A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEVELOPMENT OF GRAFTING AND ACCLIMATIZATION TECHNIQUES TO IMPROVE QUALITY OF GRAFTED TRANSPLANTS AND THEIR TOLERANCE TO SOIL-BORNE DISEASES AND LOW TEMPERATURE STRESSES IN *Capsicum annuum* L.

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UNDER THE DIRECTION OF DR. CHANGHOO CHUN
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF SEOUL NATIONAL UNIVERSITY

BY
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NOVEMBER, 2012

APPROVED AS A QUALIFIED DISSERTATION OF YOON AH JANG
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ABSTRACT

This thesis about the improvement of tolerance to biotic and abiotic stresses by grafting and the development of acclimatization techniques for high-quality grafted transplants in pepper (*Capsicum annuum* L.). It consists of two main parts. The first part consists of three chapters. Chapters I and II deal with the reduction of two major soil-borne diseases (Phytophthora blight and bacterial wilt) and the tolerance to low night temperature by the grafting. Chapter III deals with the effect of grafting on fruit quality. The second part consists of two chapters. Chapters IV and V deal with the light condition and CO_2 level during healing and acclimatization for improving the growth and quality of grafted pepper transplants.

In Chapter I, study was conducted to investigate the effect of grafting on growth and resistance to both Phytophthora blight and

bacterial wilt, and to evaluate the breeding lines as candidate rootstocks resistant to both Phytophthora blight and bacterial wilt for pepper production. Peppers grafted onto breeding lines which were selected for their resistance to Phytophthora blight and bacterial wilt showed greater resistance to both Phytophthora blight and bacterial wilt without the decrease in yield and fruit quality. Accordingly, selected breeding lines are recommended as candidates of pepper rootstock resistant to both Phytophthora blight and bacterial wilt.In order to evaluate the grafting as a tool to alleviate the impact of suboptimal temperature in pepper production, the effects of rootstock and night temperature (NT) on the growth and yield of grafted peppers in greenhouse were investigated in Chapter II. Peppers could be categorized into three types according to the response to NT. When peppers grafted onto the rootstocks which were selected for their stable relative growth rate irrespective of NT were cultivated at different NT conditions, the early growth after transplanting became greater than non-grafted peppers irrespective of temperature. Even though the growth and yield of grafted peppers were greater than those of non-grafted peppers irrespective of temperature, the commercial productivity was not improved. It has been reported that the size, shape, color, and texture of fruits of grafted peppers can be changed from those of non-grafted plants and were also affected by types of rootstock used. In Chapter III, in order to examine the effects of grafting on fruit quality of peppers, the fruit quality of peppers grafted onto different rootstocks was investigated during two harvest periods. Apparent qualities and textural properties of pepper fruits of the grafted onto different rootstocks were influenced by grafting,

harvest time, and cropping pattern. However, the fruit characteristics of rootstocks did not affect the fruit characteristics of scions grafted onto those rootstocks.

In the production of grafted transplants, healing and acclimatization are the most critical processes for survival. In Chapter IV and V, in order to determine the optimum environmental conditions for healing and acclimatization in a healing chamber with artificial lighting, grafted pepper transplants were healed and acclimatized under different light quality, intensity and atmospheric CO_2 levels.

Increasing PPF significantly increased CO_2 exchange rates during healing and acclimatization irrespective of light quality. The difference in the CO_2 exchange rate among the treatments increased as the post-graft time increased. The increase of photosynthesis led to an improvement of growth. PPF also influenced the anatomical structures of the leaves, and the palisade and spongy tissue cells of leaves irradiated with higher PPF were aligned more densely, with more chloroplasts and a small empty space.

The grafted pepper transplants irradiated with red light emitting diodes (LEDs) had lower CO_2 exchange rate, SPAD value, leaf dry weight, and dry matter content. Leaves irradiated with the red LED had the smallest leaf area and showed leaf epinasty. In addition, the palisade and spongy cells of the pepper leaves were dysplastic and exhibited hyperplasia. Grafted pepper transplants treated with red plus blue LEDs showed similar growth and morphology to those transplants irradiated with fluorescent lamps. These results suggest

that high-quality grafted pepper transplants can be obtained by healing and acclimatization in a healing chamber where optimal conditions such as high CO_2 and PPF level are maintained using fluorescent lamps or a combination of red and blue LEDs.

As a result, grafting is expected to be a tool to improve the tolerance to major soil-borne diseases and low temperature stress. However, in order to expand the purpose and use of pepper grafting, the identification and development of multi-disease resistant rootstocks with tolerance abiotic stresses should be accompanied. In addition, to control and maintain more optimal conditions during healing and acclimatization enable the production of high-quality grafted transplants which is essential and critical for the successful pepper production.

Key words: abiotic stress, biotic stress, *Capsicum annuum*, carbon dioxide, fruit quality, grafting, healing and acclimatization, light emitting diode (LED), night temperature, rootstock, soil-borne disease, photosynthesis, photosynthetic photo flux (PPF), scion

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS USED

AVRDC	The World Vegetable Center
BW	Bacterial wilt
CER	CO_2 exchange rate
DW	Dry weight
FL	Fluorescence lamps
FW	Fusarium wilt
IPM	Integrated pest management
LED	Light emitting diode
LMT	Low minimum temperature
NIHHS	National Institute of Horticultural & Herbal Science
NT	Night temperature
PPF	Photosynthetic photon flux
RDA	Rural Development Administration
RH	Relative humidity
RGR	Relative growth rate
SLA	Specific leaf area
TSS	Total soluble solids
VW	Verticillium wilt

GENERAL INTRODUCTION

Pepper (*Capsicum annuum* L., Solanaceae) is the most important and widely cultivated vegetable in Korea. The fruit is used in green or red forms by the difference of harvest time. In 2011, the cultivation area of pepper, including green pepper, was 47,388 ha and its yield was 262,257 t while the value of pepper production reached 1,533 billion won (MIFAFF, 2012). Red and green peppers have been cultivated intensively around Gyeongsangbuk-Do and Gyeongsangnam-Do, respectively. Under continuous cropping practices of peppers, salt accumulation and soil-borne diseases consequently reduce the yield and quality of the produce. Two soil-borne diseases, Phytophthora blight and bacterial wilt caused by *Phytophthora capsici* and *Ralstonia solanacearum*, respectively, are the most devastating diseases in pepper production (Kim and Kim, 2002; Myung et al., 2006).

Green pepper is mostly cultivated in greenhouse for five or six months after transplanting. It is mostly produced by retarding, semi-forcing or forcing culture when it should go through the cold season, chilling and suboptimal temperature conditions. Pepper is a thermophilic crop and requires relatively higher growth temperature than other crops. The temperature threshold for growth is about 8-12°C (Schwarz et al., 2010). The optimum temperature for maintaining productivity ranges 25 to 30°C day and 18 to 19°C night (Lim et al., 2006). In a cold season, it is recommended that the

minimum temperature is 18°C and should be above 15°C. The growth and yield of pepper were severely inhibited by low night temperature (Choi et al., 2001). Accordingly, cold-season cultivation in greenhouse requires a large energy input to keep the proper condition for a good growth and productivity. The cost for heating is above 30% of production costs (RDA, 2006). The soaring of the oil price and reducing of tax-free oil burden farmers with higher expenses for the heating.

Recently, vegetable grafting has been expanded in order to control pathogens and to enhance the plants' tolerance against abiotic stresses. Factors that have led to increased expansion of grafting include: increased pathogen inoculum densities due to intensification of production practices, reliance on susceptible cultivars to meet specific market demands (Sakata et al., 2007), global movement and/or local invasion of novel pathogens, increased use of organic practices, the rapid adoption of high tunnel production systems, use of appropriate technologies for resource-limited farmers, and the loss of methyl bromide (MeBr). The purposes of vegetable grafting are to reduce soil-borne diseases (Tables 1 and 2, Louws et al., 2010) and enhance the tolerance against abiotic stresses such as low (Venema et al., 2008) and high temperatures (Rivero et al., 2003), flooding (Yetisir et al., 2006) and drought (Rouphael et al., 2008), salt (Colla et al., 2006 a,b; Edelstein et al., 2011), alkalinity (Colla et al., 2010), and heavy metals (Savvas et al., 2010) (Table 3). Grafting is mostly practiced on fruit vegetables of the family Cucurbitaceae (watermelon, cucumber,

melon) and Solanaceae (pepper, tomato, eggplant). In Korea, about 90% of cucurbitaceous vegetables and 30% of Solanaceous vegetables are grafted onto various rootstocks (Lee et al., 2010).

In order to prevent soil-borne diseases in continuous cropping, peppers are generally grafted onto the rootstocks that are of the same species as scions (*C. annuum* L.) that have resistance to Phytophthora blight (King et al., 2010). However, few commercial rootstocks resistant to Phytophthora blight are resistant to bacterial wilt. Peppers grafted onto rootstocks resistant to Phytophthora blight are reported to be damaged by bacterial wilt in the late stage of cultivation and need additional efforts in order to control bacterial wilt. Accordingly, there is a need to develop pepper rootstocks resistant to both Phytophthora blight and bacterial wilt. It was reported that grafting of peppers also improved tolerance to high salt conditions (Chung and Choi, 2002). However, there are few reports on the effects of grafting on the growth and productivity of green peppers under high or low temperature conditions.

Regarding the changes in fruit quality by grafting, there are several conflicting reports whether grafting effects are advantageous or deleterious (Lee et al., 2010; Davis et al., 2008a). It has been reported that the size, shape, color, texture, flavor, pH, sugar, carotenoid content of fruits can be affected by grafting and the type of rootstock used (Alexopoulos et al., 2007; Bruton et al., 2009; Davis et al., 2008a, b; Tsaballa et al., 2012). More and larger fruits, the increase of firmness and rind thickness of fruit, and the improvement of soluble

solid content and titratable acidity in fruit by grafting were reported (Yetisir et al., 2003; Miguel et al., 2004; Flores et al., 2010; Gisbert et al., 2010). However, abnormal fruit qualities including reduced fruit soluble solid content, fruit fermentation, poor texture, and off-taste were also reported (Lee and Oda, 2003). However, there is no information on how grafting and rootstocks affect the fruit quality of peppers.

Most of grafted transplants are generally produced by commercial growers. In Korea, more than 200 seedlings growers, excluding individual farmers and farmers' associations, are producing plug seedlings and about half of them are producing grafted transplants (Lee et al., 2010). Grafted transplants are produced by 1) raising scions and rootstocks; 2) grafting; 3) healing and acclimatization; and 4) raising the grafted transplants before transplanting (Jang et al., 2011). In the production of grafted transplant, healing and acclimatization are very important processes that are necessary for grafted plants to survive (Lee and Oda, 2003). Grafted plants were usually healed and acclimatized in the past under specific environmental conditions such as high relative humidity (RH ≥ 95%) and low light intensity in order to produce healthy plants that survived and grew (Mun et al., 2011). Under these environmental conditions, the net photosynthesis rate of grafted transplants is almost 0 mg CO_2 m^{-2} s^{-1} (Shibuya et al., 2003). The temperature inside the closed tunnel during healing and acclimatization processes could often exceeds a threshold high temperature at midday, sometimes resulting in the

death of plants. The air humidity inside the closed tunnel is also higher than outside and often reaches saturation. Under these environmental conditions, the grafted transplants are in danger of heat stress, leading to a decrease in photosynthesis and growth (Wahid et al., 2007). The increase in unpredictable abnormal weather has also caused greater hardship for the maintenance of uniform and optimal environmental conditions for the healing and acclimatization of grafted transplants.

High-performance production systems unconstrained by weather conditions have recently been developed to produce high-quality transplants under artificial light (Kozai, 2005; Kozai, 2009). Optimizing the temperature, relative humidity, light condition, and atmospheric CO_2 concentration in these systems makes it possible to achieve rapid and uniform growth of high-quality transplants throughout the year. This optimization can also be applied to healing and acclimatization for production of grafted transplants. Recent reports have suggested that there is a higher survival rate, faster growth, and a higher quality of grafted plants under highly controlled healing conditions (Nobuoka et al., 2005; Jang et al., 2011). Reports have primarily focused on the increase of photosynthesis, which has been so far overlooked during healing and acclimatization. The increase of photosynthesis in grafted transplants during healing and acclimatization was confirmed by higher photosynthetic photo flux (PPF) conditions using fluorescent lamps under highly controlled healing conditions (Jang et al., 2011). These conditions resulted in an

improvement in the growth and quality of grafted transplants.

This thesis consists of two main parts (Fig. 1). The first part is about the improvement of tolerance to biotic and abiotic stresses by grafting in pepper (*C. annuum* L.). It consists of three chapters. Chapters I and II deal with the reduction of two major soil-borne diseases, Phytophthora blight and bacterial wilt, and the tolerance to low night temperature by the grafting. Chapter III deals with the effect of grafting on fruit quality. The second part is about the development of acclimatization techniques for high-quality grafted transplants. It consists of two chapters. Chapters IV and V deal with the light condition and CO_2 level during healing and acclimatization for improving the growth and quality of grafted pepper transplants. In Chapter IV, the effects of light intensity and CO_2 concentration during healing and acclimatization on the rate of photosynthesis, and the growth and graft-take of grafted pepper transplants were examined in order to determine the optimal environmental conditions for healing and acclimatization in a healing chamber with artificial lighting. In Chapter IV, the influence of light quality and intensity during the healing and acclimatization period on the photosynthetic characteristics, photomorphogenesis, and growth of grafted pepper transplants were evaluated, using a system for continuous measurement of the photosynthetic rate.

Table 1. Importance and relative efficacy of integrated pest management (IPM) tactics and use of grafting to manage selected biotic agents in Solanaceae crops. (from Louws et al., 2010)

Biotic stress	Seriousness	Efficacy of IPM tactic				Graft references		
		Crop rotation	Fumigant/ soil disinfection	Other IPM tactics	Graft/host resistance	Tomato	Pepper	Eggplant
Fungi								
Verticillium dahliae race 1	****	*	***	*	****	Gindrat et al. (1976), Ginoux et al. (1979), Paplomatas et al. (2002)	-	Curuk et al. (2009), Bilotto et al. (2006), Liu et al. (2009), Bletsos et al. (2003)
Verticillium alboatrum	****	*	***	*	****	Blackhurst and Wood (1963)	-	-
Sclerotium rolfsii	***	*	***	**	**	Black et al. (2003), Rivard et al. (2010)	-	-
Oomycetes								
Phytophthora (capsici, nicotianae, crytogea, parasitica)	****Pepper, *Tomato, *Eggplant	**	**	**	**	Diánez et al. (2007), Upstone (1968), Berenguer et al (2001)	Gisbert et al. (2010), Morra and Bilotto (2006), Wu et al. (2008)	-
Bacteria								
Ralstonia solanacearum	****	*	*	**	****	Balck et al. (2003), Lin et al. (2008), Wang et al. (2009)	Wu et al. (2008), Palada and Wu (2009)	Bilotto et al. (2006)
Viruses								
Tomato Spotted Wilt Virus (TSWV)	****	-	-	-	-	Rivard and Louws (2008)	-	-

Table 2. Importance and relative efficacy of integrated pest management (IPM) tactics and use of grafting to manage selected biotic agents in Cucurbitaceae crops. (from Louws et al., 2010)

Biotic stress	Seriousness	Efficacy of IPM tactic				Graft references		
		Crop rotation	Fumigant/ soil disinfection	Other IPM tactics	Graft/host resistance	Watermelon	Melon	Cucumber
Fungi								
Fusarium oxyporum	****	*	**	*	****	Huh et al. (2002), Miguel et al. (2004), Yetisir et al. (2007)	Crino et al. (2007), Bletsos (2005), Cohen et al. (2002)	Sakata et al. (2008), Cohen et al. (2007), Dianez et al. (2007)
Monosporascus cannonballus	***	**	**	**	**	Beltran et al. (2008), Jifon et al. (2008)	Cohen et al. (2005), Fita et al. (2007), Jebari et al. (2009)	-
Verticillium dahliae	****	*	**	*	****	Paplomatas et al. (2002), Paroussi et al. (2007)	Paplomatas et al. (2002)	Paplomatas et al. (2002)
Phytophthora melonis or *capsici*; *Pythium* sp.	**	**	**	**	**	Tominaga et al. (1983), Wang et al. (2004)		
Podosphaera xanthii (Powdery mildew	***	No efficacy	No efficacy	***	***	-	-	Sakata et al. (2006)
Nematodes Root knot	****	***	****	**	****	Huitron et al. (2007), Lee and Oda (2003), Siguenza et al. (2005)		
Viruses								
Melon necrotic spotted virus (MNSV)	***	****	No efficacy	**	****	Besri and Rabat (2008), Cohen et al. (2007), Sakata et al. (2008)	NA	

Table 3. Use of grafting to mitigate abiotic stresses. (from Colla et al., 2010; Louws et al., 2010, Savvas et al., 2010)

Abiotic stress	Cucurbitaceae			Solanaceae		
	Watermelon	Melon	Cucumber	Tomato	Pepper	Eggplant
Thermal Stress						
Chilling & suboptimal temperature	Davis et al. (2008)		Ahn et al. (1999), Rivero et al. (2003)	Rivero et al. (2003), Venema et al.(2008)	-	Gao et al. (2008)
Supra-optimal temperature	-	-	-	AVRDC(2009)	Palada and Wu(2008)	Wang et al. (2007)
Nutrient Stress						
Heavy metal stress	-	Edelstein and Ben-Hur (2007)	-	-	-	Arao et al. (2008), Mori et al. (2009)
Nutrient toxicity	Goreta et al. (2008), Colla et al. (2006)	Edelstein et al. (2005, 2007)	Huang et al. (2010), Rouphael et al.(2008)	Savvas et al. (2009)	Chung and Choi (2002)	Bai et al. (2005), Wei et al.(2007)
Nutrient deficiency	Pulgar et al. (2000), Rouphael et al. (2008)	Ruiz et al. (1997), Ruiz and Romero (1999)	Rouphael et al.(2008), Zhu et al. (2008)	Oztekin et al. (2009), Albacete et al. (2009)	-	Leonardi and Giuffrida (2006)
Alkalinity	Colla et al. (2010)	-	-	-	-	-
Water Stress						
Drought	Rouphael et al. (2008)	-	-	-	-	-
Flooding	Yetisir et al. (2006)	-	-	AVRDC (2003)	AVRDC (2009)	-
Etc.						
Organic pollutant stress			Otani and Seike (2007)			

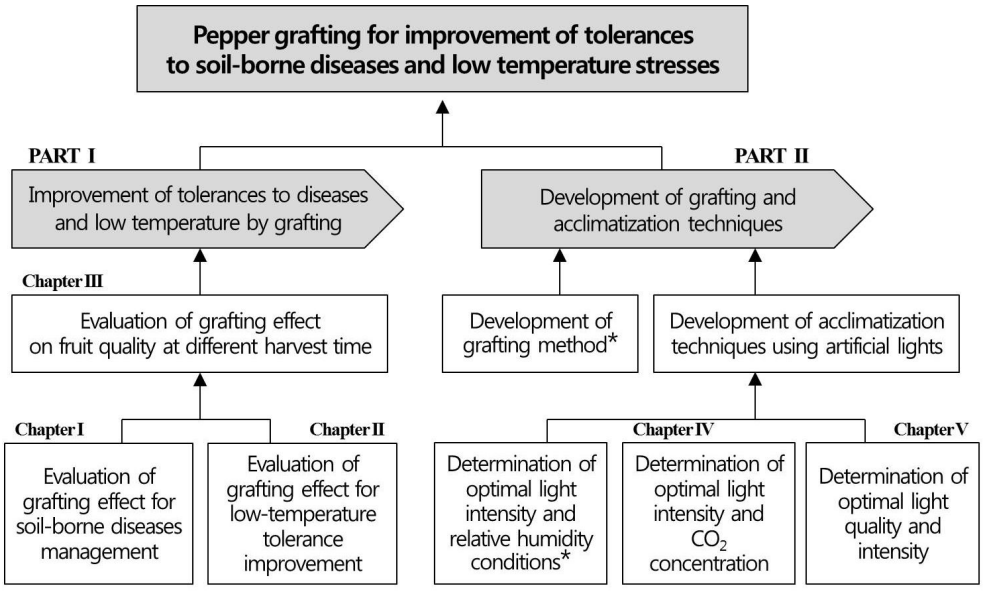

Fig. 1. Flow chart for the development of grafting and acclimatization techniques to improve quality of grafted transplants and their tolerance to soil-borne diseases and low temperature stresses in *Capsicum annuum* L. The parts marked by an asterisk were examined but not included in this thesis.

Chapter Ⅰ. Effect of grafting on growth and incidence of Phytophthora blight and bacterial wilt of pepper (*Capsicum annuum* L.)

Chapter Ⅱ. Effects of grafting on the growth and yield of peppers under low temperature

Chapter Ⅲ. Effects of grafting on fruit quality of peppers

Chapter Ⅳ. Effects of light intensity and and atmospheric CO_2 concentration on the photosynthesis and growth of grafted pepper transplants during healing and acclimatization

Chapter Ⅴ. Effects of light quality and intensity on the photosynthesis, growth, and morphogenesis of grafted pepper transplants during healing and acclimatization

LITERATURE REVIEW

Grafting to Manage Biotic Stresses

The primary purpose of vegetables grafting has been to obtain resistance to soil-borne diseases. Tateishi (1927) and Murata and Ohara (1936) reported the use of grafting in preventing disease (Fusaruium) in watermelon production. Grafting is mostly practiced on fruit vegetables of the family Cucurbitaceae and Solanaceae.

Nowadays, it has widely used to manage a broad range of pathogens including fungi (*Verticillium dahliae*, *Fusarium oxysporum, Monosporascus cannonballus*), oomycetes (*Phytophthora* spp.), bacteria (*R. solanacearum*), and nematodes (*Meloidogyne* spp.). It has also been shown in some instances to increase tolerance to foliar fungal diseases, viruses, and insects (King et al., 2008; Louws et al., 2010).

Tomato hybrids and interspecific tomato hybrids (*Solanum lycopersicon* × *S.* sp.) are the most common genetic rootstock sources for tomato. Tomato grafting has been commonly used to manage Verticillium wilt (VW) (Paplomatas et al., 2002), Fusarium wilt (FW) (Chung et al., 1997; Rivard and Louws, 2008), bacterial wilt (BW) (Lin et al., 2008; Rivard and Louws, 2008), nematode (López-Pérez et al., 2006; Barrett et al., 2012) and virus (Rivard and Louws, 2008). In the case of eggplant, grafting has been used to manage VW (Bletsos et al., 2003; Wang et al. 2003; Çürük et al., 2009; Liu et al., 2009), FW, BW (Gousset et al., 2005), and nematode (Çürük, S. et al., 2009;

Gisbert et al., 2011), mainly using *S. integrifolium* and *S. torvum* as rootstocks.

The most common rootstocks for cucurbit crops include *Lagenaria siceraria* (Molina) Standl. and *Cucurbita moshata* (Duchesne ex. Poir) × *C. maxima* (Duchense ex. Lam.) hybrids (King et al., 2008). The primary reason for grafting watermelon has been for Fusarium resistance, but it can be used to provide resistance or increase tolerance to Phytophthora blight, VW, nematodes and in some cases viruses (Yetisir et al., 2003; Miguel et al., 2004; Davis et al., 2008).

Melon grafting has mainly been conducted to manage FW (Cohen et al., 2002; Yetişir et al., 2003; Crinò et al., 2007), Monosporascus root rot and vine decline (Cohen et al., 2005). In the case of cucumber (*Cucumis sativus* L.), grafting has been used to manage VW (Bletsos et al., 2003; Çürük et al., 2009) and FW (Pavlou et al., 2002; Sakata et al., 2008). Pepper grafting is still in its beginning stages and partially used in winter greenhouse production in Korea, even though there are some reports that grafting can control *Phytophthora capsici* and nematodes (Oka et al., 2004; King et al., 2010).

Mechanism by which grafting suppresses the disease occurrence is not well known. However, the increase of disease resistance by grafting is seemed to be associated with increased root vigor and biomass (Bletsos et al., 2003), and associated increased water and nutrient uptake (Lee, 1994). In the case of bacterial wilt resistance, it has been shown that resistant rootstocks physically limit the movement of bacteria from the soil to the scion (Grimaultet al., 1994).

Allelochemicals (phytotoxic compounds such as benzoic acid, ferulic acid, vanillin) in the root exudates of grafted plants may also influence disease resistance (Liu et al., 2009).

Grafting to Manage Abiotic Stresses

Grafting is used not only to reduce infections by pathogens but also to enhance the tolerance against abiotic stresses (Flores et al., 2010). Among those are thermal stress, saline soils, drought and flooding, soil-pH (alkalinity) stress, nutrient deficiency, toxicity of heavy metals, and persistent organic pollutants (Colla et al., 2010; Savvas et al., 2010; Schwarz et al., 2010).

Grafting improved low temperature tolerance in fruit vegetables such as watermelon (Davis et al., 2008b), cucumber (Ahn et al., 1999; Zhou et al., 2007), and tomato (Venema et al., 2008). Grafting has allowed grafted plants to grow properly and maintain the yield even though they are under chilling and suboptimal condition (Edelstein, 2004; Miguel et al., 2004).

The increase of suboptimal-temperature tolerance in vegetables by grafting can make the practical benefits such as extension of the growing season, adaptation to growing areas with shorter growing seasons, less combustions of fossil fuel, and decrease in CO_2 emissions (Schwarz et al., 20108). Watermelon and Cucumber are generally grafted on *Cucurbita* species such as Shintozwa (an interspecific squash hybrid, *C. maxima* x *C. moschata*) and figleaf gourd (*C. ficifolia* Bouché) to improve the low temperature tolerance

during cool periods (Lee and Chung, 2005; Davis et al., 2008).

For tomato, rootstocks of the high-altitude accession of *S. habrochaites* (Venema et al., 2008) were able to alleviate low root-temperature stress for different scions. Contrary to the grafting at suboptimal temperature, practical applications at supraoptimal temperature are insignificant. Although the use of Solanaceae rootstocks for tomato conferred a certain degree of resistance against thermal stress, it had no advantage in yield (Rivero et al., 2003; Abdelmageed and Gruda, 2009). For pepper, comparing different lines of pepper rootstocks (*C. chacoense, C. baccatum, C. frutescens*, *C. annuum*) confirmed highest yields under high-temperature conditions for rootstocks recommended by the AVRDC (*C. annuum* cv. Toom-1 and 9852-54) (Palada and Wu, 2008).

It was also reported that grafting improved tolerance to high salt conditions including both saline and sodic soils in watermelon (Colla et al., 2006a; Goreta et al., 2008), melon (Colla et al., 2006b), cucumber (Huang et al., 2010), tomato (Estañ et al., 2005), pepper (Chung and Choi, 2002), eggplant (Wei et al., 2009). Grafted plants grown under saline conditions often exhibited better growth and yield, higher photosynthesis and leaf water content, greater root-to-shoot ratio, higher accumulation of compatible osmolytes, abscisic acid and polyamines in leaves, greater antioxidant capacity in leaves, and lower accumulation of Na^+ and/or Cl^- in shoots than ungrafted or self-grafted plants (Colla et al., 2010).

Grafting to Improve Fruit Quality

Regarding the changes in fruit quality by grafting, there are several conflicting reports whether grafting effects are advantageous or deleterious (Lee et al., 2010; Davis et al., 2008a). It has been reported that the size, shape, color, texture, flavor, pH, sugar, carotenoid content of fruits can be affected by grafting and the type of rootstock used (Alexopoulos et al., 2007; Bruton et al., 2009; Davis et al., 2008a, b; Tsaballa et al., 2012). More and larger fruits were reported in pepper (Gisbert et al., 2010), watermelon (Miguel et al., 2004) and so on when grafting was adopted. Firmness and rind thickness, which are the typical attributes used to describe the fruit texture were also reported to increase in watermelon (Yetisir et al., 2003) when it was grafted. In tomato, soluble solid content and titratable acidity were improved by grafting (Flores et al., 2010). However, abnormal fruit qualities including reduced fruit soluble solid content, fruit fermentation, poor texture, and off-taste were reported for grafted oriental melon and watermelon (Lee and Oda, 2003).

Positive effects of grafting are reported in tomato and watermelon, including the increase of fruit size, firmness, total soluble solids (TSS) content, and titratable acidity (Flores et al., 2010; Miguel et al., 2004). On the contrary, other reports showed abnormal fruit quality in eggplant, melon, and watermelon, including poor texture, reduction of contents of TSS and vitamin C and firmness (Arvanitoyannis et al., 2005; Lee and Oda, 2003; Traka-Mavrona et al., 2000). There were also reports that demonstrated that grafting had no effect on fruit

shape index, thickness of rind, texture, lycopene content or TSS in cucumber, tomato, and watermelon (Alan et al., 2007; Bruton et al., 2009; Khah et al., 2006; Sakata et al., 2008).

Healing and Acclimatization of Grafted Plants

Healing and acclimatization are critical for grafted transplants to survive and grow as healthy plants, which involve the healing of the cut surface and hardening for field or greenhouse survival (Lee and Oda, 2003). Generally, healing and acclimatization of the grafted plants are done in a tunnel, made of double-layered plastic film and shade cloth, in a greenhouse.

Environmental management during healing and acclimatization is usually done by the empirical knowledge of a grower depending on the season or weather. To prevent grafted plants from wilting by excessive transpiration and to promote healing, the tunnel is closed during the three- to four-day healing period. The opening and closing of the tunnel are controlled based on the condition of the grafted plants and the weather.

When the tunnel is closed, the air in the tunnel is saturated (Relative humidity $\geq$ 90%) and light intensity is slightly higher than the light compensation point (below a PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Kim and Park, 2001; Lee and Oda, 2003). When the air current speed in the tunnel is near 0 $m \cdot s^{-1}$, the net photosynthesis rate of the grafted plants is almost 0 mg $CO_2 \cdot m^{-2} \cdot s\text{-}^{1}$ (Shibuya et al., 2003). Under these environmental conditions, the grafted transplants scarcely grow and

are in danger of heat stress, infection by pathogens and overgrowth wherein roots are arising from the hypocotyls of the scion. Some papers have reported higher survival rate, faster growth, and higher quality of grafted plants under highly controlled healing conditions (Kim et al., 2001; Nobuoka et al., 2005; Shibuya et al., 2003). These have mainly focused on the increase in the net photosynthesis rate in grafted plants during healing and acclimatization, by increasing air current speed and light intensity. It may result in an improvement of the graft-take, growth, and quality of grafted plants.

Light Quality/Quantity of Artificial Lights and Photosynthesis

Light is an essential factor for plant growth. Many studies have shown that both the light intensity and the light quality are important for the growth, development, pigmentation, and shape of plants (McNellis and Deng, 1995; Schuerger et al., 1997; Amaki and Hirai, 2008; Fukuda et al., 2008). Various types of artificial light have been used in plant production including fluorescent, metal halide, and high-pressure sodium lamps (Fang and Jao, 2000). In recent years, light emitting diodes (LEDs) have attracted interest as light sources for plant production, because of their features such as small size, low mass, long life, narrow spectral output, and energy conversion efficiency (Brown et al., 1995; Massa et al., 2008, Goto, 2011). LEDs enable the selection of specific wavelengths for a targeted plant response.

The use of red LEDs to power photosynthesis has been widely

accepted, because red wavelengths (600 to 700 nm) are efficiently absorbed by plant pigments. Early LEDs were red, with the most efficient emitting at 660 nm, which is close to one of the absorption peaks of chlorophyll (Massa et al., 2008). When baby leaf lettuce was grown under varying light sources including red, blue, and red plus blue LEDs, and fluorescent lamps, the growth was most favorable under the red single wavelength LEDs than the other treatments (Lee et al., 2010). However, blue light also has a variety of important photomorphogenic roles in plants, including stomatal control, CO_2 exchange, stem elongation, and phototropism (Massa et al., 2008). There have been reports of a reduction in photosynthesis and growth under red LEDs alone in wheat, rice, radish, lettuce, and spinach (Goins et al., 1997; Yorio et al., 2001; Matsuda et al., 2004).

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PART I: IMPROVEMENT OF THE TOLERANCE TO BIOTIC AND ABIOTIC STRESSES BY GRAFTING

CHAPTER I

EFFECT OF GRAFTING ON GROWTH AND INCIDENCE OF PHYTOPHTHORA BLIGHT AND BACTERIAL WILT OF PEPPER

ABSTRACT

This study was conducted to investigate the effect of grafting on growth and resistance to both Phytophthora blight (*P. capsici*) and bacterial wilt (*R. solanacearum*) in pepper, and to evaluate the breeding lines as candidate rootstocks resistant to both Phytophthora blight and bacterial wilt for pepper production. 'Nokkwang' (scion) was grafted onto five commercial rootstocks and nine breeding lines that were selected for their resistance to Phytophthora blight and bacterial wilt. All the used rootstocks exhibited high germination and a good affinity with the scion except 'PR 901' (64 and 72%). Among nine breeding lines, three rootstocks ('PR 920', 'PR 921', and 'PR 922') were selected as candidate rootstocks for grafted pepper based on graft-take, growth, yield, fruit quality, and resistance to diseases. Three major pepper cultivars were grafted onto those three breeding lines and 'Tantan' (control). Peppers grafted onto breeding lines of 'PR 920', 'PR 921', and 'PR 922' showed greater resistance to both Phytophthora blight and bacterial wilt without the decrease in yield and fruit quality. Accordingly, they were considered to be used as rootstocks resistant to both Phytophthora blight and bacterial wilt for pepper production.

INTRODUCTION

Pepper (*C. annuum* L.) is the most important and widely cultivated vegetable in Korea. In 2011, the cultivation area of pepper, including green pepper, was 47,388 ha and its yield was 262,257 t while the value of pepper production reached 1,533 billion won (MIFAFF, 2012). Red and green peppers have been cultivated intensively around Gyeongsangbuk-Do and Gyeongsangnam-Do region, respectively. Under continuous cropping practices of peppers, salt accumulation and soil-borne diseases consequently reduce the yield and quality of the produce. Two soil-borne diseases, Phytophthora blight and bacterial wilt caused by *Phtytophthora capsici* and *R. solanacearum*, respectively, are the most devastating diseases in pepper production (Kim and Kim, 2002; Myung et al., 2006).

Numerous attempts have been made to improve the disease resistance of pepper crops. One of the major goals in pepper breeding is the development of a cultivar completely resistant to soil-borne diseases. However, that is very difficult to achieve and requires much time and effort. For the alleviation of soil-borne diseases, cultural practices such as crop rotation and sanitation are recommended, but pesticide is generally applied to control the diseases (Kim et al., 2010; Semi et al., 2010; Tran and Kim, 2010; Yeon et al. 2008).

Grafting is an environment-friendly alternative method for disease control (Oka et al., 2004; Rivard and Louws, 2008). Grafting scions onto resistant rootstocks makes it possible to control soil-borne

diseases and increase yield of susceptible cultivar (Lee and Oda, 2003). Recently, the cultivated area of grafted Solanaceae and Cucubitaceae has increased tremendously (Lee et al., 2010). At present, grafting is mainly used in order to reduce infections by soil-borne pathogens and to enhance the tolerance against abiotic stresses (King et al., 2008; Louws et al., 2010; Rivero et al., 2003).

In order to prevent soil-borne diseases in continuous cropping, peppers are generally grafted onto the rootstocks that are of the same species as scions (*C. annuum* L.) that have resistance to Phytophthora blight (King et al., 2010). It was reported that grafting of peppers also improved tolerance to high salt conditions (Chung and Choi, 2002). However, few commercial rootstocks resistant to Phytophthora blight are resistant to bacterial wilt. Peppers grafted onto rootstocks resistant to Phytophthora blight are reported to be damaged by bacterial wilt in the late stage of cultivation and need additional efforts in order to control bacterial wilt. Accordingly, there is a need to develop pepper rootstocks resistant to both Phytophthora blight and bacterial wilt.

Rootstock should not only have resistance to diseases but also have good compatibility with scion and be able to maintain yields and fruit quality of the scion variety. This study was conducted to investigate whether grafting affect growth and resistance to both Phytophthora blight (*P. capsici*) and bacterial wilt (*R. solanacearum*) in pepper, and to evaluate the breeding lines as candidate rootstocks resistant to both Phytophthora blight and bacterial wilt for pepper production.

MATRERIALS AND METHODS

1. The Growth and Incidence of Phytophthora Blight of the Grafted Peppers as Influenced by Different Rootstocks

1.1. Plant materials and growing seedlings

'Nokkwang' (Seminis Vegetable Seeds, Inc., Seoul, Korea) that is susceptible to Phytophthora blight (Kim et al., 2010) and bacterial wilt (NIHHS, 2009) was used as a scion. The commercial rootstocks and breeding lines which were selected for their resistance to Phytophthora blight and bacterial wilt (NIHHS, 2009) were evaluated as rootstocks. The genotypes of rootstocks are listed in Table 1.1. Seeds of scion were sown one week after sowing seeds of rootstocks in order to obtain seedlings of similar diameter with rootstocks. Seeds of scion and rootstock were sown into the 105-cell trays (*W* 280 mm × *L* 540 mm × *H* 48 mm, Bumnong Co. Ltd., Jeongeup, Korea) and 72-cell plug trays (*W* 280 mm × *L* 540 mm × *H* 45 mm, Bumnong Co. Ltd., Jeongeup, Korea), respectively, then filled with commercial growing substrate (BM 2, Berger Group Ltd., Quebec, Canada). Plants were watered daily. A nutrient solution (electric conductivity 1.5 $dS \cdot m^{-1}$ with 'Hanbang' for seedling, N-P-K-Ca-Mg=8.0-2.4-2.4-4.8-1.6 $me \cdot L^{-1}$, Coseal Co. Ltd., Gunsan, Korea) was applied and the application frequency was determined depending on growth stage (RDA, 2008b).

1.2. Grafting, healing and acclimatization

Scions (5-6 leaf stage) were grafted onto rootstocks 36 days after sowing. The epicotyls of scion and rootstock were cut below 1cm from first true leaf using a razor blade. After placing the scion on the rootstock, the grafted position was fixed with ordinary grafting clip by slice grafting method. After grafting, plants were healed and acclimatized in the tunnel covered with double-layered plastic film and shade cloth in the greenhouse for one week (Lee et al., 2010). In order to prevent grafted plants from wilting due to the excessive transpiration and to promote healing, the tunnel was closed for the first three or four days of healing and acclimatization period. For the next three or four days, the opening and closing of the tunnel were done based on the condition of grafted plants and weather. This is for the acclimatization of grafted plants to environmental condition outside the tunnel (RDA, 2008b). Non-grafted and auto-grafted (scion and rootstock were of the same plants or cultivars) 'Nokkwang' trasnplants served as controls. After the end of healing and acclimatization, grafted transplants were grown on the bench in a glasshouse. Before transplanting, the graft-take and the growth parameters of sampled grafted transplants (n=9) were measured. Dry weight was determined after drying at 80°C. For the determination of mineral contents in shoot, dried shoots (n=3) were grounded and digested in mixed solution of HNO_3 and $HClO_4$ (3:1). Total nitrogen and phosphorus content were determined according to the Kjeldahl method and vanadate method, using nitrogen anlayzer (Kjeltec 2300,

Foss, Hilleroed, Denmark) and UV-visible spectrophotometer (Shimadzu UV-3150, Shimadzu Co. Kyoto, Japan), respectively. Potassium, calcium, and magnesium content were determined according to an atomatic absorption spectrophotometric method using atomic absorption spectrophotometer (Shimadzu AA-6800, Shimadzu Co. Kyoto, Japan).

1.3. Cultivation of grafted peppers in a greenhouse

The grafted and non-grafted transplants were transplanted in a greenhouse (*W* 8 × *L* 3 m) covered with polyethylene film 46 days after grafting (April). After soil test, pre-plant broadcast N-P-manure were applied at a rate of 72-61-20,000 $kg \cdot ha^{-1}$ to soil. Three rows were made and each row was mulched with black plastic film prior to planting. Irrigation and additional fertilizer application (fertigation) was carried out using standard procedures for pepper cultivation (RDA, 2008a). Additional fertilizer (‘Hanbang’ for green pepper cultivation, N-P-K-Ca-Mg=10.8-3-7-4-2 $me \cdot L^{-1}$, Coseal Co. Ltd., Gunsan, Korea) was applied depending on the growth conditions through a drip irrigation system. The experiment was arranged in a randomized complete block design with three replicates. Each replicate consisted of five plants. Peppers were cultivated for about five months (from April to August) and green fruits were harvested weekly from the 69th day after transplanting. All the fruits from each plant were harvested, counted and weighed. Fruits (n=30) were selected for the characterization 76 days after transplanting. Each fruit

(n=15) was longitudinally sliced into two and the flesh thickness was measured at the center of the fruit with a caliper gauge. Textural property of the fruit was measured using a texture analyzer (EZ Test-100N, Shimadzu Co. Kyoto, Japan) equipped with a 5-mm diameter plunger. Compression force into the flesh at 7 mm depth was monitored at 2 $mm \cdot s^{-1}$ speed.

1.4. Inoculation of pathogen

The Phytophthora blight pathogens, *P. capsici* were obtained from National Academy of Agricultural Science, Rural Development Administrations (NAAS, RDA). 'Nokkwang' was grafted onto five commercial varieties and four selected breeding lines ('PR 919', 'PR 920', 'PR 921', and 'PR 922') based on resistance to Phytophthora blight, bacterial wilt, bacterial spot, anthracnose, and virus in the field were used as rootstocks (NIHHS, 2009). Non-grafted and auto-grafted 'Nokkwang' transplants were used as control. Sixty two-day-old transplants (31 days after grafting) grafted onto different rootstocks were inoculated by dipping method with 10^5 zoospores/mL^{-1} suspension. The grafted transplants inoculated with *P. capsici* were transplanted in the plant pot filled with commercial growing substrate. The experiment was arranged in a randomized block design with three replicates. Each experimental unit consisted of three plants. The survival rate was examined 23 days after inoculation.

2. The Growth and Incidence of Phytophthora Blight and Bacterial Wilt of the Grafted Peppers as Influenced by Different Rootstocks

2.1. Plant materials and cultivation of grafted peppers

Among nine breeding lines, three rootstocks ('PR 920', 'PR 921', and 'PR 922') were selected based on resistance to pathogens such as *P. capsici*. 'Nokkwang', 'Saengsaeng Matkkwari' (Nongwoo Bio Co., Ltd.), and 'Shinhong' (Nongwoo Bio Co., Ltd.) were used as scions. 'Nokkwang', 'Saengsaeng Matkkwari', and 'Shinhong' transplants and transplants that were grafted onto 'Tantan' rootstocks were served as controls. Grafting was done 32 days after sowing the seeds of scions.

The grafted and non-grafted pepper transplants were transplanted in a greenhouse (244 m^2) covered with polyethylene film 32 days after grafting (March). After soil test, pre-plant broadcast manure and rice straw were applied at a dose of 20,000 and 5,000 $kg \cdot ha^{-1}$ to soil. Green fruits were harvested from May for about six months.

Growing scions and rootstocks, grafting, managing of grafted peppers, sampling, and examination were achieved using the same procedure as the above experiment.

2.2. Inoculation of pathogen

The Phytophthora blight pathogens, *P. capsici* and bacterial wilt pathogens, *R. solanacearum* were used. Bacterial wilt pathogens were

obtained from Kyungpook National University, Korea. Sixty three-day-old transplants (26 days after grafting) planted in a plug tray were used. Six non-grafted transplants and 15 grafted transplants grafted onto each rootstock were inoculated by dipping method with 10^5 zoospores/mL^{-1} of Phytophthora blight pathogens and 10^8 cfu/mL^{-1} suspension of bacterial wilt pathogens, respectively (Yang et. al., 2010). Disease evaluations were done 12-34 days after inoculation.

2.3. Statistical analysis

The data were subjected to analysis of variance (ANOVA). Statistical computations were carried out using the SigmaPlot version 11 (Systat Software Inc., San Jose, CA, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) software.

RESULTS

1. The Growth and Incidence of Phytophthora Blight of the Grafted Peppers as Influenced by Different Rootstocks

1.1. Graft-take, growth, and mineral content of grafted transplants

Commercial varieties exhibited high germination (≥96%). Among breeding lines, 'PR 901' (63.9%) and 'PR 930' (72.2%) had low percent germination (Tables 1.2). The other breeding lines showed similar percent germination with commercial varieties. Graft-take was over 80% in all grafted transplants except those grafted onto 'PR 901' (72.0%). Growth and mineral content of pepper transplants were influenced by grafting (Tables 1.3 and 1.4). The number of leaves, stem diameter, leaf area, and dry weight of shoot of grafted transplants were greater than those of non-grafted transplants. They varied among different rootstocks. The dry weight was highest in the transplants grafted onto 'PR 921' followed by 'PR 928' and 'PR 919'. Mineral contents in the shoot of grafted transplants were significantly different, depending on rootstock genotypes. With respect to total N, pepper transplants grafted onto 'PR 929' (4.20%), 'PR 901' (3.98%), and 'Kataguruma' (3.91%) presented values exceeding those of control plants (non-graft 3.52% and auto-graft 3.47%). The calcium content in seedling grafted onto 'PR 922' (6.82%) was 1.9 times higher that of non-grafted transplants (3.57%). In contrast,

phosphorus content in grafted transplants was lower than that of non-grafted transplants (non-graft 1.51% and auto-graft 1.39%).

1.2. Yield and fruit quality of grafted peppers

Marketable yield ranged from 1.35 to 1.95 kg per plant depending on rootstock genotypes but there were no significant difference between rootstocks (Table 1.5). Differences among treatments were found for fruit length, weight, flesh thickness, and textural property (Table 1.6). Fruit length in grafted peppers (average of 12.4 cm) was longer than that of non-grafted peppers (average of 12.0 cm). Fruits from peppers grafted onto 'PR 901' (12.9 cm) were more elongated than those from non-grafted peppers. With respect to textural property, the value of strength ranged from 529 to 623 $kN \cdot m^{-2}$, and was highest in fruits grafted onto 'PR 919', and lowest in those grafted onto 'PR 928'. The value of hardness ranged from 5,135 to 6,397 $kN \cdot m^{-2}$, and was highest in fruits grafted onto 'PR 919', and lowest in those grafted onto 'Tantan'.

1.3. Survival of grafted transplants after inoculation of *P. capsici*

Survival rate of pepper transplants after inoculation with *P. capsici* as influenced by different rootstocks is presented in Fig. 1.1. 'Nokkwang' was highly susceptible and all of the inoculated non-grafted and auto-grafted transplants died. The transplants showed typical symptoms of Phytophthora blight such as browninsh lesions on stem, plant wilt, leaf defoliation, and damping-off. Among

transplants grafted onto commercial rootstocks, the transplants grafted onto ‘Tantan’ and ‘Kataguruma’ showed high resistance. The survival rates of the transplants were 100 and 89%, respectively. Among transplants grafted onto breeding lines, these transplants grafted onto ‘PR 920’, ‘PR 921’, and ‘PR 922’ showed high resistance. The survival rates of these transplants were 100, 100 and 89%, respectively.

2. The Growth and Incidence of Phytophthora Blight and Bacterial Wilt of the Grafted Peppers Influenced by Different Rootstocks

2.1. Graft-take and growth of grafted transplants

Different scions or rootstocks did not affect the graft-take (Fig. 1.2). The graft-take ranged from 79 to 96% according to the combinations of scions and rootstocks. The graft-take of transplants grafted onto ‘Tantan’ averaged 94%. Those of transplants grafted onto ‘PR 920’, ‘PR 921’, and ‘PR 922’ averaged 88, 86, and 85%, respectively.

The growth of pepper transplants were influenced by grafting (Table 1.7). Shoot length, stem diameter, and leaf area of non-grafted transplants were greater than those of grafted transplants. The growth varied among combinations of scions and rootstocks. In ‘Nokkwang’, the dry weight was highest in the non-grafted transplants followed by transplants grafted onto ‘Tantan’ and ‘PR 922’. In ‘Saengsaeng Matkkwari’, the dry weight was highest in the transplants grafted onto

'PR 921'. In 'Shinhong', the dry weight was highest in the non-grafted transplants followed by transplants grafted onto 'Tantan' and 'PR 921'.

2.2. Yield and fruit quality of grafted peppers

Cumulative yields of marketable fruits are presented in Fig. 1.3. The yields were different depending on scion varieties, but significant differences among treatments were not found. Moreover, no significant differences in fruit quality were found (Table 1.8).

2.3. Incidence of grafted transplants after inoculation of *P. capsici* and *R. solanacearum*

Incidence of pepper transplants after inoculation with *P. capsici* and *R. solanacearum* as influenced by different rootstocks are presented in Fig. 1.4. The inoculated scion varieties were susceptible to *P. capsici*, and non-grafted transplants exhibited infection symptom in five days after and then all died. On the other hand, the incidence of Phytophthora blight on grafted transplants were markedly lower than those of non-grafted transplants, and showed high resistance to *P. capsici*. The incidence of transplants grafted onto breeding lines ('PR 920', 'PR 921', and 'PR 922') was delayed and lower than those grafted onto commercial rootstock 'Tantan'.

Incidence of pepper transplants inoculated with *R. solanacearum* was different depending on scion varieties. In scion 'Nokkwang', the incidence of transplants grafted onto breeding lines ('PR 920', 'PR

921', and 'PR 922') was delayed and lower than non-grafted transplants and those grafted onto commercial rootstock 'Tantan'. In scion 'Saengsaeng', the incidence was highest in transplants grafted onto commercial rootstock 'Tantan'. The survival rates of non-grafted 'Nokkwang', 'Saengsaeng Matkkwari', and 'Shinhong' were 67, 83, and 100%, respectively. 'Shinhong' showed high resistance to *P. capsici*. In 'Nokkwang', the survival rates increased by grafting and those of transplants grafted onto breeding lines were greater than that of commercial variety 'Tantan'. In 'Saengsaeng Matkkwari', the survival rate was highest in the transplants grafted onto 'PR 922' followed by 'PR 920'. In 'Shinhong', the survival rate was highest in the non-grafted transplants followed by transplants grafted onto 'PR 922' and 'PR 921'. The average survival rates of pepper transplants grafted onto 'Tantan', 'PR 920', 'PR 921', and 'PR 922' were 78, 82, 91 and 100%, respectively, and 'PR 922' showed the highest resistance.

DISCUSSION

Vegetable grafting is globally conducted because it can increase disease resistance, abiotic stress tolerance, yield and quality of a number of vegetable crops (Davis et al., 2008b; King et al., 2008, 2010; Lee and Oda, 2003; Lee et al., 2010; Rivero et al., 2003). This can be achieved by using rootstocks that have resistance to soil-borne diseases or pests, tolerance to abiotic stress, selective absorption of available nutrients, high vigor and good compatibility with the scion (Davis et al., 2008b; Gisbert et al., 2011; Lee and Oda, 2003; Rivero et al., 2003). In this study, *C. annuum* commercial rootstocks and breeding lines for rootstock were tested for resistance to both Phytophthora blight and bacterial wilt as well as their graft compatibility to commercial pepper cultivars, and have confirmed that the new pepper rootstock is resistant to both Phytophthora blight and bacterial wilt.

Seed germination and grafting success are very important in using grafted crops. Some rootstocks of wild species such as wild *Solanum* are known to emerge slowly and have poor germination (Gisbert et al., 2011). In this study, commercial varieties exhibited high germination, but some breeding lines showed lower germination. Grafting success does not only depend on environmental factors and skill of the grafter but also on graft compatibility between rootstock and scion (Davis et al., 2008b; Gisbert et al., 2011). Even though the scion and rootstock are same species (*C. annuum* L.) in grafted pepper production,

grafting success is affected by the combination of scion and rootstock. Different graft-takes were achieved between different scion-rootstock combinations. The graft-takes of selected breeding lines (‘PR 920’, ‘PR 921’, and ‘PR 922’) averaged over 80%.

Grafting also affects the growth and mineral contents of grafted plants (Edelstein et al., 2010; Ruiz et al., 1997; Savvas et al., 2010). In this study, growth parameters such as shoot length, number of leaves, dry weight, and mineral contents were different according to the combinations of scions and rootstocks. Since both the root structure and the uptake efficiency of the root cells are determined by the rootstock, plants grafted onto different rootstocks may exhibit dissimilar abilities to take up and translocate nutrients (Savvas et al, 2010).

It is reported that grafting can increase yield since grafted plants are resistant to soil-borne disease, have strong root systems, and increased photosynthesis (Davis et al., 2008b). It has been also reported that pH, flavor, sugar, color, carotenoid content, and texture can be affected by grafting and the type of rootstock used and the rootstock/scion combination must be carefully chosen for optimal fruit quality (Davis et al., 2008a; Gisbert et al., 2011). In this study, we found no differences for yield and apparent fruit quality among non-grafted peppers or peppers onto ‘Tantan’ and selected breeding lines (‘PR 920’, ‘PR 921’, and ‘PR 922’). However, there were some differences for apparent fruit quality among commercial and breeding rootstocks. For example, rootstocks affected fruit length, possibly due

to changes in the concentration of growth regulators induced by the rootstock (Gisbert et al., 2011).

Phytophtora blight caused by *P. capsici* and bacterial wilt caused by *R. solanacearum* are the most economically important and destructive diseases in pepper production (Kim et al., 2010; Tran and Kim, 2010). When pepper transplants grafted onto commercial rootstocks and selected breeding lines were inoculated with *P. capsici*, the grafted transplants showed variation in resistance according to rootstock genotypes (Fig. 1.1). Some commercial rootstocks were found to have low level of resistance or susceptibility to the disease, although their names were initialed with 'PR' meaning Phytophthora resistance or they were known as resistant cultivar (Kim et al., 2010). Breeding lines 'PR 920', 'PR 921', and 'PR 922' originated from CM334 which has been reported to be highly resistant to *P. capsici* (Oelke et al., 2003) were found to be highly resistant to Phytophthora blight. These lines also showed higher resistance to bacterial wilt than commercial rootstock 'Tantan' (Fig. 1.4).

Among non-grafted transplants (scion varieties), 'Shinhong' showed very high resistant to bacterial wilt followed by 'Saengsaeng Matkkwari'. This result agreed with the report about resistance to bacterial wilt of commercial peppers (NIHHS, 2009). The NIHHS reported that 'Shinhong' has the highest resistance followed by 'Saengsaeng Matkkwari', while 'Nokkwang' was susceptible to bacterial wilt. In 'Nokkwang', the resistance to bacterial wilt was increased by grafting using commercial rootstock and breeding lines.

However, in ‘Shinhong’ and ‘Saengsaeng Matkkwari’, the resistance of grafted transplants was influenced by scion/rootstock combinations and the specific combination negatively impacted the resistance. Thus, the rootstock/scion combination should be carefully chosen for obtaining resistance, optimal yield and fruit quality.

Peppers grafted onto breeding lines of ‘PR 920’, ‘PR 921’, and ‘PR 922’ had shown high resistance to both Phytophthora blight and bacterial wilt without decrease in yield and fruit quality. Accordingly, they were considered to be used as rootstocks resistant to Phytophthora and bacterial wilt for pepper production.

Greater resistance to both Phytophthora blight and bacterial wilt can be obtained when peppers were grafted onto resistant breeding lines such as ‘PR 920’, ‘PR 921’, and ‘PR 922’ without the reduction in yield and apparent fruit quality, and these resistance breeding lines could effectively be used as a source of rootstock.

In conclusion, grafting using rootstocks resistant to both Phytophthora blight and bacterial wilt seems to be effective tool for disease resistance. Pepper scions grafted onto breeding lines (‘PR 920’, ‘PR 921’, and ‘PR 922’) resistant to both Phytophthora blight and bacterial wilt showed greater survival rate when they were inoculated with *P. capsici* and *R. solanacearum*. The grafted peppers did not exhibit any detrimental effect on yield and apparent fruit quality. Accordingly, selected breeding lines are recommended as candidates of pepper rootstock resistant to both Phytophthora blight and bacterial wilt.

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Table 1.1. Pepper accessions used as rootstocks in experiments.

Accession/cultivar	Description	Source
Kataguruma	Commercial variety	Sakada Korea Seed Co., Ltd.
Konesianhot	Commercial variety	Seminis Inc.
Koregon PR-380	Commercial variety	Koregon Seed Co.
PR-power	Commercial variety	Nongwoo Bio Co.
Tantan	Commercial variety	Nongwoo Bio Co.
PR901	Breeding line (LV2323/YCM334, F_5)	NIHHS[z]
PR919	Breeding line (LV2319, F_7)	NIHHS
PR920	Breeding line (CM334-7, F_3)	NIHHS
PR921	Breeding line (CM334-8, F_3)	NIHHS
PR922	Breeding line (CM334-9, F_3)	NIHHS
PR927	Breeding line (ICPN9#9-1, F_6)	NIHHS
PR928	Breeding line (ICPN12#2-1, F_6)	NIHHS
PR929	Breeding line (ICPN12#8-3, F_6)	NIHHS
PR930	Breeding line (ICPN12#10-4, F_6)	NIHHS

[z]National Institute of Horticultural & Herbal Science, Korea.

Table 1.2. Germination of rootstocks and graft-take of peppers grafted onto these rootstocks when using 'Nokkwang' as scion.

Rootstock	Percent Germination	Graft-take (%)	
		12 days after grafting	30 days after grafting
Commercial varieties			
Kataguruma	97.2a[z]	84.7a	81.4a
Konesianhot	100.0a	94.4a	94.4a
Koregon PR-380	95.8ab	92.8a	92.8a
PR-power	100.0a	97.2a	90.3a
Tantan	98.6a	91.5a	91.5a
Breeding lines			
PR901	63.9c	84.5a	72.0a
PR919	98.6a	97.2a	82.7a
PR920	93.1ab	86.6a	86.6a
PR921	81.9a-c	97.9a	80.3a
PR922	93.1ab	85.0a	85.0a
PR927	89ab	84.4a	82.9a
PR928	98.6a	100.0a	87.4a
PR929	97.2a	100.0a	88.6a
PR930	72.2bc	83.0a	81.5a

[z]Mean separation within columns by Duncan's multiple range test ($P \leq 0.05$).

Table 1.3. Growth of grafted pepper transplants 'Nokkwang' as influenced by rootstocks.

Rootstock	Shoot length (cm)	Number of leaves	Stem diameter of scion[z] (mm)	Leaf area (cm^2)	Dry weight (mg)	
					Root	Shoot
Commercial varieties						
Kataguruma	29cd[y]	14.0b-d	3.62a	153a	83cd	698c-f
Konesianhot	29cd	13.7d	3.58a	127b	78cd	658ef
Koregon PR-380	30bc	14.7b-d	3.62a	141ab	91b-d	684d-f
PR-power	33a	14.3b-d	3.61a	152a	113ab	767a-e
Tantan	30bc	15.7ab	3.66a	152a	100bc	724b-f
Breeding lines						
PR901	25f	16.4a	4.00a	154a	107a-c	799a-d
PR919	31b	14.7b-d	3.85a	137ab	86b-d	847ab
PR920	27e	13.8cd	3.46a	138ab	89b-d	618f
PR921	30b-d	14.2b-d	3.86a	152a	132a	869a
PR922	28de	15.4a-c	4.03a	152a	100bc	706c-f
PR927	31bc	15.7ab	3.48a	155a	98bc	747a-f
PR928	34a	15.4a-c	4.12a	148a	106a-c	827a-c
PR929	33a	15.2a-d	3.83a	141ab	93b-d	810a-d
PR930	28de	13.8cd	3.86a	153a	67d	644ef

[z]Measurement at 1 cm above the graft union.

[y]Mean separation within columns by Duncan's multiple range test ($P \leq 0.05$).

Table 1.4. Mineral content (% D.W.) of grafted peppers transplants 'Nokkwang' as influenced by rootstocks.

Rootstock	T-N	P_2O_5	K	Ca	Mg
Commercial varieties					
Kataguruma	3.91a-c[z]	1.33a	8.83a	4.05a	2.01b-f
Konesianhot	3.51de	1.03de	7.68a	3.87a	1.92c-f
Koregon PR-380	3.87a-d	1.33a	8.85a	4.58a	2.09a-d
PR-power	3.76b-d	1.29ab	8.58a	4.35a	2.03b-e
Tantan	3.80b-d	1.13c-e	8.14a	4.03a	2.22ab
Breeding lines					
PR901	3.98ab	1.07c-e	8.77a	5.61a	2.32a
PR919	3.59c-e	1.05c-e	8.31a	3.61a	1.75f
PR920	3.85a-d	1.17b-d	8.70a	4.32a	2.16a-c
PR921	3.34e	1.15c-e	7.76a	5.74a	1.92b-f
PR922	3.83b-d	1.18bc	9.03a	6.82a	2.06b-d
PR927	3.73b-d	1.01e	9.06a	4.71a	1.77ef
PR928	3.53de	1.04de	8.77a	5.43a	1.83d-f
PR929	4.20a	1.18bc	9.91a	5.25a	1.97b-f
PR930	3.73b-d	1.37a	9.74a	4.63a	2.33a

[z]Mean separation within columns by Duncan's multiple range test ($P \leq 0.05$).

Table 1.5. Cumulative yield of grafted peppers 'Nokkwang' cultivated in uninfested soil as influenced by rootstocks.

Rootstock	Marketable yield (g/plant)	Unmarketable yield (g/plant)	Gross yield (g/plant)
Commercial varieties			
Kataguruma	1,529a[z]	337a	1,866a
Konesianhot	1,783a	396a	2,636a
Koregon PR-380	1,533a	331a	1,864a
PR-power	1,784a	306a	2,090a
Tantan	1,668a	376a	2,044a
Breeding lines			
PR901	1,945a	374a	2,320a
PR919	1,784a	335a	2,119a
PR920	1,525a	316a	1,841a
PR921	1,557a	290a	1,847a
PR922	1,611a	339a	1,951a
PR927	1,918a	376a	2,294a
PR928	1,352a	266a	1,618a
PR929	1,592a	306a	1,898a
PR930	1,625a	356a	1,981a

[z]Mean separation within columns by Duncan's multiple range test ($P \leq 0.05$).

Table 1.6. Fruit quality of grafted peppers 'Nokkwang' cultivated in uninfested soil as influenced by grafting and rootstocks.

Rootstock	Fruit length (cm)	Fruit width (mm)	Flesh thickness (mm)	Fresh weight (g)	Textural property	
					Strength ($kN \cdot m^{-2}$)	Hardness ($kN \cdot m^{-2}$)
Commercial varieties						
Kataguruma	12.4b-d[z]	14.5a	1.57a	11.7ab	545a	5,446b-d
Konesianhot	12.7ab	14.7a	1.66a	12.1a	568a	5,331cd
Koregon PR-380	12.5a-d	14.4a	1.50a	11.3b-e	569a	5,275cd
PR-power	12.4a-d	14.8a	1.59a	11.1b-e	550a	5,577a-d
Tantan	12.3b-d	14.6a	1.62a	11.5a-d	546a	5,135d
Breeding lines						
PR901	12.9a	14.8a	1.66a	11.8ab	547a	5,337cd
PR919	12.5a-d	14.6a	1.52a	11.5a-d	623a	6,397a
PR920	12.5a-d	14.4a	1.54a	10.9c-e	596a	6,249ab
PR921	12.1d	14.5a	1.54a	10.8de	584a	5,502b-d
PR922	12.6a-c	15.0a	1.50a	11.7ab	564a	5,521b-d
PR927	12.6a-c	14.8a	1.53a	11.4a-d	567a	5,934a-d
PR928	12.3cd	14.6a	1.42a	10.5e	529a	5,216cd
PR929	12.1d	15.0a	1.54a	10.8de	562a	6,011a-c
PR930	12.3cd	14.8a	1.49a	11.3b-e	577a	5,798a-d

[z]Mean separation within columns by Duncan's multiple range test ($P \leq 0.05$).

Table 1.7. Growth of grafted pepper transplants 'Nokkwang', 'Saensaeng', and 'Shinhong' as influenced by grafting and rootstocks.

Treatment		Shoot length (cm)	Number of leaves	Stem diameter of scion[z] (mm)	Leaf area (cm^2)	Dry weight (mg)	
Scion	Rootstock					Root	Shoot
Nokkwang	Non-graft	36a[y]	16.4a	4.22a	192a	148a	966a
	Tantan	32b	13.0b	3.20b	188b	86b	798b
	PR920	24c	12.8b	2.71c	141c	50c	534c
	PR921	25c	12.6b	2.74c	149c	50c	596c
	PR922	25c	12.8b	2.80c	157c	66c	620c
Saengsaeng	Non-graft	41a	22.6a	3.06a	218a	130a	886a
	Tantan	29b	17.4b	2.76b	187bc	84b	758ab
	PR920	29c	17.2b	2.98b	162c	72b	622b
	PR921	30c	21.2ab	2.90b	190a-c	138a	928a
	PR922	32c	23.2a	2.99b	207ab	66b	620b
Shinhong	Non-graft	42a	21.8a	3.44a	210a	134a	1,050a
	Tantan	32b	16.0b	3.01b	178ab	78cd	688b
	PR920	30b	17.4b	3.17ab	170b	102bc	774b
	PR921	25c	18.8ab	3.46a	174b	108b	804b
	PR922	30b	16.4b	3.02b	184ab	72d	656b
Scion (A)		*** [x]	***	***	***	***	*
Rootstock (B)		***	*	ns	**	***	***
A x B		***	**	**	ns	***	***

[z]Stem diameter was measured at 1cm above the graft union.

[y]Mean separation within columns for each scion variety by Duncan's multiple range test (P≤0.05).

[x]ns indicates nonsignificant; *significant at P≤0.05; **significant at P≤0.01; ***significant at P≤0.001.

Table 1.8. Fruit quality of grafted peppers ‘Nokkwang’, ‘Saensaeng’, and ‘Shinhong’ as influenced by grafting and rootstocks.

Treatment		Fruit length[z] (cm)	Fruit width (mm)	Flesh thickness (mm)	Fruit weight (g)
Scion	Rootstock				
Nokkwang	Non-graft	12.04	14.98	2.36	10.39b[y]
	Tantan	11.95	14.69	2.29	10.26b
	PR920	12.42	15.18	2.20	11.47a
	PR921	11.97	14.45	2.24	9.99b
	PR922	12.16	14.74	2.29	10.57b
Saengsaeng	Non-graft	9.69	10.53	1.49	4.33
	Tantan	9.73	10.86	1.38	4.52
	PR920	9.77	10.59	1.52	4.39
	PR921	9.89	11.12	1.46	4.70
	PR922	9.36	10.92	1.48	4.15
Shinhong	Non-graft	8.53	10.55ab	1.76	4.60
	Tantan	8.73	10.60a	1.62	4.53
	PR920	8.13	10.17ab	1.67	4.12
	PR921	7.75	10.34ab	1.67	4.22
	PR922	8.04	9.90b	1.70	4.11
Scion (A)		*** [x]	***	***	***
Rootstock (B)		ns	ns	ns	ns
A x B		ns	*	ns	***

[z]Fruits (n=30) were selected 76 days after transplanting.

[y]Mean separation within columns for each scion variety by Duncan’s multiple range test (P≤0.05).

[x]ns indicates nonsignificant; *significant at P≤0.05; **significant at P≤0.01; ***significant at P≤0.001.

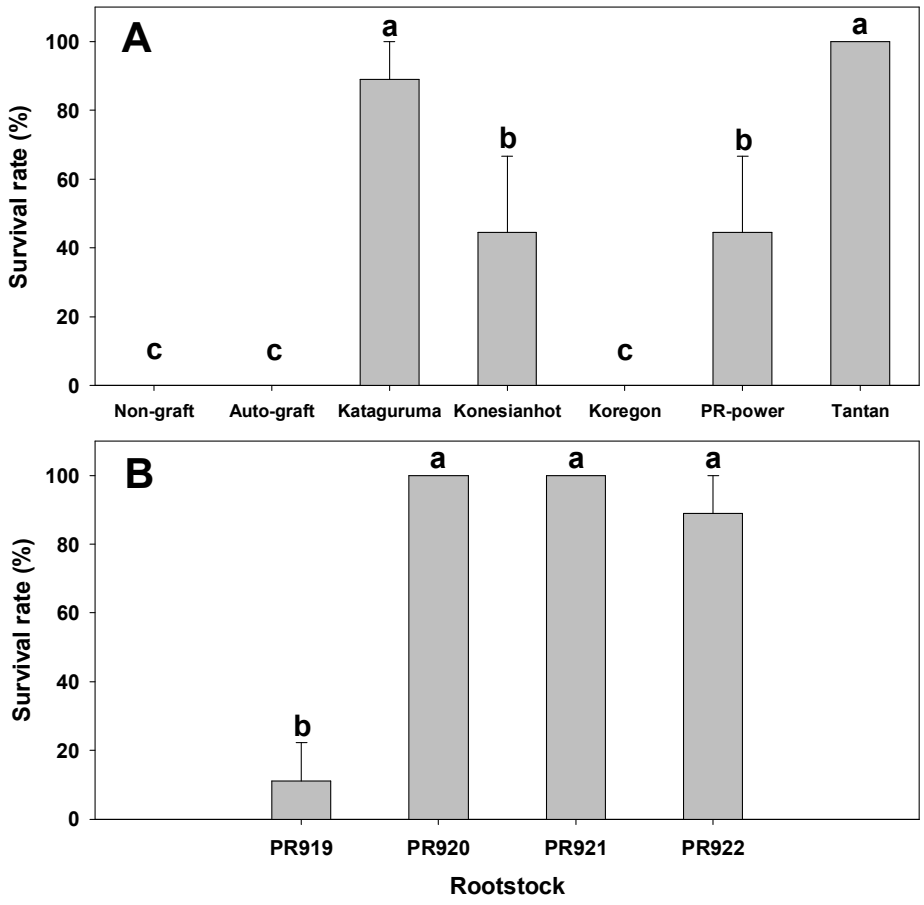

Fig. 1.1. Survival rate of peppers after inoculation with *Phytophthora capsici* as influenced by different rootstocks (A:commercial varieties, B:breeding lines). Vertical bars represent standard errors. Different letters are significantly different at P≤0.05 (Duncan's multiple range test).

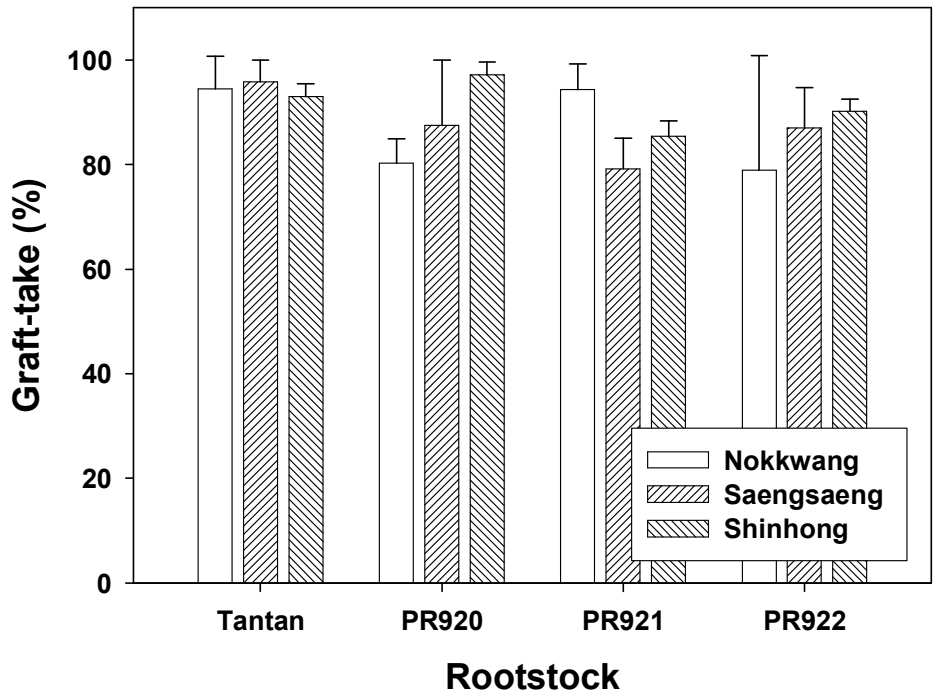

Fig. 1.2. Graft-take of peppers grafted onto different rootstocks 10 days after grafting. Vertical bars represent standard errors.

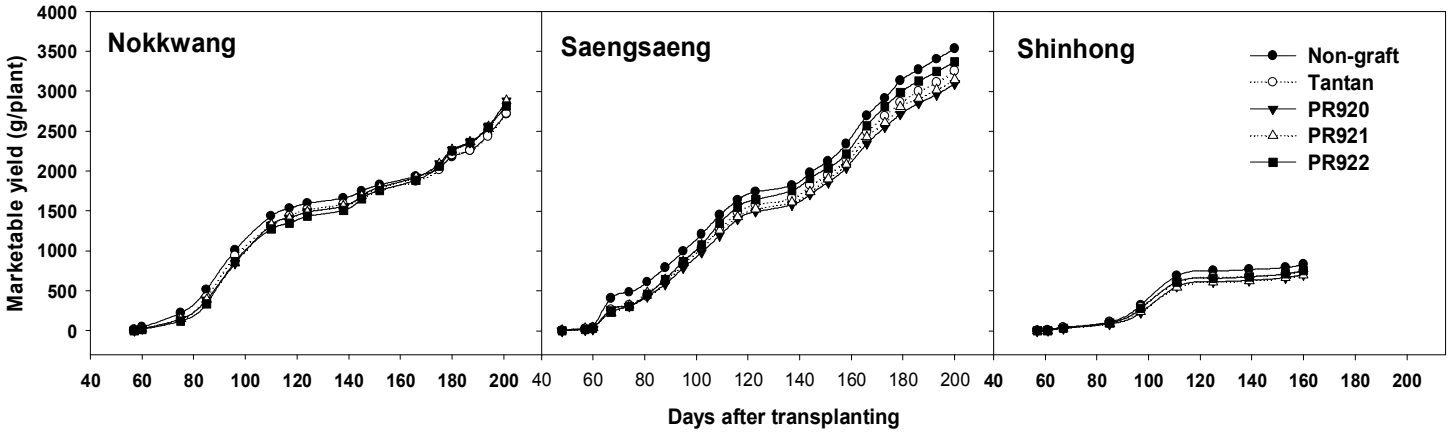

Fig. 1.3. Cumulative yield of marketable fruits of grafted peppers ‘Nokkwang’, ‘Saengsaeng’, and ‘Shinhong’ as influenced by grafting and rootstocks. Pepper was cultivated in uninfested soil.

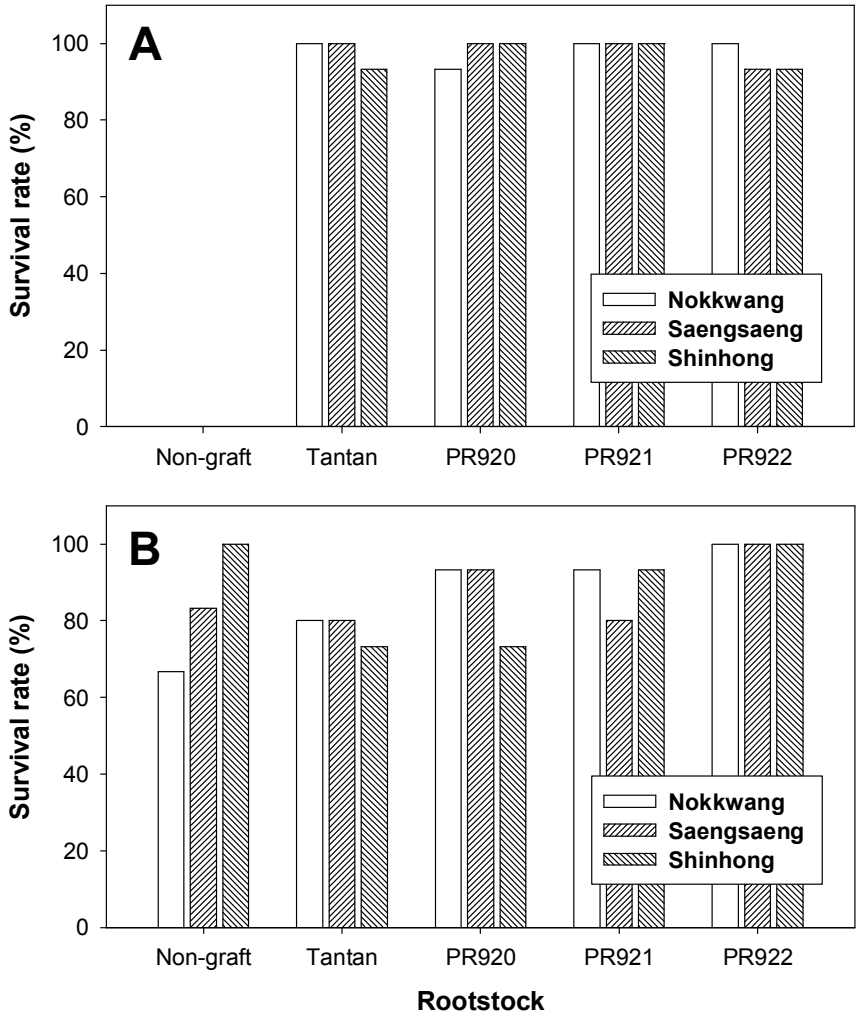

Fig. 1.4. Survival rate of pepper transplants after inoculation with *Phytophthora capsici* (A) and *Ralstonia solanacearum* (B) as influenced by grafting and rootstocks.

CHAPTER II

EFFECTS OF GRAFTING ON THE GROWTH AND YIELD OF PEPPER UNDER LOW TEMPERATURE CONDITION

ABSTRACT

The objective of this study was to investigate the effects of rootstock and night temperature on the growth and yield of grafted pepper (*C. annuum* L.) in greenhouse. Four commercial pepper varieties, six commercial rootstocks, four breeding lines, and one eggplant rootstock were grown at three levels of NT (10, 15, and 20°L). At 10°C NT, the total dry weight was the greatest in 'Kataguruma' followed by 'Koregon PR-380' and 'PR-Power'. They could be categorized into three types according to the response to night temperature. The relative growth rate (RGR) of genotypes that belonged to type I ('Asaggy', 'PI', 'Saengreok 211', 'Saengreok 213', and 'Tantan') were reduced approximately 0.01 to 0.03 $g \cdot g^{-1} \cdot day^{-1}$ as NT decreased. In type II ('Shinhong'), they were similar between 15 and 20°C NT but rapidly decreased at 10°C NT, whereas they were stable irrespective of NT in type III ('A7', 'Kataguruma', 'Konesianhot', 'Koregon PR-380', and 'Manitta'). Among 15 genotypes, four rootstocks (Kataguruma, Koregon PR-380, PR-Power, and Tantan) were selected based on differential responses to

different NT conditions; ‘Kataguruma’ and ‘Koregon PR-380’ for higher growth and ‘PR-Power’ and ‘Tantan’ for mild growth under low temperature. When ‘Nokkwang’ grafted onto the selected rootstocks were cultivated at different NT conditions (8, 13, and 20°C), the early growth after transplanting became greater than non-grafted peppers irrespective of temperature. Even though the growth and yield of grafted peppers were greater than those of non-grafted peppers irrespective of temperature, the commercial productivity was not improved.

INTRODUCTION

Pepper is the most economically important and widely cultivated crop in Korea. The fruit is used in green or red forms by the difference of harvest time. Recently, the production of green pepper in greenhouse continues to increase. Green pepper is cultivated for five or six months after transplanting. It is mostly produced by retarding, semi-forcing or forcing culture when it should go through the cold season.

Pepper is a thermophilic crop and requires relatively higher growth temperature than other crops. The optimum temperature for maintaining productivity ranges 25 to 30°C day and 18 to 19°C night (Lim et al., 2006). In a cold season, it is recommended that the minimum temperature is 18°C and should be above 15°C. The growth and yield of pepper were severely inhibited by low NT (Choi et al., 2001). Cold-season cultivation in greenhouse requires a large energy input to keep the proper condition for a good growth and productivity. The cost for heating is above 30% of production costs (RDA, 2006). The soaring of the oil price and reducing of tax-free oil burden farmers with higher expenses for the heating.

The purposes of vegetable grafting are to improve not only the resistance to soil-borne pest and pathogen but also the adaptation to abiotic stresses (Ahn et al., 1999; Lee and Oda. 2003; Rivero et al., 2003). Grafting improved the tolerance to low temperature in fruit vegetables such as watermelon, cucumber, tomato, and eggplant.

Accordingly, it suggests that grafted plants can grow properly and maintain the yield even though they are under the condition below the optimum temperature (Edelstein, 2004). Cucumber (*Cucumis sativus* L.) is generally grafted on *Cucurbita* species such as figleaf gourd (*Cucurbita ficifolia* Bouché) to improve the low temperature tolerance during winter culture (Lee and Chung, 2005). Venema et al.(2008) reported that grafting tomato onto the rootstock of a cold-tolerant high-altitude accession of *Solanum habrochaites* improved suboptimal-temperature tolerance.Green pepper has been cultivated intensively for many years around Gyeongsangnam-Do region. In order to prevent soil-borne diseases in continuous cropping, green peppers are generally grafted on the rootstocks that have resistance to phytophthora blight and bacterial wilt. Rootstocks for green pepper production are the same species (*C. annuum* L.) as pepper and limited within ten varieties. It was reported that some of them also have a good tolerance to high salt conditions (Chung and Choi, 2002). However, there are few reports on the effects of grafting on the growth and productivity of green peppers under high or low temperature conditions.

The objective of this work was to investigate whether the grafting using a rootstock with tolerance to low temperature is able to lower the minimum temperature for growth and productivity. It was conducted 1) to verify the level of tolerance to low temperature conditions of commercial peppers, rootstocks, and breeding lines and 2) to examine the effect of grafting on the growth and yield of green pepper under different minimum temperature conditions.

MATERIALS AND METHODS

The growth responses of genotype seedlings to different night temperature

The genotypes used in the experiment are listed in Table 2.1. They were sown into the 105-cell plug trays filled with commercial growing substrate (BM 2, Berger Group Ltd., Quebec, Canada). They were grown under natural light conditions in a greenhouse after germination. From two weeks after sowing, they were placed in growth chambers at temperatures of 10, 15, or 20°C, respectively, during night time (from 18:00 to 09:00, 12-h dark). Plants were watered daily. A nutrient solution (electric conductivity 1.4 $dS \cdot m^{-1}$ with 'Hanbang' for seedling, Coseal Co. Ltd., Gunsan, Korea) was applied depending on growth conditions. Three seedlings were sampled every week for growth measurement for shoot length, stem diameter, number of leaves, leaf area, fresh weight, and dry weight. RGR was calculated from the logarithmically transformed equation of exponential growth equation ($w=w_0 \cdot e^{(rm \cdot t)}$, where w=dry mass, w_0=initial dry mass, rm=RGR, and t=growth time) by logarithmic transformation described by Goudriaan and van Laar(1994).

The growth and yield of the grafted peppers influenced by rootstock and night temperature

Among 15 genotypes, four rootstocks (Kataguruma, Koregon PR-380, PR-Power, and Tantan) were selected based on differential

response to different NT; ‘Kataguruma’ and ‘Koregon PR-380’ for higher growth and ‘PR-Power’ and ‘Tantan’ for mild growth under low temperature. ‘Nokkwang’ was used as scions for all stocks in this experiment. They were sown into the 72-cell plug trays filled with commercial growing substrate (BM 2, Berger Group Ltd., Quebec, Canada). After sowing, Epicotyl of scion was cut above 1cm from cotyledons and grafted to the similar position of rootstocks by slice grafting method at 4 weeks after sowing. After grafting, plants were healed and acclimatized in the tunnel covered with double-layered plastic film and shade cloth in the greenhouse for a week. Non-grafted

‘Nokkwang’ seedlings served as controls. Before transplanting, the graft-take and the growth parameters of sampled grafted transplants were measured.

The grafted and non-grafted transplants were transplanted in three greenhouses (244 m^2) covered with polyethylene film 68 days after sowing (18 September 2004). Pre-plant broadcast N-P-K-lime fertilizer and manure were applied at a dose of 122-64-61-2,000-20,000 $kg \cdot ha^{-1}$ to soil. Additional nutrient solution (‘Hanbang’ for pepper, Coseal Co. Ltd., Gunsan, Korea) was applied depending on the growth conditions through a drip irrigation system. The experiment was arranged in a randomized block design with three replicates. Each experimental unit consisted of ten plants. The greenhouses were heated by a warm air furnace system from Oct. 21 2004 until the end of experiment (Feb. 24 2005), but underground

was not heated. The minimum temperatures of three greenhouses were set to 8, 13, or 18°C respectively during night. For growth analysis, three plants of each treatment were sampled 50th day after transplanting. Plant height, stem diameter, leaf area, number of branching, fresh weight, and dry weight were measured. Fruits were harvested from Nov. 10 2004 to Feb. 24 2005. The number of fruits, the mean fruit weights, and yield were determined on five plants per plot. For the characterization of fruits, we select them between 13th and 18th node of each plant.

Statistical analysis

Collected data were analyzed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

The growth responses of genotype seedlings to different night temperature

Table 2.2 shows the dry weight of each genotype grown at different NT conditions. Low NT caused the reduction of dry matter accumulation rate of the genotypes except 'Manitta', 'PI', and 'Shinhong'. In these genotypes, the dry weights were reduced below half when the NT was lowered from 20 to 10°C. The total dry weights of 'Manitta', 'PI', and 'Shinhong' were the greatest individually at 15°C NT. The total dry weight under 10°C NT was the greatest in 'Kataguruma' followed by 'Koregon PR-380' and 'PR-Power'. That of 'PI' was smallest. The root dry weight at 10°C NT was also the greatest in 'Kataguruma' followed by 'PR-Power'.

In all genotypes, plants grown at 10°C NT showed smaller RGRs than those grown at 20°C NT (Table 2.3 and Fig. 2.1). But the differences in RGRs of genotypes greatly varied among 10, 15, and 20°C NT. So they could be categorized into three types according to the response to NT. The RGRs of genotypes that belonged to type I were reduced approximately 0.01 to 0.03 $g \cdot g^{-1} \cdot day^{-1}$ as NT decreased. In type II, they were similar between 15 and 20°C NT but rapidly decreased under 10°C NT, whereas they were stable irrespective of NT in type III. When they were subdivided in detail, 'Asaggy', 'PI', 'Saengreok 211', 'Saengreok 213', and 'Tantan' belonged to type I, 'Shinhong' belonged to type II, and 'A7',

‘Kataguruma’, ‘Konesianhot’, ‘Koregon PR-380’, and ‘Manitta’ belonged to type III. However, ‘Nokkwang’, ‘R-Safe’, and ‘PR-Power’ showed different responses between total and root growth rate. ‘Nokkwang’ and ‘R-Safe’ belonged type III in total plant but the RGRs in root rapidly decreased under 10°C NT. On the contrary, ‘PR-Power’ belonged type II in total plant but the RGR in root was stable irrespective of NT. The ratio of shoot to root (T/R ratio) in the type III was comparatively constant in the wide range of temperature.

The RGR of total dry weight at 10°C NT was the greatest in eggplant rootstock ‘Taibyo VF’. However, the peppers grafted to ‘Taibyo VF’ could not survive (data not shown).

The growth and yield of the grafted peppers by rootstock and night temperature

The growth of grafted peppers transplants: Table 2.4 shows the graft-take and growth of grafted pepper transplants grafted onto different rootstocks. Graft-take was over 90% in all grafted transplants, but that of ‘Koregon PR-380’ was slightly lower than the others. Among grafted transplants, the growth of peppers grafted onto ‘Kataguruma’ was better. The leaf area and dry weight of leaves were the smallest in the pepper transplants grafted onto ‘Koregon PR-380’.

The growth and yield of grafted peppers: Fig. 2.2 shows the air temperature and relative humidity in greenhouses during the part of

cultivation period. Air temperature was affected by outer temperature but differences of NT among treatments were made by 3~5°C, with each temperature being maintained constant to their set point. Soil temperature in10cm depth was fluctuated similarly to air temperature but it was more stable. The increase of NT by heating lowered relative humidity in the greenhouse and it went down by 60, 50, and 40% in the treatments of minimum temperature 8, 13, or 18°C, respectively.

Table 2.5 shows the growth of peppers 50 days after transplanting and simultaneously 20 days after starting of the heating. The growth of peppers was inhibited by low NT: smaller plant height, leaf area, number of branch, and total dry weight. Stem diameter, number of branch, and root dry weight were affected by grafting. Stem diameter and root dry weight of branch of grafted peppers were smaller than those of non-grafted peppers before transplanting. After transplanting, however, they became greater than those of non-grafted peppers irrespective of temperature. Grafting and higher NT increased the number of nodes with sympodial branch.

The total weight, number, and mean fruit weight of marketable and total fruit decreased rapidly with the decline of NT (Table 2.6). The ratio of abnormal fruit to total increased with the decline of NT. The total weight and number of abnormal fruits at 13°C NT were bigger than those of at 18°C NT.

The cumulative yield of marketable fruits during cultivation period is shown in Fig. 2.3. The yield decreased rapidly with the

decline of NT. At 8°C NT, pepper suffered seriously even though they did not perish. The yields of peppers were below 10% of that at 18°C NT. There was no yield in the late production period. It reduced below half when the NT was lowered from 18 to 13°C. The yield at 8 and 18°C NT was the lowest in non-grafted peppers.

Higher NT and grafting slightly advanced the days to harvest, and increased fresh weight, and fruit width (Table 2.7). Grafting and NT did not affect the fruit length.

DISCUSSION

The growth responses of genotype seedlings to different night temperature

RGRs of genotypes could be categorized into three types according to the response to NT. They were stable irrespective of NT in type III. Accordingly the root growth of the type III was considered to be little affected by low temperature. It means that they have higher adaptability to the low temperature than other types. However, T/R ratio in the type I was constant under low and mild temperature but it decreased at higher temperature. Therefore, it was considered that the root growth of the genotypes in type I was more stimulated than the shoot by high temperature. But the RGRs in the both types were linear under the range of temperatures. While the genotypes in type I showed different values in the slope, root growth was promoted more than shoot growth under higher temperature in the view of the T/R ratio.

It was reported that low temperature interfered with water and nutrient uptake of plants and caused the reduction of growth (Ahn et al., 1999). However, some plants or cultivars grow well at relatively low temperature (Lee and Chung, 2005). The low-root temperature resistance of cucumbers grafted onto figleaf gourd is exerted through the water absorption capacity of its root system (Ahn et al., 1999). The different tolerance levels among cucumber cultivars are the result of changes in the characteristic of leaf surface structure,

different absorption levels, and rapid metabolism of toxic compounds (Kuk and Shin, 2007). Some cultivars of rice and wheat with high respiratory homeostasis (the ability to maintain similar respiratory rates, even when grown at different temperatures) showed a tendency to maintain their RGR, irrespective of growth temperature (Kurimoto et al., 2004).

'Kataguruma' and 'Koregon PR-380' in type III showed higher RGR than the other genotypes except 'Taibyo VF', irrespective of growth temperature. Unlike other genotypes, 'Kataguruma' did not show the wilting of leaves, common response to low temperature. Accordingly, 'Kataguruma' and 'Koregon PR-380' are considered to be suitable rootstocks for the cultivation of low temperature condition.

The RGR of total dry weight at 10°C NT was the greatest in eggplant rootstock 'Taibyo VF'. Gao et al. (2005) reported that the tolerance of eggplant seedlings to low temperature was verified in five rootstocks and 'Taibyo VF' showed moderate cold tolerance. Furthermore, eggplants grafted onto 'Taibyo VF' grew better at low temperature of 18 and 21°C than non-grafted plants (Edelstein, 2004). Grafting between different species (interspecific) and even genera (inter-generic) is well accepted in vegetable crops grown from grafted transplants (Lee and Oda, 2003). However, the peppers grafted to 'Taibyo VF' could not survive.

The growth and yield of the grafted peppers by rootstock and night temperature

The growth of grafted transplants is generally about one-week slower than non-grafted transplants because the process of grafting and acclimatization causes the delay of growth. Among grafted transplants, the growth of peppers grafted onto 'Kataguruma' was better. After transplanting, the growth of peppers 50 days after transplanting was inhibited by low NT. It was also affected by grafting. Stem diameter and root dry weight of branch of grafted peppers became greater than those of non-grafted peppers irrespective of temperature.

The yield decreased rapidly and the ratio of abnormal fruit to total increased with the decline of NT (Table 2.6). Choi et al (2001) reported that low minimum temperature (LMT) caused the rapid decrease of total and marketable yield and the increase of small and abnormal fruits. They considered that the reason was because the number of flower, pollination and fertilization decreased and the growth rate of fruit slowed down by LMT. Néji et al. (2003) reported that low NT of 12°C delayed flowering and reduced fruit set. Shaked et al. (2004) also reported that the low NT of 10±2°C caused a decrease in the number of pollen grains and the reduction of their germinability in pepper and that the fruits were smaller, seedless and misshapen in plants grown at low NT. Therefore, the growth and productivity of peppers require proper shoot growth and the fertilization of flowers by warming the air as well as the root

activity by underground heating or grafting.

The cumulative yield of marketable fruits also decreased rapidly with the decline of NT. It was interesting that no significant effects of rootstocks on the production of marketable fruit were found. Nevertheless, it was approved that the grafting onto rootstocks yielded more marketable fruits compared to non-grafted pepper at any temperature except 'PR-Power' at 13°C NT.

We expected that rootstocks with higher adaptability to low temperature would yield good growth and productivity. However, rootstocks with tolerance to low temperature did not significantly improve the growth and productivity under low temperature condition though the grafting did. One of possible explanation is that low temperature tested in the latter was 8°C which was lower than the temperature of resistance screening of rootstock, and it might be too low to exhibit their ability because critical temperature for growth of pepper at the lower limit is known to be 15°C (Lim et al., 2006). Therefore, the effect of rootstocks might be offset by lower temperature below critical limit. On the other hand, it was reported that grafted plant showed less activity than the own-rooted in figleaf gourd (Ahn et al., 1999). Ahn et al. (1999) reported that grafted figleaf gourd roots showed less activity than own-rooted ones. It was considered that the root activity was affected by the grafting and scions. Moreover, because all of rootstocks used for green pepper production are the same species (*C. annuum* L.) as pepper, they require similar temperature condition and their root

ability may be not as strong as that of figleaf gourd which is different genus from cucumber. In that paper, the effect of grafting against non-grafting was positive.

Liebig (1984) suggested that overcoming low temperature by grafting was suitable for short-term period only. As shown in Fig. 2.3, peppers were harvested covering the four-month period, and the different adaptability to low temperature among rootstocks would be offset by long harvest period. An intensive examination must be taken to elucidate the effect of rootstocks on the growth under economically low temperature as shown in the prior experiment. Further study will also be needed to assess a detailed examination of both the species of scions and the cropping pattern.

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Table 2.1. Genotypes tested for the tolerance to low temperature.

Genotype	Species	Commercial use[z]	Source
Asaggy	*C. annuum*	F	Nongwoo Bio Co., Ltd., Korea
A7	*C. annuum*		NIHHS[y]
Kataguruma	*C. annuum*	RS	Sakada Korea Seed Co., Ltd., Korea
Konesianhot	*C. annuum*	RS	Heungnong Seed Co. (Seminis Inc.), Korea
Koregon PR-380	*C. annuum*	RS	Koregon Seed Co., Ltd., Korea
Manitta	*C. annuum*	F	Nongwoo Bio Co., Ltd., Korea
Nokkwang	*C. annuum*	F	Heungnong Seed Co. (Seminis Inc.), Korea
PI	*C. annuum*		NIHHS
PR-power	*C. annuum*	RS	Nongwoo Bio Co., Ltd., Korea
R-safe	*C. annuum*	RS	Heungnong Seed Co. (Seminis Inc.), Korea
Saengreok 211	*C. annuum*	F	NIHHS
Saengreok 213	*C. annuum*	F	NIHHS
Shinhong	*C. annuum*	F	Nongwoo Bio Co., Ltd., Korea
Tantan	*C. annuum*	RS	Nongwoo Bio Co., Ltd., Korea
Taibyo VF	*Solanum melongena*	RS	Takii & Co., Ltd., Japan

[z]Fruit production(F) or Rootstock (RS)

[y]National Institute of Horticultural & Herbal Science, Korea.

Table 2.2. Dry weight (DW) of genotype seedlings grown at different night temperatures (NT) 70 days after sowing.

Genotype	NT / DW (g)	10°C		15°C		20°C	
		Total	Root	Total	Root	Total	Root
Asaggy		0.387ab[z]	0.083a-c	0.723b-d	0.137a-d	0.887ab	0.237a
A7		0.333bc	0.073a-c	0.433g	0.100ef	0.713c-e	0.233a
Kataguruma		0.497a	0.113a	0.633de	0.127b-e	0.760a-d	0.147c
Konesianhot		0.390ab	0.087a-c	0.587e	0.117d-f	0.833a-d	0.177bc
Koregon PR-380		0.450ab	0.090a-c	0.757bc	0.140a-d	0.920ab	0.177bc
Manitta		0.417ab	0.100ab	0.870a	0.167a	0.710c-e	0.163bc
Nokkwang		0.347bc	0.073a-c	0.640de	0.123c-f	0.893ab	0.230a
PI		0.270c	0.053c	0.690cd	0.153a-c	0.687de	0.170bc
PR-Power		0.437ab	0.103ab	0.753bc	0.160ab	0.870a-c	0.217ab
R-Safe		0.423ab	0.093a-c	0.863a	0.163a	0.947a	0.187a-c
Saengreok 211		0.423ab	0.097ab	0.563ef	0.140a-d	0.627e	0.137c
Saengreok 213		0.397bc	0.073a-c	0.470fg	0.093f	0.720c-e	0.173bc
Shinhong		0.397ab	0.080a-c	0.800ab	0.150a-d	0.757b-e	0.153c
Tantan		0.343bc	0.070bc	0.810ab	0.143a-d	0.893ab	0.213ab

[z]Means separation within columns by Duncan's multiple range test ($P \leq 0.01$)

Table 2.3. Estimated coefficients (CE) of linearized growth curve[z] logarithmically transformed from exponential

equation of genotype seedlings grown at different NTs 70 dayd after sowing.

Genotype	NT / CE	10°C			15°C			20°C		
		$\ln(w_0)$	rm	R^2	$\ln(w_0)$	rm	R^2	$\ln(w_0)$	rm	R^2
Asaggy		-4.921 [y]	0.057	0.998	-4.587	0.062	0.971	-5.004	0.069	0.985
A7		-5.811	0.068	0.973	-5.623	0.073	0.973	-5.847	0.078	0.977
Kataguruma		-5.539	0.069	0.995	-5.477	0.075	0.954	-5.376	0.076	0.993
Konesianhot		-5.562	0.068	0.989	-5.079	0.070	0.974	-5.239	0.075	0.988
Koregon PR-380		-5.539	0.069	0.991	-5.318	0.073	0.984	-5.385	0.079	0.961
Manitta		-5.548	0.066	0.986	-4.947	0.068	0.987	-5.125	0.070	0.990
Nokkwang		-5.244	0.061	0.984	-5.267	0.072	0.984	-5.389	0.078	0.989
PI		-4.566	0.046	0.951	-4.806	0.063	0.997	-5.007	0.068	0.991
PR-Power		-5.083	0.060	0.994	-5.159	0.072	0.980	-5.061	0.073	0.984
R-Safe		-5.111	0.061	0.962	-5.128	0.073	0.982	-5.310	0.079	0.975
Saengreok 211		-5.365	0.065	0.994	-5.407	0.074	0.960	-5.478	0.078	0.976
Saengreok 213		-5.317	0.064	0.989	-5.178	0.070	0.959	-5.288	0.075	0.981
Shinhong		-5.382	0.064	0.993	-5.526	0.079	0.985	-5.402	0.079	0.977
Tantan		-4.593	0.052	0.982	-5.071	0.073	0.980	-5.288	0.077	0.989

[z] $\ln(y) = \ln(w_0) + rm \cdot t$, where y=dry mass (g), rm=RGR ($g \cdot g^{-1} \cdot day^{-1}$), and t=growth time (day).

[y] All coefficients are significant at level $P \leq 0.001$.

Table 2.4. The graft-take and growth of grafted peppers 'Nokkwang' 65 days after sowing (before transplanting) as influenced by grafting.

Rootstock	Growth type	Graft-take (%)	Shoot length (cm)	Stem diameter (mm)	No. of leaves	Leaf area (cm^2)	Dry weight (g/plant)		
							Leaves	Stem	Root
Non-graft	Control	-	48.3a[z]	3.54a	13.7a	133.7a	0.407a	0.500a	0.201a
Tantan	I	98	36.5c	2.91b	11.7bc	109.6b	0.310bc	0.337b	0.140b
PR-Power	II	98	39.3b	2.79b	10.3c	113.4b	0.293bc	0.320b	0.137b
Kataguruma	III	95	39.3b	2.85b	12.7ab	108.3b	0.340b	0.343b	0.120b
Koregon PR-380	III	92	37.7c	2.78b	11.3bc	90.0c	0.277c	0.300b	0.110b

[z] Mean separation within columns for each scion variety by least significant test ($P \leq 0.05$).

Table 2.5. The growth of grafted peppers 'Nokkwang' 50 days after transplanting[z] as influenced by rootstocks and temperature conditions.

Rootstock	NT (°C)	Stem diameter[y] (mm)	Leaf area (cm^2)	No. of nodes[x]	Total dry weight (g)
Non-graft	8	9.3±2.0[w]	1168± 618	6.3±0.8	18.1± 8.8
	13	7.9±1.5	903± 371	6.0±1.0	12.2± 7.5
	18	8.5±0.6	1899± 755	7.7±0.6	18.9± 8.6
Tantan	8	11.6±1.3	1308± 264	7.7±0.6	19.3± 1.5
	13	10.7±0.9	2148± 308	8.0±0.0	25.4± 2.4
	18	12.7±1.2	2858±1272	8.3±0.6	29.1±10.7
PR-Power	8	9.2±0.8	1201± 629	7.0±0.0	17.4± 5.1
	13	10.8±1.0	2244± 541	7.3±1.2	25.7± 5.4
	18	9.7±1.3	3089± 744	8.3±0.6	28.8± 3.7
Kataguruma	8	10.1±0.5	1240± 212	7.3±0.6	20.4± 1.4
	13	11.0±1.2	2107± 526	8.3±0.6	27.2± 6.2
	18	10.2±1.2	2309±1119	7.3±1.2	23.2±11.5
Koregon PR-380	8	11.0±2.8	1279± 600	7.3±0.6	19.6± 6.9
	13	11.7±2.1	2132± 720	8.0±0.0	25.1± 9.2
	18	12.2±0.9	3174±635	9.3±1.5	30.7± 6.4
Rootstock (RS)		***	ns	**	ns
Temperature (T)		ns	***	**	*
RS*T		ns	ns	ns	ns

[z]20 days after heating.

[y]Measurement at 1 cm below the graft union.

[x]Number of nodes with sympodial branch of one side.

Mean ± standared deviation (n=3).

Table 2.6. The yield of grafted peppers 'Nokkwang' as influenced by rootstocks and NT condition.

Rootstock	NT (°C)	Fruit yield (g/plant)		No. of fruit		Mean fruit weight (g/fruit)	
		Marketable	Total	Marketable	Total	Marketable	Total
Non-graft	8	61± 7[z]	96± 10	6± 1	12± 2	8.1±0.9	10.7±1.1
	13	540±329	1,041±377	44±26	104±21	9.8±1.6	12.5±1.1
	18	1,034±241	1,445±307	83±25	122±34	12.1±1.0	12.7±1.1
Tantan	8	96± 29	132± 30	7± 2	13± 3	9.8±0.7	12.8±1.3
	13	641±262	1,325±454	56±21	127±41	10.4±0.3	11.4±0.5
	18	1,186±120	1,666±205	91± 8	136±19	12.2±0.3	13.0±0.2
PR-Power	8	84± 7	117± 10	8± 1	15± 2	7.9±1.5	10.2±1.3
	13	480±176	1,060±336	45± 9	116±20	9.0±1.4	10.7±2.8
	18	1,255±183	1,618±185	96±21	132±23	12.3±0.7	13.2±0.9
Kata-guruma	8	88± 18	133± 21	8± 1	15± 1	8.6±0.8	10.1±1.2
	13	696±312	1,272±271	62±21	132± 4	9.6±2.0	10.9±1.6
	18	1,171±154	1,560±142	92±12	132±10	11.8±0.4	12.7±0.2
Koregon PR-380	8	80± 28	119± 27	8± 2	14± 2	8.4±0.7	10.3±1.8
	13	574± 61	1,083±190	52± 4	109±27	10.1±1.3	11.1±1.0
	18	1,174±147	1,577±213	91±10	129±15	12.2±0.5	12.9±0.5
Rootstock(RS)		ns	ns	ns	ns	ns	ns
Temperature (T)		***	***	***	***	***	***
RS*T		ns	ns	ns	ns	ns	ns

[z]Mean ± standared deviation (n=3).

Table 2.7. The fruit characteristics of grafted peppers 'Nokkwang' as influenced by rootstocks and night temperature conditions.

Rootstock	NT (°C)	Days to harvest[z]	Fresh weight (g)	Fruit length (cm)	Fruit width (mm)
Non-graft	13	45± 7[y]	11.98±3.74	11.06±2.76	15.74±1.50
	18	43± 3	18.12±5.53	12.70±2.17	17.73±1.77
Tantan	13	41± 9	11.27±3.16	10.28±1.94	15.99±1.13
	18	41± 7	14.42±2.66	11.50±1.41	17.09±1.23
PR-Power	13	42± 8	12.94±4.83	10.71±1.70	16.00±1.29
	18	42± 4	11.18±4.52	9.79±2.90	15.76±1.88
Kataguruma	13	41±10	14.99±5.05	11.79±1.89	16.52±2.23
	18	40± 6	18.93±2.87	12.33±0.93	18.26±1.58
Koregon PR-380	13	43± 8	10.49±3.21	10.30±2.73	15.84±0.80
	18	39± 6	16.75±3.97	11.86±1.03	18.69±2.56
Rootstock (RS)		ns	*	ns	ns
Temperature (T)		***	***	ns	***
RS*T		ns	ns	ns	ns

[z]Days from anthesis to harvest.
[y]Mean ± standared deviation.

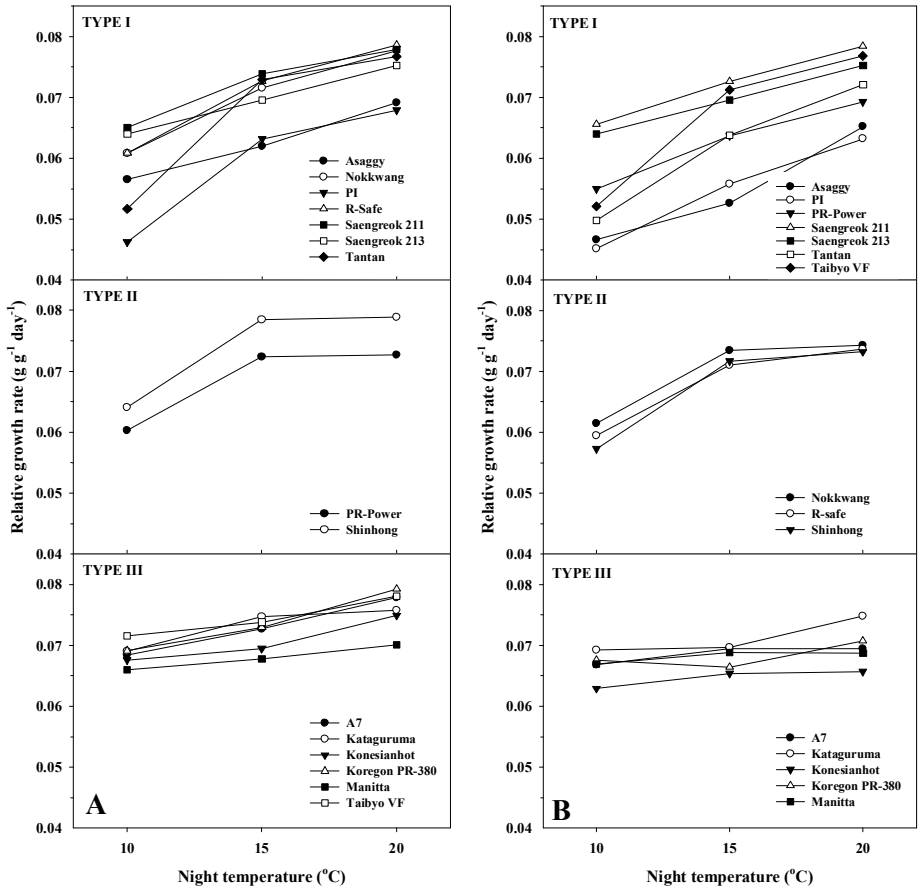

Fig. 2.1. Relative growth rate of the total plant (A) and the root (B) of pepper seedlings grown at different night temperatures.

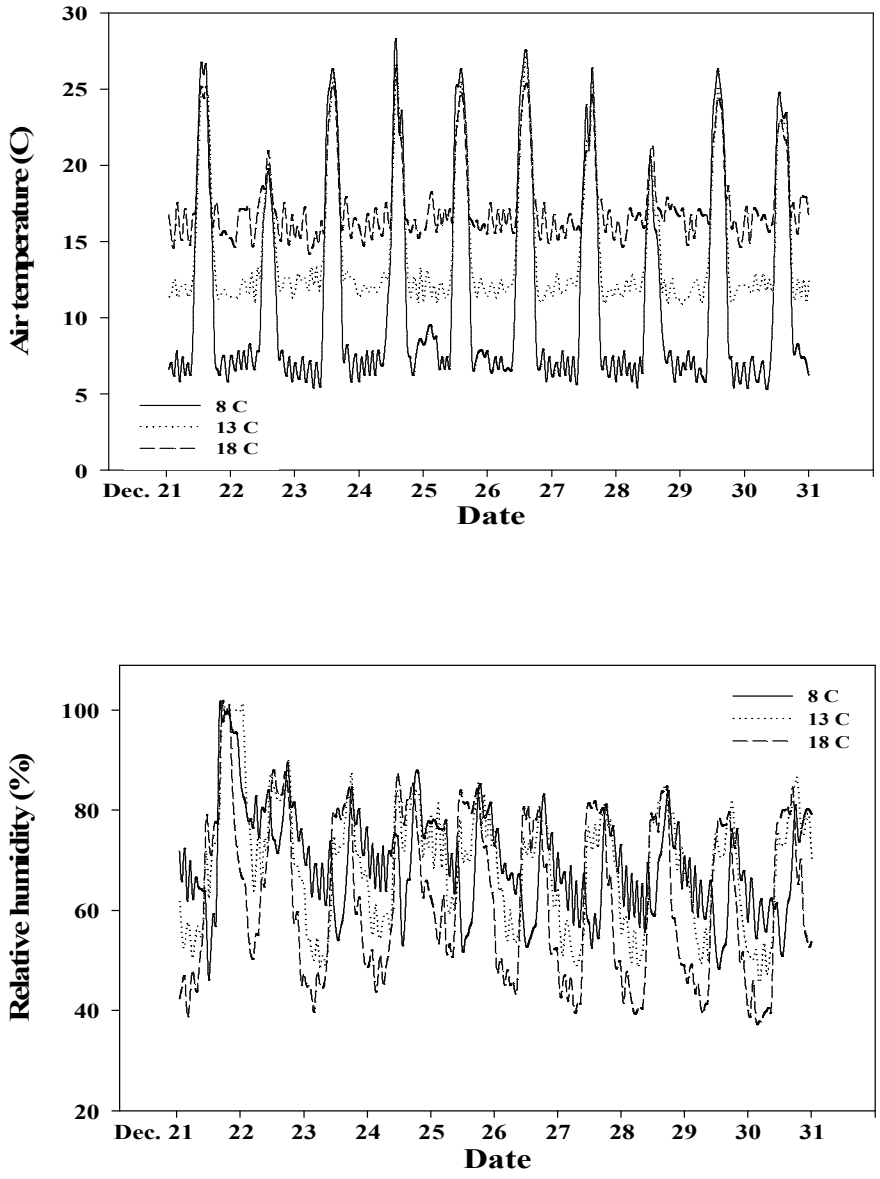

Fig. 2.2. Air temperature and relative humidity in the greenhouse during the cultivation period (from Dec. 21 to Dec. 31 2004).

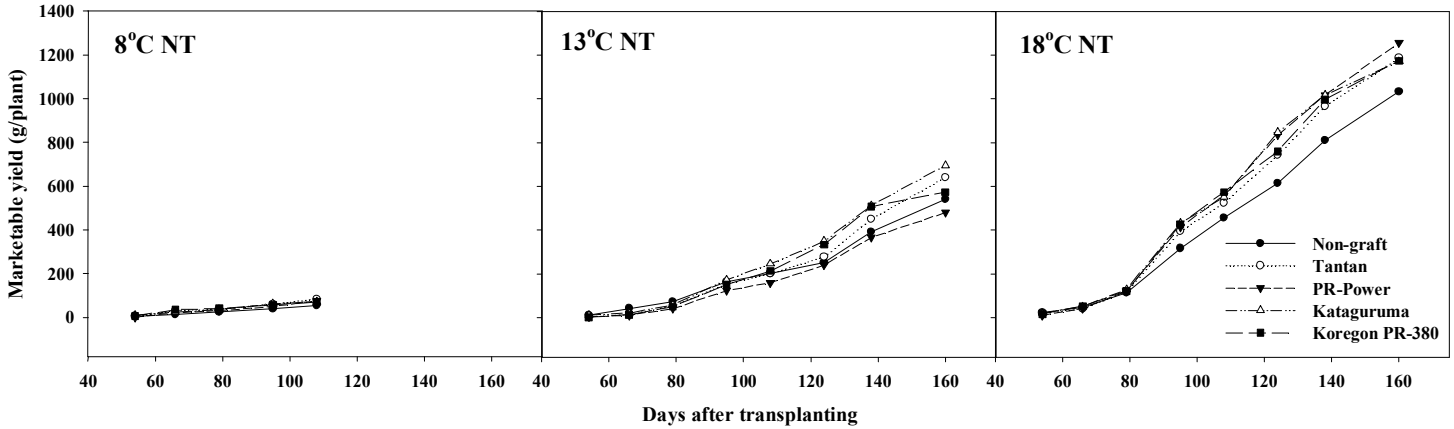

Fig. 2.3. Cumulative yield of marketable fruits of pepper 'Nokkwang' as influenced by grafting and night temperature conditions.

CHAPTER III

EFFECTS OF GRAFTING ON FRUIT QUALITY OF PEPPER

ABSTRACT

The objective of this study was to examine the effects of grafting on fruit quality of peppers. The fruit quality of peppers grafted onto different rootstocks was investigated during two harvest periods (semi-forcing culture and retarding culture). Three cultivars of pepper were used as scions and five commercial rootstocks that are known to be resistant to phytophtora blight were used as rootstocks. The apparent fruit quality (fruit size, weight, flesh thickness) and texture property were influenced by grafting. However, the fruit characteristics of rootstock did not affect the fruit characteristics of scion grafted onto that rootstock. Fruit quality parameters were different as affected by scion variety and cropping pattern. Fruit quality was also affected by harvest time. In conclusion, apparent quality and textural property of pepper fruits grafted onto different rootstocks were influenced by grafting, harvest time, and cropping pattern. Accordingly, rootstock/scion combination, the scion variety and the cropping pattern must be carefully chosen to get the desired optimal fruit quality.

INTRODUCTION

Grafting is the union of two or more pieces of living plant tissues that grow as a single plant (Lee and Oda, 2003; Rouphael et al., 2010). It is usually used to reduce infections by soil-borne pathogens and to enhance the tolerance against abiotic stresses such as low and high temperatures, salt, flooding, etc. (Colla et al., 2010; King et al., 2008; Louws et al., 2010; Rivero et al., 2003; Venema et al., 2008; Yetisir et al., 2006). It has been reported that grafting using proper rootstocks can minimize the problems associated with successive cropping and stress tolerance. Vegetable grafting is now common in Asia, parts of Europe, and the Middle East. In Korea, about 90% of cucurbitaceous vegetables and 30% of Solanaceous vegetables are grafted onto various rootstocks (Lee et al., 2010).

Regarding the changes in fruit quality by grafting, there are several conflicting reports whether grafting effects are advantageous or deleterious (Lee et al., 2010; Davis et al., 2008a). Vegetable fruit quality for fresh consumption can be determined in terms of appearance (size, shape, color, absence of defects and decay), firmness, texture, flavor (sugars, acids and aroma volatiles) and health-related compounds (desired compounds: minerals, vitamins, and carotenoids as well as undesired compounds: heavy metals, pesticides and nitrates) (Rouphael et al., 2010). It has been reported that the size, shape, color, texture, flavor, pH, sugar, carotenoid content of fruits can be affected by grafting and the type of rootstock used (Alexopoulos et al., 2007; Bruton et al., 2009;

Davis et al., 2008a, b; Tsaballa et al., 2012). More and larger fruits were reported in pepper (Gisbert et al., 2010), watermelon (Miguel et al., 2004) and so on when grafting was adopted. Firmness and rind thickness, which are the typical attributes used to describe the fruit texture were also reported to increase in watermelon (Yetisir et al., 2003) when it was grafted. In tomato, soluble solid content and titratable acidity were improved by grafting (Flores et al., 2010). However, abnormal fruit qualities including reduced fruit soluble solid content, fruit fermentation, poor texture, and off-taste were reported for grafted oriental melon and watermelon (Lee and Oda, 2003).

Pepper is the most important and widely cultivated vegetable in Korea. It is successively produced in the same field or greenhouse. In order to prevent soil-borne diseases in continuous cropping, peppers are grafted onto the rootstocks that have resistance to phytophthora blight. The rootstocks used for pepper grafting were the same species. The present ratio of graft utilization in peppers is 5-10% (Lee et al., 2010) and the use of grafted peppers is expected to increase. Grafted peppers are mostly cultivated in greenhouse for five to six months, and fruits are harvested continuously in green and unripe form. Some greenhouse pepper growers noted that the fruit length of grafted peppers is shorter or longer than non-grafted peppers, depending on rootstock genotypes. However, there is no information on how grafting and rootstocks affect the fruit quality of peppers. This study was conducted to examine the effects of grafting and the use of different rootstocks on fruit quality of peppers.

MATERIALS AND METHODS

Plant materials

Three cultivars of pepper, 'Nokkwang' (Seminis Inc.), 'Saengsaeng Matkkwari', and 'Shinhong' (Nongwoo Bio Co., Ltd.) were used as scions. Five commercial rootstocks resistant to Phytophthora blight, 'Kataguruma' (Sakada Korea Seed Co., Ltd.), 'Konesianhot' (Seminis Inc), 'Koregon PR-380' (Koregon Seed Co., Ltd.), 'PR-power' (Nongwoo Bio Co., Ltd.), and 'Tantan' (Nongwoo Bio Co., Ltd.) were used as rootstocks. Non-grafted or auto-grafted (scion and rootstock were same species) scion seedlings served as controls.

Growing grafted pepper transplants

Experiments were conducted during the two harvest periods (semi-forcing culture and retarding culture). Grafted peppers were cultivated from April to August in semi-forcing and from September to March the following year in retarding culture.

Seeds of scion were sown seven days (Semi-forcing culture) and four days (Retarding culture) after sowing seeds of rootstocks in order to obtain seedlings of similar diameter with rootstocks. Seeds of scion and rootstock were sown into the 72-cell plug trays (*W* 280 × *L* 540 × *H* 45 mm, Bumnong Co., LTD., Jeongeup, Korea) filled with commercial growing substrate (BM 2, Berger Group Ltd., Quebec, Canada). To promote germination, the plug trays were

wrapped with vinyl chloride resin film and then placed in the germination chamber at a temperature of 28°C. After four days, the germinated seedlings were moved to a greenhouse and placed on the bench. Seedlings were watered daily. A nutrient solution (electric conductivity 1.5 $dS \cdot m^{-1}$ with 'Hanbang' for seedling, N-P-K-Ca-Mg=8.0-2.4-2.4-4.8-1.6 $me \cdot L^{-1}$, Coseal Co., Ltd., Gunsan, Korea) was applied. Application frequency of the nutrient solution was determined depending on the plants growth stage (RDA, 2008b).

When seedlings have already developed five or six true leaves at 36 days (semi-forcing culture) and 31 days (retarding culture) after sowing of scion seeds, scions were grafted onto rootstocks. The epicotyl of scion and rootstock were cut below 1cm from first true leaf using a razor blade. After placing the scion on the rootstock, the grafted position was fixed with ordinary grafting clip by slice grafting method. After grafting, plants were healed and acclimatized in the tunnel covered with double-layered plastic film and shade cloth in the greenhouse for one week (Lee et al., 2010). In order to prevent grafted plants from wilting by the excessive transpiration and to enhance healing, the tunnel was closed for the first three or four days of healing and acclimatization period. For the next three or four days, the opening and closing of the tunnel were done based on the condition of grafted plants and weather. This was done for the acclimatization of grafted plants to environmental condition outside tunnel (RDA, 2008b). After the end of healing and acclimatization, grafted transplants were grown on the bench inside

a glasshouse.

Cultivation of grafted peppers in a greenhouse

Experiments were conducted during the two harvest periods (semi-forcing culture and retarding culture). Grafted peppers were cultivated from April to August in semi-forcing culture and from September to March the following year in retarding culture. The grafted and non-grafted transplants were transplanted in a greenhouse covered with polyethylene film at 38 days (April 6) and 31 days (September 27) after grafting, respectively. After soil test, pre-plant broadcast N-P-K-manure was applied to soil according to fertilizer recommendation for pepper (NAAS, 2006). Three rows were made and each row was mulched with black plastic film prior to planting. Two rows of drip irrigation tubing were placed under the plastic mulch in each row. One row of pepper transplants were transplanted between drip irrigation tubing (35 cm between plants within row). The experiment was arranged in a randomized complete block design with three replicates.

Plants were pruned to a 4-stem training system. Irrigation and additional fertilizer application was carried out using standard procedures for pepper cultivation (RDA, 2008a). Additional fertilizer (‘Hanbang’ for green pepper cultivation, N-P-K-Ca-Mg=10.8-8-3-7-4-2 $me \cdot L^{-1}$, Gunsan, Korea) was applied depending on the growth conditions through a drip irrigation system. Air temperature and relative humidity data in a greenhouse were

collected at an interval of 30 minute with a data logger (TR-72U, T&D Co., Matsumoto, Japan). The greenhouses were heated by a warm air furnace system containing soil heating with electrothermal wire from 6 April to 6 May in semi-forcing culture, and from 10 December until the end of experiment in retarding culture, respectively. The minimum temperature of the greenhouse was set to 15°C.

The sized green fruits were harvested weekly from June to August (Semi-forcing culture) and from December to March of the following year (Retarding culture). All the fruits from each plant were harvested, counted and weighed. Fruits (ten fruits per each replicate) were selected and used for physical property test of fruit every month. Each fruit (five fruits per each replicate) was sliced into two, (longitudinal) and the flesh thickness at the center of the fruit was measured using a caliper gauge. Textural property of the fruit was measured by using a texture analyzer (EZ Test-100N, Shimadzu Co., Kyoto, Japan) equipped with a 5 mm-diameter plunger. Compression force into the flesh at 7 mm depth was monitored at 2 $mm \cdot s^{-1}$ speed.

Statistical analysis

The data were subjected to one-way and two-way analyses of variance (ANOVA). Statistical computations were carried out using the SigmaPlot version 11 (Systat Software Inc., San Jose, CA, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) software.

RESULTS

Temperature and relative humidity during cultivation

The average daily maximum, mean, and minimum temperatures were recorded at 33.0, 23.8, and 17.6°C during semi-forcing culture, and 30.4, 19.0, and 13.0°C during retarding culture, respectively (Fig. 3.1). In semi-forcing culture, the daily mean and minimum temperature went up steadily. The daily mean temperature from late May and the daily minimum temperature from late June rose above 20°C. In retarding culture, the temperatures hovered around average values. The average daily maximum, mean, and minimum relative humidity were 94.2, 73.6, and 41.0% during semi-forcing culture, and 83.7, 60.6, and 36.0% during retarding culture, respectively. In semi-forcing culture, relative humidity went up steadily. The daily maximum relative humidity reached 100% from late June until the end of experiment. May, and the daily minimum temperature from late June rose above 20°C. In retarding culture, relative humidity fluctuated around average values.

Yield of grafted peppers

There were no significant differences in marketable yield among peppers grafted onto different rootstocks (Fig. 3.2). The average marketable yields of ‘Nokkwang’ were 1,706g per plant in semi-forcing culture, and 1,036g in retarding culture. The average marketable yields of ‘Saengsaeng Matkkwari’ were 1,871 g per

plant in semi-forcing culture, and 709 g in retarding culture. The average marketable yields of 'Shinhong' were 458 g per plant in semi-forcing culture, and 699 g in retarding culture.

Apparent fruit quality of grafted peppers

The apparent quality of pepper fruit was influenced by grafting and harvest time. The average fruit length, fruit width, flesh thickness, fruit weight, and dry matter of 'Nokkwang' were 114.3, 15.0, and 1.99 mm, 10.58 g, and 9.23%, respectively (Tables 3.1 and 3.2). Fruit size and fruit dry matter in semi-forcing culture were a little bigger than those in retarding culture. In semi-forcing culture, fruit width and flesh thickness were affected by grafting. The width and flesh of fruits of non-grafted peppers were thickest. length, shape index and weight of fruits decreased but width and flesh thickness increased at later stage of cultivation. In retarding culture, fruit length, weight, and dry matter were affected by grafting, and ranged from 108.9 to 122.4 mm, from 7.3 to 13.99 g, and from 7.7 to 12.08%, respectively, depending on rootstock genotypes. Fruit length and weight of pepper grafted onto 'Konesianhot' were found to be the highest. Fruit size, flesh thickness, fruit weight, and dry matter increased at later stage of cultivation.

The average fruit length, fruit width, flesh thickness, fruit weight, and dry matter of 'Saengsaeng Matkkwari' were 89.0, 12.2 mm, and 1.29 mm, 4.29 g, and 9.74%, respectively (Tables 3.3 and 3.4). Fruit

size, weight and flesh thickness in semi-forcing culture were greater than those in retarding culture. In semi-forcing culture, fruit width and dry matter were affected by grafting. Fruit width, fruit shape index, flesh thickness, and weight were affected by harvest time. Fruit width decreased but fruit shape index and weight increased at later stage of cultivation. In retarding culture, fruit size, fruit shape index, and dry matter were affected by grafting. Fruit length of non-grafted pepper and width of pepper grafted onto 'PR-power' were greatest. Fruit width, flesh thickness, and fruit weight increased at later stage of cultivation.

The average fruit length, fruit width, flesh thickness, fruit weight, and dry matter of 'Shinhong' were 84.6, 12.4, and 1.8 mm, 5.54 g, and 10.7%, respectively (Tables 3.5 and 3.6). Fruit size, weight and flesh thickness in semi-forcing culture were greater than those in retarding culture. In semi-forcing culture, flesh thickness and dry matter were affected by grafting. The flesh of pepper fruits grafted onto 'Kataguruma' was thickest, but the dry matter was lower. Fruit length and weight decreased at later stage of cultivation. In retarding culture, fruit length, weight, and dry matter were affected by grafting. Fruit size and weight were greatest in pepper grafted onto 'PR-power', and smallest in pepper grafted onto 'Kataguruma'. Fruit size, flesh thickness, fruit weight, and dry matter increased at later stage of cultivation.

Textural property of grafted peppers

The average hardness and strength of ‘Nokkwang’, ‘Saengsaeng Matkkwari’, and ‘Shinhong’ were 676, 7068, 419, 3880, 497, and 5375 $kN \cdot m^{-2}$, respectively (Tables 3.1, 3.2, 3.3, 3.4, 3.5, and 3.6). The hardness and strength of ‘Nokkwang’ in retarding culture were greater than those in semi-forcing culture. However, the hardness and strength of ‘Saengsaeng Matkkwari’ in semi-forcing culture were greater than those in retarding culture. The hardness of ‘Nokkwang’ pepper fruit semi-forcing culture was affected by grafting, and ranged from 534 to 574 $kN \cdot m^{-2}$, depending on rootstock genotypes. The hardness and strength of ‘Saengsaeng Matkkwari’ increased at later stage of cultivation. In retarding culture, the hardness of ‘Nokkwang’ and the hardness and strength of ‘Shinhong’ also increased at later stage of cultivation.

DISCUSSION

The fruit quality of vegetables is affected by cultivar genotype, environmental conditions (light, temperature, humidity, atmospheric CO_2, and air pollutants), and agricultural practices such as water management, fertilization strategies, growth and development regulators, pruning, growing systems, harvesting stage, and grafting (Rouphael et al., 2010). There are several conflicting reports on changes in fruit quality due to grafting and whether grafting effects are advantageous or deleterious (Davis et al., 2008a; Flores et al., 2010).

Positive effects of grafting are reported in tomato and watermelon, including the increase of fruit size, firmness, total soluble solids (TSS) content, and titratable acidity (Flores et al., 2010; Miguel et al., 2004). On the contrary, other reports showed abnormal fruit quality in eggplant, melon, and watermelon, including poor texture, reduction of contents of TSS and vitamin C and firmness (Arvanitoyannis et al., 2005; Lee and Oda, 2003; Traka-Mavrona et al., 2000). There were also reports that demonstrated that grafting had no effect on fruit shape index, thickness of rind, texture, lycopene content or TSS in cucumber, tomato, and watermelon (Alan et al., 2007; Bruton et al., 2009; Khah et al., 2006; Sakata et al., 2008).

In this study, the fruit qualities of three pepper varieties grafted onto different rootstocks were investigated during two harvest

periods (semi-forcing culture and retarding culture). The apparent fruit quality (fruit size, weight, flesh thickness) and texture property were influenced by grafting (Tables 3.1, 3.2, 3.3, 3.4, 3.5, and 3.6). However, fruit quality parameters were affected by grafting with different scion varieties and cropping patterns. In the case of 'Nokkwang', fruit width and flesh thickness were affected by grafting matter in semi-forcing culture, however, fruit length, weight, and dry matter were the ones affected in retarding culture. In the case of 'Saengsaeng Matkkwari', dry matter in semi-forcing culture and fruit length, width, fruit shape index, and dry matter in retarding culture were affected by grafting. In the case of 'Shinhong', flesh thickness and dry matter in semi-forcing culture and, fruit length and dry matter in retarding culture were affected by grafting. Regarding the textural property, only the hardness of 'Nokkwang' pepper fruit in the semi-forcing culture was affected by grafting.

Some greenhouse pepper growers mentioned that fruit characteristics of rootstock such as fruit length affect the fruit characteristics of scion grafted onto that rootstock. According to them, pepper grafted onto rootstock of which fruit length is longer has longer fruits than the non-grafted ones. Tsaballa et al. (2012) also reported that grafting the round shaped pepper (scion) on the long shaped pepper (rootstock) changed the fruit shape of the scion, giving rise to elongated fruits different from the normal round shape fruit expected in the scion.

Among the rootstocks used in this experiment, the fruit of ‘Konesianhot’ was slender with a pointed apex and the fruit length was longest (Fig. 3). The fruit of ‘Kataguruma’ was bell pepper type with inverted-blunt and two-lobed apex, and its fruit length was shortest. The fruit length of ‘Tantan’ was shorter than that of ‘Koregon PR-380’ and ‘PR-power’. However, the fruit length of grafted peppers had no difference among rootstocks in semi-forcing culture, though the fruit of ‘Nokkwang’ grafted onto ‘Konesianhot’ was longest (118.5 mm) in retarding culture. In retarding culture, the fruit length of ‘Saengsaeng Matkkwari’ or ‘Shinhong’ grafted onto ‘Konesianhot’ was shorter or similar than that of non-grafted pepper. Accordingly, it is considered that grafting affects the apparent fruit quality, but the fruit characteristics of rootstock do not affect the fruit characteristics of scion grafted onto that rootstock (Tables 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, and Fig. 3.4).

There was a report that fruit length and weight of ‘Nokkwang’ decreased for later harvest time (Kwon et al., 2009). In this study, apparent fruit quality of ‘Nokkwang’ in semi-force culture was consistent with that report. However, in retarding culture, fruit length was not affected by harvest time and fruit weight increased for later harvest time (Tables 3.1 and 3.2). The apparent fruit quality of ‘Saengsaeng Matkkwari’ and ‘Shinhong’ was also different according to harvest time and harvest pattern (Tables 3.3, 3.4, 3.5, and 3.6). The differences in fruit quality by grafting may be attributable partly to different production environments (e.g.

light intensity, air temperature, vapor pressure deficit) and methods (e.g. soilless vs. soil culture, irrigation, and fertilization), type of rootstock/scion combinations used, rootstock/scion incompatibility, and harvest time (Davis et al., 2008; Lee et al., 2010; Rouphael et al., 2010). Exposure to high temperature often causes the reduction of yield and quality of many crops including vegetables (Erickson and Markhart, 2001; Firon et al., 2006). Temperature is the key factor in the development of pepper fruit and high temperature (38/30°C day/night) during the development of pepper fruit caused the poor quality including fruit size and weight (Pagamas and Nawata, 2008). Temperature and relative humidity condition were different between semi-forcing and retarding culture (Fig. 3.1). In semi-forcing culture, high temperature from late June was considered to be a reason for the decrease of fruit length and weight.

In semi-forcing culture, the cumulative yields of 'Nokkwang' and 'Saengsaeng Matkkwari' were similar (1,706 and 1,871 g/plant, respectively). However, the cumulative yield of 'Nokkwang' (1,036 g/plant) was greater than that of 'Saengsaeng Matkkwari' (709 g/plant) in retarding culture. 'Nokkwang' was known to be tolerant to low temperature (Lim et al., 2006) and each scion variety requires its own optimal environmental conditions. Rootstocks also have optimal temperature and moisture ranges (Davis et al., 2008a). Therefore, each rootstock/scion combination may respond differently to environmental conditions such as temperature and relative humidity.

In conclusion, apparent quality and textural property of pepper fruits grafted onto different rootstocks were influenced by grafting, the time of harvest, and cropping pattern. Accordingly, rootstock/scion combination, the scion variety and the cropping pattern must be carefully chosen to get the desired optimal fruit quality.

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Table 3.1. Analysis of variance (ANOVA) of rootstocks on the apparent quality and textural property of 'Nokkwang' pepper fruits at different harvest time in semi-forcing culture.

Rootstock	apparent fruit quality						textural property	
	Fruit length (mm)	Fruit width (mm)	Fruit shape index[z]	Flesh thickness (mm)	Fruit weight (g)	Dry matter (%)	Hardness ($kN \cdot m^{-2}$)	Strength ($kN \cdot m^{-2}$)
First harvest[y]								
Non-graft	123.4	14.6	8.47	1.58	10.86c[x]	8.90	569	5,551
Auto-graft	123.9	14.9	8.33	1.54	11.29bc	8.70	537	5,363
Kataguruma	123.8	14.5	8.56	1.57	11.68ab	8.67	545	5,446
Konesianhot	127.4	14.7	8.73	1.66	12.13a	8.49	568	5,331
Koregon PR-380	124.8	14.4	8.69	1.50	11.31bc	8.92	569	5,275
PR-power	124.3	14.6	8.53	1.59	11.09bc	8.90	550	5,577
Tantan	123.2	14.8	8.39	1.62	11.54a-c	8.75	547	5,135
Second harvest								
Non-graft	122.2a	16.0	7.77	2.12a	11.73a	9.11b	558ab	5,122a-c
Auto-graft	123.0a	15.5	7.93	1.85bc	11.53a	9.45ab	520b	5,082a-c
Kataguruma	119.4ab	15.3	7.88	2.03ab	11.26a	9.11b	609a	5,666a
Konesianhot	119.9ab	15.6	7.73	1.78cd	11.03a	9.65ab	616a	5,457ab
Koregon PR-380	115.7b	15.4	7.56	1.62 d	10.68ab	8.96b	525b	5,337ab
PR-power	115.8b	15.8	7.38	1.83bc	11.15a	9.08b	546ab	4,892bc
Tantan	118.5ab	15.4	7.74	1.97a-c	10.01b	9.87a	511b	4,562c
Third harves								
Non-graft	102.3	16.3	6.31b	2.42	9.76	9.80	581	5,676
Auto-graft	100.7	15.7	6.49ab	2.33	8.86	9.69	546	5,451
Kataguruma	103.3	15.3	6.83a	2.29	8.75	10.26	568	5,647
Konesianhot	99.5	15.8	6.34b	2.27	8.83	9.95	555	5,186
Koregon PR-380	100.5	15.8	6.39b	2.17	9.02	9.61	565	5,768
PR-power	102.3	16.4	6.32b	2.23	9.12	9.38	550	5,133
Tantan	105.3	15.7	6.75ab	2.30	8.80	9.27	551	5,320
P value								
Rootstock(A)	0.2479	0.0155	0.0689	<0.001	0.0603	0.6247	0.0344	0.0866
Harvest time(B)	0.0002	0.0082	<0.001	0.0002	0.0049	0.1987	0.9828	0.5503
A*B	0.0004	0.4687	0.0508	0.0566	0.0001	<0.001	0.2072	0.4894

[z]Fruit shape index= fruit width/fruit length.

[y]In semi-forcing culture, first, second, and third harvest were done on 21 June, 19 July, and 23 August, respectively.

[x]Mean separation within columns for each harvest time by Duncan's multiple range test ($P \leq 0.05$).

Table 3.2. Analysis of variance (ANOVA) of rootstocks on the apparent quality and textural property of 'Nokkwang' pepper fruits at different harvest time in retarding culture.

Rootstock	apparent fruit quality						textural property	
	Fruit length (mm)	Fruit width (mm)	Fruit shape index[z]	Flesh thickness (mm)	Fruit weight (g)	Dry matter (%)	Hardness ($kN \cdot m^{-2}$)	Strength ($kN \cdot m^{-2}$)
First harvest[y]								
Non-graft	108.9b[x]	13.4	8.2	1.80	7.95ab	8.07	694	8,912
Auto-graft	109.6b	13.2	8.3	1.79	7.77b	7.88	673	8,210
Kataguruma	109.2b	12.9	8.5	1.75	7.93ab	7.97	650	8,171
Konesianhot	116.9a	13.4	8.8	1.72	8.76a	7.70	640	7,219
Koregon PR-380	110.8b	13.3	8.4	1.80	7.67b	8.01	655	7,885
PR-power	110.2b	12.7	8.7	1.65	7.53b	8.06	646	7,560
Tantan	107.6b	12.9	8.4	1.90	7.30b	8.30	695	8,984
Second harvest								
Non-graft	112.0bc	15.4	7.3	2.18	11.62	8.84a	779	9,502
Auto-graft	109.9bc	15.5	7.1	2.16	11.49	8.60ab	759	10,232
Kataguruma	108.9c	15.6	7.0	2.21	11.18	8.83a	779	9,550
Konesianhot	116.1a	15.7	7.5	2.23	12.34	8.52ab	765	9,913
Koregon PR-380	111.8bc	15.4	7.3	2.24	11.45	8.94a	752	10,060
PR-power	111.1bc	15.3	7.3	2.32	11.38	8.14b	763	9,582
Tantan	113.8ab	15.3	7.5	2.23	11.59	8.53ab	770	9,879
Third harves								
Non-graft	118.7ab	15.3	7.8	2.24	11.91c	10.91c	888	8,354
Auto-graft	117.6ab	15.5	7.6	2.15	12.26bc	10.17c	937	7,723
Kataguruma	116.9b	15.5	7.6	2.17	12.13bc	11.81ab	980	9,571
Konesianhot	122.4a	15.9	7.7	2.25	13.99a	10.25c	908	7,710
Koregon PR-380	113.8b	15.3	7.5	2.12	11.98c	12.08a	953	8,248
PR-power	117.5ab	15.3	7.7	2.11	13.03b	10.37c	1,120	9,868
Tantan	118.0ab	15.4	7.7	2.17	12.88bc	11.06bc	940	7,947
P value								
Rootstock(A)	<0.001	0.0739	0.0872	0.6882	<0.001	<0.001	0.3875	0.7626
Harvest time(B)	0.1033	<0.001	0.0007	<0.001	0.0002	0.0010	0.0008	0.1878
A*B	0.3274	0.8355	0.4222	0.0742	0.0625	<0.001	0.0538	0.1648

[z]Fruit shape index= fruit width/fruit length.

[y]In retarding culture, first, second, and third harvest were done on 5 December, 9 January, and 20 February, respectively.

[x]Mean separation within columns for each harvest time by Duncan's multiple range test ($P \leq 0.05$).

Table 3.3. Analysis of variance (ANOVA) of rootstocks on the apparent quality and textural property of 'Saengsaeng' pepper fruits at different harvest time in semi-forcing culture.

Rootstock	apparent fruit quality						textural property	
	Fruit length (mm)	Fruit width (mm)	Fruit shape index[z]	Flesh thickness (mm)	Fruit weight (g)	Dry matter (%)	Hardness ($kN \cdot m^{-2}$)	Strength ($kN \cdot m^{-2}$)
First harvest[y]								
Auto-graft	99.9	14.1	7.1	1.53	4.26	-	394	3,987
Kataguruma	102.9	14.4	7.2	1.36	4.50	-	407	3,940
Konesianhot	100.4	14.5	6.9	1.72	4.39	-	401	3,731
Koregon PR-380	100.8	13.9	7.3	1.48	4.28	-	379	3,451
PR-power	98.3	14.3	6.9	1.59	4.46	-	372	3,084
Tantan	102.4	14.1	7.3	1.55	4.55	-	419	3,587
Second harvest								
Auto-graft	93.8	12.0	7.9	1.11	5.25a[x]	8.81	439ab	4,320
Kataguruma	94.0	12.1	7.8	1.04	4.67b	8.71	450ab	4,426
Konesianhot	96.0	12.4	7.8	1.05	5.39a	9.14	468a	4,446
Koregon PR-380	93.4	12.2	7.7	0.99	4.97ab	8.95	482a	4,463
PR-power	92.4	12.0	7.7	1.00	4.96ab	8.81	439ab	4,335
Tantan	95.3	11.8	8.2	1.10	5.30a	8.83	414b	4,238
Third harves								
Auto-graft	97.5	12.5ab	7.9ab	1.65	5.03	9.05ab	529	4,841
Kataguruma	97.0	12.2a-c	8.0ab	1.51	5.03	9.01ab	536	5,026
Konesianhot	98.4	11.8c	8.4a	1.58	4.78	8.72bc	488	5,012
Koregon PR-380	97.2	12.4ab	7.9ab	1.55	5.10	8.56bc	503	4,794
PR-power	98.2	12.0bc	8.2a	1.52	4.90	9.50a	497	5,055
Tantan	97.0	12.8a	7.7b	1.53	5.23	8.04c	509	4,567
P value								
Rootstock(A)	0.3572	0.9769	0.9299	0.1085	0.0926	0.0141	0.4004	0.4074
Harvest time(B)	0.1236	0.0001	0.0118	0.0038	0.0448	0.8304	0.0014	<0.001
A*B	0.5305	0.0047	0.0048	0.4052	0.0095	0.0020	0.0456	0.5569

[z]Fruit shape index= fruit width/fruit length.

[y]In semi-forcing culture, first, second, and third harvest were done on 12 June, 10 July, and 14 August, respectively.

[x]Mean separation within columns for each harvest time by Duncan's multiple range test ($P \leq 0.05$).

Table 3.4. Analysis of variance (ANOVA) of rootstocks on the apparent quality and textural property of 'Saengsaeng' pepper fruits at different harvest time in retarding culture.

Rootstock	apparent fruit quality						textural property	
	Fruit length (mm)	Fruit width (mm)	Fruit shape index[z]	Flesh thickness (mm)	Fruit weight (g)	Dry matter (%)	Hardness ($kN \cdot m^{-2}$)	Strength ($kN \cdot m^{-2}$)
First harvest[y]								
Non-graft	85.7	10.4bc[x]	8.3a	0.81	3.42b	9.91	329	2,932
Auto-graft	84.1	10.7bc	7.9ab	0.79	3.54ab	9.82	350	3,055
Kataguruma	84.0	11.2ab	7.5b-d	0.73	3.72ab	9.96	345	3,266
Konesianhot	81.7	11.0ab	7. 4cd	0.77	3.38b	9.98	313	2,909
Koregon PR-380	84.6	11.3a	7. 5b-d	0.73	3.93a	9.87	332	2,917
PR-power	81.7	11.3a	7.3d	0.77	3.59ab	9.85	328	3,033
Tantan	84.3	10.8a-c	7.8bc	0.71	3.68ab	9.58	322	2,809
Second harvest								
Non-graft	77.4	11.4b-d	6.8	1.30b	3.73	9.90b	392	2,923
Auto-graft	74.2	11.1cd	6.7	1.52a	3.53	9.96b	393	3,062
Kataguruma	75.2	11.5a-d	6.6	1.62a	3.54	10.25ab	383	2,927
Konesianhot	75.2	11.5a-c	6.6	1.52a	3.69	10.65a	362	2,459
Koregon PR-380	76.5	11.8ab	6.5	1.40ab	3.60	10.43ab	372	2,833
PR-power	74.8	12.0a	6.3	1.52a	3.61	10.49ab	427	3,184
Tantan	73.9	11.0d	6.8	1.45ab	3.34	10.49ab	347	2,631
Third harves								
Non-graft	83.6	11.5	7.3	1.40	4.13	11.62	453	3,977
Auto-graft	84.2	11.9	7.1	1.32	4.23	11.34	460	4,842
Kataguruma	78.7	11.7	6.8	1.28	3.77	12.41	450	4,472
Konesianhot	80.2	12.4	6.5	1.32	4.09	12.19	415	4,348
Koregon PR-380	80.9	11.9	6.8	1.26	3.94	12.16	449	4,949
PR-power	83.4	12.0	7.0	1.41	4.24	11.10	438	4,687
Tantan	83.7	12.1	6.9	1.39	4.17	11.70	448	4,394
P value								
Rootstock(A)	0.0427	<0.001	<0.001	0.0920	0.7234	0.0123	0.2898	0.2635
Harvest time(B)	0.0036	0.0141	0.0002	0.0002	0.0059	0.0947	0.0005	0.0029
A*B	0.1005	0.0089	0.0549	0.0004	0.0014	0.1207	0.9185	0.8613

[z]Fruit shape index= fruit width/fruit length.

[y]In retarding culture, first, second, and third harvest were done on 10 December, 7 January, and 18 February, respectively.

[x]Mean separation within columns for each harvest time by Duncan's multiple range test ($P \leq 0.05$).

Table 3.5. Analysis of variance (ANOVA) of rootstocks on the apparent quality and textural property of 'Shinhong' pepper fruits at different harvest time semi-forcing culture.

Rootstock	apparent fruit quality						textural property	
	Fruit length (mm)	Fruit width (mm)	Fruit shape index[z]	Flesh thickness (mm)	Fruit weight (g)	Dry matter (%)	Hardness ($kN \cdot m^{-2}$)	Strength ($kN \cdot m^{-2}$)
First harvest[y]								
Auto-graft	96.7ab[x]	12.0b	8.1	1.49b	6.27ab	8.49c	533	5,552ab
Kataguruma	94.2b	12.0b	7.9	1.74a	6.66a	8.55c	505	4,766c
Konesianhot	93.3b	12.0b	7.8	1.76a	5.92b	10.05a	538	5,787a
Koregon PR-380	99.4a	12.5ab	8.0	1.54b	6.90a	8.40c	478	4,740c
PR-power	96.6ab	12.7a	7.7	1.52b	6.52ab	8.91bc	517	4,923bc
Tantan	96.9ab	12.1b	8.0	1.51b	6.19ab	9.92ab	504	4,878bc
Second harvest								
Auto-graft	86.7	14.1	6.2	1.99b	6.38	11.16	541	5,298
Kataguruma	89.5	14.5	6.2	1.98b	6.39	10.62	528	5,332
Konesianhot	88.1	14.6	6.0	1.89b	6.21	11.11	495	4,910
Koregon PR-380	85.5	14.2	6.1	1.97b	5.95	10.46	513	5,401
PR-power	87.5	14.4	6.1	2.20a	6.43	10.73	517	5,151
Tantan	89.8	14.9	6.1	1.88b	6.47	10.91	521	5,097
Third harves								
Auto-graft	83.9	11.7	7.2	1.78	5.33	12.51	460	5,226
Kataguruma	86.7	11.4	7.7	1.90	5.43	11.96	503	5,229
Konesianhot	85.1	11.6	7.4	1.76	5.36	12.35	463	5,025
Koregon PR-380	84.7	11.5	7.4	1.76	5.41	12.84	471	5,143
PR-power	83.4	11.4	7.4	1.80	5.28	11.42	485	5,102
Tantan	86.3	12.0	7.3	1.76	5.62	11.88	473	4,960
P value								
Rootstock(A)	0.2694	0.2397	0.3095	0.0015	0.4632	0.0353	0.3686	0.2682
Harvest time(B)	0.0306	0.0005	<0.001	0.0051	0.0563	0.0004	0.1541	0.7065
A*B	0.0129	0.0791	0.2258	<0.001	0.1133	0.0121	0.0379	0.0208

[z]Fruit shape index= fruit width/fruit length.

[y]In semi-forcing culture, first, second, and third harvest were done on 27 June, 26 July, and 8 August, respectively.

[x]Mean separation within columns for each harvest time by Duncan's multiple range test ($P \leq 0.05$).

Table 3.6. Analysis of variance (ANOVA) of rootstocks on the apparent quality and textural property of 'Shinhong' pepper fruits at different harvest time in retarding culture.

Rootstock	apparent fruit quality						textural property	
	Fruit length (mm)	Fruit width (mm)	Fruit shape index[z]	Flesh thickness (mm)	Fruit weight (g)	Dry matter (%)	Hardness ($kN \cdot m^{-2}$)	Strength ($kN \cdot m^{-2}$)
First harvest[y]								
Non-graft	72.5	10.6	6.84	1.62	3.8	9.87	413a[x]	5,397
Auto-graft	73.8	11.1	6.68	1.57	3.9	10.02	416a	5,305
Kataguruma	70.9	10.9	6.54	1.55	3.6	9.81	426a	5,147
Konesianhot	72.2	10.8	6.72	1.54	3.7	9.81	413a	5,319
Koregon PR-380	75.7	11.2	6.82	1.56	4.1	9.88	413a	5,107
PR-power	75.1	11.2	6.73	1.55	4.1	10.06	436a	5,225
Tantan	72.3	10.8	6.77	1.49	3.7	9.93	368b	4,984
Second harvest								
Non-graft	73.6b	11.2	6.61	1.46	4.1c	10.09	405	4,906
Auto-graft	76.5a	11.5	6.70	1.54	4.4a-c	9.91	421	4,942
Kataguruma	75.3ab	11.6	6.52	1.41	4.3bc	10.12	415	5,133
Konesianhot	73.9b	11.5	6.45	1.55	4.4a-c	10.01	412	5,290
Koregon PR-380	77.7a	11.6	6.70	1.44	4.7a	9.91	448	4,795
PR-power	77.6a	11.3	6.90	1.43	4.5ab	10.27	400	4,534
Tantan	77.5a	11.6	6.72	1.57	4.6ab	9.87	440	4,683
Third harves								
Non-graft	88.0c	13.9	6.36	2.05	7.0	12.42	616	6,914
Auto-graft	88.8a-c	13.9	6.44	1.99	6.8	11.79	558	6,419
Kataguruma	87.4c	13.8	6.35	1.94	6.6	13.05	575	6,278
Konesianhot	87.9c	13.8	6.40	1.88	6.9	12.42	574	6,413
Koregon PR-380	88.4bc	13.8	6.43	2.09	6.6	12.42	633	6,898
PR-power	92.0a	14.1	6.54	2.05	7.1	11.73	585	6,811
Tantan	91.5ab	13.8	6.67	2.04	7.1	11.16	598	7,322
P value								
Rootstock(A)	<0.001	0.3549	0.1106	0.5794	0.0183	0.0040	0.2030	0.8916
Harvest time(B)	<0.001	<0.001	0.1739	0.0020	<0.001	0.0031	0.0007	0.0008
A*B	0.4303	0.4964	0.8613	0.1511	0.0216	<0.001	0.0220	0.0454

[z]Fruit shape index= fruit width/fruit length.

[y]In retarding culture, first, second, and third harvest were done on 11 December, 8 January, and 19 February, respectively.

[x]Mean separation within columns for each harvest time by Duncan's multiple range test ($P \leq 0.05$).

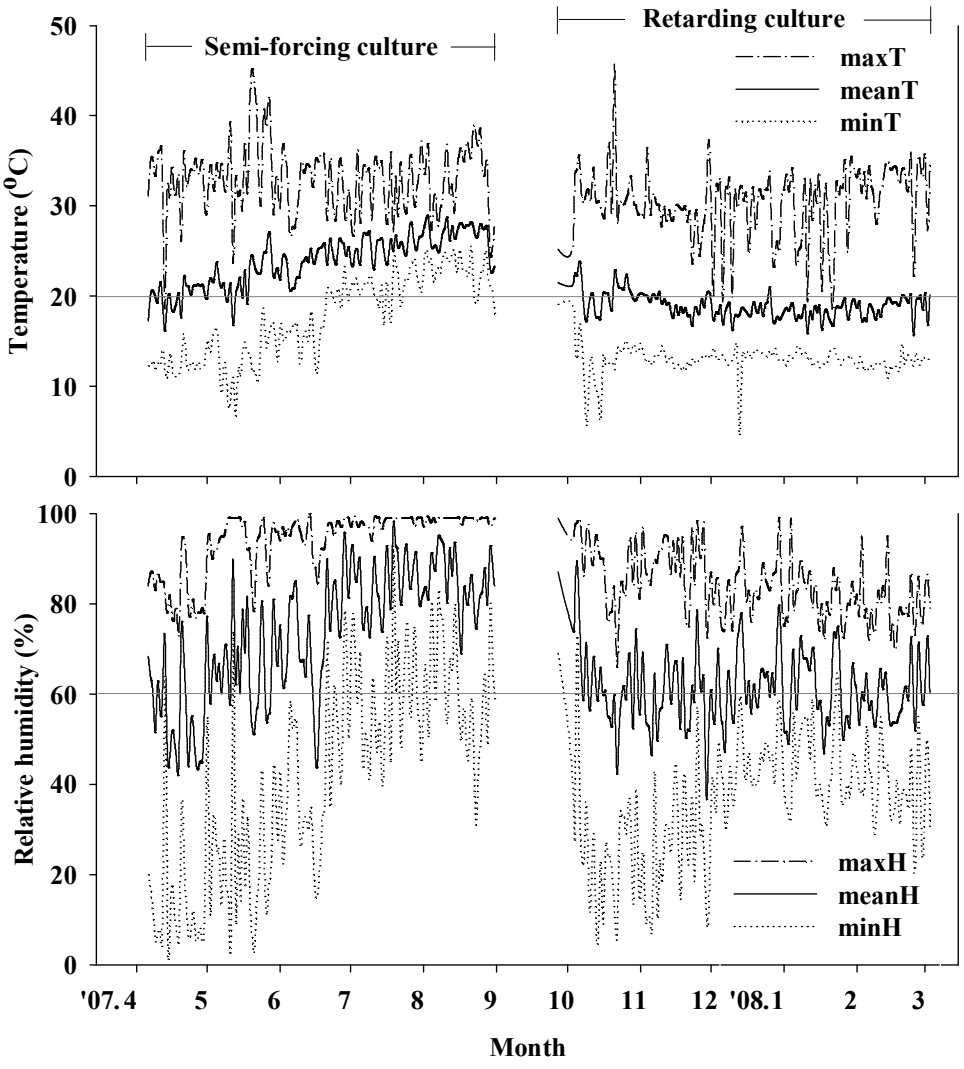

Fig. 3.1. Changes in daily maximum (max), mean, and minimum (min) temperature (upper) and relative humidity (bottom) during the cultivation of peppers.

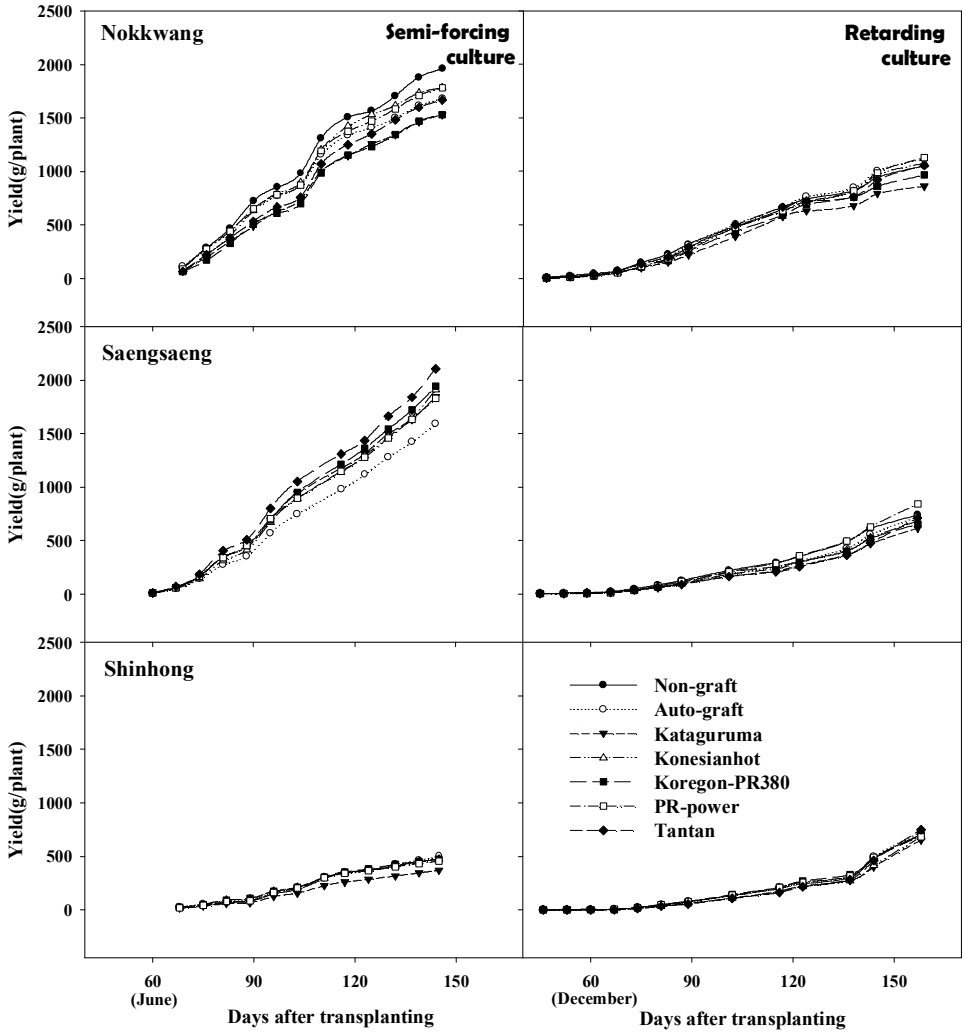

Fig. 3.2. Cumulative yield of marketable fruits of grafted peppers ‘Nokkwang’, ‘Saengsaeng’, and ‘Shinhong’ as influenced by grafting and rootstocks.

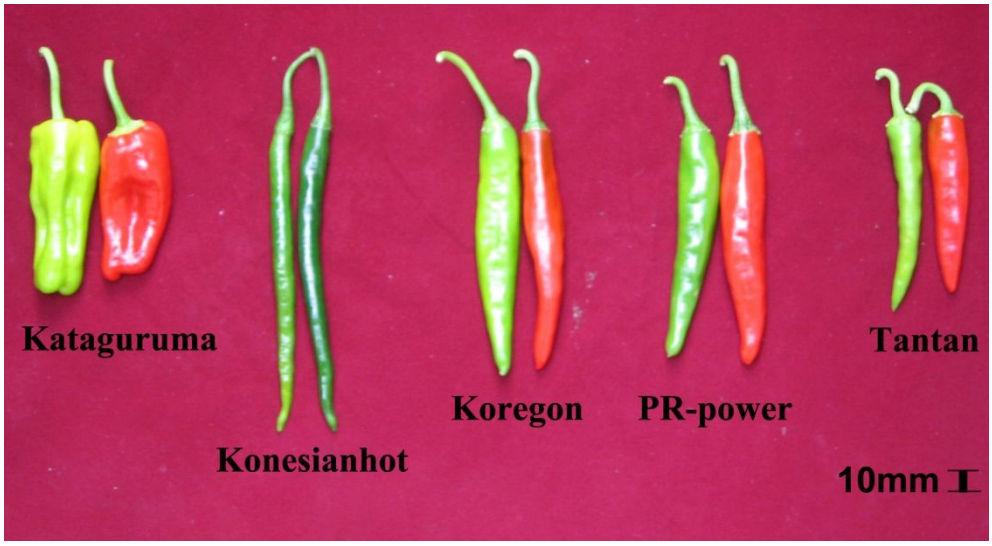

Fig. 3.3. Fruit shapes of different rootstock genotypes.

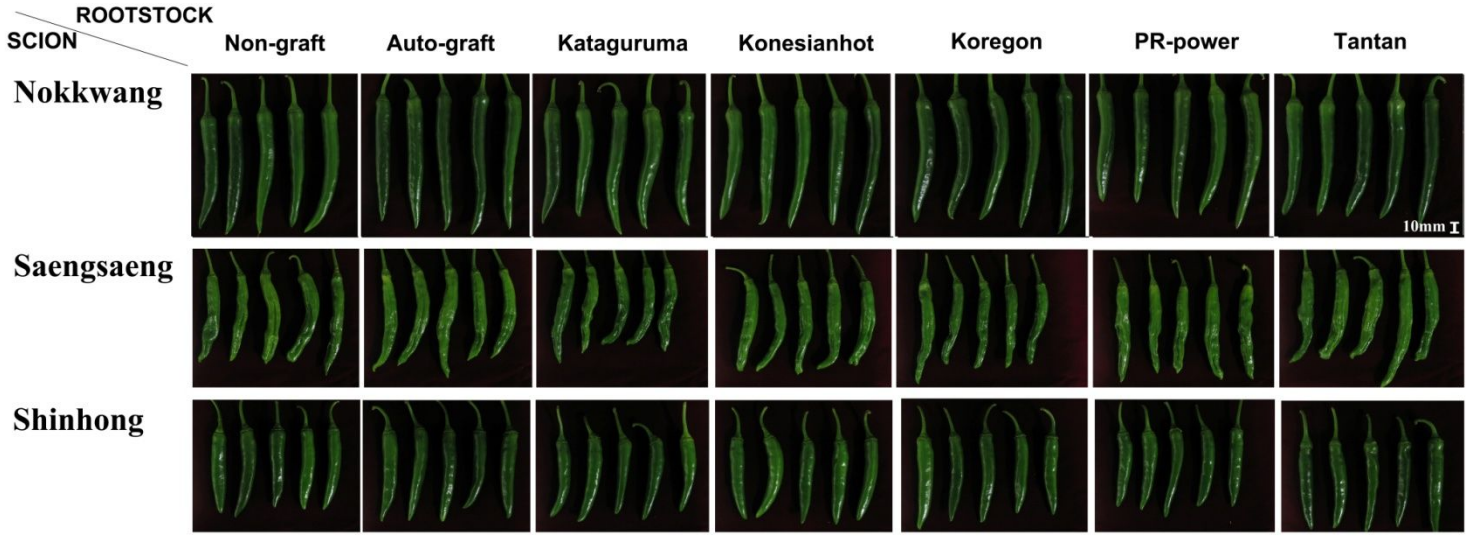

Fig. 3.4. Fruits of peppers ‘Nokkwang’, ‘Saensaeng’, and ‘Shinhong’ grafted onto different rootstocks.

PART II: DEVELOPMENT OF ACCLIMATIZATION TECHNIQUES TO IMPROVE QUALITY OF GRAFTED TRANSPLANTS

CHAPTER IV

EFFECTS OF LIGHT INTENSITY AND ATMOSPHERIC CO_2 CONCENTRATION ON THE PHOTOSYNTHESIS AND GROWTH OF GRAFTED PEPPER TRANSPLANTS DURING HEALING AND ACCLIMATIZATION

ABSTRACT

In the production of grafted transplants, healing and acclimatization are the most critical processes for survival. Generally, healing and acclimatization are performed in a tunnel in a greenhouse, and environmental management is performed according to the empirical knowledge of the grower, depending on the season or weather. This study investigated the influence of light intensity and the atmospheric CO_2 concentration during healing and acclimatization on the photosynthetic characteristics and growth of grafted pepper transplants to determine the optimum environmental conditions for healing and acclimatization in a healing chamber with artificial lighting. Grafted pepper transplants were healed and acclimatized under two levels of atmospheric CO_2 (374 or 1,013 $\mu mol \cdot mol^{-1}$) and four levels of PPF (0, 50, 98 or 147 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for six days. Increasing PPF significantly increased CO_2 exchange rates during healing and acclimatization. The difference in the CO_2

exchange rate among the treatments increased as the post-graft time increased. Under ambient CO_2 (374 $\mu mol \cdot mol^{-1}$), the CO_2 exchange rates in PPF treatments of 98 and 147 $\mu mol \cdot m^{-2}$ s^{-1} were 3.6 (3.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 6.6 times (5.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$) higher than that observed for PPF 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively, on the sixth day. Under an elevated CO_2 concentration (1,013 $\mu mol \cdot mol^{-1}$), the CO_2 exchange rate under PPF 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was 4.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and those of the PPF treatments of 98 and 147 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were 1.8 (8.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 2.1 times (9.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$) higher, respectively. The increase of photosynthesis led to an improvement of growth. Dry weight and leaf area were greater under higher PPF and CO_2 concentrations. PPF also influenced the anatomical structures of the leaves, and the palisade and spongy tissue cells of leaves irradiated with higher PPF were aligned more densely, with more chloroplasts and a small empty space. When compared to the tunnel in a greenhouse with natural light, healing and acclimatization under high CO_2 (1,000 $\mu mol \cdot mol^{-1}$) and PPF (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) conditions in the healing chamber promoted the growth and graft union formation of grafted pepper transplants. The results suggest that high-quality grafted pepper transplants can be achieved by healing and acclimatization in a healing chamber where optimal conditions such as high CO_2 and PPF are maintained.

INTRODUCTION

Vegetable grafting is practiced over the world (Lee et al., 2010) and its purposes are to reduce soil-borne diseases (Louws et al., 2010) and enhance the tolerance against abiotic stresses such as low (Venema et al., 2008) and high temperatures (Rivero et al., 2003), flooding (Yetisir et al., 2006) and drought (Rouphael et al., 2008), salt (Colla et al., 2006 a,b; Edelstein et al., 2011), alkalinity (Colla et al., 2010), and heavy metals (Savvas et al., 2010). Grafting is mostly practiced on fruit vegetables of the family Cucurbitaceae and Solanaceae. Grafted transplants are produced according to the procedures like1) raising scions and rootstocks; 2) grafting; 3) healing and acclimatization; and 4) raising the grafted transplants before transplanting (Jang et al., 2011).

In those production procedures, healing and acclimatization are critical for grafted transplants to survive and grow as healthy plants, which involve the healing of the cut surface and hardening for field or greenhouse survival (Lee and Oda, 2003). Healing and acclimatization have been practiced by focusing on the survival of grafted transplants rather than growth. Generally, the healing and acclimatization processes are performed in a tunnel made of double-layered plastic film on a greenhouse bench. Shade cloth is installed on or above the tunnel to prevent the wilting of grafted transplants by transpiration and avoid excessive heat build-up in the tunnel. The tunnel is kept sealed for three or four days to prevent the

wilting of grafted transplants and to promote the formation of graft union. The tunnel is then gradually opened over the following three or four days to acclimate grafted transplants to normal conditions. When the tunnel is closed, the air in the tunnel is apt to be saturated (Relative humidity ≥ 90%), the air current speed is near 0 m s^{-1}, and light intensity is near the light compensation point (below a PPF of 50 μmol·m^{-2}·s^{-1}) (Kim and Park, 2001; Lee and Oda, 2003; Shibuya et al., 2003). Under these environmental conditions, the net photosynthesis rate of grafted transplants is almost 0 mg CO_2·m^{-2}·s^{-1} (Shibuya et al., 2003).

In general, the use of greenhouses enables faster and year-round production of various crops. Nevertheless, greenhouse environmental conditions such as temperature, humidity, and light intensity vary by day, season, and years and are often unfavorable to crop growth. The air temperature in greenhouses is especially apt to exceed the optima for crops at midday or in summer (Choi et al., 2000; Max et al., 2009; Villarreal-Guerrero et al., 2012). The temperature inside the closed tunnel during healing and acclimatization processes could often be higher than the outside temperature. It often exceeds a threshold high temperature at midday, sometimes resulting in the death of plants. The air humidity inside the closed tunnel is also higher than outside and often reaches saturation. Under these environmental conditions, the grafted transplants are in danger of heat stress, leading to a decrease in photosynthesis and growth (Wahid et al., 2007). Plants are also in

danger of infection by pathogens and overgrowth, wherein roots arise from the hypocotyls of the scion, leading to lower plant quality and weaker plants. Environmental management during healing and acclimatization is usually performed based on the empirical knowledge of the grower, depending on the season or weather. A grower decides the opening and closing of the tunnel based on the condition of the grafted transplants and the weather (Jang et al., 2011). Recently, the increase in unpredictable abnormal weather has caused greater hardship for the maintenance of uniform and optimal environmental conditions for the healing and acclimatization of grafted transplants.

High-performance production systems unconstrained by weather conditions have recently been developed to produce high-quality transplants under artificial light (Kozai, 2005; Kozai, 2009). Optimizing the temperature, relative humidity, PPF, and atmospheric CO_2 concentration in these systems makes it possible to achieve rapid and uniform growth of high-quality transplants throughout the year. This optimization can also be applied to healing and acclimatization for production of grafted transplants. Recent reports have suggested that there is a higher survival rate, faster growth, and a higher quality of grafted plants under highly controlled healing conditions (Nobuoka et al., 2005; Jang et al., 2011). Reports have primarily focused on the increase of photosynthesis, which has been so far overlooked during healing and acclimatization. The increase of photosynthesis in grafted

transplants during healing and acclimatization was confirmed by higher PPF conditions using fluorescent lamps under highly controlled healing conditions (Jang et al., 2011). These conditions resulted in an improvement in the growth and quality of grafted transplants.

The objective of this study was to investigate the photosynthetic characteristics and growth of grafted pepper transplants during healing and acclimatization and to determine the optimal environmental conditions for healing and acclimatization in a healing chamber with artificial lighting. Specifically, this study was conducted to examine the effects of PPF and CO_2 concentration during healing and acclimatization on the rate of photosynthesis, and the growth and graft-take of grafted pepper transplants.

MATERIALS AND METHODS

1. Plant material and growing scions and rootstocks

The peppers 'Nokkwang' (Seminis Vegetable Seeds, Inc., Seoul, Korea) and 'Tantan' (Nongwoo Bio Co. Ltd., Suwon, Korea) were used as scions and rootstocks, respectively, for producing grafted transplants. Pepper s eeds were sown into 72-cell plug trays (*W* 280 × *L* 540 × *H* 45 mm, Bumnong Co. Ltd., Jeongeup, Korea) filled with commercial growing substrate (BM 2, Berger Group Ltd., St. Modeste, QC, Canada). Seeds of rootstocks were sown two days before sowing seeds of scions to obtain scions and rootstock with similar stem diameter. To promote germination, the plug trays were wrapped with vinyl chloride resin film and placed in a germination chamber at 28°C. After four days, the germinated seedlings were watered by overhead watering and moved to a growth chamber with artificial light (Hanbaek Co. Ltd., Bucheon, Korea), where the temperature was set at 25/18°C (photo / dark periods). The light period was 14 hours·d^{-1}, and PPF was approximately 200 μmol·m^{-2}·s^{-1}, provided by high pressure sodium, metal halide, and fluorescent lamps. They were bottom-irrigated two times with water and one time a week with a nutrient solution (EC 1.4 dS·m^{-1}, 'Hanbang' for seedling, Coseal Co., Ltd., Seoul, Korea). The dry weight, the number of true leaves, leaf area, and stem diameter of the scion and rootstock before grafting were 119.7±3.9 mg, 4.98±0.09, 30.16±0.95 cm^2, 1.53±0.02 mm, and 118.8±3.9 mg,

5.32±0.08, 30.27±1.16 cm^2, 1.62±0.02 mm, respectively.

2. Grafting

Grafting was performed at four weeks after the sowing of rootstocks. The epicotyls of the scions and rootstocks were cut below 1 cm from the first true leaf using a razor blade. After placing the scion on the rootstock, the grafted position was fixed tightly together with an ordinary grafting clip by the slice grafting method (Lee and Oda, 2003).

3. Healing and acclimatization of grafted pepper transplants and the measurement of whole-canopy CO_2 exchange rate

A continuous CO_2 measurement system using a semi-open multi-chamber was used for the healing and acclimatization of grafted pepper transplants and the measurement of the whole-canopy CO_2 exchange rate (van Iersel and Bugbee, 2000; Jang et al., 2011; Mun et al., 2011). Four light-transmitting boxes (inside dimension of *W* 350 × *L* 780 × *H* 220 mm, volume of 60 L) made of 10 mm-thick acryl plastic were placed in the growth chamber (Hanbaek Co. Ltd., Bucheon, Korea), where the temperature was set at 22°C. Each healing chamber had air inlets and outlets and an air drawing tube. It was also equipped with thermocouple (T-types), a humidity sensor (CHS-UPS, TDK, Japan), a heater (hair dryer heater, Kaiser KHD-5207i, My Friend Co. Ltd., Goyang, Korea) and a humidifier (nebulizer, CT-24, Techsin Electronic Co. Ltd., Foshan, China) to

measure and maintain the temperature and relative humidity inside the box.

Atmospheric air was drawn in through the inlet of each healing box at an airflow rate of 13 $L \cdot min^{-1}$ using air pumps (LP80VC, Youngnam Air Pump Inc., Busan, Korea) and flow meters (15 $L \cdot min^{-1}$, Kofloc, Kojima Instruments Inc., Kyoto, Japan), and air flowed out through the outlet (Shibuya et al., 2006; Mun et al., 2011). The individual gas from each box was pulled by a 3-way solenoid valve (VD3, Korcon, Seoul, Korea) every two minutes with a switching power relay (SDM-CD16AC 16-channel AC/DC controller, Campbell Scientific Inc., Logan, UT, USA). Two flow meters (1.0 $L \cdot min^{-1}$, Kofloc, Kojima Instrruments Inc., Kyoto, Japan) were also used at 0.5 L min^{-1} for the sample and reference gas measurement. The CO_2 concentrations of the air at the inlet and the outlet were measured using a CO_2/H_2O analyzer (LI-7000, Li-Cor Bioscience, St. Lincoln, NE, USA) after moisture in the air was removed with a dehumidifying tube (SWG-A01-18/PP, Asahi Glass Engineering Co., Ltd., Chiba, Japan). The CO_2 concentration of the air in each chamber was measured for 2 minutes during a 10-minute cycle. The data were recorded for the last five seconds.

All sensors were attached to a data logger (CR23X, Campbell Scientific Inc., Logan, UT, USA) with a switching power relay (SDM-CD16AC 16-channel AC/DC controller, Campbell Scientific Inc., Logan, UT, USA) that switched the heaters, humidifiers, fluorescent lamps, and solenoid valves. The temperature and

relative humidity data inside the box were also collected every hour using a thermocouple (T-types) and a humidity sensor (CHS-UPS, TDK, Tokyo, Japan), respectively. The air temperature in the box was kept at 27°C, and the relative humidity was set at 90%.

Eleven fluorescent lamps (FL30SSD/29, Dooyoung Lighting Industrial Co., Ltd., Seoul, Korea) were installed approximately 20 cm above the box, and the distance between two lamps was approximately 1 cm. The light levels were adjusted by the number of lamps and measured above the top of each healing box using a light meter with 6 quantum light sensor bars (Field Scout external light senor meter, Spectrum Technologies, Inc., Plainfield, IL, USA). The light period was 12 hours d^{-1}.

Healing and acclimatization of grafted pepper transplants were conducted in the healing chamber for 6 days. Irrigation was not applied during healing and acclimatization.

4. Treatments

4.1. Effects of PPF and atmospheric CO_2 during healing and acclimatization in a healing chamber with artificial lighting

The experiment was conducted in the semi-open multi-chamber. Six treatments were designed by the combination of two (2) levels of atmospheric CO_2 and four (4) levels of PPF during healing and acclimatization (Table 4.1). For treatment code abbreviation, ambient and elevated atmospheric CO_2 were abbreviated to A and E,

respectively for the first letter, whereas the dark condition and low, medium, and high PPF were abbreviated to D, L, M, and H, respectively, for the second letter. The experimental design was a split-plot with atmospheric CO_2 as the main plot and PPF as the sub plot. The experiment was repeated twice. In each replication, one 72-cell plug tray with 48 plants was measured.

To elevate the atmospheric CO_2 concentration, a buffer (87 L) was made of 10 mm-thick acryl plastic (0.32 × 0.32 × 0.85 m, L × W × H). Atmospheric air and additional CO_2 from a CO_2 bombe were drawn in through the inlet of the buffer using a oil-less piston pump (100RND, G&M Tech. Inc., Gunpo, Korea) and flow meters (100 $L \cdot min^{-1}$, Dwyer Instruments, Inc., Michigan City, IN, USA), and air flowed out through the outlet. The CO_2 level in the buffer was controlled using flow meter and needle valves (100 $mL \cdot min^{-1}$, Dwyer Instruments, Inc., Michigan City, IN, USA). Four micro fans (Suntronix fan SJ1238HA2, Sanju Electric Machinery, Co. Ltd., Shenzhen, Guangdong, China) inside the buffer mixed the air to maintain a stable CO_2 concentration.

4.2. Comparison of the growth of grafted pepper transplants that were healed and acclimatized in a tunnel in a greenhouse vs. a healing chamber with artificial lighting

The grafted pepper transplants were healed and acclimatized in a tunnel in a greenhouse with natural light or in a healing chamber with artificial lighting for 6 days. The experiment included three

treatments:(i) a control of normal atmospheric CO_2 and light conditions, in which healing and acclimatization were performed in a tunnel on a greenhouse bench; (ii) a high CO_2 and medium PPF treatment, with an increased CO_2 concentration of 1,000 $\mu mol \cdot mol^{-1}$ and a PPF of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the healing chamber; and (iii) a high CO_2 and PPF treatment, with an increased CO_2 concentration of 1,000 $\mu mol \cdot mol^{-1}$ and a PPF of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the healing chamber. Each treatment had two replications. In each replication, one 72-cell plug tray with 48 plants was measured.

The healing and acclimatization of the control were performed in a tunnel made of double-layered plastic film on a greenhouse bench. Shade cloth was installed on the tunnel. For the first three days, the humidity inside the tunnel was kept high by closing the tunnel and using a humidifier (H-650C, LG Electronics, Seoul, Korea). Then, the tunnel was gradually opened over the following three days to acclimate grafted transplants to normal conditions. Temperature, relative humidity, and PPF in the tunnel and greenhouse were measured using a temperature/humidity data logger (TR-75U Thermo Recorder, T and D Corp., Matsumoto, Japan) and a data logger (WatchDog 1000 Series, Spectrum Technologies, Inc., Plainfield, IL, USA) with a quantum light sensor (LightScout Quantum light sensor, Spectrum Technologies, Inc., Plainfield, IL, USA).

5. Measurement

5.1. CO_2 exchange rate

The CO_2 exchange rate of each healing box was estimated using the equation below with the following parameters:1) CO_2 concentration; 2) air flow rate into the box; and 3) area of the plug tray. The CO_2 generation rate from the growing media and roots was neglected because it was small when compared with the exchange rate of transplants (Shibuya and Kozai, 1998).

$CER = F (C_i - C_o) / A$

where CER is the CO_2 exchange rate in the healing box (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), F is the air flow rate in the healing box ($mol \cdot s^{-1}$), C_i and C_o are the CO_2 concentrations in the inlet and outlet of the healing box (μmol $CO_2 \cdot mol^{-1}$), and A is the area of the plug tray (m^2).

5.2. Microscopic observation

Cross sections of specimens for microscopic observation were prepared as described by Luft (1973). Leaf pieces for anatomy measurements were cut off from the scion 6 days after grafting and graft union pieces were cut off 2 and 6 days after grafting. They were infiltrated and fixed in 2.5% glutaraldehyde in 100 mM phosphate buffer (pH 7.2) for 2 hours at 4°C. Then, they were rinsed and post-fixed in 1% osmium tetroxide for 2 hours at 4°C and held overnight in phosphate buffer. After fixation, they were dehydrated in a graded series of ethyl alcohol (40, 60, 80, 90, 95

and 100% in distilled water [v/v]). The tissues were further processed with three changes of propylene oxide for 15, 15, and 30 minutes per change and gradually infiltrated (3 hours each at 30, 50, and 100% embedding media in propylene oxide) with Epon (EMS, Hatfield PA, U.S.A.) embedding media to ensure complete dehydration. They were held overnight in 100% Epon before polymerization at 60°C for 72 hours. They were sectioned (1500 nm), stained with periodic acid staining, and viewed with a light microscope (Axioskop 2, Carl Zeiss AG, Oberkochen, Germany).

5.3. Growth parameters

Graft-take and growth parameters such as dry weight were measured 6 days after grafting. Ten plants in each treatment were sampled. Data were analyzed using SAS v.9.1 software (SAS Institute, Cary, NC, USA).

RESULTS

1. Environmental condition during healing and acclimatization

When the air temperature and relative humidity inside the tunnel were monitored from July to September, those fluctuated from day to day and changed depending on the month (Fig. 4.1). Those were apt to be lower or higher than those in a greenhouse. The maximum air temperatures in the tunnel in July, August, and September were 40, 47, and 41°C, while those in the greenhouse were 33, 38, and 37°C, respectively. At the highest temperature in the tunnel, the relative humidity decreased. The minimum relative humidity in the tunnel in July, August, and September were 41, 46, and 31%, while those in the greenhouse were 24, 26, and 24%, respectively. The maximum PPF values in the greenhouse were 1,649, 1,871, and 1,783 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in July, August, and September, respectively. The intensity of the incoming radiation, however, was significantly reduced inside the tunnel, and the maximum PPF values in the tunnel were 39, 39, and 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in July, August, and September, respectively.

On the other hand, air temperature and relative humidity in the healing box were maintained near the set point (27°C and 90%), while they were slightly higher under light conditions (Fig. 4.2).

2. Effects of PPF and CO_2

2.1. CO_2 exchange rate

Just after grafting, grafted pepper transplants wilted and then gradually recovered as they healed and acclimatized. During healing and acclimatization, the CO_2 exchange rates of grafted pepper transplants under light conditions were positive and gradually increased with time, while the CO_2 exchange rate was negative under dark conditions (Fig. 4.3). Increasing PPF significantly increased the CO_2 exchange rates during healing and acclimatization. Under the ambient CO_2 concentration, the CO_2 exchange rate on the first day of treatment AL was negative even in daytime and increased by 0.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on day 6 after grafting. The CO_2 exchange rates on the sixth day of treatments AM and AH were 3.6 (3.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 6.6 times (5.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$) higher than that of treatment AL, respectively. Under the elevated CO_2 concentration (1,013 $\mu mol \cdot mol^{-1}$), the CO_2 exchange rates ranged from -0.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (ED) to 9.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (EH) under different light conditions on the sixth day. However, atmospheric CO_2 concentrations of 373 to 1,013 $\mu mol \cdot mol^{-1}$ did not significantly affect the CO_2 exchange rates during healing and acclimatization. The CO_2 exchange rates during the dark period were positively correlated with PPF.

2.2. Leaf anatomical differences

The distribution of palisade and spongy mesophyll cells and chloroplasts in young upper leaves of the grafted pepper transplants were different under different light conditions during the six-day healing and acclimatization (Fig. 4.4). The palisade and spongy mesophyll cells irradiated with higher PPF were aligned more densely, and there was a small empty space among the cells. More chloroplasts in the leaf healed and acclimatized under the high PPF condition compared with the medium PPF or dark conditions.

2.3. Plant growth

At day 6 after grafting, the shoot length, number of true leaves, SPAD value, leaf area, and dry weight of roots, stems, and leaves increased with an increase in PPF during healing and acclimatization (Table 4.2). The percent dry matter also increased, but the specific leaf area (SLA) decreased as the PPF increased. Moreover, an atmospheric CO_2 concentration at 373 to 1,013 $\mu mol \cdot mol^{-1}$ did not significantly affect the growth of grafted pepper transplants. The percentages of graft-take were close to 100% in all treatments (data not shown).

3. Comparison of growth of grafted pepper transplants healed and acclimatized in a tunnel in a greenhouse and a healing chamber

3.1. Plant growth

On sixth day after grafting, the number of true leaves, the SPAD value, and the dry weight of stems and leaves increased in the grafted pepper transplants that were healed and acclimatized under high CO_2 (1,000 $\mu mol \cdot mol^{-1}$) and medium (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (EM) or high PPF (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (EH) conditions in the healing chamber compared with those in the tunnel in a greenhouse (Table 4.3). The percent dry matter of stems and leaves increased, but SLA decreased in the healing chamber.

3.2. Graft union anatomical differences

Microscopic observations showed the graft union healed and acclimatized in the tunnel in a greenhouse and in the healing chamber under high CO_2 and medium or high PPF condition two and six days after grafting (Fig. 4.5). On the second day after grafting, the graft union in treatments C and EM had not formed (Fig. 4.5C2 and EM2), although the graft union in treatment EH was starting to partly form. On the sixth day after grafting, the connection of the graft union surrounding the vascular bundle was processed. The graft union that was healed and acclimatized under high CO_2 and PPF conditions (EH) (Fig. 4.5EH6) had a closer connection between the scion and rootstock compared with medium light conditions (EM) (Fig. 4.5EM6) or the tunnel in a greenhouse (Fig. 4.5C6).

DISCUSSION

4.1. Environmental conditions during healing and acclimatization

In the production of grafted transplants, healing and acclimatization are the most critical processes for survival. The successful production of grafted transplants depends on healing and acclimatization. The healing and acclimatization of grafted vegetable transplants are generally performed in a tunnel on a bench in a greenhouse. During healing and acclimatization, keeping the temperature, relative humidity, and light intensity at optimal levels is essential to prevent wilting, promote graft union formation and harden transplants for field conditions. However, such optimization is difficult because environmental conditions inside the tunnel are apt to be influenced external environmental and exceed the optima, especially at midday and in summer.

In a greenhouse with only roof vents on the ridge line and an exhaust fan on the sidewall side without extra devices for cooling, the air temperature, relative humidity, PPF, and photoperiod fluctuated and varied with time and season (Fig. 4.1). The maximum air temperature in the greenhouse ranged from 33 to 38°C from July to September. The temperature inside the tunnel was approximately 10°C higher than that in the greenhouse. High air temperature in a greenhouse severely limits crop production, reducing the yield and lowering the quality of the crop (Villarreal-Guerrero et al., 2012).

The increase of the mean global temperature also increases temperatures in greenhouses, leading to adverse effects on the photosynthesis, growth, and yield of crops due to heat stress (Dannehl et al., 2012). The excess increase in the temperature may simultaneously cause reduction of the relative humidity. Just after grafting, the water supply from the root to cut scions may be limited, and grafted pepper transplants may wilt until the vascular bundles between scion and rootstock are connected. In general, plants tend to maintain stable tissue water status regardless of temperature when moisture is sufficient. However, high temperatures severely impair this tendency when water is limited (Machado and Paulsen, 2001; Wahid et al., 2007). Accordingly, grafted transplants in a tunnel in a greenhouse are in danger of heat and water stress, especially in daytime when the temperature is high and evapotranspiration is accelerated. To reduce heat and water stress by heat build-up and excess evapotranspiration, plastic film and shading materials were used, and these remarkably decreased the light intensity in the tunnel. The PPF in the tunnel was below 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$, fluctuating with day and season.

Recently, high-performance production systems such as closed-type transplant production systems with artificial lighting have been developed as a way to produce high quality transplants regardless of the weather (Kozai et al., 2000; Kozai, 2005; 2007). The air temperature, relative humidity, PPF, and CO_2 concentration in these systems can be controlled easily and accurately, leading to rapid

and uniform growth of high-quality transplants throughout the year, regardless of the outside weather. These systems can also be applied to the production of grafted transplants. The air temperature and relative humidity in the healing box in this experiment were maintained within the optimal range during healing and acclimatization (Fig. 4.2). The CO_2 concentration, PPF, and photoperiod in the healing box were also controlled and constant during healing and acclimatization.

4.2. PPF and CO_2 Effects

The stable maintenance of environmental conditions at the optimal range in the healing box reduces the danger of heat and water stress to which the grafted transplants in the tunnel in greenhouse are exposed. Furthermore, in the healing box with artificial lighting, the photosynthesis of grafted pepper transplants during healing and acclimatization increased because of the increase of PPF (Fig. 4.3). This result concurred with a previous finding that the photosynthesis of grafted cucumber transplants during healing and acclimatization increased due to an increase in PPF (Jang et al., 2011).

The measurement of plant photosynthesis is very important for the understanding of physiological responses to environmental factors. Generally, plant photosynthesis is measured using equipment such as an LI-6400 (LI-COR Biosciences, Lincoln, NE, USA). These meters generally measure only the short-term

photosynthetic rate of a single leaf under different conditions, and they are sensitive to microenvironment changes by clamping onto the leaf disc and, therefore, may not be representative of the canopy (van Iersel and Bugbee, 2000; Mun et al., 2011). The measurement of CO_2 exchange rates during healing and acclimatization requires high relative humidity (≥ 85%), and it is very difficult and rarely reported. However, in this experiment, the CO_2 exchange rates of whole plants were measured continuously during healing and acclimatization using a continuous CO_2 measurement system.

Under the dark condition, the CO_2 exchange rate was below zero throughout the healing and acclimatization, and the assimilation product was assumed to be consumed (Fig. 4.4). Under light conditions, grafted transplants withered for the first 2 days after grafting. During this period, the CO_2 exchange rates of grafted pepper transplants were low, approximately 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$, although positive. From day 3 after grafting, the CO_2 exchange rates increased more rapidly. At this transition point, it is assumed that water transport from rootstock to scion had begun (Mun et al., 2011). On the sixth day, the CO_2 exchange rates in treatments AM and AH were 3.6 (3.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) times and 6.6 times (5.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$) higher than that of the AL treatments. Under elevated CO_2 concentrations (1,013 $\mu mol \cdot mol^{-1}$), the CO_2 exchange rate in treatment EL was 4.6 $\mu mol\ m^{-2}\ s^{-1}$, and those of treatments EM and EH were 1.8 (8.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 2.1 times (9.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$) higher, respectively. Although CO_2 treatment was not statistically

significant, the CO_2 exchange rates under the same PPF condition were higher under elevated CO_2 concentrations than the ambient CO_2 concentration. Many studies have reported that elevated CO_2 concentrations increase photosynthesis. Moreover, it is reported that elevated CO_2 increases water use efficiency by decreasing transpiration (Jianlin et al., 2008). During healing and acclimation, it is important to decrease transpiration because there is no additional irrigation, and there is poor water transport from roots to shoots. Therefore, higher PPF and CO_2 are necessary for promoting photosynthesis and reducing transpiration during healing and acclimatization, although the CO_2 exchange rates of grafted transplants during healing and acclimatization are smaller than those of non-grafted transplants (Mun et al., 2011).

The increased photosynthesis led to an improvement in growth. With the PPF range evaluated in this experiment, the shoot length, number of true leaves, SPAD value, leaf area, and dry weight increased (Table 4.2). The percent dry matter also increased, but SLA decreased as the PPF increased. Although the CO_2 treatment was not statistically significant, the SPAD value, leaf area, and dry weight under the same PPF condition were greater under elevated CO_2 concentrations.

The increased PPF influenced also the leaf anatomical structures (Fig. 4.4). The palisade and spongy mesophyll cells of leaves irradiated with higher PPF were aligned more densely, with a small empty space and more chloroplasts, compared with the medium PPF

or dark conditions. The leaf anatomical structures under higher PPF during healing and acclimatization were close to the sun leaf type. This similarity is expected to increase the transplants' adaptability to the outside environment of high PPF.

4.3. Healing and acclimatization in the healing chamber with artificial lighting

When comparing the healing chamber with artificial lighting to the tunnel in a greenhouse with natural light, healing and acclimatization under high CO_2 (1,000 $\mu mol \cdot mol^{-1}$) and medium (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (EM) or high PPF (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (EH) in the healing chamber improved growth (Table 4.3). This result can be attributed to the increase of photosynthesis and the decrease of exposure to stressful conditions.

Graft union formation was also influenced by environmental conditions during healing and acclimatization. The graft union is the part of a grafted plant where the scion is joined to the rootstock. The graft union is formed through several developmental stages: (1) development of a necrotic layer, (2) callus proliferation, (3) differentiation of new vascular tissues, and (4) a full vascular graft union formation between the scion and rootstock (Jeffree and Yeoman, 1983; Fernández-García et al., 2004; Flaishman et al., 2008). In tomatoes, the major hydraulic connections within the graft union were reported to be functional on day 5 after grafting (Turquois and Malone, 1996). Fernández-García et al. also reported

that the differentiation of the callus parenchyma to form new cambial initials and the subsequent union of the newly formed vascular strand with the original vascular bundle in both the rootstock and scion begins between day 4 and 8 and is fully developed by day 15 after grafting. In this experiment, the connection of the vascular bundle between the scion and rootstock was observed by day 6 after grafting. In treatment EH, the graft union formation progressed earlier and was more tightly connected. Therefore, it is thought that the management of the healing and acclimatization conditions influences graft union formation, and such formation can be accelerated by improving the healing and acclimatization conditions through an increase in photosynthesis and a decrease in the exposure to stressful conditions.

From results in this chapter, we found that the PPF and atmospheric CO_2 concentration during healing and acclimatization affect the photosynthesis and growth of grafted pepper transplants, and higher PPF conditions improved the photosynthesis and growth of the transplants, including the graft union formation. Healing and acclimatization in a healing box enable the grower to control and maintain more optimal conditions for grafted transplants than those in a tunnel in a greenhouse, resulting in the production of high-quality grafted transplants.

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Table 4.1. Atmospheric CO_2 concentration and photosynthetic photon flux (PPF) during healing and acclimatization in each treatment.

Treatment code	CO_2 concentration ($\mu mol \cdot mol^{-1}$)	PPF ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
AD[z]	373.7 ± 1.9[y]	-
AL	373.7 ± 1.9	50.0 ± 4.7
AM	373.7 ± 1.9	97.5 ± 4.7
AH	373.7 ± 1.9	148.6 ± 4.3
ED	1,013.0 ± 11.3	-
EL	1,013.0 ± 11.3	50.0 ± 4.7
EM	1,013.0 ± 11.3	97.5 ± 4.7
EH	1,013.0 ± 11.3	148.6 ± 4.3

[z]For treatment codes, A and E on the left represent the ambient and elevated atmospheric CO_2 concentration, respectively; D, L, M, and H on the right represent the dark condition and low, medium, high PPF, respectively.

[y]Mean ± standard error.

Table 4.2. Growth of grafted pepper transplants affected by the atmospheric CO_2 concentration and photosynthetic photon flux (PPF) during healing and acclimatization 6 days after grafting.

Treatment code	Shoot length (cm)	Number of leaves	SPAD value	Leaf area (cm^2)	SLA ($cm^2 \cdot g^{-1}$)	Shoot to root ratio	Dry weight (mg)			Percent dry matter (%)		
							Root	Stem	Leaf	Root	Stem	Leaf
AD	6.72	4.9	22.4	24.1	542	4.7	16.3	29.9	44.6	8.0	7.3	8.4
AL	7.28	5. 5	24.9	24.7	573	5.4	14.2	30.4	43.7	7.9	6.9	8.7
AM	7.34	5.8	26.4	31.6	565	5.8	16.0	31.7	57.2	8.8	6.8	9.2
AH	7.38	6.1	26.3	34.9	488	6.5	18.5	36.0	72.8	7.9	7.6	10.0
ED	6.99	4.6	27.2	30. 9	574	5.0	18.2	26.7	54.4	6.6	6.0	7.7
EL	8.06	5.9	29.2	44.3	517	5.6	22.6	37.7	86.8	6.8	7.2	9.7
EM	7.67	5.9	28.8	41.0	461	6.3	22.3	38.6	89.7	7.0	7.4	10.5
EH	7.67	6.2	29.5	42.8	425	5.9	26.3	45.7	103.3	7.9	8.9	11.2
P value												
CO_2 conc. (A)	0.0700	0.4939	0.1647	0.1212	0.0971	0.5632	0.1360	0.3420	0.0510	0.2897	0.5389	0.4094
PPF (B)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0045	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A x B	0.0300	0.0573	0.1412	<0.0001	0.0002	0.5164	0.0300	<0.0001	<0.0001	0.0110	<0.0001	0.0002

Table 4.3. Growth of grafted pepper transplants healed and acclimatized in a tunnel in a greenhouse and a healing chamber with artificial lighting 6 days after grafting.

Treatment code[z]	Shoot length (cm)	Number of leaves	SPAD value	Leaf area (cm^2)	SLA ($cm^2 \cdot g^{-1}$)	Shoot to root ratio	Dry weight (mg)			Percent dry matter (%)		
							Root	Stem	Leaf	Root	Stem	Leaf
control	7.11	5.9b[y]	32.1b	35.20	447a	6.9	18.1	39.3b	79.5b	11.4	8.6b	10.9b
EM	7.07	6.3a	32.8b	34.20	348b	7.0	22.8	44.5ab	103.7a	12.0	9.1b	14.1a
EH	6.90	6.5a	35.7a	35.20	324b	7.8	21.3	46.2a	110.2a	11.2	10.1a	14.9a
P value	0.3348	0.0072	0.0040	0.8810	<0.0001	0.3274	0.0721	0.0324	0.0038	0.2704	0.0004	<0.0001

[z]For treatment codes, control represent the treatment with normal atmospheric CO_2 and light condition, in which healing and acclimatization of grafted transplants are performed in a tunnel on a greenhouse bench. EM and EH represent a high CO_2 (1,000 μmol mol^{-1}) and medium photosynthetic photon flux (PPF) (100 μmol m^{-2} s^{-1}) treatment and a high CO_2 and PPF (150 μmol m^{-2} s^{-1}) treatment in the healing chamber.

[y]Different letters correspond to significantly different values at $P \leq 0.05$ according to Fisher's least significant difference test.

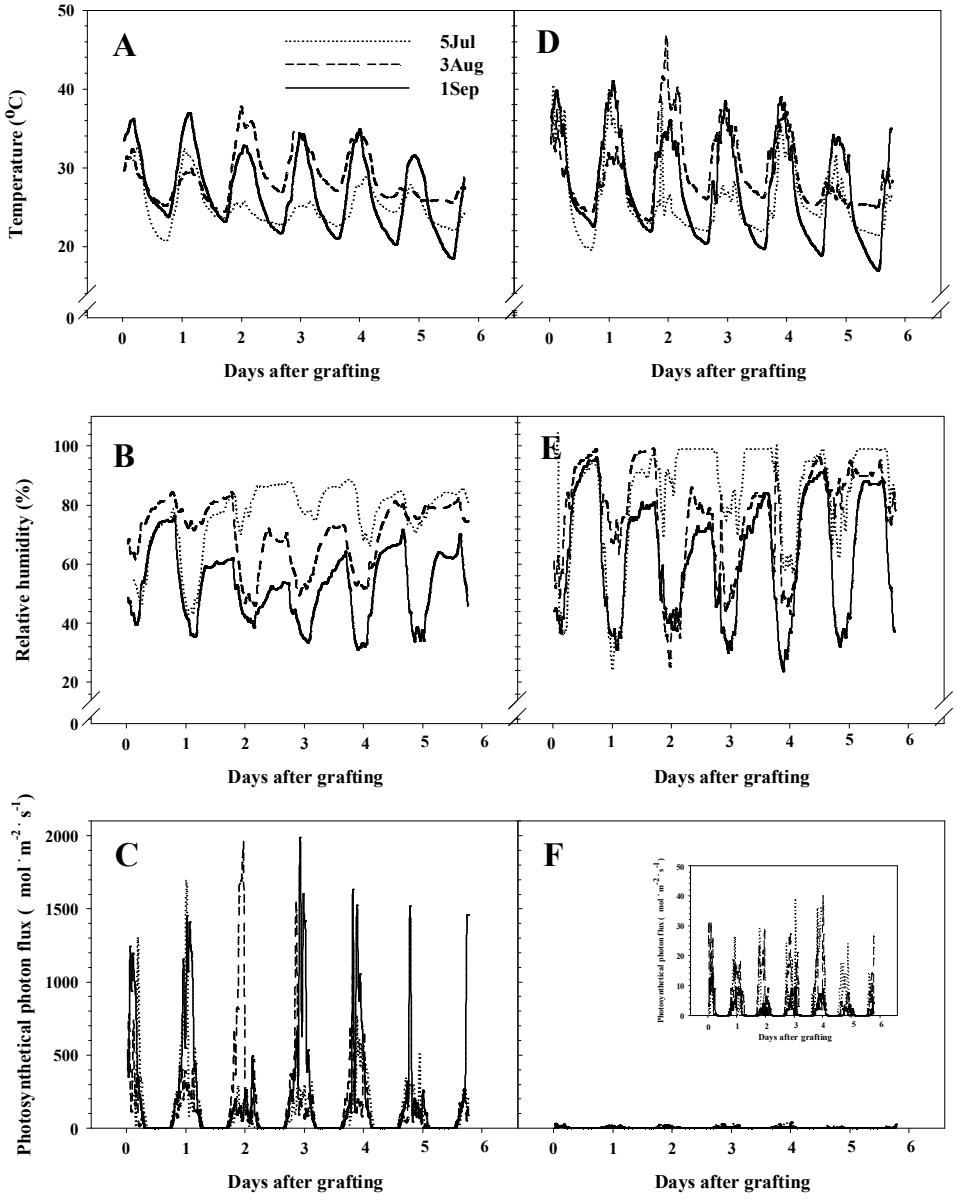

Fig. 4.1. Time course of air temperature, relative humidity, and photosynthetic photon flux (PPF) in a tunnel (D, E, and F) in a greenhouse (A, B, and C) during the healing and acclimatization of grafted pepper transplants.

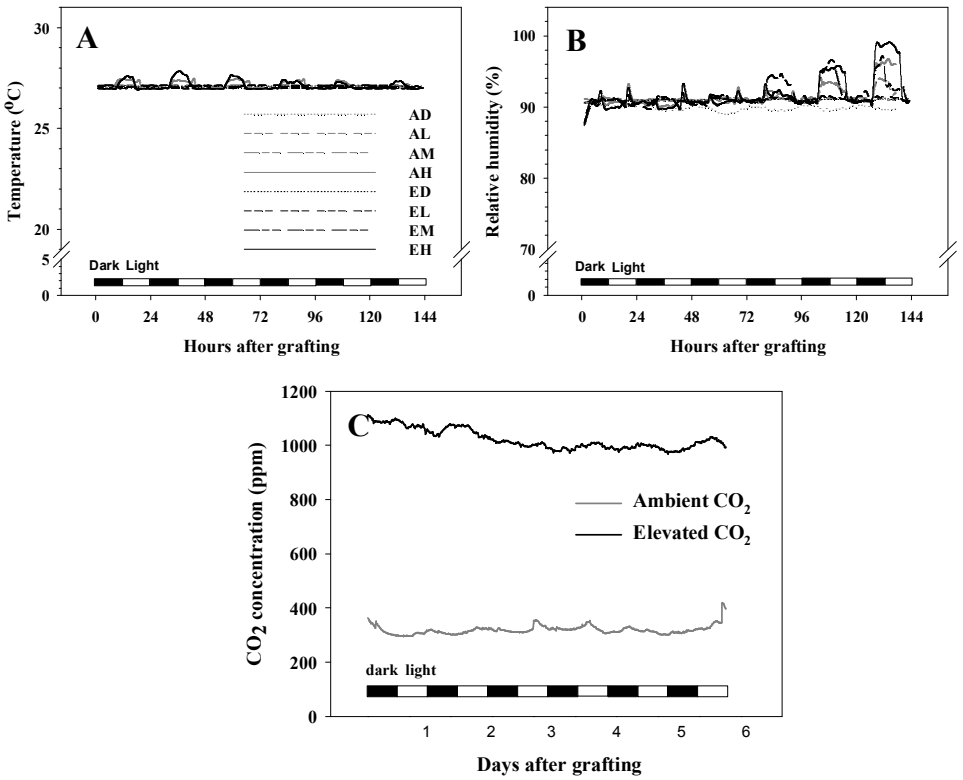

Fig. 4.2. Time course of air temperature, relative humidity, and CO_2 concentration in a healing chamber with artificial lighting during healing and acclimatization of grafted pepper transplants.

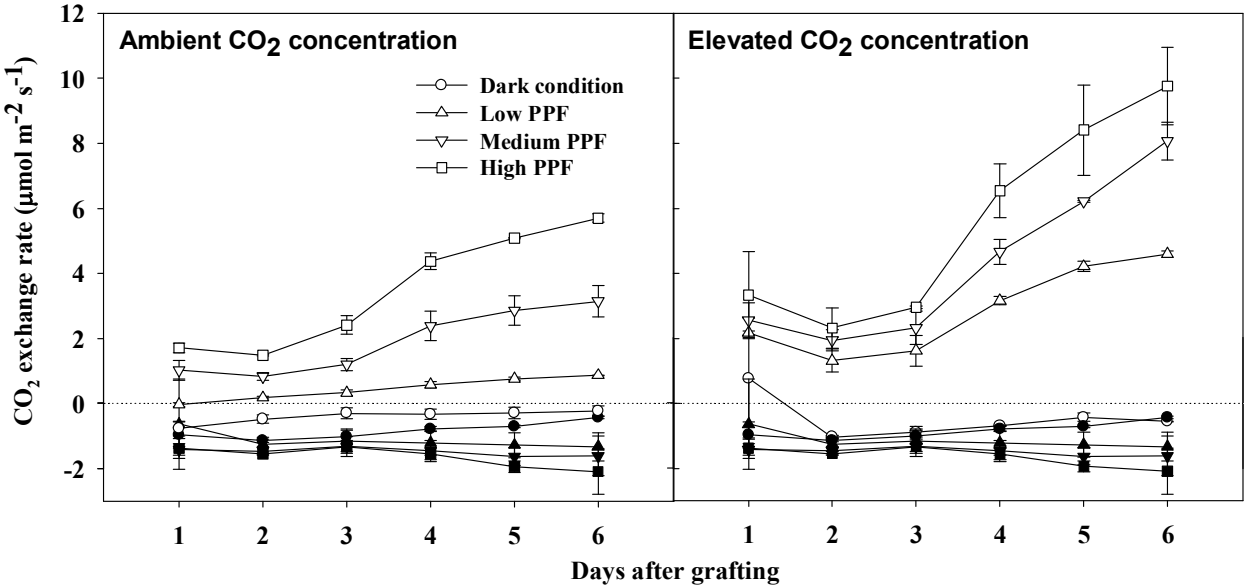

Fig. 4.3. CO_2 exchange rates of grafted pepper transplants during healing and acclimatization as affected by atmospheric CO_2 concentration and photosynthetic photon flux. Open and closed circles represent the CO_2 exchange rates during photo- and dark period, respectively. The results are shown as means ± standard error.

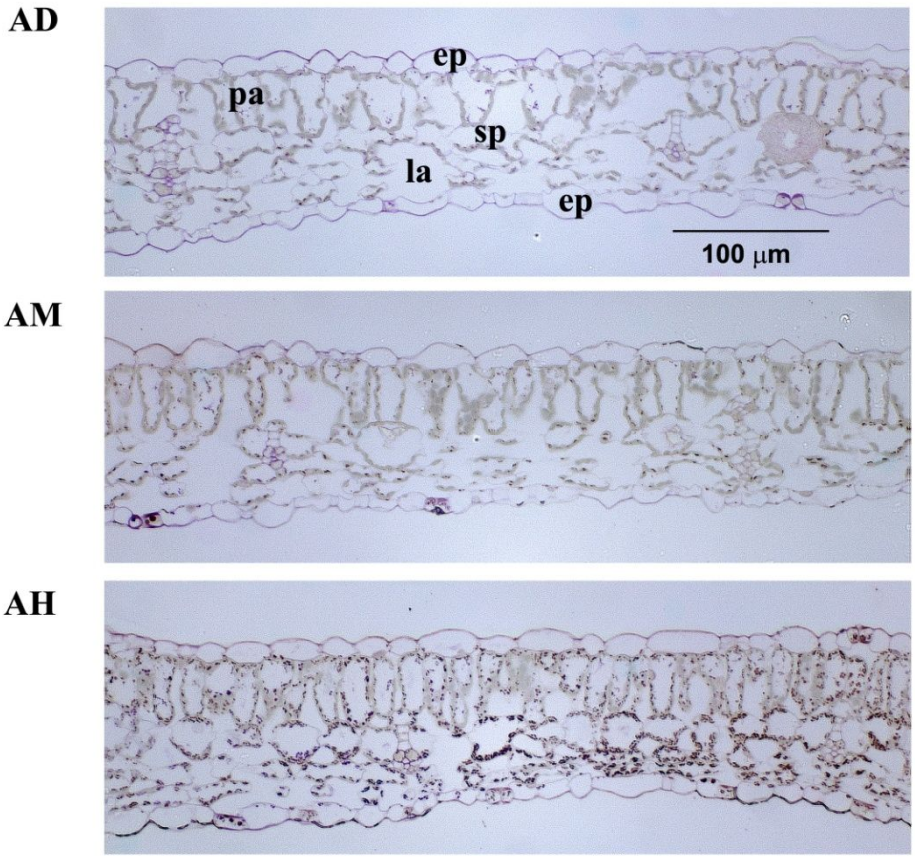

Fig. 4.4. Leaf cross-sections of scions healed and acclimatized under dark, medium, and high light conditions 6 days after grafting.

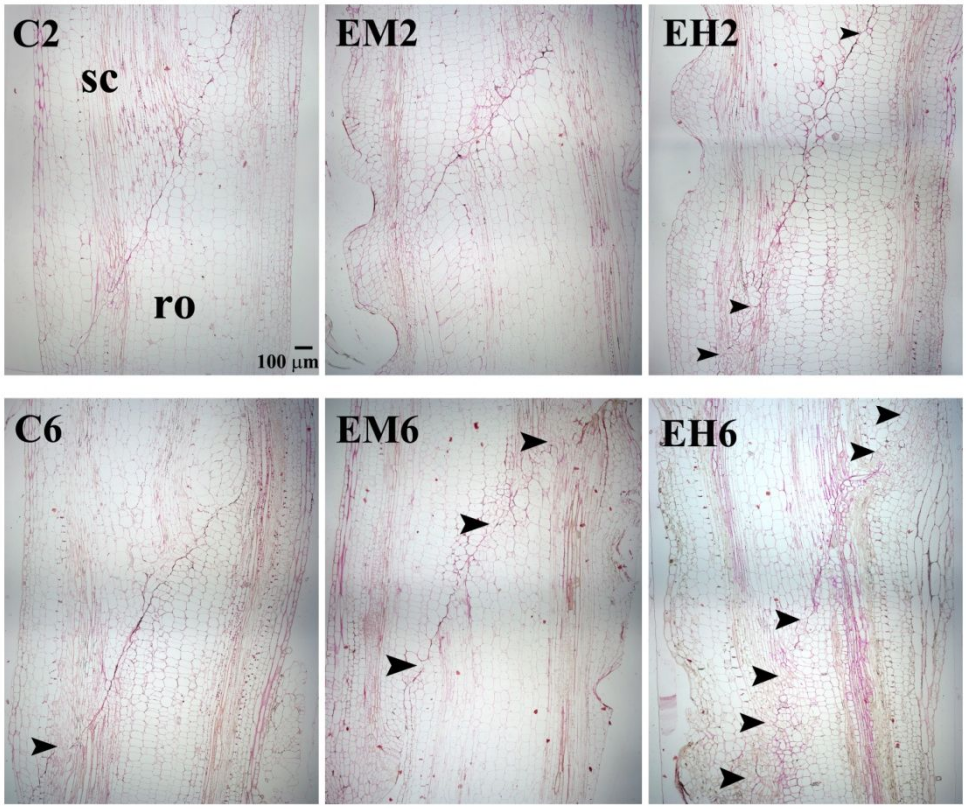

Fig. 4.5. Longitudinal sections through the graft union of the grafted pepper transplants healed and acclimatized in a tunnel on a greenhouse bench (C2-C6), and under high CO_2 concentration (1,000 µmol mol^{-1}) and medium photosynthetic photon flux (PPF) (100 µmol m^{-2} s^{-1}) conditions (EM2-EM6), and high CO_2 and PPF (150 µmol m^{-2} s^{-1}) conditions (EH2-EH6) in the healing chamber. Number in each treatment codes represents the number of days after grafting. The arrow points the juncture where scion and rootstock were united.

CHAPTER V

EFFECTS OF LIGHT QUALITY AND INTENSITY ON THE PHOTOSYNTHESIS, GROWTH, AND MORPHOGENESIS OF GRAFTED PEPPER TRANSPLANTS DURING HEALING AND ACCLIMATIZATION

ABSTRACT

This study evaluated the influence of light quality and intensity during healing and acclimatization on the CO_2 exchange rate, growth, and morphogenesis of grafted pepper (*C. annuum* L.) transplants, using a system for the continuous measurement of the CO_2 exchange rate. The grafted pepper transplants were then healed and acclimatized under different light quality conditions using fluorescent lamps (control) and red, blue, and red plus blue light-emitting diodes (LEDs). All of the transplants were irradiated for 12 hours per day, for six days, at PPF of 50, 100, or 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The higher PPF levels increased the CO_2 exchange rate during the healing and acclimatization. A smaller increase in the CO_2 exchange rates was observed in the transplants under red LEDs. The graft-take was not affected by the light quality. The grafted pepper transplants irradiated with red LEDs had lower SPAD value, leaf dry weight, and dry matter content. The transplants irradiated with blue LEDs had longer shoot length and heavier stem fresh weight

than those irradiated with the other treatments. Leaves irradiated with the red LED had the smallest leaf area and showed leaf epinasty. In addition, the palisade and spongy cells of the pepper leaves were dysplastic and exhibited hyperplasia. Grafted pepper transplants treated with red plus blue LEDs showed similar growth and morphology to those transplants irradiated with fluorescent lamps. These results suggest that high-quality grafted pepper transplants can be obtained by healing and acclimatization under a combination of blue and red lights at a high PPF level.

INTRODUCTION

The use of grafting in fruit and vegetable production has been expanded in order to control pathogens and to enhance the plants' tolerance against abiotic stresses (Louws et al., 2010; Savvas et al., 2010; Schwarz et al., 2010). Highly technical grafting skills and environmental controls during the healing and acclimatization period are required for the successful production of grafted plants. Grafted plants were usually healed and acclimatized in the past under specific environmental conditions such as high relative humidity (RH $\geq$ 95%) and low light intensity in order to produce healthy plants that survived and grew (Mun et al., 2011). However, several recent papers have reported that the photosynthesis, growth, and quality of grafted plants were improved by increasing the light intensity under highly controlled conditions during the healing and acclimatization periods (Nobuoka et al., 2005; Jang et al., 2011).

Light is an essential factor for plant growth. Many studies have shown that both the light intensity and the light quality are important for the growth, development, pigmentation, and shape of plants (McNellis and Deng, 1995; Schuerger et al., 1997; Amaki and Hirai, 2008; Fukuda et al., 2008). Various types of artificial light have been used in plant production including fluorescent, metal halide, and high-pressure sodium lamps (Fang and Jao, 2000). In recent years, light emitting diodes (LEDs) have attracted interest as light sources for plant production, because of their features such

as small size, low mass, long life, narrow spectral output, and energy conversion efficiency (Brown et al., 1995; Massa et al., 2008, Goto, 2011). LEDs enable the selection of specific wavelengths for a targeted plant response. The use of red LEDs to power photosynthesis has been widely accepted, because red wavelengths (600 to 700 nm) are efficiently absorbed by plant pigments. Early LEDs were red, with the most efficient emitting at 660 nm, which is close to one of the absorption peaks of chlorophyll (Massa et al., 2008). When baby leaf lettuce was grown under varying light sources including red, blue, and red plus blue LEDs, and fluorescent lamps, the growth was most favorable under the red single wavelength LEDs than the other treatments (Lee et al., 2010). However, blue light also has a variety of important photomorphogenic roles in plants, including stomatal control, CO_2 exchange, stem elongation, and phototropism (Massa et al., 2008).

Our study was conducted in order to evaluate the influence of different light qualities of LEDs during the healing and acclimatization period on the photosynthetic characteristics, photomorphogenesis, and growth of grafted pepper transplants using a system for continuous measurement of the photosynthetic rate.

MATERIALS AND METHODS

Plant material and growing scions and rootstocks

Pepper 'Nokkwang' (Seminis Vegetable Seeds, Inc., Seoul, Korea) and 'Tantan' (Nongwoo Bio Co. Ltd., Suwon, Korea) were used as the scions and rootstocks, respectively, for producing grafted transplants. Scion seeds were sown two days after the rootstock seeds were sown, in order to obtain scion and rootstock with similar stem diameters. Seeds were sown in 72-cell plug trays (*W* 280 × *L* 540 × *H* 45 mm, Bumnong Co. Ltd., Jeongeup, Korea) filled with commercial growing substrate (BM 2, Berger Group Ltd., St. Modeste, QC, Canada). The planting density was approximately 476 plants m^{-2}. The plug trays were wrapped with vinyl chloride resin film and then placed in a germination chamber set at a temperature of 28°C in order to promote germination. Five days after sowing, the germinated seedlings were overhead-watered and moved to a growth chamber with artificial light (Hanbaek Co. Ltd., Bucheon, Korea). The temperature of the chamber was set at 25/18°C (photo/dark periods), where the light period was 14 hours·d^{-1} and PPF was approximately 200 μmol·m^{-2}·s^{-1} provided by high pressure sodium, metal halide, and fluorescent lamps. The seedlings were bottom-irrigated twice at weekly intervals, once with water and then with a nutrient solution (EC 1.4 dS m^{-1}, 'Hanbang' for seedling, Coseal Co. Ltd., Seoul, Korea). The scion seedlings had a dry weight of 103.5±3.47 mg, an average of 5.28 true leaves, a leaf area

of 29.42±0.67 cm^2, and a stem diameter of 1.64±0.03 mm. The rootstock seedlings had a dry weight of 115.6±3.09 mg, an average of 4.48 true leaves, a leaf area of 24.65±0.79 cm^2, and a stem diameter of 1.54±0.02 mm.

Grafting

Grafting was completed four weeks after sowing the rootstock. Both the scions and rootstocks had five to six unfolded true leaves. Using a razor blade, the epicotyls of the scion and rootstock were cut 1 cm below the first true leaf. After placing the scion on the rootstock, the grafted position was tightly secured together with an ordinary grafting clip following the slice grafting method (Lee and Oda, 2003).

Healing and acclimatization of grafted pepper transplants

A continuous CO_2 measurement system using a semi-open multi-chamber was used for the healing and acclimatization of the grafted pepper transplants and the measurement of the CO_2 exchange rate (van Iersel and Bugbee, 2000; Jang et al., 2011; Mun et al., 2011). Four light-transmitting boxes (inside dimension of W 350 × L 780 × H 220 mm, volume of 60 L) made of 10 mm-thick acryl plastic were placed in the growth chamber (Hanbaek Co. Ltd., Bucheon, Korea) where the temperature was set at 22°C. Each healing box had air inlets and outlets, and a tube for drawing air. Each box was also equipped with a heater (hair dryer heater, Kaiser KHD-5207i,

My Friend Co. Ltd., Goyang, Korea) and a humidifier (nebulizer, CT-24, Techsin Electronic Co. Ltd., Foshan, China) in order to maintain the temperature and relative humidity inside.

Atmospheric air was drawn in through the inlet of the healing box at an airflow rate of 13.5 L min^{-1} using the air pumps (LP80VC, Youngnam Air Pump Inc., Busan, Korea) and flow meters (15 L·min^{-1}, Kofloc, Kojima Instruments Inc., Kyoto, Japan), and the air flowed out through the outlet (Shibuya et al., 2006; Mun et al., 2011). Moisture in the air was removed using a dehumidifying tube (SWG-A01-18/PP, Asahi Glass Engineering Co. Ltd., Chiba, Japan). The CO_2 concentrations of the air at the inlet and the outlet were measured using the CO_2/H_2O analyzer (LI-7000, Li-Cor Bioscience, St. Lincoln, NE, USA). The gas exchange in each chamber was measured for 2 minutes during a 10-minute cycle and the data were recorded for the last five seconds.

All of the sensors were attached to a data logger (CR23X, Campbell Scientific Inc., Logan, UT, USA) with a power relay (SDM-CD16AC 16-channnel AC/DC controller, Campbell Scientific Inc., Logan, UT, USA), switching heaters, humidifiers, fluorescent lamps, and solenoid valves. The temperature and relative humidity inside the box were also measured every hour using a thermocouple (T-types) and a humidity sensor (CHS-UPS, TDK Corp., Tokyo, Japan), respectively. The air temperature in the box was kept at 27°C and the relative humidity was maintained at approximately 88 to 94% (Fig. 5.1). The healing and acclimatization period lasted for

6 days, during which irrigation was not applied. Afterwards, the grafted pepper transplants were transferred and grown in a glasshouse.

The lighting system

The characteristics of the three LED types: red, blue, and red plus blue are described in Table 5.1. Panel type light sources (*W* 320 × *L* 700 × *H* 45 mm, WISE Sensor Inc., Yongin, Korea) of red, blue, or red plus blue LEDs were installed approximately 7 cm above the box. The representative peak wave length and bandwidth, respectively, at half peak height of the red, blue, and red plus blue LEDs were 639 and 19 nm for the red LEDs, 469 and 24 nm for the blue LEDs, and 640 + 468 and 20 + 24 nm for the red plus blue LEDs (Fig. 5.2). Fluorescent lamps (FL30SSD/29, Dooyoung Lighting Co. Ltd., Seoul, Korea) were used as the control and installed approximately 20 cm above the box.

The light levels were adjusted by changing the number of lamps used. The light intensity and spectral quantum distribution of the light sources were measured above the top of each healing box using a light meter with a quantum light and six sensor bars (Field Scout external light sensor meter, Spectrum Technologies, Inc., Plainfield, IL, USA) and a spectroradiometer (Black Comet CXR-SR-50, StellarNet Inc., Tampa, FL, USA), respectively. The photo period was 12 hours·d^{-1}.

Treatment

During healing and acclimatization, the grafted pepper transplants were irradiated with light with different qualities from the LEDs or fluorescent lamps. All of the transplants were irradiated for 12 hours per day at PPF 50, 100, or 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table 5.2). The experimental design was a split-plot with PPF as the main plot and light quality as the sub plot. The experiment was repeated twice. In each replication, one 72-cell plug tray with 48 plants was measured.

Measurement of CO_2 exchange rate and growth parameters

The CO_2 exchange rate of each healing box was estimated using the equation noted below with the following parameters: CO_2 concentrations, air flow rate to the box, and area of the plug tray.

$CER = F (C_i - C_o) / A$

where CER is the CO_2 exchange rate in the healing box ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), F is the air flow rate in the healing box ($mol \cdot s^{-1}$), C_i and C_o are the CO_2 concentrations ($\mu mol\ CO_2 \cdot mol^{-1}$) in the inlet and outlet of the healing box, respectively, and A is the area of the plug tray (m^2).

The CO_2 generation rates from the growing media and roots were ignored, because they were negligible compared to the exchange rate of the transplants (Shibuya and Kozai, 1998). The graft-take and growth parameters, such as fresh weight and stem diameter, were measured on the sixth day after grafting. The leaf area of the

true leaves was measured with an area meter (LI-3000, Li-Cor Bioscience, Lincoln, Nebraska, USA). The dry weight was measured after drying the samples at 80°C. Ten plants in each treatment group were sampled. The data was analyzed using SAS v.9.1 software (SAS Institute, Cary, NC, USA).

Microscopic observation

The cross sections of the specimens for microscopic observation were prepared as described by Luft (1973). Leaf pieces for measurement of the anatomy were cut off from the young upper leaves of the scions 6 days after grafting. They were infiltrated and fixed in 2.5% glutaraldehyde in 100 mM phosphate buffer (pH 7.2) for 2 hours at 4°C. They were then rinsed and post-fixed in 1% osmium tetroxide for 2 hours at 4°C and kept overnight in the phosphate buffer. After fixation, they were dehydrated through a series of graded ethyl alcohol solutions (40, 60, 80, 90, 95, and 100% in distilled water (v/v)). The tissues were further processed with three changes of propylene oxide for 15, 15, and 30 minutes per change, and gradually infiltrated for 3 hours each in 30, 50, and 100% Epon embedding media in propylene oxide to ensure complete dehydration. They were kept overnight in 100% Epon and polymerized at 60°C for 72 hours. They were sectioned (1,500 nm), stained with periodic acid-Schiff staining (PAS), and viewed under a light microscope (Axioskop 2, Carl Zeiss AG, Oberkochen, Germany).

RESULTS

CO_2 exchange rate of grafted pepper transplants

The CO_2 exchange rates of the grafted pepper transplants were near or above zero for two or three days after grafting. Then they gradually increased, averaging 2.9 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ on the sixth day (Fig. 5.3). The CO_2 exchange rates were influenced by the light quality and intensity during healing and acclimatization and the rates varied under the different light treatments. During healing and acclimatization, the CO_2 exchange rates were higher and increased faster with higher PPF levels.

At a PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the CO_2 exchange rates on the sixth day ranged from 1.2 (red LED) to 1.7 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ (fluorescent lamps) under different light quality conditions. They ranged from 2.4 (blue LED) to 3.5 (fluorescent lamps) at a PPF of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and from 3.2 (red LED) to 6.1 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ (blue LED) at a PPF of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Compared with of the plants irradiated with fluorescent lamps, the CO_2 exchange rates on the sixth day of those plants irradiated with red, blue, and red plus blue LEDs at a PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, were 29, 7, and 15% and the rates were 22, 31, and 11% lower, respectively, at a PPF of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At a PPF of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the CO_2 exchange rate of samples irradiated with blue LEDs (6.1 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) was the highest among the treatments and it was 20% higher than for those samples irradiated

with fluorescent lamps. The CO_2 exchange rate of the samples irradiated with red LEDs (3.2 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) was 37% lower than those irradiated with fluorescent lamps.

Plant growth and morphology

The grafted pepper transplants irradiated with the red LEDs had significantly lower leaf areas and leaf dry matter content than the transplants irradiated with the other treatments at PPFs of 100 and 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At a PPF of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the leaf dry weight of the transplants irradiated with the red LEDs was the lowest among the treatments and it was 19% lower than for the transplants irradiated with fluorescent lamps. The SPAD value of the leaves irradiated with the red LEDs was also lower than that of the leaves irradiated with fluorescent lamps, irrespective of the light intensity.

No difference was observed in the shoot length at a PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, but the shoot length of the samples irradiated with the blue LEDs was higher than the samples treated with other light sources at PPFs of 100 and 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table 5.3). The fresh weight of the stem irradiated with the blue LEDs was also higher than the samples irradiated with the other treatments at PPFs of 50 and 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. No differences were observed in the case of the graft-take, as all of the treatments were close to 100% (data not shown).

The grafted pepper transplants treated with different light quality and intensity showed different morphologies on the sixth day (Figs. 5.4 and 5.5). The transplants irradiated with the red LEDs

developed abaxially curled (epinastic), young, upper leaves on the third day after grafting. The epinasty became more pronounced over time and under high PPF conditions. Blue LEDs elongated the stem of the plants especially at a PPF of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The characteristics of the leaves treated with red plus blue LEDs were similar with those under fluorescent lamps.

The RGRs of the leaf, shoot, and the entire plant increased under high PPF conditions (Table 5.4). At a PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the RGRs of the entire plants irradiated with blue and red plus blue LEDs were higher than those irradiated with fluorescent lamps and red LEDs. A difference in the RGRs was not observed at a PPF of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, but the RGRs of the leaves irradiated with the red LEDs were the lowest at a PPF of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

DISCUSSION

Healing and acclimatization are critical processes for grafted plants to survive. Light intensity, relative humidity, and temperature are the key environmental factors that influence the healing and acclimatization of grafted transplants. During healing and acclimatization, PPF affected the photosynthesis, growth, and quality of the grafted transplants, with a high PPF improving these characteristics (Jang et al., 2011).

At the present time, LEDs are used in plant production because of their various merits). Early LEDs were red because they were regarded to have great potential for driving photosynthesis (Massa et al., 2008). However, there have been reports of a reduction in photosynthesis and growth under red LEDs alone in wheat, rice, radish, lettuce, and spinach (Goins et al., 1997; Yorio et al., 2001; Matsuda et al., 2004). These reports noted that the combination of red and blue light was an effective light source for several crops.

In this study, the CO_2 exchange rates of grafted pepper transplants irradiated with red LEDs were lower than those irradiated with other treatments at PPFs of 50 and 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At a PPF of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the CO_2 exchange rates of the grafted pepper transplants irradiated with blue LEDs was the highest. Liu et al. (2011) reported that the net photosynthesis of cherry tomato plants decreased under red LEDs and increased under the light treatments with blue, red plus blue, and red plus blue plus green

LEDs with a PPF of 320 μmol·m^{-2}·s^{-1}.

Tennessen et al. (1994) reported that the photosynthesis of *Pueraria lobata* (Willd) Ohwi. was greater in red light at lower light intensities (175 μmol·m^{-2}·s^{-1}) but slightly lower at high light intensities (500 μmol·m^{-2}·s^{-1}). In rice, the photosynthesis under red LEDs was also lower than under red plus blue LEDs (Matsuda et al., 2004). The difference in the photosynthesis between the light treatments of red and red plus blue LEDs was greater under higher PPF conditions. In this study, the increase in the CO_2 exchange rates of grafted pepper transplants by higher PPFs was lower under red LEDs. Light quality may affect light compensation or the saturation point and light-limited or light-saturated photosynthetic rates (Fig. 5.3).

These different photosynthetic responses were attributed to changes in stomatal development and behavior as well as the structure and chlorophyll content in leaves (Tennessen et al., 1994; Hogewonign et al., 2010; Liu et al., 2011). Leaves of cherry tomato irradiated with spectra containing blue light were reported to be thicker, have well-developed structure in the palisade tissue cells, and have more stomata (Liu et al., 2011). Tennessen et al. (1994) suggested that monochromatic red light caused an imbalance in the available light energy required for optimal functioning of photosystems I and II.

Kim et al. (2004) reported that the CO_2 assimilation rates of a single leaf at a single point could not fully explain the effect of

light quality on dry weight accumulation. They suggested that diurnal net photosynthesis and dark respiration measurements of single leaves or whole canopies would be useful in order to determine the fate of carbon in plants grown under different qualities of light. In this study, the CO_2 exchange rates of whole pepper transplants were measured continuously during the six day healing and acclimatization period (Fig. 5.3).

Right after grafting, the CO_2 exchange rates during the light period were near zero and increased as time passed. The CO_2 exchange rates during the dark period were very low (approximately -2 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) on the first day, but they were constant thereafter (about -1 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). At a PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the photosynthetic rates during the light period and the respiration rates during the dark period were almost the same. As the PPF increased, the photosynthetic rates increased, but the respiration rates remained constant. These responses to light intensity were considered to influence the RGRs of the grafted pepper transplants. At a PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the RGRs were near zero and eventually increased as the PPF increased (Table 5.4).

The grafted pepper transplants irradiated with red LEDs showed low photosynthesis rates and had low leaf area, leaf dry weight, and dry matter content (Table 5.3). The SPAD value of the leaves was also low. These results correspond to the inferior photosynthetic ability shown in the CO_2 exchange rates for red LEDs versus other light treatments. Macedo et al. (2011) reported that the growth

parameters, including specific leaf mass, thickness, leaf density, and leaf area, of *Alternanthera brasiliana* Kuntze were lowest in plants grown under red light. Goins et al. (1997) also reported that wheat grown under red LEDs alone had significantly lower amounts of dry matter than when other light treatments were used. They suggested that the lower dry matter accumulation in wheat grown under red LEDs alone might be related to the lower CO_2 assimilation rate.

Amaki and Hirai (2008) classified horticultural crops into two types according to hypocotyl or stem internode elongation in response to monochromatic light (red alone or blue alone). The first type was where the stem internode irradiated with red light was longer than that irradiated with blue light and the elongation was inhibited by blue light. Lettuce, tomato, radish, and chrysanthemum were reported to belong to this first type. The second type was where the stem internode irradiated with blue light was longer than that irradiated with red light and the elongation was inhibited by red light. Eggplant, sunflower, broccoli, rose, and pepper were reported to belong to this second type.

The inhibition of stem internode elongation by red light is theoretically mediated by phytochrome, red-light photoreceptors, and the inhabitation by blue light is supposed to be mediated by cryptochrome, blue-light photoreceptors. In this study, the shoot length of the grafted pepper transplants irradiated with blue LEDs was longer than those irradiated using other treatments. The stem

fresh weight was also greater under blue LEDs.

Under red LEDs, the young upper leaves became abaxially curled (epinastic). The epinasty became more pronounced over time and under higher PPF conditions (Figs. 5.4 and 5.5). However, this was not the case for plants grown under blue LEDs, red plus blue LEDs, or fluorescent lamps. Downward curling of the leaf margins and spiral growth of the rosette appeared in Arabidopsis plants grown under red light alone. The inclusion of blue light at any level, however, restored normal leaf morphology (Massa et al., 2008). Fukuda et al. (2008) also reported that geranium leaves showed one type of epinasty under red LEDs.

Under a combination lamp with red and blue LEDs, the geranium produced normally shaped leaves. Fukuda et al. (2008) proposed that the leaf epinasty in the geranium was controlled by cell elongation of the abaxial epidermis triggered by blue light irradiation and that these responses were shade avoidance responses. In their study, the length of the epidermal cells of the abaxial sides irradiated with blue light was 7 to 13% longer than those under red irradiation alone. The effect of light quality on the leaf epinasty was dependent on the light intensity. Fukuda et al. (2008) also reported that young leaves clearly responded to changes in the light quality, while older leaves that had already stopped growing and had no further growth capacity showed less response to the light quality.

The light quality also influenced the leaf anatomical structure (Fig. 5.6). The palisade and spongy cells of pepper leaves irradiated

with red LEDs were dysplastic and exhibited hyperplasia. The cells were aligned more densely and there was barely any empty space (lacunae) between the spongy cells. The leaf thickness was a little bit thicker in the plants irradiated under red LEDs. This result did not agree with previous reports that the thickness of the palisade and spongy parenchyma of leaves was significantly reduced in plants grown under red light (Schuerger et al., 1997; Fukuda et al., 2008; Macedo et al., 2010; Liu et al., 2011). Smaller empty space (lacunae) in the spongy tissue in leaf, especially in plants irradiated under red LEDs, may inhibit the supply of CO_2 for photosynthesis. The decrease in photosynthesis under red light seems to be related to the structure of the palisade and spongy tissue in leaves. Additional experiments need to be carried out in order to examine the structural differences in leaves grown under various types of LEDs.

In conclusion, this study showed that the light quality and intensity during the healing and acclimatization periods influenced the photosynthesis, growth, and morphology of grafted pepper transplants. Red LEDs alone decreased the photosynthesis and growth and also caused abnormal morphology in the leaves. Higher PPF levels increased the photosynthesis and growth, irrespective of light quality. The results of this suggest that high-quality grafted pepper transplants can be obtained by healing and acclimatizing plants under a combination of red and blue light and high PPF levels.

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Table 5.1. Characteristics of the LEDs and fluorescent lamps.

Light source	Relative quantum distribution (%)					Relative quantum ratio	
	300-400	400-500	500-600	600-700	700-800	R/FR ratio	B/R ratio
FL	2.5	29.8	44.0	20.0	3.7	5.4	1.490
Red LED	0.0	0.1	1.4	98.2	0.3	290.9	0.001
R+B LED	0.3	28.1	2.5	68.7	0.4	172.1	0.410
Blue LED	0.1	95.0	4.9	0.0	0.0	4.9	1291

Table 5.2. Photosynthetic photon flux (PPF) in each light treatment.

PPF ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	FL[z]	Red LED	Blue LED	R + B LED
50	56.9±2.3[y]	56.5±0.9	51.5±3.7	55.7±0.5
100	93.0±4.6	97.3±3.1	90.3±1.1	97.8±0.0
180	182.7±8.7	183.5±2.5	177.5±5.5	-

[z]FL = fluorescent lamps.

[y]Mean ± standared error.

Table 5.3. The effects of light quality and intensity on the growth of grafted pepper transplants during healing and acclimatization 6 days after grafting.

Treatment		Shoot length (cm)	SPAD value	Leaf area (cm^2)	Fresh weight (mg/plant)			Dry weight (mg/plant)			Percent dry matter (%)		
PPF ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Light source				Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
50	FL[z]	7.9a[z]	28.5a	33.2a	240b	460b	730a	18.0a	32.5a	67.1a	7.6a	7.0a	9.2a
	Red LED	7.8a	25.6b	30.6a	296a	477b	716a	20.7a	32.9a	63.8a	7.0a	6.9a	8.9a
	Blue LED	8.2a	25.6b	32.1a	297a	523a	705a	20.3a	35.7a	63.7a	6.9a	6.8a	8.9a
	R + B LED	7.6a	26.4b	32.8a	308a	473b	719a	21.0a	32.3a	65.9a	6.9a	6.8a	9.1a
100	FL	6.8b	24.3a	31.9a	271a	465a	641a	19.8a	35.1a	59.9a	7.4a	7.6a	9.4a
	Red LED	7.1ab	22.8b	27.0b	295a	492a	659a	20.2a	35.0a	55.3a	6.9a	7.1a	8.4b
	Blue LED	7.4a	23.8ab	29.6ab	265a	466a	632a	18.7a	33.2a	58.7a	7.4a	7.1a	9.3a
	R + B LED	7.3a	24.9a	31.2a	246a	477a	638a	17.9a	34.6a	59.4a	7.3a	7.3a	9.3a
180	FL	8.2b	28.4ab	42.2a	265a	536b	832a	18.8a	41.6a	83.8a	7.0a	7.8a	10.1a
	Red LED	8.6b	27.4b	33.1b	257a	573ab	786a	20.4a	41.6a	68.7b	8.1a	7.2ab	8.7b
	Blue LED	9.3a	29.6a	41.0a	309a	621a	845a	18.2a	41.1a	80.2a	6.3a	6.7b	9.6a

[z]FL = fluorescent lamps.

[y]Different letters indicated a significant difference within a PPF treatment at P≤0.05 according to Duncan's multiple range test.

Table 5.4. The effects of light quality and intensity on the relative growth rate ($g \cdot g^{-1} \cdot day^{-1}$)[z] of grafted pepper transplants during healing and acclimatization.

Treatment		Root	Stem	Leaf	Shoot	Entire plant
PPF ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Light source					
50	FL[y]	-0.014a[x]	-0.004a	0.010a	0.006a	0.003b
	Red LED	0.010a	-0.002a	0.001a	0.000a	0.003b
	Blue LED	0.006a	0.009a	0.005a	0.007a	0.017a
	R + B LED	0.010a	-0.005a	0.005a	0.002a	0.020a
100	FL	0.046a	0.023a	0.021a	0.023a	0.027a
	Red LED	0.052a	0.024a	0.007a	0.014a	0.021a
	Blue LED	0.037a	0.014a	0.017a	0.017a	0.020a
	R + B LED	0.030a	0.022a	0.020a	0.021a	0.023a
180	FL	0.006a	0.018a	0.086a	0.060a	0.053a
	Red LED	0.017a	0.018a	0.044b	0.035b	0.033a
	Blue LED	0.007a	0.018a	0.079a	0.055a	0.048a

[z]Relative growth rate = $(\ln w_2 - \ln w_1) / (t_2 - t_1)$, where w_2 and w_1 represent the last and initial dry weight (g) and $(t_2 - t_1)$ represents the experimental time (day).

[y]FL = fluorescent lamps.

[x]Different letters indicate a significant difference within a PPF treatment at $P \leq 0.05$ according to Duncan's multiple range test.

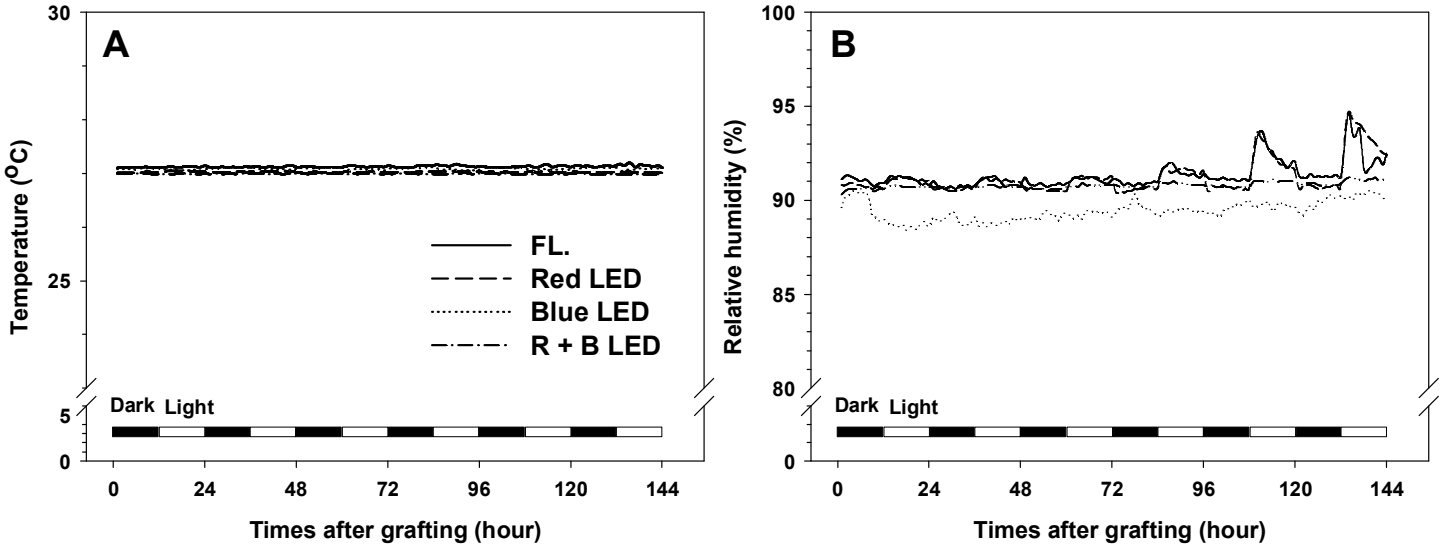

Fig. 5.1. Time course of the air temperature and relative humidity conditions in light quality treatments during the healing and acclimatization of grafted pepper transplants. The photosynthetic photon flux was 100 μmol·m^{-2}·s^{-1}. FL = fluorescent lamps.

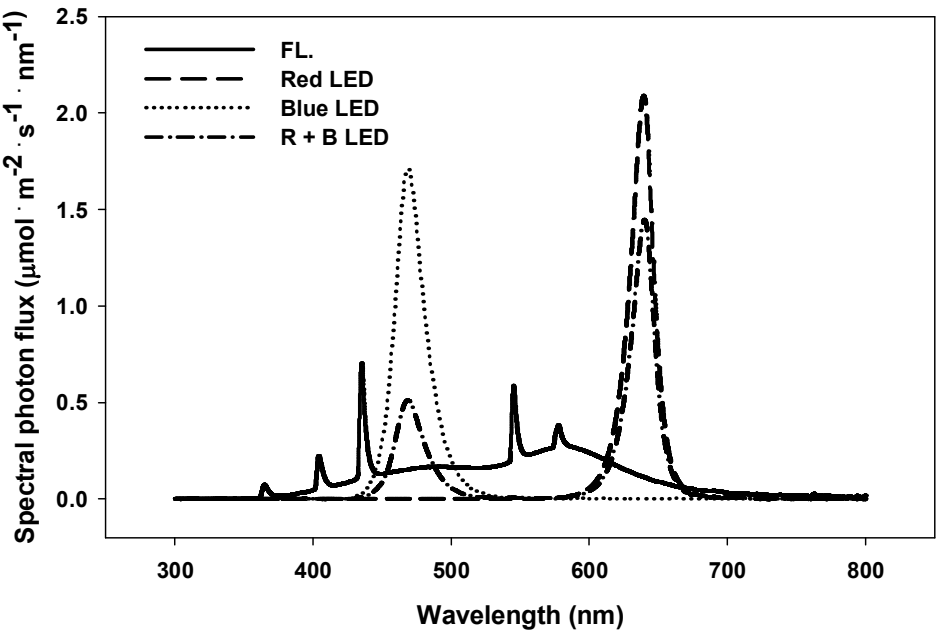

Fig. 5.2. Spectral distributions of the light emitting diodes (LEDs) and fluorescent lamps (FL).

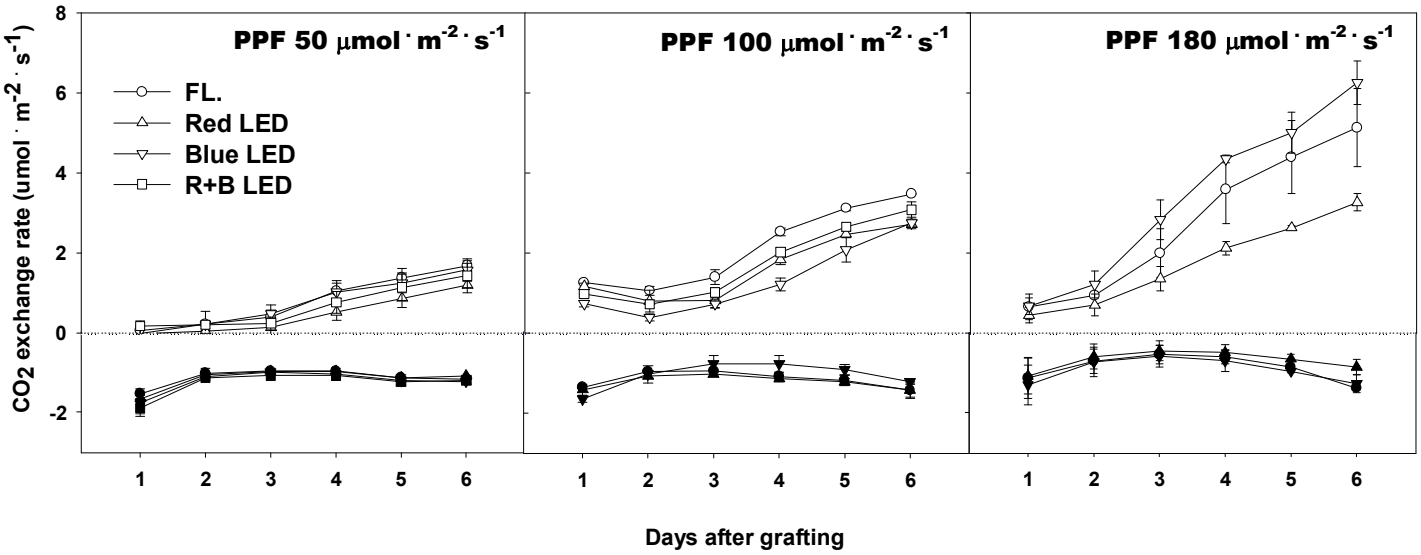

Fig. 5.3. The effects of light quality and intensity on the CO_2 exchange rates of grafted pepper transplants during healing and acclimatization. Open circles represent the CO_2 exchange rates during the photoperiod and closed circles represent the CO_2 exchange rates during the dark period. Results are means ± standard error. FL = fluorescent lamps.

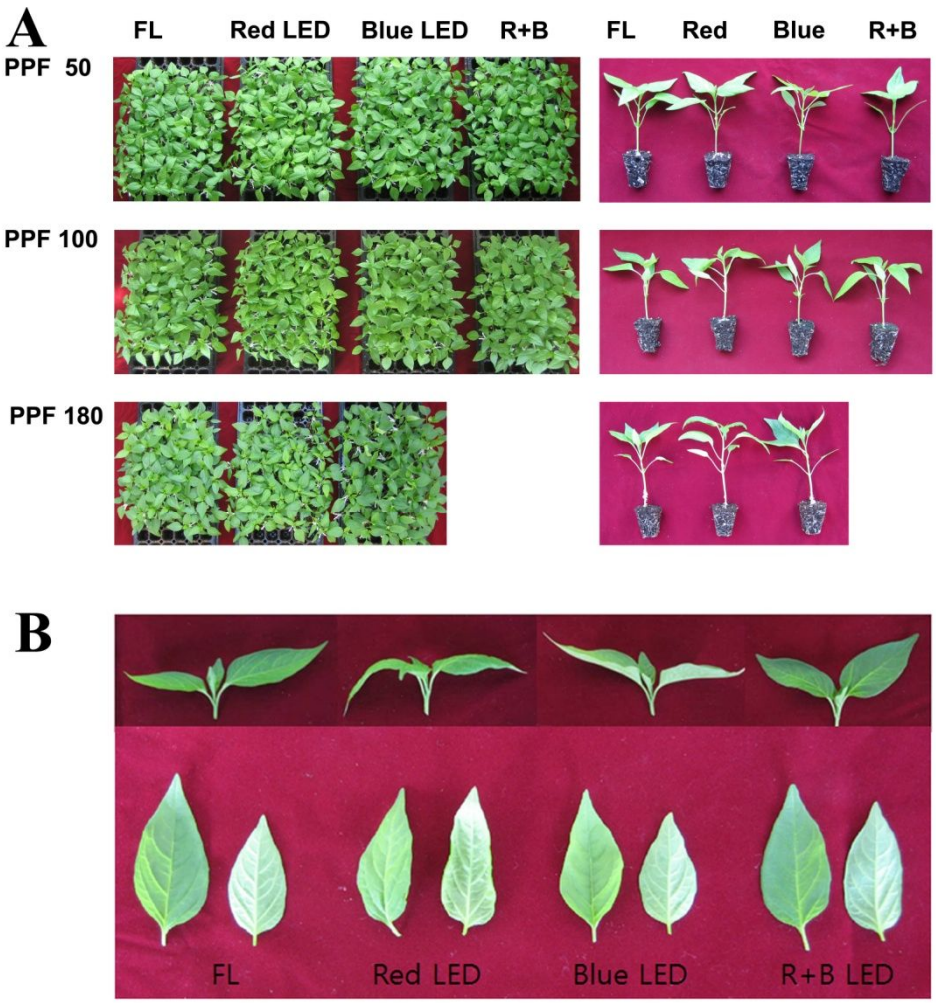

Fig. 5.4. The effects of light quality and intensity on the morphology of grafted pepper transplants (A) and young upper leaves treated with different light qualities (B) during the six day healing and acclimatization period. The photosynthetic photon flux in (B) was 100 μmol·m^{-2}·s^{-1}. FL = fluorescent lamps.

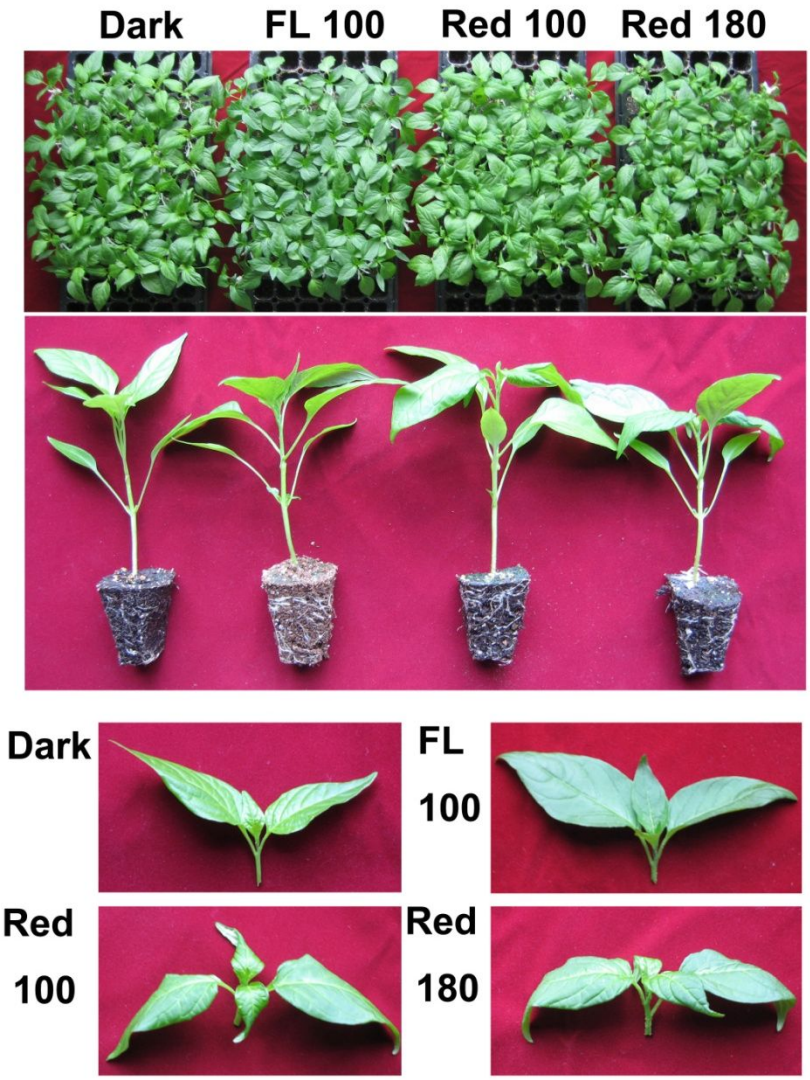

Fig. 5.5. Cross-sections of young upper leaves of grafted pepper transplants treated with different light qualities during the six-days healing and acclimatization period. The photosynthetic photon flux during the healing and acclimatization period was 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. FL = fluorescent lamps; pa = palisade tissue cells; sp = sponge tissue cells; la = lacunae.

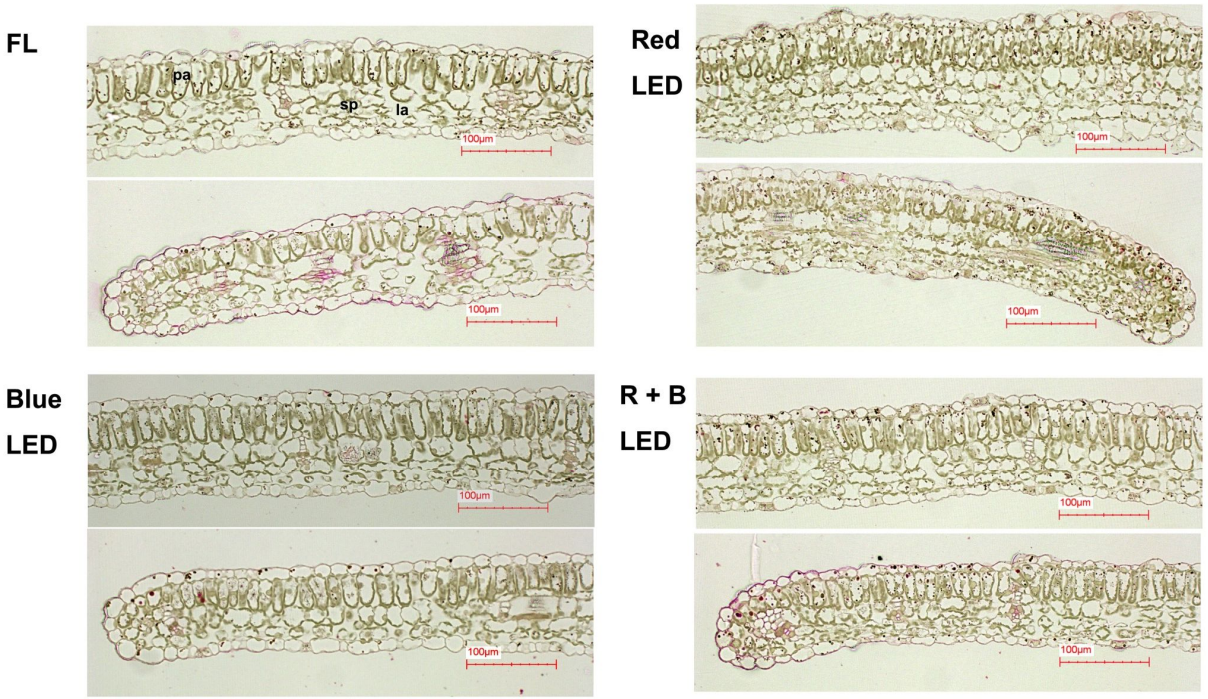

Fig. 5.6. Cross-sections of young upper leaves of grafted pepper transplants treated with different light qualities during the six-days healing and acclimatization period. The photosynthetic photon flux during the healing and acclimatization period was 100 µmol·m^{-2}·s^{-1}. FL = fluorescent lamps; pa = palisade tissue cells; sp = sponge tissue cells; la = lacunae.

GENERAL CONCLUSION

Improvement of the Tolerance to Biotic and Abiotic Stresses by Grafting

Grafting is a promising tool to control pathogens and to enhance the tolerance against abiotic stresses. Pepper has been grafted onto the rootstocks resistant to Phytophthora blight. In this thesis, grafting using rootstocks resistant to both Phytophthora blight and bacterial wilt improved the resistance of pepper to the major two diseases. Pepper grafting using rootstocks with high resistance can be applied to obtain resistance to various diseases including foliar fungal diseases and viruses in pepper production (Fig. 2).

When pepper was grafted onto rootstocks selected for their tolerance to low temperature, the early growth after transplanting was greater than those of non-grafted peppers. However, the yield of marketable and total fruit decreased rapidly with the decline of night temperature (NT). Even though the growth and yield of grafted peppers were greater than those of non-grafted peppers irrespective of temperature, the commercial productivity was not improved in NT 8°C or 15°C. In cucumber, the grafting using rootstocks tolerant to low temperature such as figleaf gourd has been reported to improve vegetative growth and early yield at suboptimal temperatures. The different adaptability to low temperature among rootstocks would be offset by four-month harvest period. Moreover, because all of rootstocks used for green

pepper production are the same species (*C. annuum* L.) as pepper, they require similar temperature condition and their rooting ability may be not as strong as that of figleaf gourd which is different genus from cucumber. Accordingly, in order to expand the purpose and use of pepper grafting, the development of suitable tolerant or resistant rootstocks should be accompanied. Further study will also be needed to assess related species (e.g. *Capsicum baccatum, C. pubescens*) as rootstocks in order to widen the tolerance or resistance (Fig. 2).

Pepper grafting for the improvement of tolerance to biotic and abiotic stresses affected the apparent fruit quality (fruit size, weight, flesh thickness) and texture property. However, fruit quality parameters were affected by grafting with different scion varieties and cropping patterns. The fruit characteristics of rootstock did not affect the fruit characteristics of scion grafted onto that rootstock. Accordingly, rootstock/scion combination, the scion variety and the cropping pattern must be carefully chosen to get the desired optimal fruit quality.

Development of Acclimatization Techniques to Improve Quality of Grafted Transplants

In the grafted transplant production procedures, healing and acclimatization are critical for grafted transplants to survive and grow as healthy plants, which involve the healing of the cut surface and hardening for field or greenhouse survival. Healing and

acclimatization which are performed in a tunnel made of double-layered plastic film on a greenhouse bench have been practiced by focusing on the survival of grafted transplants rather than growth. However, it is found that the PPF and atmospheric CO_2 concentration during healing and acclimatization affect the photosynthesis and growth of grafted pepper transplants, and higher PPF conditions improved the photosynthesis and growth of the transplants, including the graft union formation.

The light quality and intensity during the healing and acclimatization periods influenced the photosynthesis, growth, and morphology of grafted pepper transplants. Red LEDs alone decreased the photosynthesis and growth and also caused abnormal morphology in the leaves. The transplants irradiated with blue LEDs had longer shoot length and heavier stem fresh weight. Grafted pepper transplants treated with red plus blue LEDs showed similar growth and morphology to those transplants irradiated with fluorescent lamps. The results of this suggest that high-quality grafted pepper transplants can be obtained by healing and acclimatizing plants under a combination of red and blue light and high PPF levels.

Healing and acclimatization in a healing box are expected to enable the vegetable nursery grower to control and maintain more optimal conditions for grafted transplants than those in a tunnel in a greenhouse, resulting in the production of high-quality grafted transplants. Healing and acclimatization in a healing chamber using

a healing chamber with artificial lighting are recommended as follows;

1. Healing and acclimatization period: 6 days
2. Environmental conditions
   1) Air temperature: 27°C
   2) Relative humidity: ≥85%
   3) Atmospheric CO_2 concentration: 1,000 μmol·mol^{-1}
   4) Light condition
      - Light source: fluorescence lamps,
        or the red plus blue LEDs (640 + 468 nm)
      - PPF 100-200 μmol m^{-2}·s^{-1}

   (* Higher PPF promotes the photosynthesis and growth of grafted transplants)

      - Light period: 12 hours·d^{-1}

✓ Factors which should be examined additionally: air flow rate, DIF

Grafting is expected to be a tool to improve the tolerance to major soil-borne diseases and low temperature stress. However, in order to expand the purpose and use of pepper grafting, the identification and development of multi-disease resistant rootstocks with tolerance to abiotic stresses should be accompanied. In addition, to control and maintain more optimal conditions during healing and acclimatization enable the production of high-quality grafted transplants which is essential and critical for the successful pepper production. Healing and acclimatization in a healing

chamber using a healing chamber with artificial lighting can solve the problems arising from conventional healing and acclimatization. It can be used for commercial plug seedling growers to produce high-quality vegetable transplants.

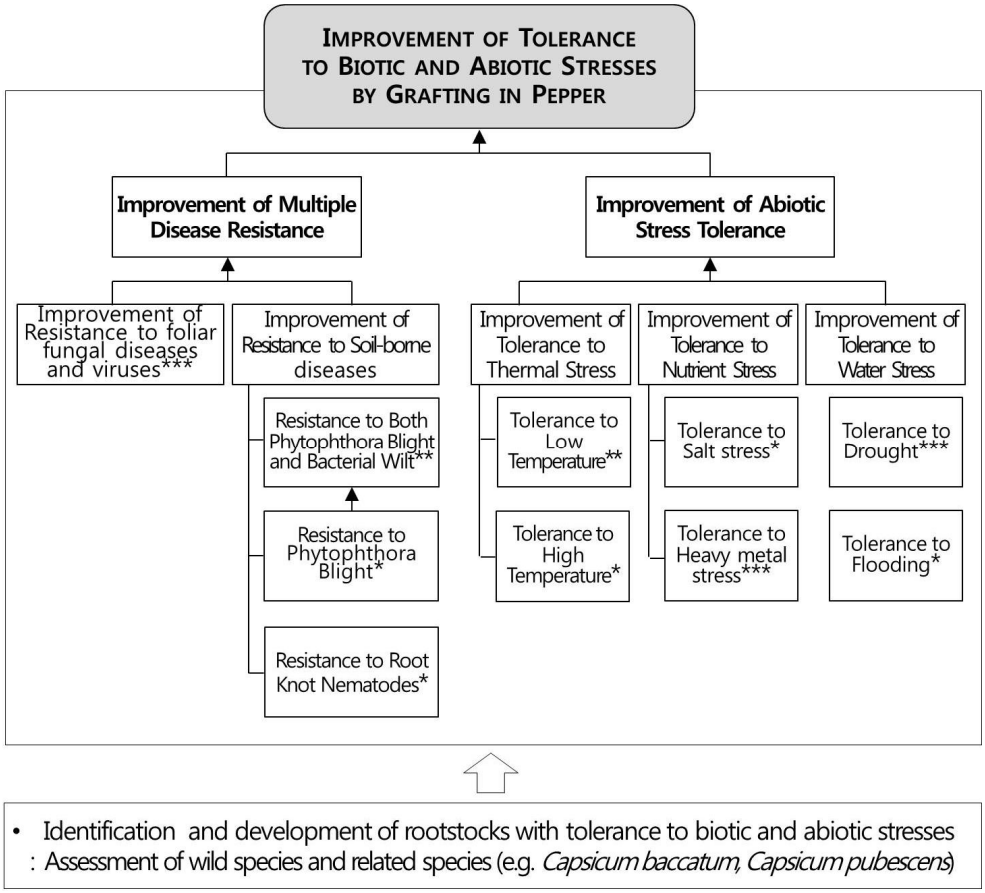

Fig. 2. The concept of improvement of the tolerance to biotic and abiotic stresses by grafting in pepper. * indicates the grafting effects which have been examined or applied commercially; ** indicates the grafting effects which have been examined in this thesis; *** indicates the grafting effects which should be examined.

ABSTRACT IN KOREAN

본 논문은 고추(*Capsicum annuum* L.)에 있어서 접목을 통한 생물·비생물적 스트레스에 대한 내성 향상과, 이러한 접목에 있어서 접목묘 생산의 성공여부를 좌우하는 접목활착기술 개발에 관한 것이다. 본 논문은 5개의 장으로 이루어져 있다. 제1장과 제2장은 고추 접목의 주요 토양병 저항성 및 저온 내성 향상효과에 관한 연구이며, 제3장은 접목이 고추의 과실에 미치는 영향에 관한 연구이다. 제4장과 제5장은 고추 접목묘의 생육촉진 및 품질향상을 위한 접목활착기술 개발에 관한 연구로, 인공광을 이용한 접목활착시스템 내에서의 광(광질 및 광량) 및 이산화탄소 조건 구명에 관한 연구이다.

제1장에서의 연구는 고추 접목이 역병·풋마름병 저항성 및 생육에 미치는 영향을 구명하고, 역병·풋마름병 복합저항성 육성계통의 대목으로서의 이용가능성을 검토하기 위하여 수행되었다. 역병·풋마름병 복합저항성 육성계통을 대목으로 이용시, 수량 및 품질의 저하 없이 역병·풋마름병에 시판 대목 이상의 저항성을 나타내어, 대목으로서의 이용가능성을 확인하였으며, 복합 병 저항성 대목을 이용한 접목을 통하여 고추의 병 저항성을 증진시킬 수 있음을 확인하였다.

제2장에서는, 겨울철 등 저온기 고추재배시 문제가 되는 저온에 대하여 접목을 통한 내성 증진 가능성을 검토하기 위하여, 대목종류 및 시설 내 야간온도 조건이 고추 접목묘의 생육 및 수량에 미치는 영향을 검토하였다. 검토된 고추 품종은 야간온도 조건에 따른 상대생장률에 근거하여, 3개의

유형으로 분류할 수 있었다. 처리온도 조건에 상관없이 일정한 상대생장률을 나타냈던 품종들을 선발, 대목으로 하여 고추에 접목한 후, 야간 최저온도 조건이 다른 시설에서 재배하였을 때, 정식 후 초기생육은 온도조건에 상관없이 접목처리구가 무접목구에 비해 우수하였다. 고추의 수량은 접목시 무접목에 비해 다소 우수하였으나, 온도가 낮을수록 수량감소는 급격하여, 8 또는 13°C의 낮은 온도조건에서도 안정적인 수량을 얻을 수 있는 저온 내성효과는 얻을 수 없었다.

접목재배시 이용되는 대목의 종류에 따라 접수의 과실 크기, 형태 등이 영향을 받는 것으로 보고되고 있다. 제3장에서는 고추 접목 및 대목의 종류가 과실에 미치는 영향을 검토하기 위하여, 대목 종류를 달리한 고추 접목묘의 반촉성 및 억제재배시 과실품질을 조사하였다. 고추 과실의 외관상의 특성 및 물성은 대목의 종류, 수확시기, 재배작형의 영향을 받았다. 그러나 대목품종의 과실특성이 접목재배시 접수품종의 과실특성에 영향을 미치지는 않았다.

접목활착은 접목묘 생산에 있어서 매우 중요하다. 제4장과 제5장에서는 인공광을 이용한 활착챔버에서의 최적 활착환경조건(광 및 이산화탄소 조건) 구명을 위하여, 광질·광량 및 이산화탄소 농도 조건을 달리하여 고추 접목묘를 활착시켰을 때 활착기간 중 광합성 및 생육을 검토하였다. 활착기간 중 검토된 광질에 상관없이 광량이 증가함에 따라 고추 접목묘의 광합성량이 증가하였으며, 그 차이는 시간이 지남에 따라 더 커졌다. 이러한 광합성량의 증가는 생육증가로 이어졌으며, 접목부위의 접수와 대목간 연결도 촉진되었다.

활착기간 중 광량에 따라 고추 잎의 해부학적 특성도 영향을 받아, 광량이 높을수록 잎의 책상조직 및 해면조직이 더 치밀해졌으며, 엽록체의 함량도 증가하였다.

활착기간 중 적생광 조사구는 다른 광질 조사구보다 고추 접목묘의 광합성량, SPAD 값, 엽중, 건물률이 낮았다. 또한 잎은 상편생장(epinasty)을 하여 잎이 아랫쪽으로 말리는 증상을 보였으며, 엽내 책상조직과 해면조직의 세포수가 이상적으로 증가하는 것을 관찰할 수 있었다. 적색과 청색의 혼합광하에서는 형광등에서와 유사한 생육을 보였다. 따라서 환경조절이 용이한 활착챔버 내에서 형광등 또는 적색 LED와 청색 LED를 혼합 조사하여 높은 광량과 이산화탄소 농도를 높게 유지함으로써, 접목묘의 생육및 품질을 향상시킬 수 있음을 확인할 수 있었다.

요약하면, 접목을 통하여 고추의 주요 토양병 및 저온 등 불량환경에 대한 내성을 높일 수 있었으며, 이를 위해서는 강한 내성을 갖는 적절한 대목의 개발에 관한 지속적인 연구가 필요하다. 아울러 고추 접목에 의해 과실 특성이 영향을 받으나, 접목 외 여러 가지 요인이 복합적으로 영향을 미치는 것으로 판단된다. 또한 고추 접목묘 생산에 있어서 매우 중요한 활착기간 중 광량 및 이산화탄소 농도를 높여주는 등 환경개선 및 정밀한 환경관리를 통하여 접목묘의 광합성, 생육 및 활착을 촉진할 수 있음을 확인하였다. 이와 같은 활착환경 관리 기술은 묘 생산을 전문으로 하는 공정육묘장에서의 과채류 접목묘를 연중 안정적으로 대량생산하는 데에 적용할 수 있을 것으로 기대된다.