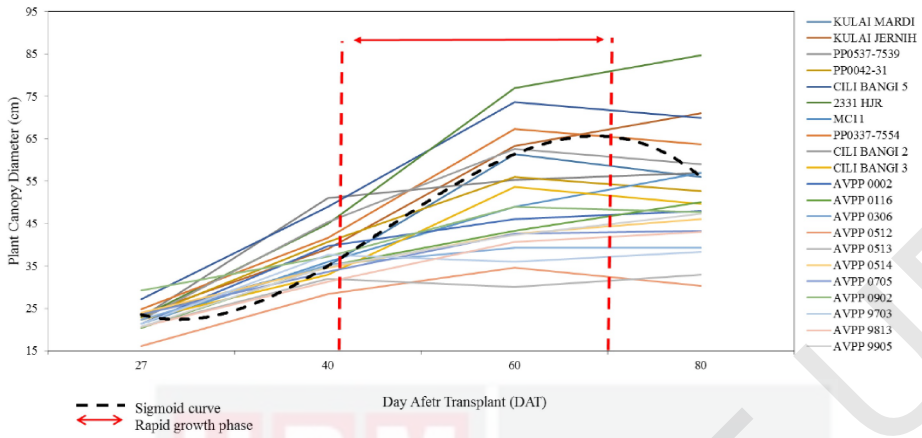
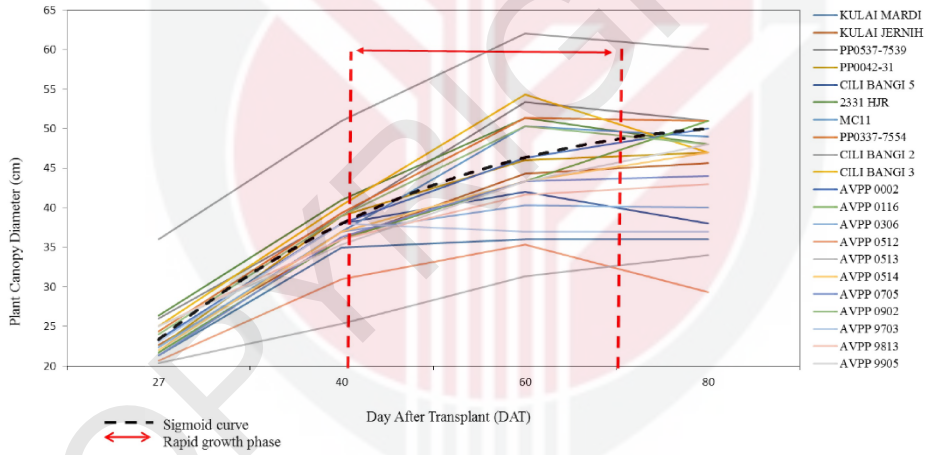


Plant canopy diameter of 21 chilli genotypes planted on Feb until June 2015



Plant canopy diameter of 21 chilli genotypes planted on July until December 2015

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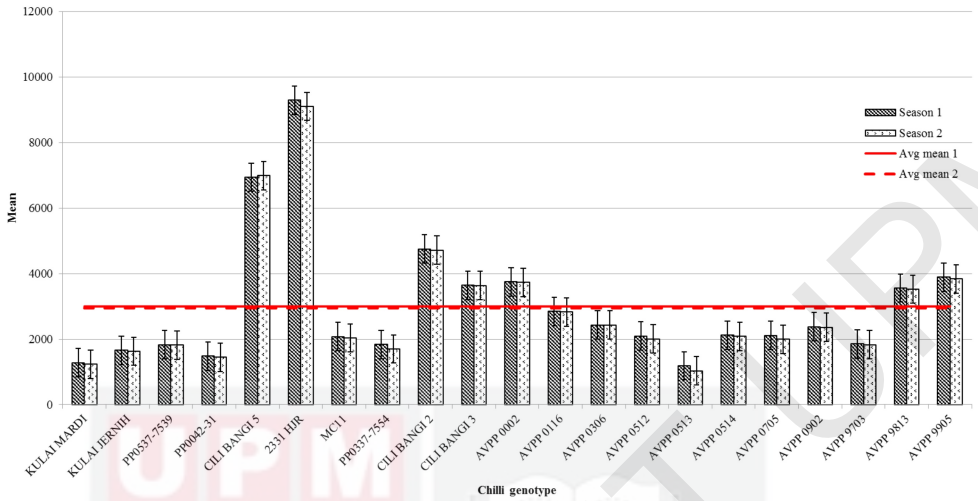
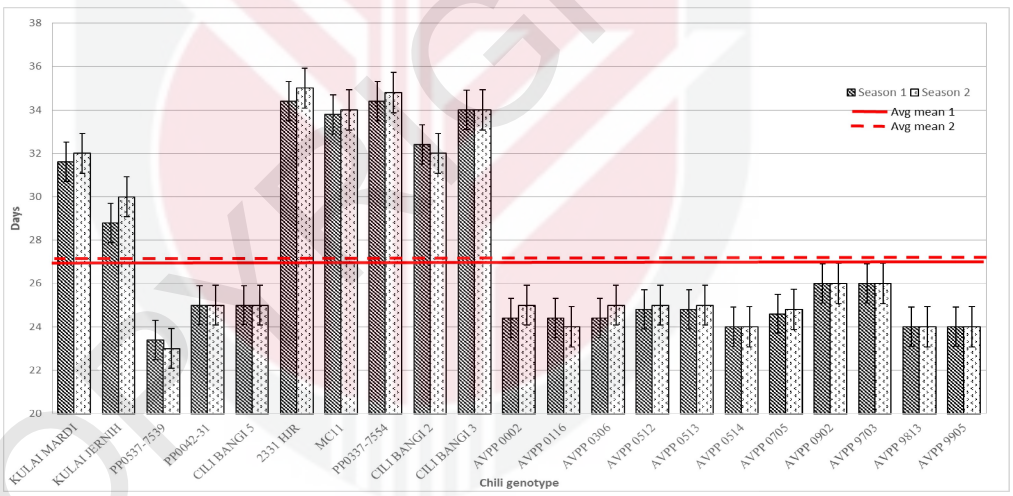


Total leaf area of 21 chilies genotypes from both season 1 and season 2



Day to flowering of 21 chili genotypes between two planting seasons

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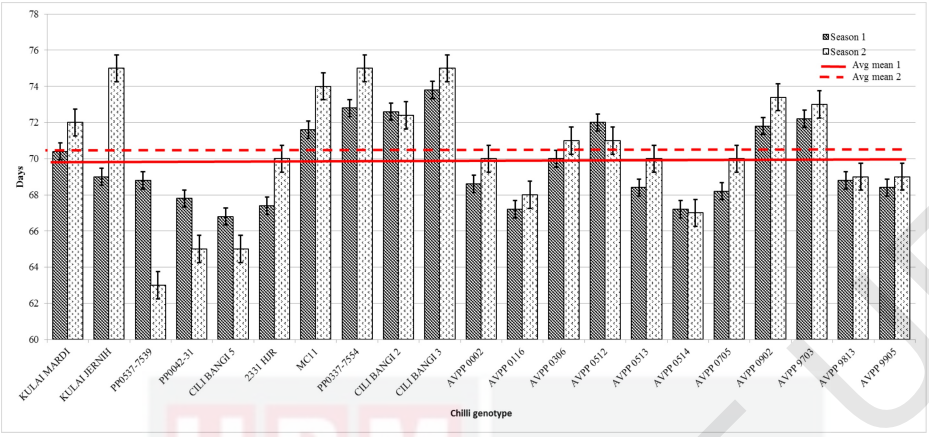
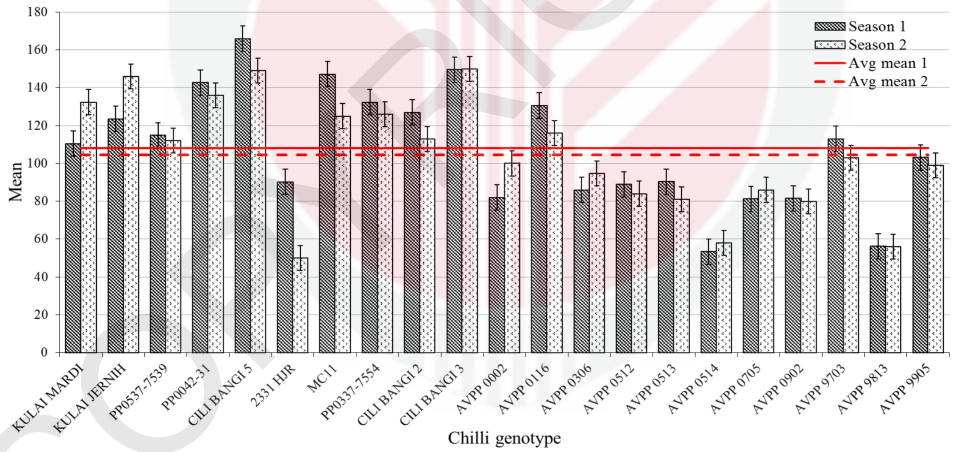
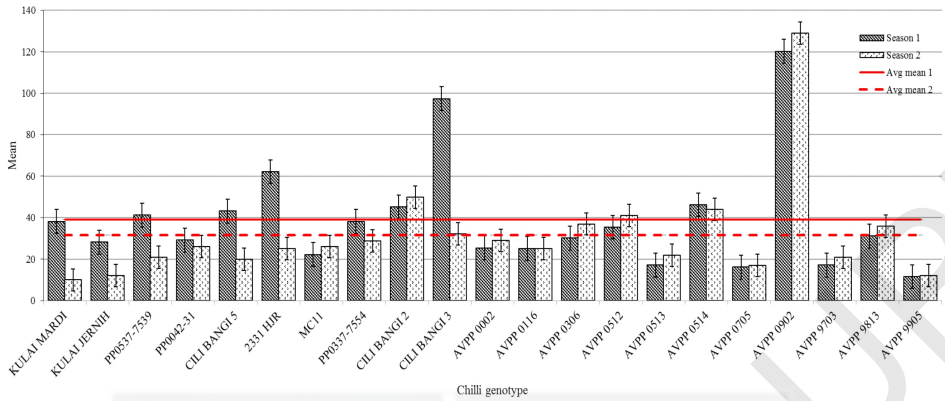


Day to maturity of 21 chili genotypes between two planting seasons

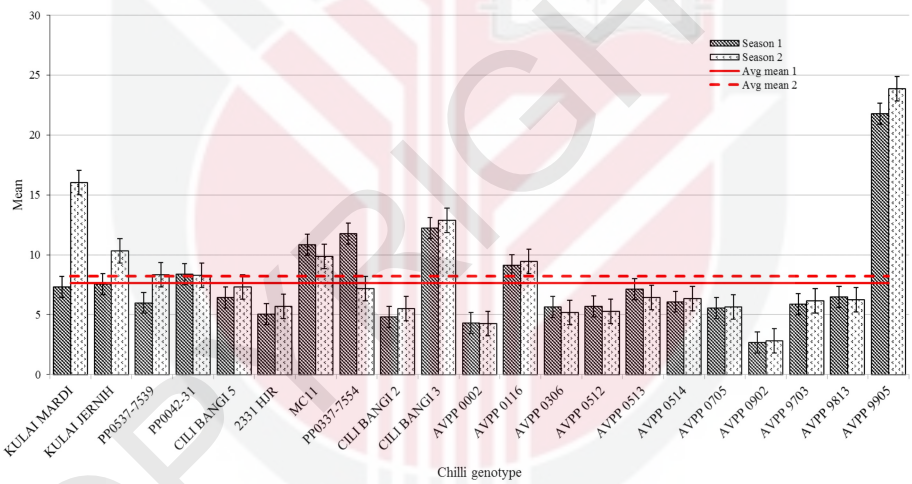
C2) Mean comparison on yield and yield components of 21 *C. annuum L.* over two different planting seasons



Total seed of 21 selected chili genotypes from season 1 and season 2



Number of fruits of 21selected chili genotypes from season 1 and season 2



Fruit weight of 21 selected chili genotypes from season 1 and season 2

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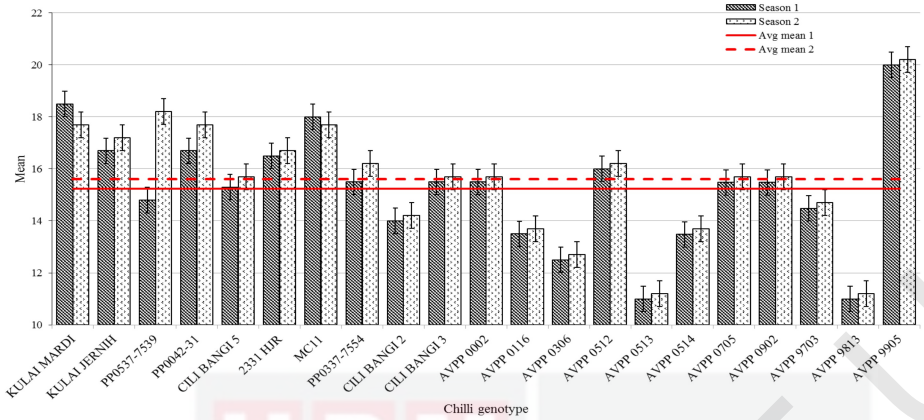
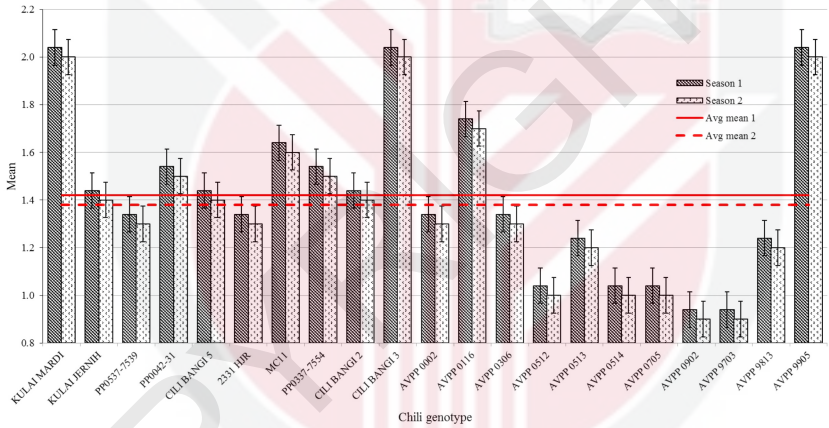


Fruit length of 21 selected chili genotypes from season 1 and season 2



Fruit diameter of 21 selected chili genotypes from season 1 and season 2

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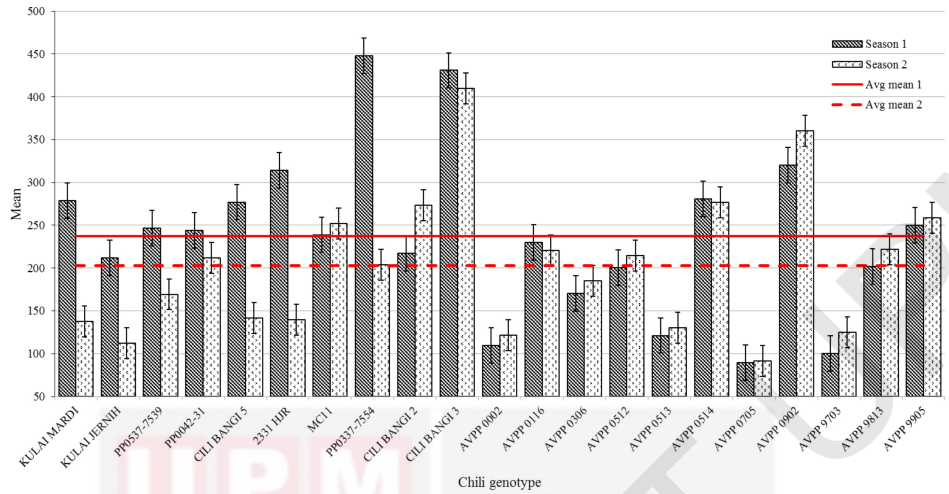
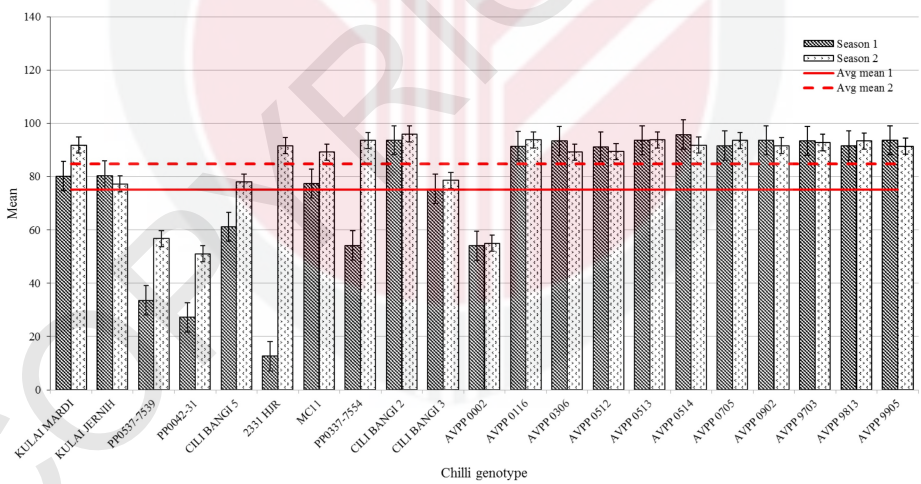


Yield per plant of 21 selected chili genotypes from season 1 and season 2

C3) Mean comparison on disease severity index of 21 *C. annuum L.* over two different planting seasons



Disease severity index in 21 chilies genotypes from season 1 and season 2

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BIODATA OF STUDENT

Mr. Abdul Azim bin Adnan was born on the 5th March 1988 in Sungai Udang, Melaka. He is the eldest child of six children of Adnan bin Omar and Roslina binti Haron. He received his primary school and secondary school education in Sekolah Kebangsaan Rawang, Selangor and Sekolah Agama Menengah Rawang, Selangor. Then he went for his foundation center in International Islamic University (IIUM) Foundation Center, Petaling Jaya, Selangor, in 2010. In July 2012, he continued his bachelors in IIUM and graduated with Bachelor of Biotechnology with Honors in the year 2014. Later he enrolled his master degree in Master of Genetic and Plant Breeding in 2014 under supervision of Professor Dr. Mohd Rafii Bin Yusop in Institute of Tropical Agriculture and Food Security (ITAFOS), UPM. The title of his research was "Genetics and heritability of chili (*Capsicum annuum* L.) germplasm on cucumber mosaic virus and agro-morphological traits".

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