Article

The Effect of Night Low Temperature on Agronomical Traits of Thirty-Nine Pepper Accessions (*Capsicum annuum L.*)

Sherzod Nigmatullayevich Rajametov†, Kwanuk Lee†, Hyo-Bong Jeong, Myeong-Cheoul Cho, Chun-Woo Nam, and Eun-Young Yang*

National Institute of Horticultural & Herbal Science, Rural Development Administration, Wanju, 55365, Republic of Korea; sherzod_2004@list.ru (S.N.R.); kwanuk01@korea.kr (K.L.); bong9846@korea.kr (H.B.J.); chomc@korea.kr (M.C.C.); cwsky1004@daum.net (C.W.N.)

* Correspondence: yangyang2@korea.kr (E.Y.Y.); Tel: +82-(0)63-238-6613; Fax: +82-(0)63-238-6605

† Equal contribution

Abstract: Pepper plants experience complex environmental factors including abiotic and biotic stresses in field and the importance of climate changes including low and high temperatures has been emerged. Low temperature stress in the growth and development is one of the most critical issues, which directly impact on the crop yield and productivity of pepper plants. It is essential to select and breed low temperature-(LT) tolerant pepper (*Capsicum annuum L.*) cultivars. The research was performed to assess the agronomical traits of 39 pepper accessions belonging to chili and bell fruit types which cultivated two different night temperature set-points at 15°C for suboptimal temperature (CT) and at 10°C for low temperature (LT), respectively. Plant heights (PH) of most pepper accessions in LT were significantly decreased compared to those in CT. The stem diameter (SD) and the length of main axis (LMA) were various depending on the genotypes under LT. Moreover, the number of flowers (NFL), the total number of fruits (NFR), fruit yield (FY), fruit fresh weight (FFW), fruit length (FL), fruit diameter (FD), and the number of seeds in a fruit (NSF) were notably declined in LT compared to CT. The evaluated agronomical traits between LT and CT were further applied for the correlation analysis, the principal component analysis (PCA), and the hierarchical cluster analysis. Notably, FY trait was correlated with other reproductive traits including NFR, FFW, FD, and FL on positive directions and LT treated-39 pepper accessions were clustered into seven groups by the clustering analysis. The selected accessions were primarily involved with the positive trends with the reproductive index including NFR, FL, FD, and FW traits and would be used for pepper breeding programs on developing LT-tolerant cultivars.

Keywords: chili and bell pepper; low temperature stress; vegetative and reproductive traits; pepper breeding; PCA; hierarchical cluster analysis

1. Introduction

Pepper plants (*Capsicum annuum L.*) are originated from American tropics and classified into Solanaceae family, which are considered as an essential horticultural crop. Among 30 species in *Capsicum*, around 5 species including *C. annum*, *C. baccatum*, *C. chinense*, *C. frutescens mill*, and *C. pubescens* have been broadly domesticated by plant breeders and farmed in agricultural area [1]. The pepper fruits of chili and bell peppers are utilized in diverse cuisines as a source of basic ingredients contributing to a variety of vitamins, phytochemicals, minerals, food colors, and capsaicin [2-4]. The importance of peppers in agriculture has been increased and the cultivation area and production of peppers have been continuously risen all over world. According to Food and Agriculture Organization (FAO) and Korean Statistical Information Service (KOSIS) in 2019, the cultivation area and the production have attained approximately 4.5 million ha and 61 million tonnes of total green and dried peppers in the world (http://www.fao.org/faostat/) as well as around 36,600 ha and 334,280 tonnes including green and dried peppers of chili and bell types in Korea (https://kosis.kr/eng/), respectively.

Climate changes including low and high temperatures, strong wind, drought, flood, and havy rainfall have been severely influenced the crop yield and productivity [2,5]. Particularly, reports have been demonstrated in the impact of the night low temperature (NLT) with different temperature regimes during the period of entire growth and development [6,7]. The NLT remarkably affect seed germination, seedling growth, leaf morphology, stem diameter (SD), and plant height (PH) during vegetative stages of tomato, cucumber, and peppers [7-14], causing poor growth and development. The temperature stress has also affected reproductive index including agronomical traits of the number of flowers (NFL) and fruits (NFR), fruit set (FS), as well as fruit yield (FY) during reproductive stages of pepper plants [15-17]. the pepper plants grown in temperatures below 15°C resulted in abnormal flower shapes, reduced pollen activity and quality [18-20], parthenocarpic fruits [21], and decrease in the number of seeds in fruit (NSF) [21-23]. Moreover, since the flower development is highly associated with NFR, FS, and FY in response to LT, many researches have focused on the development of floral organs. In comparison with those in normal and low temperature, flower development was shown in malformation of unexpanded petals, stunted stamens containing a few of pollen, and reduced pollen germination activity in androecium [19,22] and impaired with swelling ovary and shorter styles in gynoecium, resulting in poor quaility of fruit development such as smaller, flatted, and irregular shaped fruits [15-22]. Although the effects of NLT on aforementioned vegetative and reproductive parameters have been determined in pepper plants, the impact of NLT are only characterized in limited accessions and certain pepper type mainly in sweet peppers.

The temperature regulation is one of the most important factors for pepper growth and development and is preferentially considered for the cultivation in winter greenhouse. According to Korea Meteorological Administration (KMA) (https://www.weather.go.kr/w/index.do (accessed on 9 August 2021)) and previous our publication [19], in Wanju where this study was performed in Korea, the minimum and maximum temperature has been fluctuated from −6.3 to 8.7 °C. As well as, the average temperature has been maintained from −1.7 to 3.9 °C for the winter season since 1970, indicating that the climate of Wanju is fluctuated from -7 to 10 °C during the period of winter season. Generally, the heating demand for greenhouse cultivation is significantly inclined at night time in winter [24] and the heating utility spent around 19–23 % from the operating cost of 2017–2019 in Korea [25]. Importantly, the several researches have been studied on sub-optimal temperatures in greenhouse that could result in the decrease in approximately 16% of energy cost by declining by 2 °C [26]. The studies have reported that the sub-optimal temperature ranging from 15-20 °C was considered for the minimal low temperature without serious damage to plant growth and development [27,28]. The LT led to more increased fruit yield than optimal temperature (> 20 °C) [22], whereas LT (below 10 °C) resulted in severe defective growth and development of pepper plants [29,30]. However, a few studies have been recently elucidated in developing the breeding systems for selecting low temperature (LT)-tolerant pepper accessions by economically regulating NLT at 10 °C and optimal temperature set-points at 15-20 °C in winter greenhouse.

In this work, we analyzed the agronomical traits including chili peppers (n = 27) and bell peppers (n = 12) in response to low night temperature in the greenhouse condition. The vegetative parameters of PH, SD, and LMA and the reproductive parameters of NFL, FY, FW, FD, NFR, and NSF were investigated among 39 pepper genotypes between 15 °C and 10 °C in greenhouse. On the basis of the correlation, PCA, and cluster analysis with 10 agronomical traits, we selected 4 genotypes of chili peppers and 4 genotypes of bell peppers exhibiting low or high performance of vegetative and reproductive parameters which is associated with high FY triat between LT and CT. The characterized and identified pepper genotypes in the present study will be used as a good resource for pepper breeders to breed LT stress-tolerant cultivars with the consideration of the high FY index in winter greenhouse.

2. Materials and Methods

2.1 Plant material and growth conditions

Total 39 pepper breeding lines including chili (n = 27) and bell peppers (n = 12) from National Institute of Horticultural and Herbal Science (NIHHS, Korea, 35°83′ N, 127°03′ E) were utilized in the experiments (Table S1). The seeds of the 39 pepper lines were sown on 28 September, 2020 in plastic trays which were 52 x 26 cm in pot size and 6 x 6 cm in cell size. The trays were transferred into a glasshouse that maintained 26/18 °C (day/night) and relative humidity within 65-70% and the water was daily given with a liter. 14-d-old seedlings were transplanted on 13 November, 2020 into two plastic film greenhouses. Pepper seedlings of ten plants per accession were planted with a distance of 35 cm by 35 cm between plants in both LT and CT greenhouses. To help the pepper seedlings adapt new environment conditions, night temperature set-point was initially maintained at 15 °C for 2 weeks in both greenhouses and then was modulated for LT and CT, respectively.

2.2 Soil preparations

The soil preparations in tray and two greenhouses were followed as previously described in [16]. The soli contained 1:1 of sand and commercial bed soil (Bio Sangto, Seoul, Korea) which consist of coco peat (47.2%), peat moss (35%), zeolite (7%), vermiculite (10.0%), dolomite (0.6%), humectant (0.006%), and fertilizers (0.194%) which are made of 270 mg kg^{-1} of N, P, and K, respectively. The soil preparations in two greenhouses were prepared as followed by the recommendations of the Korea Soil Information System (KSIS) (https://soil.rda.go.kr (accessed on 28 August 2021)) equally with pre-plant broadcast manure at a dose of 1 kg m^{-2} and basal fertilizer containing 16 g m^{-2} N, 8 g m^{-2} K_2O, and 16 g m^{-2} P_2O_5, which were regularly fertigated with the mixture of solution A (5.5% nitrogen, 4.5% potassium, 4.5% calcium, 0.00014% boron, 0.05% iron, 0.0001% zinc, and 0.0002% molybdenum) and B (6% nitrogen, 2% phosphorus, 4% potassium, 1% magnesium, 0.05% boron, 0.01% manganese, 0.005% zinc, and 0.0015% copper) in 1200 L water (Mulpure, Daeyu Co. Ltd., Gyeongsan, Korea)

2.3 Temperature regulations

The winter climate where this experiment was conducted was shown in previous our report [16]. Briefly, the temperature was monitored and recorded in LT and CT greenhouses during the period of the pepper cultivation using data logger (Figure S1) (WatchDog 1450, Spectrum Technologies Inc., Aurora, USA). Night time temperature was maintained by heating machine (Model TKP-800, Tae Kwang Machine Co. LTD., Daegu, South Korea) whenever the temperature went down below 10 °C and 15 °C set-points. The relative humidity (RH) was kept within approximately 40%-60% in both greenhouses, respectively.

2.4 Diseases and pest controls

Diseases and pest controls were conducted as previously described in [16]. Briefly, 20% of Spiromesifen (Farmhannong, Korea) was diluted with the ratio of 1 to 2,000 for controlling whitefly and 5% of Rampage (Hankooksamgong, Korea) was diluted with the ratio of 1to1,000 for controlling thrips and 30% of Iminoctadine tris (Farmhannong, Korea) was diluted with the ratio of 1 to 1,000 for controlling leaf and gray molds. 50% of Polyoxin B (Farmhannong, Korea) was diluted with 1 to 5,000 for controlling powderly mildew and fungi.

2.5 Data collections

The pepper accessions were planted with the same arrangement in LT and CT greenhouses from three independent biological plants which were randomly selected among ten plants for measurment the reproductive parameters the number of flowers (NFL) which were determined from the second to fifth internodes and the vegetative parameters including plant height (PH), stem diameter (SD), and length of main axis (LMA) at 120 day after transplanting (DAT) of seedlings. Total number of fruits per plant (NFR) and fruit yield per plant (FY) were measured from three individual plants, randomly. Five fruits of each accession were collected for measurement of fruit fresh weight (FFW), fruit length (FL), fruit diameter (FD), and the number of seeds in a fruit (NSF) using a digital electron Micro Weighing Scale MW-II (CAS), a ruler, and a caliper, respectively.

2.6 Data analysis

The significant difference in vegetative parameters of PH, SD, and LMA, and reproductive parameters of NFL, NFR, FY, FD, FL, FFW and NSF were assessed as described in the figure legends with Student's *t*-test using EXCEL 2016 program (Microsoft Co. Ltd., USA). To figure out the effects of LT on evaluated traits, the score of agnomical traits was calculated by dividing LT by CT and multiplying 100 (%). The analysis of correlation coefficients was performed among total populations (n = 39) with EXCEL 2016 (Microsoft, WA, USA). The multivariate analysis including correlation analysis, principal components analysis (PCA), and hierarchical cluster analysis were assessed using SPSS program (IBM SPSS v27.0, Chicago, IL, USA). The adequacy of the samples was carried out by The Kasier-Meyer-Olkin (KMO > 0.5) and the Bartlett's test of Sphericity (BTS < 0.001) were utilized as an indicator in the proper construct of the PCA model to evaluate the relationship between variables.

3. Results

3.1. The vegetative traits with chili and bell peppers

In order to understand the response of pepper plants to night low temperature (NLT), the vegetative parameters including PH, SD, and LMA were investigated among of 39 pepper accessions at 120 DAT in LT and CT greenhouses. Most accessions of the growth rate in PH were significantly reduced in LT compared to that in CT (Figure 1A). Only one accession C22 of chili pepper was observed with no significant difference in both LT and CT. The lowest significant difference ($p \leq 0.05$) was identified with C17 of chili pepper and P06 of bell pepper.

In order to evaluate the effect of LT on the stem growth, the plant stem diameter (SD) was measured among 39 pepper accessions. 16 accessions of chili pepper and 7 accessions of bell pepper were observed with no significant differences between LT and CT (Figure 1B). The significant difference ($p \leq 0.01$) was identified with C02, C05, C17, and C20 of chili peppers and P01 of bell pepper, respectively. And the SD of C05 accession was higher in LT than that in CT. In addition to this, the growth of LMA was investigated and 13 accessions of chili peppers and 2 accessions of bell peppers were observed with no remarkable difference between LT and CT (Figure 1C). The significant difference ($p \leq 0.001$) was determined in C13, C16, C21, and C23 of chili peppers and P01 and P02 of bell peppers. Taken together, the observations indicate that the effect of LT on vegetative traits was widely varied from genotypes among 39 pepper accessions.

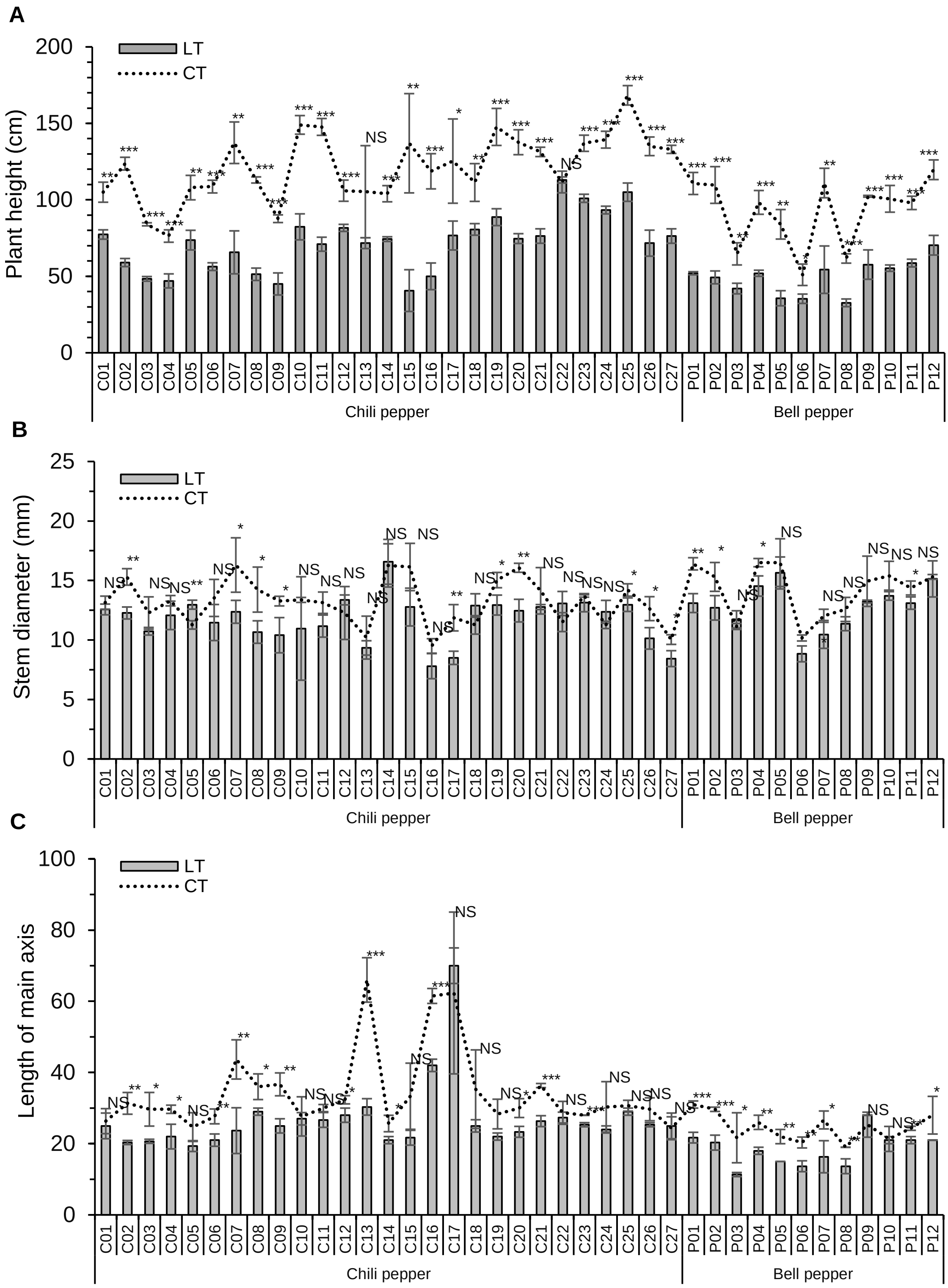

Figure 1. The evaluation of vegetative traits on (A) plant height, (B) plant stem diameter, and (C) length of main axis among 39 pepper accessions in LT and CT greenhouses. Plant height, stem diameter, and length of main axis were measured at 120 days after transplanting. Significant differences were evaluated with Student's *t*-test with $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ and denoted by *, **, and ***, respectively. NS means not significant and bars indicate ± standard deviation (n = 3).

3.2. The reproductive traits of NFL, NFR, and FY with chili and bell peppers

 doi:10.20944/preprints202109.0089.v1

In order to determine the response of pepper accessions to NLT with the reproductive traits, the reproductive parameters including NFL, NFR, and FY were investigated among 39 pepper accessions at 120 DAT in LT and CT greenhouses. The effect of LT on NFL was different depending on pepper accessions (Figure 2A). The floral organs were not developed in C04, C13, and C16 accession in both LT and CT conditions and the flowers of C03, C17, and P11 were developed in CT but not in LT. No significant difference in NFL was observed except for P06 and P10 accessions between both LT and CT. In addition, the effect of LT on NFR per plant at 120 DAT were subsequently evaluated and observed in the significant reduction in most pepper accessions in LT (Figure 2B). Notably, C04, C08, C13, and C16 of chili peppers were not shown in any fruit in both LT and CT and C22, P08, and P09 were not different between LT and CT. Because high fruit yield is one of the most important parameters to determine LT-tolerant pepper cultivars on breeding programs, the evaluation of FY was implemented and it was drastically decreased in LT compared to CT except for P08 and P09 of bell peppers (Figure 2C). Interestingly, the highest index of FY over 500 gram was identified in C01, C02, C05, C18, C19, C23, and C24 of chili peppers and in P01, P02, P03, and P04 of bell peppers in CT, whereas it was observed in C22 (226.7 gram) of chili pepper and P04 (215.0 gram) of bell pepper in LT.

3.3. The fruit traits of FD, FL, FFW, and NSF with chili and bell peppers

In order to explore the impact of fruit traits in response to LT, the fruit traits-related FD, FL, FFW, and NSF were evaluated among 39 pepper accessions at 120 DAT in LT and CT greenhouses. FD was dramatically declined in most pepper accessions in LT in comparison with CT (Figure 3A). However, no appreciable differences in FD were found in C23 of chili peppers and P01 and P04 of bell peppers between LT and CT. In detail, the widest FD over 25 mm and 60 mm was found in C02, C05, C14, C15, C21, and C22 of chili peppers and P02, P03, P5, and P10 of bell peppers in CT, respectively. The widest FD over 15 mm and 50 mm were observed in C02, C05, C14, C22, and C23 accessions, and in P01, P02, P05, P08, and P09 accessions in LT.

A previous study has reported that FD is highly associated with FL [31,32]. In order to validate the effect of LT on FL, together with FD, FL was measured among 39 pepper accessions and FL was noticeably reduced in most pepper genotypes in LT than that in CT (Figure 3B). However, no appreciable differences in FL were observed in P06 and P08 of bell peppers between LT and CT. In detail, the longest FL over 15 cm and 10 cm in CT were determined in C02, C05, C06, C14, C18, C19, C22, C23, and C24 of chili peppers, and in P01 and P02 in bell peppers. Moreover, the longest FL over 10 cm and 5 cm in LT was observed in C18, C19, C22, and C23 of chili peppers and P02, P04, and P09 of bell peppers. In addition to this, FFW was investigated and decreased in most pepper accessions in LT compared to that in CT except for P08 and P09 of bell pepper that exhibited no significant differences between LT and CT conditions (Figure 3C). A study has been determined in the effect of LT on the seed development in the pepper fruit, causing the seedless fruit (referred to as parthenocarphy) and the reduced fruit marketability [18]. To further confirm whether the effect of LT on seed development in a fruit, the number of seeds per fruit was counted. The result showed that NSF was reduced in a variety of pepper accessions and even all fruits of bell peppers did not develop any seed in LT condition (Figure 3D). NSF of C12 accession increased in LT than that in CT and no appreciable difference in NSF was found in C25 and P05 accessions between LT and CT. In detail, the highest NSF over 80, 70, and 40 seeds in CT was identified in C20, C21, and C24 of chili peppers and P01, P02, and P09 of bell peppers. Also, the highest NSF over 40 in LT were observed in C12, C24 and C25 of chili peppers.

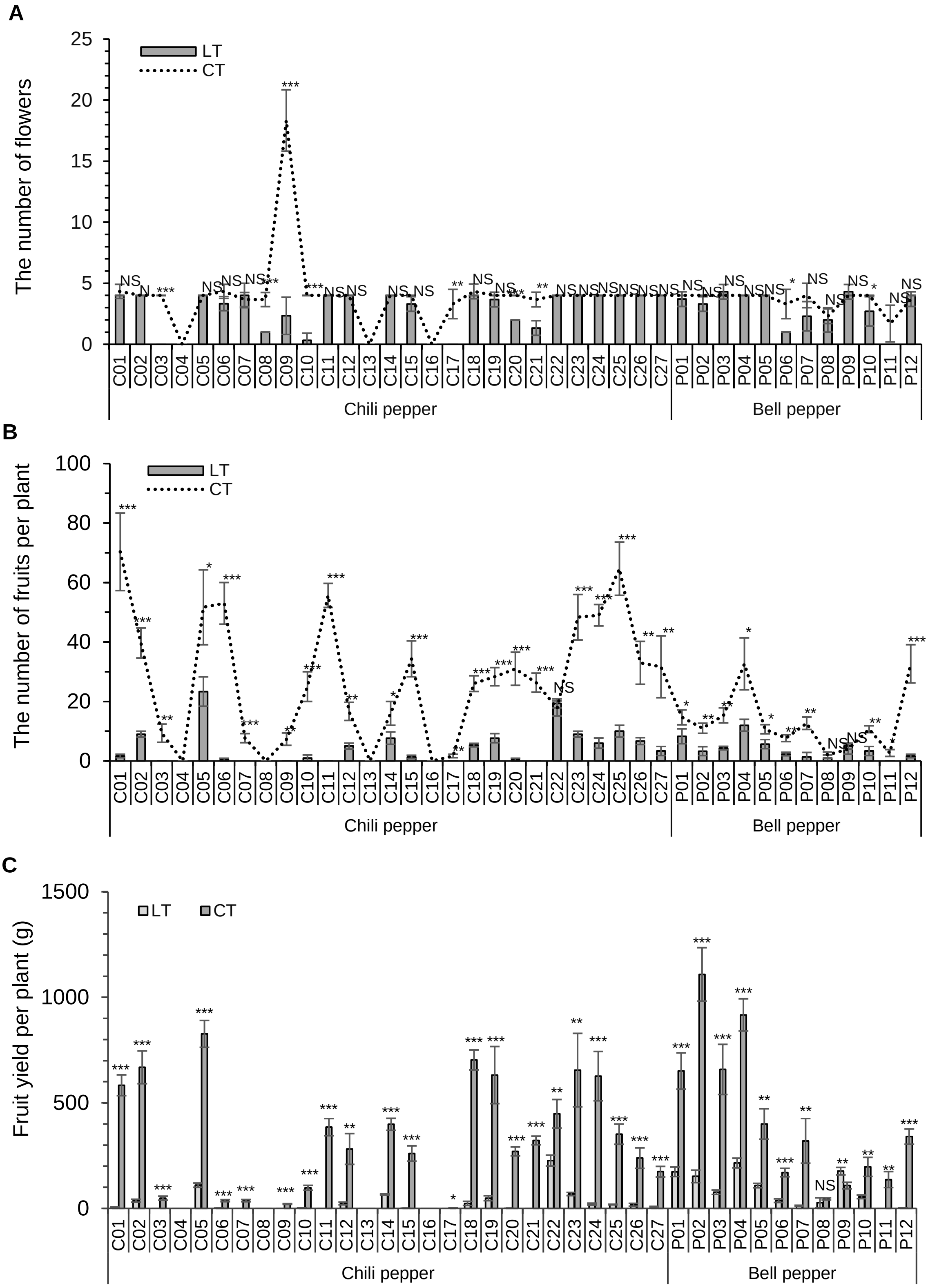

Figure 2. The evaluation of reproductive traits on (A) the number of flowers, (B) the number of fruits, and (C) fruit yield among 39 pepper accessions in LT and CT greenhouses. The reproductive parameters were measured at 120 days after transplanting. Significant differences were evaluated with Student's *t*-test with $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ and denoted by *, **, and ***, respectively. NS means not significant and bars indicate ± standard deviation.

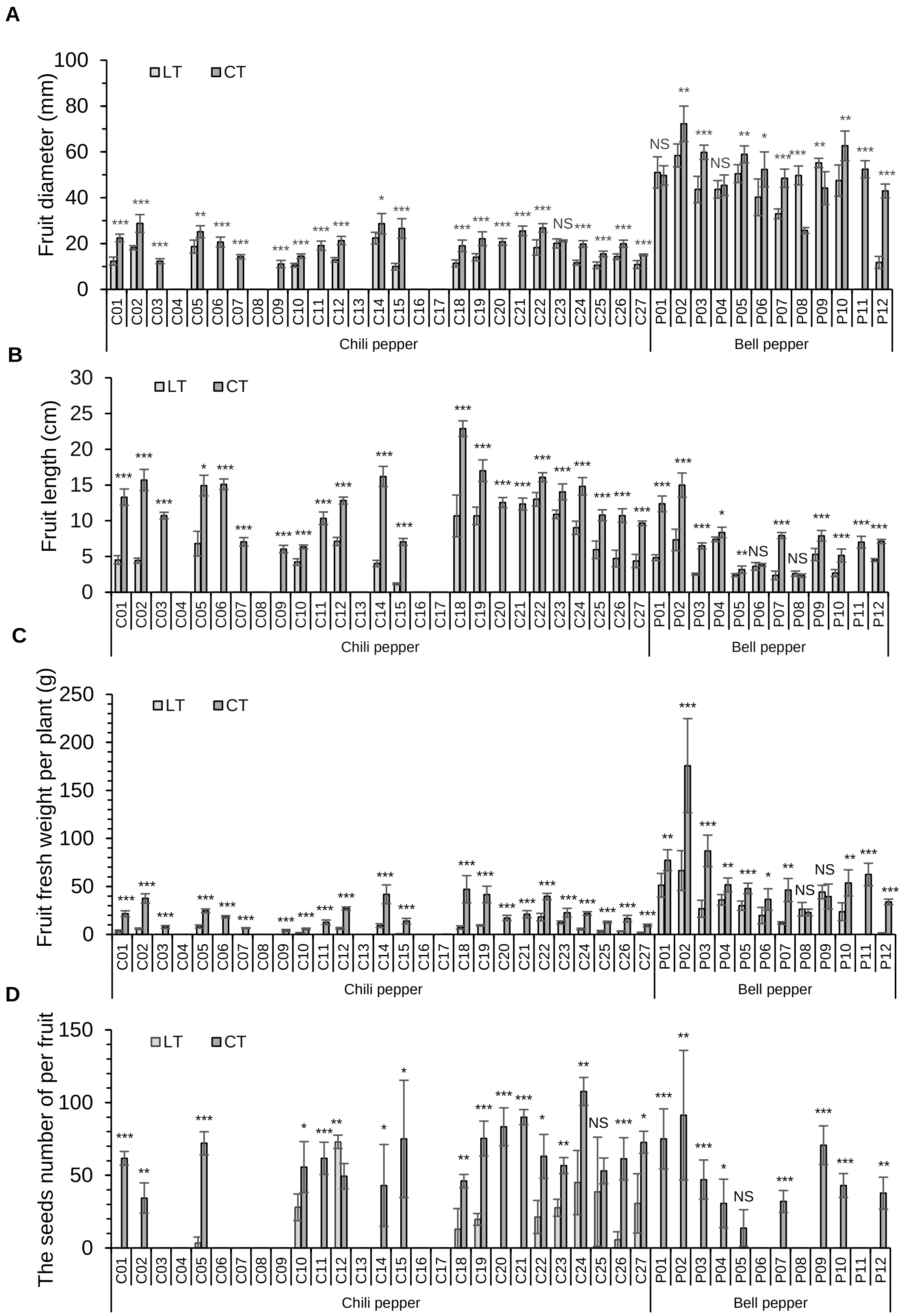

Figure 3. The evaluation of reproductive traits on (A) fruit diameter, (B) fruit length (cm), (C) fruit fresh weight, and (D) the number of seeds among 39 pepper accessions in LT and CT greenhouses. The reproductive parameters were measured at 120 days after transplanting. Significant differences were evaluated with Student's *t*-test with $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ and denoted by *, **, and ***, respectively. NS means not significant and bars indicate ± standard deviation (n = 3).

 doi:10.20944/preprints202109.0089.v1

3.4. The principal component analysis (PCA) of agronomical traits

In order to understand the relationship among the multiple variables including aformentioned parameters, the principal component analysis (PCA) was conducted (Figure 4). The correlation matrix of 10 variables were produced in LT and CT conditions (Figure S2). The Kaisere-Meyere-Olkin (KMO) was calculated for the adequacy of measured samples on vegetative and reproductive traits and the score was 0.720. Bartlett's Test of Sphericity (BTS) was significantly lower than 0.001, indicating that the samples and PCA construct were appropriate for PCA analysis. 5 PCs were extracted from 10 studied agronomical traits and the eigenvalues were greater than 1 from the first three (Table S2). To reduce the dimensions of the data space, the correlation matrix with the first two components of PCA were applied. Total variance of the acquired data was explained with the 65.76%, which represented 46.63% from component factor 1 (PC1) and 19.13% from component 2 (PC2), respectively. The traits (socres > 0.30) were loaded onto PC1 and PC2 (Table S2A). The first component PC1 contributed to multiple traits, concluding that FL has a major contribution towards positive loading vectors (0.885), followed by FD (0.878) and FW (0.791) within the first component and PC2 exhibited FY (0.914) and NFR (0.815). It would indicate that three major and two variables have a strong correlation with the first PC and second PC, respectively.

3.5. Clustering analysis

In order to analyze the association with PCs and 39 pepper accessions, a scatter plot matrix was drawn using the component factor 1 (PC1) and factor 2 (PC2), exhibiting no clear pattern for grouping of pepper fruit types (Figure 4). The PC1 and PC2 factors were further justified to agglomerative hierarchical clustering utilizing the Euclidean distance matrix through Ward's method and the dendrogram was produced on the basis of the results. Seven major groups were distinctly clustered (Figure 5A). The results showed the group 1 was primarily clustered with very low value of LMA and PH, the group 2 with moderate low value of SD and NFL, the group 3 with low value of both vegetative and reproductive traits, the group 4 with the moderate value of SD in vegetative traits and high value of FL and FD in reproductive traits, the group 5 with high value of PH and SD in vegetative traits and NFR and FL in reproductive traits, the group 6 with high value of FW, FL, and FD in reproductive traits, and the group 7 with high value of LMA in vegetative traits and NFL, NFR, FY, FFW, and FD in reproductive traits. Depending on the clustering and the studied agronomical traits, the plants displaying low performance of the agronomical traits were selected in two accessions (C01 and C02) of chili peppers and two accessions (P01 and P02) of bell peppers. Also, the plants showing the high performnce of them were selected in two accessions (C22 and C23) of chili peppers and two accessions (P08 and P09) of bell peppers (Figure 5B).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 6 September 2021 doi:10.20944/preprints202109.0089.v1

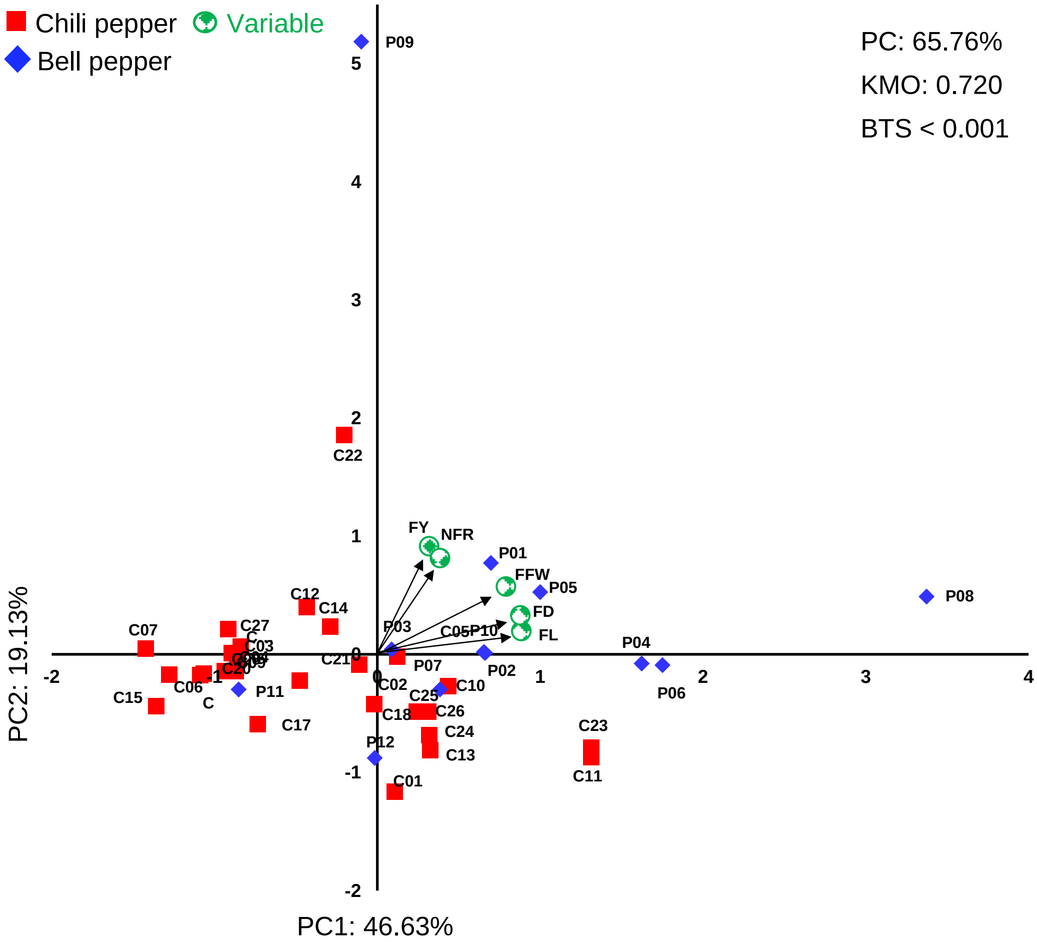

Figure 4. Biplot for first two principal components was analyzed using the principal component analysis (PCA) for 10 agronomical traits among 39 pepper accessions with chili and bell pepper type between LT and CT. The number of fruits (NFR), fruit yield (FY), fruit fresh weight (FFW), fruit diameter (FD), and fruit length (FL).

doi:10.20944/preprints202109.0089.v1

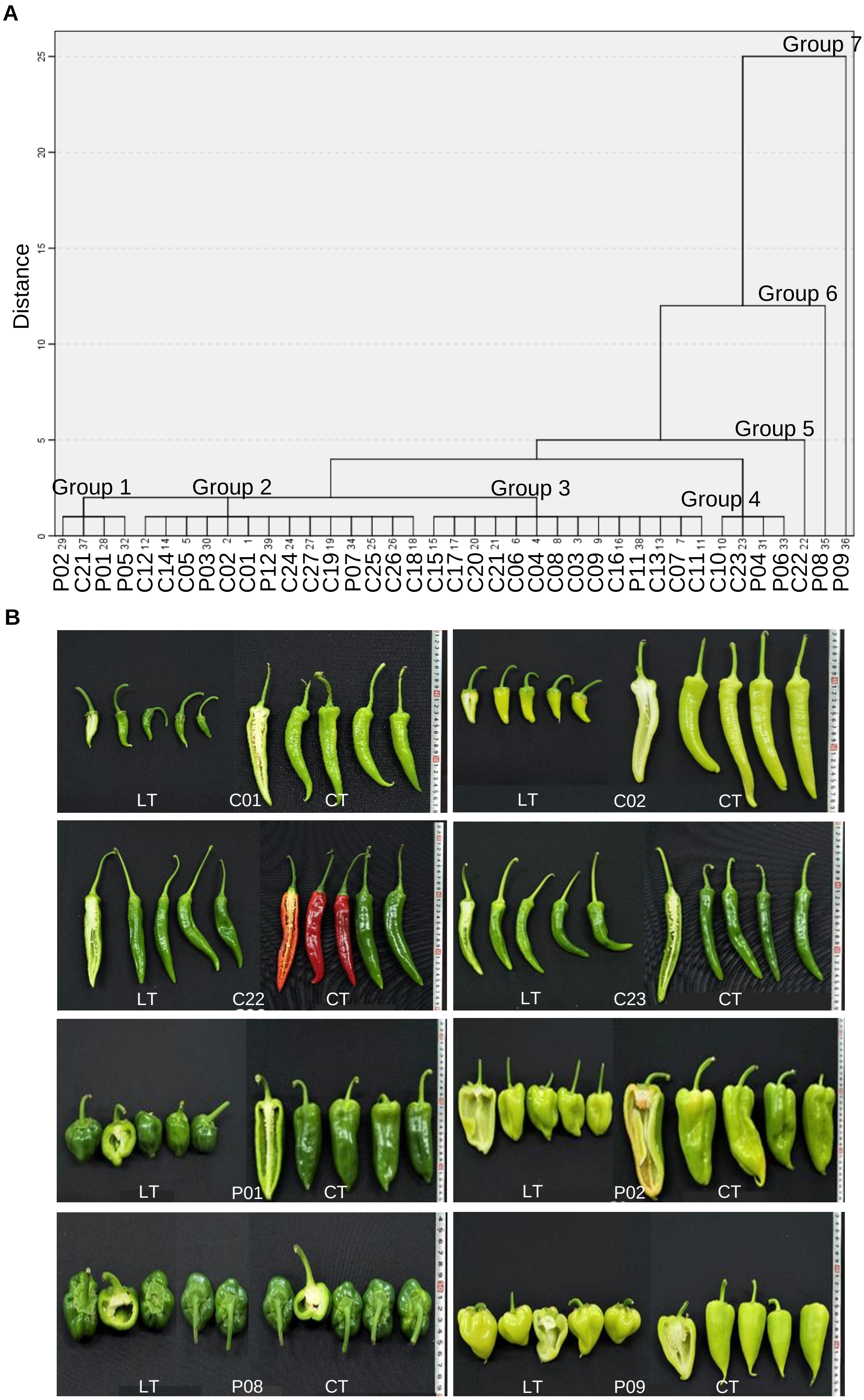

Figure 5. (A) Dendrogram was performed by the cluster analysis of 39 pepper accessions on the basis of the Euclidean distance using components factors 1 and factors 2. (B) 4 pepper accessions in chili and bell pepper were selected and the pictures were shown in the fruits of pepper plants taken at 120 days between LT and CT greenhouse conditions.

4. Discussion and conclusion

Pepper plants have been naturally exposed to harsh cold stress during winter season in agriculture and possess the cellular and molecular mechanisms to acclimate and overcome the low temperature stress [33-36]. Previous reports have been demonstrated in the response to LT with limited accessions and mainly in reproductive traits including flower morphologies, fruit shapes, fruit types, and fruit yield [6,18,19]. In addition, the effect of LT on the agronomical traits has been reported primarily in bell pepper. In this study, we assessed 39 pepper genotypes including chili (n = 27) and bell fruit types (n = 12) and evaluated vegetative and reproductive traits during the entire period of pepper growth and developmental stages under LT, which can economically reduce the heating demand of the pepper cultivation in winter greenhouse.

The effect of LT showed that the PH was notably decreased in almost all accessions except for one accession, C32, which was not significant different among chili peppers grown between LT and CT greenhouse (Figure 1A). In line with our current result, previous studies have determined that the effect of LT on PH influences the retarded growth of plant height in pepper and cucumber plants with different low temperature regimes [7,9,37]. In addition to this, previous researches have also reported that the reduction of tomato plant height in LT could be correlated to the number of leaves, which influence relative growth rate and net assimilation rate with the modulated photosynthetic ability [9]. Next, it is of our interest to further investigate how the number of perpper leaves, leaf length, leaf width, as well as photosynthetic parameters are involved in PH in LT.

The effect of LT on SD exhibited the reduction of 21 accessions (40.8%) of chili peppers and 5 accessions (41.6%) of bell peppers, respectively (Figure 1B), whereas other accessions in LT did not significantly decrease in SD except for C05 which showed the higher growth of SD in LT than that in CT. The result is in agreement with a previous study which has determined the difference in the SD of tomato plants under LT [15,16]. The finding showed that the SDs are varied depending on tomato genotypes without fruit types [16]. Moreover, the effects of LT on LMA displayed the reduction of 13 accessions (48.2%) of chili peppers and 10 accessions (83.3%) of bell peppers, respectively (Figure 1C), indicating the effects of LT on SD and LMA might be also raging among pepper genotypes irrelevant of fruit types (Figure 1). In the current study, the positive high temperature (day temperature – night temperature > 0) in tomato plants leads to the increased stem thickness and enlogation as well as the number of xylem vessels via the modulation of genes involved in cell wall, GA, and auxin biosynthesis [38]. Further our studies are essential to provide factors affecting the pepper stem diameter and enlogation with the phytohormones under diverse LT regimes. Collectively, we do not completely understand how LT affects vegetative index such as SD and LMA in pepper plants. Nevertheless, our current results clearly suggest that LT influences the pepper growth and development during vegetative stages. Furthermore, the bell peppers would be very sensitive to LT on the vegetative traits compared to those in CT as previous study mentioned [18].

We conducted the investigation of reproductive index including NFL, NFR, and FY. A study has reported that NFL in first truss of tomato plant displayed no significant difference under low temperature in atmosphere, while the NFL was promoted by low temperature in root area [39]. Moreover, our previous research explored that the LT does not affect the NFL of tomato plant or LT influenced the NFL with a genotype-specific interaction [15,16]. In the agreement with our previous and other researches, our current finding also shows that NFL of most pepper accessions is no remarkable difference between LT and CT, whereas NFL is reduced in several accessions of chili and bell peppers (Figure 2). Despite that we do not understand why the effect of LT on NFL in most accessions are not influenced, our further studies need to focus on elucidating the mechanistic role of the impact of LT on NFL in pepper plants with consideration of air and root low temperature.

Previous studies have shown that NFR is closely associated with FY in LT [13, 39] and NFR and fruit setting are key determinants to select LT-tolerant tomatoes and peppers with high fruit yields [16,40]. In line with a previous study, the effect of LT on NFR and FY results in the drastic decrease compared to those in CT (Figure 2B,C), suggesting that NFR and FY are closely correlated in LT. Moreover, LT affected the reduction of FD, FL, and NSF (Figure 3), resulting in irregular fruit shapes (Figure 5B). The findings are cocodant with previous effects of LT on the flower morphology and the fruit development [18]. The studies have assessed that the impact of LT on anther and ovary shapes, causing the malformation of floral organs including the stunted stamen, the decreased number of pollen, and the reduced pollen activity by hindering pollination and fertilization [18,22], which further led to the abnormal formations of fruit development, irregular fruit shapes, and parthenocarpic fruits, together with reduced NSF [18,41]. The malformation of fruit shapes was produced by swollen ovary and shorten style in LT as previous studies mentioned in rice, mango, and pepper plants [2,6,42,43], indicating that the development of floral organs with stamen and ovary are very sensitive to LT. Moreover, the parthenocarpic fruits and the declined NSF are associated with the balance of plant hormones including auxin, gibberillin, and cytokinin due to the lack of fertilization, but might not be the defect of pollination [44,45]. Next, our important endeavor is to dissect the involvement of plant hormones and anatomic structures of floral organs during the period of flower development under different low temperature regimes.

We utilized the correlation matrix to perform PCA analysis determining the crucial factors with 10 variables of agronomical traits between LT and CT and 5 traits (scores > 0.30) were loaded into the plot. The first two PCA explained 65.76% of agronomical variables (Figure 4). The angle between the vectors of traits including FFW, FD, and FL in PC1 and NFR in PC2 with FY was less than around 80°, indicating that the positive correlations were exhibited in the variables which in line with previously published statistics [16]. Our finding shows that FFW, FD, and FL are closely correlated in LT effect. The studies have not been reported in the effects of LT as our LTN condition on fruit shapes such FFW, FD, and FL. On the one hand, a study has reported that the FFW is positively associated with FFW and pericarp thickness, but not in FL [46,47] in normal condition. Moreover, some researches have determined that quantative triat locus (QTL) governing FFW and FD are linked on choromosome P12, indicating that the positive trends between FFW and FD could be described from our PCA result [48]. Also, studies have assumed that the mechansim of fruit development factors including FD, FL, and FFW are probably shared [31,32]. Notably, in our current study, the effect of LT on the parameters of fruit development uncovered that FL is highly correlated with FD. Given that a study have mentioned that the FL is governed by from 3 to 10 pairs of genes and probably is also affected by environmental conditions [46], further study is to investigate the mecanistic role of how the fruit developmental factors are governed by the gene clusters.

As the pulication mentioned, the decreaed NFR of bell pepper in LT is associated with FY, showing the close correlation between the traits. In line with our current result, previous PCA analysis of tomato plants also exhibits the strong correlation with FY and NFR in LT effect [16], suggesting that the NFR plays an important role in determining the yield-related parameters such as fruit set and fruit yield in pepper plants for breeding programs in selecting LT-tolerant peppers. The biplot analysis was further conducted to understand the multivariate relationships with 39 pepper accessions containing 27 chili and 12 bell peppers accessions (Figure 4). The evaluated agronomical traits and correlation matrix were applied to the cluster analysis and classified into seven groups (Figure 5A). Our finding shows that the group 1 to the group 3 tend to be negative trends with vegetative and reproductive parameters, but some accessions still exhibit moderate high and low values of them. On the one hand, the group 4 to group 7 tend to be positive trends mainly with reproductive parameters and with one or two of vegetative parameter(s). Previous our studys have been determined in selection criteria for LT-tolerant tomatoes which display different vegetative or reproductive index depending on fruit types [16]. Given that the clusters groupped with reproductive index still exhibit one or two vegetative parameters in some accessions or in different clusters vice versa, it is therefore likely that the screening on the pepper accessions tolerant to LT is also taken into consideration with different selection index among pepper accessions in greenhouse. On the basis of our clustering among 39 pepper accessions (Figure 5A), we selected that LT-sensitive two chili and bell pepper accessions (C01 and C02 in group 2, P01 and P02 in group 1) which show the low value of vegetative and reproductive index, respectively. As well as, LT-tolerant two chili and bell peppers (C23 in group 4 and C22 in group 5, P08 in group 6 and P09 in group 7) show mainly the high value of reproductive index such as FY, FFW, FL, and FD. Intriguingly, the fruit shapes of LT-sensitive chili and bell pepper genotypes are extremely smaller than those in CT, whereas the the fruit shapes of LT-tolerant ones are almost similar or small to some exent in comparison with those in CT (Figure 5B). Our results indicate that FL and FD would play an crucial role in selecting LT-tolerant cultivars. Taken together, we assume that PCs and cluster analysis can be importantly considered as a measure to the effect of LT on fruit shape-related traits with FY traits although the left traits would be shown with a minimal contribution with positive or negative directions. Moreover, the selected accessions could be further used for pepper breeding programs to develop LT-tolerant chili and bell pepper cultivars with the selection criteria in winter greenhouse.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Air temperature was measured in LT and CT greenhouse during the period of pepper growth and development, respectively. Figure S2: The correlations coefficients between vegetative and reproductive traits in total population of pepper between LT and CT, Table S1: The information of chili and bell pepper accessions for evaluating agronomical traits under night low temperature in winter 2020-2021. Table S2: Loading matrix associated with the principal components analysis (PCA) for 10 agronomical traits.

Author Contributions: Conceptualization, M.C.C and E.Y.Y; methodology, S.N.R., M.C.C., C.W.N., and E.Y.Y.; investigation, S.N.R., K.L., H.B.J., and E.Y.Y.; writing—original draft preparation, S.N.R. and K.L.; writing—review and editing, S.N.R., K.L., E.Y.Y., and M.C.C; visualization, S.N.R. and K.L.; supervision, M.C.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by a grant (Project No: PJ01267102 "Study on the physiological mechanism of temperature adaptable pepper lines") from the National Institute of Horticultural and Herbal Science, Rural Development Administration.

Data Availability Statement: The datasets presented in this study are available upon request to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kothari, S.; Joshi, A.; Kachhwaha, S.; Ochoa-Alejo, N. Chilli peppers—a review on tissue culture and transgenesis. *Biotechnol. Adv.* **2010**, *28*, 35-48.
2. Bhutia, K.; Khanna, V.; Meetei, T.; Bhutia, N. Effects of climate change on growth and development of chilli. *Agrotechnology* **2018**, *7*, 2.
3. Prohens J, Nuez F. Handbook of Plant Breeding. Vegetables II: Fabaceae, Liliaceae, Solanaceae and Umbelliferae; Springer, 2008; New York; Volume 3, pp. 30-40.
4. Sun, T.; Xu, Z.; Wu, C.T.; Janes, M.; Prinyawiwatkul, W.; No, H. Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum L.*). *J. Food Sci.* **2007**, *72*, 98-102.
5. Sarada, C.; Ratnam, M.; Naidu, L.; Ramana, C.; Rajani, A.; Vijaya, T. Chilli production and productivity in relation to Seasonal weather conditions in Guntur District of Andhra Pradesh. *Int. J. Pure Appl. Biosci.* **2015**, *3*, 207-213.

6. Cruz-Huerta, N.; Williamson, J.G.; Darnell, R.L. Low night temperature increases ovary size in sweet pepper cultivars. *HortScience* **2011**, *46*, 396-401.
7. Toki, T.; Ogiwara, S.; Aoki, H. Effect of varying night temperature on the growth and yields in cucumber. *Acta Hortic*. **1978,** *87*, 233–238.
8. Horie, T.; De Wit, C.d.; Goudriaan, J.; Bensink, J. A formal template for the development of cucumber in its vegetative stage (I, II and III). *Proc. K. Ned. Akad. Wet. C.* **1979**, *82*, 433-479.
9. Nilwik, H. Growth analysis of sweet pepper (*Capsicum annuum L.*) 1. The influence of irradiance and temperature under glasshouse conditions in winter. *Ann. Bot.* **1981**, *48*, 129-136.
10. Ji, L.; Li, P.; Su, Z.; Li, M.; Guo, S. Cold-tolerant introgression line construction and low-temperature stress response analysis for bell pepper. *Plant Signal. Behav.* **2020**, *15*, 1773097.
11. Foolad, M.; Lin, G. Relationship between cold tolerance during seed germination and vegetative growth in tomato: germplasm evaluation. *J. Am. Soc. Hortic. Sci.* **2000**, *125*, 679-683.
12. O'SULLIVAN, J.; Bouw, W. Pepper seed treatment for low-temperature germination. *Can. J. Plant Sci.* **1984**, *64*, 387-393.
13. Seo, J.-U.; Hwang, J.-M.; Oh, S.-M. Effects of night temperature treatment of raising seedlings before transplanting on growth and development of pepper. *J. Bio-Env. Con.* **2006**, *15*, 149-155.
14. Bhatt, R.; Srinivasa Rao, N. Response of bell-pepper (*Capsicum annuum*) photosynthesis, growth, and flower and fruit setting to night temperature. *Photosynthetica* **1994,** *28*, 127-132.
15. Sherzod, R.; Yang, E.Y.; Cho, M.C.; Chae, S.Y.; Kim, J.H.; Nam, C.W.; Chae, W.B. Traits affecting low temperature tolerance in tomato and its application to breeding program. *Plant Breed. Biotechnol.* **2019**, *7*, 350-359.
16. Rajametov, S.N.; Lee, K.; Jeong, H.-B.; Cho, M.-C.; Nam, C.-W.; Yang, E.-Y. Physiological Traits of Thirty-Five Tomato Accessions in Response to Low Temperature. *Agriculture* **2021**, *11*, 792.
17. Oh, S.-Y.; Koh, S.C. Fruit Development and Quality of Hot Pepper (*Capsicum annuum L.*) under Various Temperature Regimes. *Hortic. Sci. Technol.* **2019**, *37*, 313-321
18. Mercado, J.; Mar Trigo, M.; Reid, M.; Valpuesta, V.; Quesada, M. Effects of low temperature on pepper pollen morphology and fertility: evidence of cold induced exine alterations. *J. Hortic. Sci.* **1997**, *72*, 317-326.
19. Pressman, E.; Moshkovitch, H.; Rosenfeld, K.; Shaked, R.; Gamliel, B.; Aloni, B. Influence of low night temperatures on sweet pepper flower quality and the effect of repeated pollinations, with viable pollen, on fruit setting. *J. Hortic. Sci. Biotechnol.* **1998**, *73*, 131-136.
20. Shaked, R.; Rosenfeld, K.; Pressman, E. The effect of low night temperatures on carbohydrates metabolism in developing pollen grains of pepper in relation to their number and functioning. *Sci. Hortic.* **2004**, *102*, 29-36.
21. KATO, K. Flowering and fertility of forced green peppers at lower temperatures. *J. Jpn. Soc. Hortic. Sci* **1989**, *58*, 113-121.
22. Rylski, I. Effect of night temperature on shape and size of sweet pepper *(Capsicum annuum L.)*; 1973.
23. Aloni, B.; Pressman, E.; Karni, L. The effect of fruit load, defoliation and night temperature on the morphology of pepper flowers and on fruit shape. *Ann. Bot.* **1999**, *83*, 529-534.
24. De Koning, A. The effect of different day/night temperature regimes on growth, development and yield of glasshouse tomatoes. *J. Hortic. Sci.* **1988**, *63*, 465-471.
25. Rural Development Administration (RDA). *Data Book of Agricultural Products Income for the Improvement of Agricultural Management in 2019*; Rural Development Administration: Jeonju, Korea, **2020**.
26. Elings, A.; Kempkes, F.; Kaarsemaker, R.; Ruijs, M.; Van De Braak, N.; Dueck, T. The Energy Balance and Energy-Saving Measures in Greenhouse Tomato Cultivation. *Acta Hortic.* **2005**, *691*, 67–74,
27. Rylski, I.; Spigelman, M. Effects of different diurnal temperature combinations on fruit set of sweet pepper. *Sci. Hortic.* **1982**, *17*, 101-106.

28. Rylski, I. Investigations on the influence of suboptimal temperatures on the flowering, fruit setting and development of sweet pepper (*Capsicum annum L.*). Ph. D. Thesis, Hebrew University of Jerusalem, Israel, **1971;** pp 1-96
29. Rylski, E.; Kempler, H. Fruit set of sweet pepper (*Capsicum annuum L.*) under plastic covers. *HortScience* **1972**, 7, 422-423.
30. Wang, C. Alleviation of chilling injury in tropical and subtropical fruits. In Proceedings of the III International Symposium on Tropical and Subtropical Fruits 864, 2004; pp. 267-273.
31. Barchi, L.; Lefebvre, V.; Sage-Palloix, A.-M.; Lanteri, S.; Palloix, A. QTL analysis of plant development and fruit traits in pepper and performance of selective phenotyping. *Theor. Appl. Genet.* **2009**, *118*, 1157-1171.
32. Yarnes, S.C.; Ashrafi, H.; Reyes-Chin-Wo, S.; Hill, T.A.; Stoffel, K.M.; Van Deynze, A. Identification of QTLs for capsaicinoids, fruit quality, and plant architecture-related traits in an interspecific Capsicum RIL population. *Genome* **2013**, *56*, 61-74.
33. Yang, S.; Tang, X.-F.; Ma, N.-N.; Wang, L.-Y.; Meng, Q.-W. Heterology expression of the sweet pepper CBF3 gene confers elevated tolerance to chilling stress in transgenic tobacco. *J. Plant Physiol.* **2011**, *168*, 1804-1812.
34. Hou, X.-m.; Zhang, H.-f.; Liu, S.-y.; Wang, X.-k.; Zhang, Y.-m.; Meng, Y.-c.; Luo, D.; Chen, R.-g. The NAC transcription factor CaNAC064 is a regulator of cold stress tolerance in peppers. *Plant Sci.* **2020**, *291*, 110346.
35. Kong, X.-M.; Zhou, Q.; Zhou, X.; Wei, B.-D.; Ji, S.-J. Transcription factor CaNAC1 regulates low-temperature-induced phospholipid degradation in green bell pepper. *J. Exp. Bot.* **2020**, *71*, 1078-1091.
36. Chinnusamy, V.; Zhu, J.-K.; Sunkar, R. Gene Regulation During Cold Stress Acclimation in Plants. *Methods Mol. Biol.* **2010**, *639*, 39–55.
37. Bakker, J.; Van Uffelen, J. The effects of diurnal temperature regimes on growth and yield of glasshouse sweet pepper. *Neth. J. Agri. Sci.* **1988**, *36*, 201-208.
38. Ohtaka, K.; Yoshida, A.; Kakei, Y.; Fukui, K.; Kojima, M.; Takebayashi, Y.; Yano, K.; Imanishi, S.; Sakakibara, H. Difference Between Day and Night Temperatures Affects Stem Elongation in Tomato (*Solanum lycopersicum*) Seedlings via Regulation of Gibberellin and Auxin Synthesis. *Front. Plant Sci.* **2020**, *11*, 1947.
39. Phatak, S. Top and root temperature effects on tomato flowering. *J. Am. Soc. Hortic. Sci.* **1966**, *88*, 527–531.
40. Goodstal, F.J.; Kohler, G.R.; Randall, L.B.; Bloom, A.J.; Clair, D.A.S. A major QTL introgressed from wild Lycopersicon hirsutum confers chilling tolerance to cultivated tomato (*Lycopersicon esculentum*). *Theor. Appl. Genet.* **2005**, *111*, 898-905.
41. Patterson, B.D.; Reid, M.S. Genetic and environmental influences on the expression of chilling injury. *Chilling injury of horticultural crops. CRC Press, Boca Raton, FL* **1990**; pp. 87-112.
42. Issarakraisila, M.; Considine, J. Effects of temperature on pollen viability in mango cv.'Kensington'. *Ann. Bot.* **1994**, *73*, 231-240.
43. Satake, T.; Hayase, H. Male sterility caused by cooling treatment at the young microspore stage in rice plants: V. Estimations of pollen developmental stage and the most sensitive stage to coolness. *Jpn. J. Crop Sci.* **1970**, *39*, 468-473.
44. Polowick, P.; Sawhney, V. Temperature effects on male fertility and flower and fruit development in *Capsicum annuum L. Sci. Hortic.* **1985**, *25*, 117-127.
45. Sawhney, V.K.; Shukla, A. Male sterility in flowering plants: are plant growth substances involved? *Am. J. Bot.* **1994**, *81*, 1640-1647.
46. Zhigila, D.A.; AbdulRahaman, A.A.; Kolawole, O.S.; Oladele, F.A. Fruit morphology as taxonomic features in five varieties of *Capsicum annuum L.* Solanaceae. *J. Bot.* **2014**, *2014*, 1-6.
47. Paran, I.; Van Der Knaap, E. Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. *J. Exp. Bot.* **2007**, *58*, 3841-3852.
48. Vilarinho, L.B.O.; da Silva, D.J.H.; Greene, A.; Salazar, K.D.; Alves, C.; Eveleth, M.; Nichols, B.; Tehseen, S.; Khoury, J.K.; Johnson, J.V. Inheritance of fruit traits in Capsicum annuum: Heirloom cultivars as sources of quality parameters relating to pericarp shape, color, thickness, and total soluble solids. *J. Am. Soc. Hortic. Sci.* **2015**, *140*, 597-604.