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Article

Physiological Traits of Thirty-Five Tomato Accessions (*Solanum lycopersicum L.*) in Response to Low Temperature

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Abstract: Tomato is exposure to diverse abiotic stresses. Cold stress is one of harsh environmental stresses. Abnormal low temperature affects tomato growth and development including physiological disorders, flower drops, and abnormal fruit morphology, causing the decrease of tomato yield and a fruit quality. It is important to identify low temperature-(LT) tolerant tomato (*Solanum lycopersicum L.*) cultivars. This study focused on analyzing physiological traits of thirty-five tomato accessions with three fruit types (cherry, medium, and large sizes) under night temperature set-points of 15°C for normal temperature (NT) and 10°C for LT, respectively. Plant heights (PH) of most tomato accessions in LT were remarkably decreased compared to those in NT. The growth of leaf length (LL) and leaf width (LW) was reduced depending on the genotypes under LT. The number of fruits (NFR), fruit set (FS), fruit yield (FY), and marketable yield (MY) was negatively affected in LT. The FS in LT was significantly correlated with FY in LT in total populations (n = 35), cherry fruit sub-populations (n = 20), and medium fruit sub-populations (n = 11). Moreover, the relevance of NFL in LT with FY in LT was related to total populations (n = 35), cherry fruit sub-populations (n = 20), but not medium fruit sub-populations (n = 11). The results indicate the physiological traits of FS in LT and FY in LT are crucial factors for selecting and determining LT-tolerant cultivars for breeding programs in tomato plants depending on different fruit types.

Keywords: Tomatoes; Night low temperature; Physiological traits; Fruit yield; Tomato breeding; correlation coefficients

1. Introduction

Tomato plant (*Solanum lycopersicum* L.) is one of sessile organisms, which experiences multiple abiotic stresses including cold stress, heat stress, high salinity stress, and drought stress during the periods of vegetative and reproductive growth [1-4]. The importance of tomato crops has been gradually increasing and the cultivation area of tomatoes is widely expanded among agricultural crops. According to Food and Agriculture Organization (FAO, http://www.fao.org/faostat/) in 2019 and Korean Statistical Information Service (KOSIS, https://kosis.kr/eng/) in 2021, the cultivation area and tomato production reached approximately 5 million ha and 180 million metric tons in the world and around 6.4 thousand ha and 420 thousand metric tons in South Korea, respectively. However, owing to global warming and climate changes, the unpredictable agriculture weather such as low and high temperatures have critically limited the yields and the area of agricultural cultivation in tomato plants [5-8].

Low temperature (LT) is a critical factor for maintaining and improving the crop yield of tomato plants (*Solanum lycopersicum* L.) during the periods of growth and development stages [5,7]. LT (0-20°C) above the freezing temperature (below 0°C), which referred to sub-optimal temperature [5,9], play an important role in the leaf morphology

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[10], the truss appearance and growth [10-13], and the fruit development [14-17] during vegetative and reproductive stages. Recent diverse studies have demonstrated that LT remarkably influenced plant height (PH), plant stem diameter (SD), leaf length (LL), and leaf width (LW) in vegetative parameters [10,12,18,19] and flowering time (FT) [18,20], the number of flowers (NFL) and fruits (NFR) [18,20,21], fruit set (FS) [21], and fruit yield (FY) [18,21,22] during plant growth and development. Moreover, the relationships of the same traits during either vegetative or reproductive stages have been investigated under high temperature (HT) conditions [23,24] but the correlation of vegetative traits with reproductive traits remain unexplored under LT condition.

The temperature control is one of the essential factors for the tomato cultivation in greenhouse condition and approximately 15°C in winter is maintained for the optimal temperature set-points, which provide tomatoes to grow healthy without severe cold stress [10,25,26]. The studies on optimal temperature set-point have reported that the reduction of temperature by around 2°C in greenhouse was able to decline around 16% of winter heating cost in tomato cultivation [18,27], implying that the temperature lowering from 15°C to 10°C in winter greenhouse would lead to the significant decrease in the heating cost of tomato cultivation in agriculture. As well as, heating demand is remarkably increased at night time in winter greenhouse compared to the daytime [28,29]. However, a few studies have been dissected in the relationship of physiological traits and night low temperature (NLT) [18,28,29]. Thus, it is reasonable that practical breeding programs for low temperature (LT)-tolerant tomato cultivars economically considers keeping low temperature (10°C) during the night.

In this work, we investigated the physiological traits of thirty-five tomato genotypes, which were grown in two different greenhouse conditions with night temperature set-points at 10°C for LT and 15°C for normal treatment (NT), respectively. We analyzed the vegetative parameters of PH, SD LL, and LW and the reproductive parameters of NFL, NFR, FS, FY, and MY with different fruit types. Furthermore, we identified the correlation coefficient of vegetative and reproductive parameters in LT and NT.

2. Results

2.1. Growth conditions for thirty-five tomato accessions

Thirty-five tomato accessions were cultivated in two plastic NT and LT greenhouses. The seedlings were kept for 14 days under night temperature set-point 20°C to adapt new environmental conditions as previously described [18]. And then, night temperature set up was for 15°C in NT and 10°C in LT greenhouse, respectively (Supplemental Figure 1). Overall the relative humidity (RH) was approximately within 40% to 50% in both greenhouses.

2.2. The analysis of the vegetative traits among thirty-five accessions with different fruit type in LT and NT

To study the vegetative traits including plant height (PH), plant stem diameter (SD), leaf length (LL), and leaf width (LW) in tomato plants under LT condition, we analyzed thirty-five tomato accessions with different fruit types classified into wild, cherry, medium, and large fruit size (Supplemental Table 1). The data showed that the PHs of most tomato accessions in LT were remarkably reduced at 70 days after transplanting (DAT) compared to those in NT except for T32 accession in medium size (Figure 1A). SDs in LT were not significantly different from those in NT except for T21 accession in cherry size (Figure 1B). Additionally, the LL and LW were investigated at 70 DAT and the data exhibited that LL and LW of twenty-five tomato accessions were decreased in LT, whereas ten tomato accessions such as T04, T07, T09, T12, T13, T19, and T22 in cherry size, T28 and T31 in medium size, and T35 in large size were not influenced by LT (Figure 2A and 2B). The results suggest that the influence of low temperature in LL and LW was widely ranging from thirty-five accessions without fruit type.

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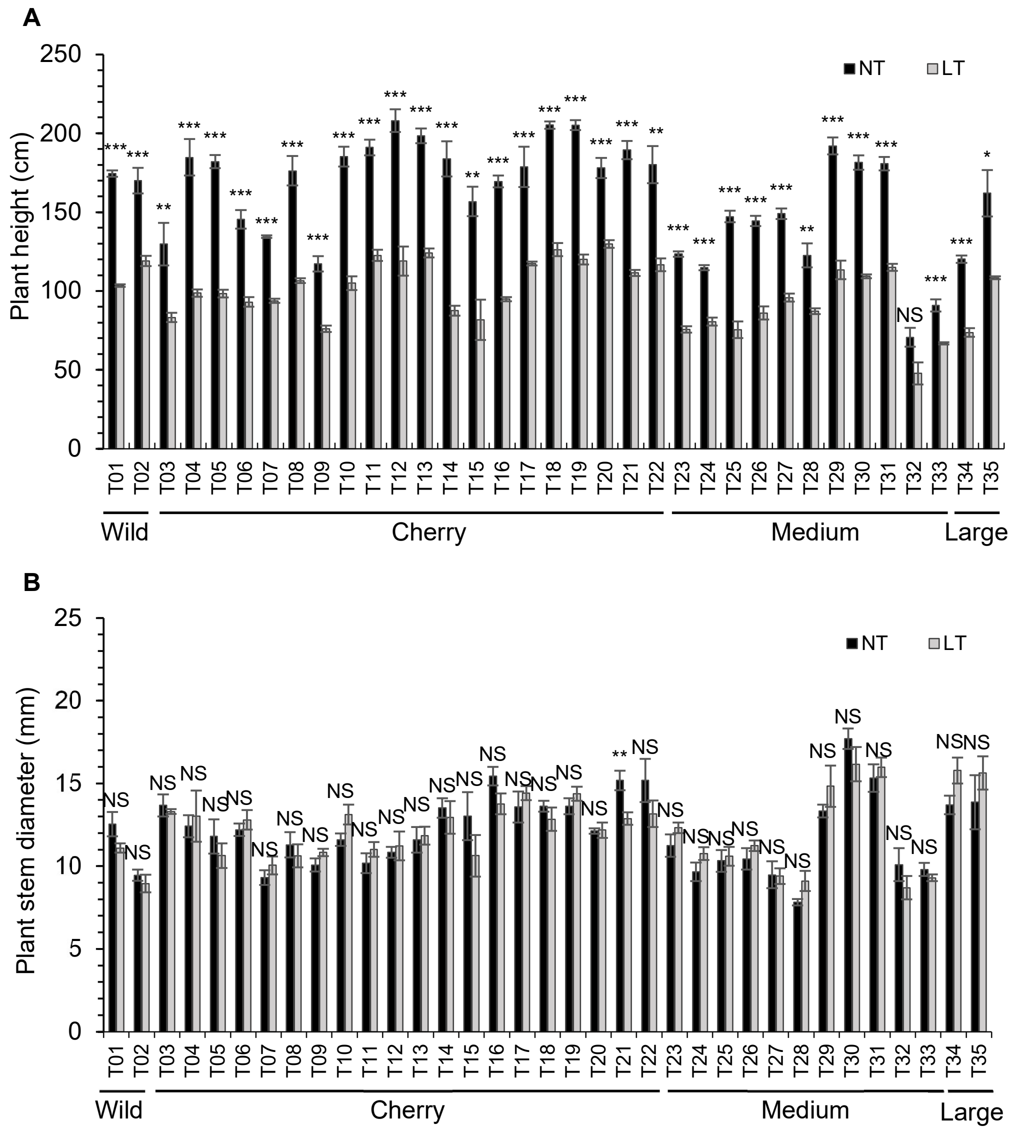

Figure 1. The analysis of vegetative traits on (A) plant height and (B) plant stem diameter among 35 tomato accessions with different fruit types under LT and NT greenhouses. Plant height and stem diameter were measured at 70 days after transplanting (DAT). Average values (n = 5) are provided with ± standard deviation and significant differences were evaluated with student's t-test ($p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$) and denoted by *, **, and ***, respectively and NS indicates not significant. Bars indicate ± standard deviation. .

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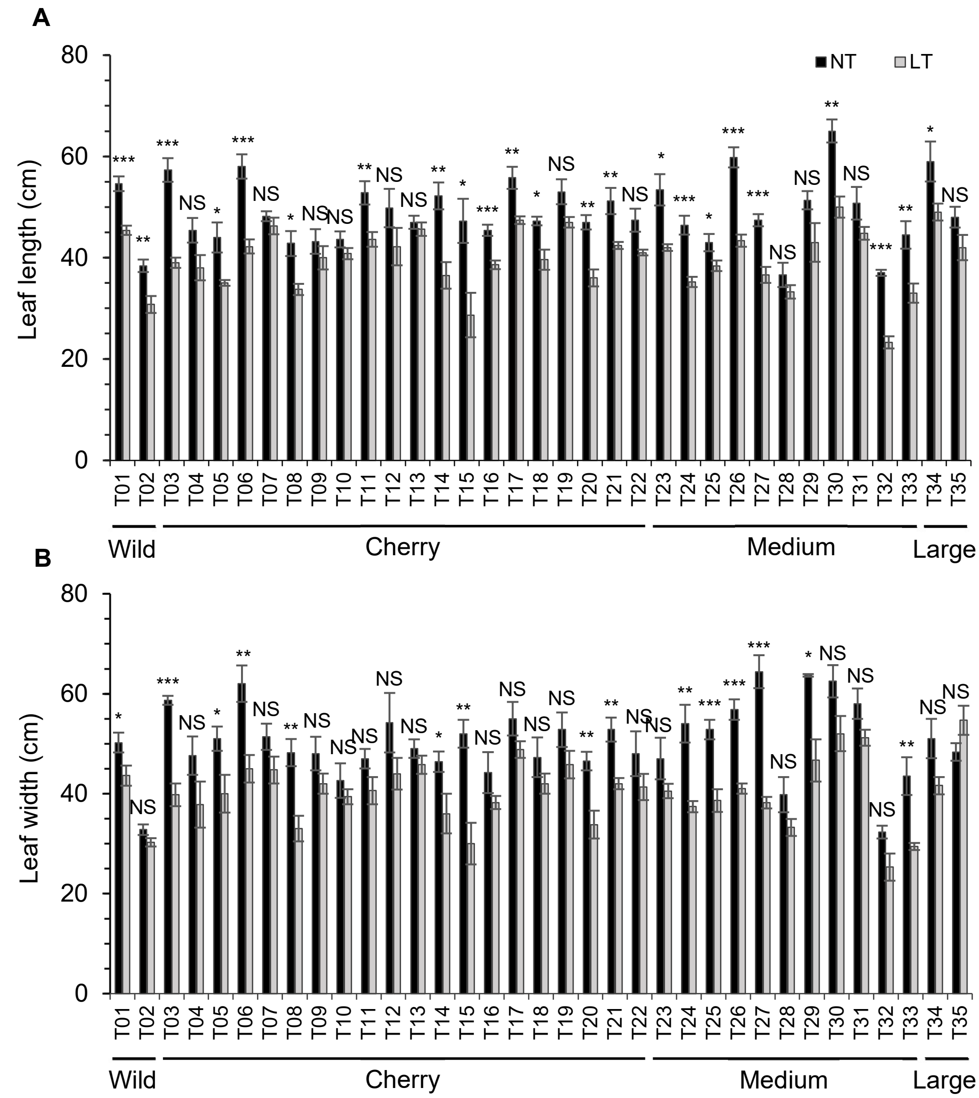

Figure 2. The analysis of vegetative traits on (A) leaf length and (B) leaf width among 35 tomato accessions with different fruit types under LT and NT greenhouses. Leaf length and width were measured at 70 DAT. Average values (n = 5) are provided and significant differences were evaluated with student's t-test ($p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$) and denoted by *, **, and ***, respectively. NS indicates not significant and bars indicate ± standard deviation.

2.3. The analysis of the reproductive traits among thirty-five accessions with different fruit type in LT and NT

It is also important to analyze whether thirty-five tomato accessions under LT condition is involved in the reproductive traits including the number of flowers (NFL), the number of fruits (NFR), fruit set (FS), fruit yield (FY), and marketable yield (MY). The data showed that the NFR of T04 and T20 accessions in LT, around 5.7% among thirty-five tomato accessions, positively increased, whereas the NFR of T15, T29, and T31 accessions in LT, around 8.6% among thirty-five tomato accessions, negatively decreased compared to that in NT condition (Figure 3A). NFR was also investigated among thirty-five tomato populations and T11 accession in cherry type was increased in LT more than that in NT (Figure 3B). In addition to this, NFR of T03, T05, T06, T07, T12, and T13 accessions in cherry type, T23, T24, T25, T26, T28, T29, and T32 in medium and T34 in large size type tomatoes were identified in no significant difference between LT and NT conditions (Figure 3B). Moreover, NFR of T01 and T02 in wild, T04, T08, T09, T10, T14, T15, T16, T17, T18, T19, T20, T21, and T22 in medium size and T27, T30, T31, T33 as well as T35 in large size under LT were remarkably reduced compared to that of NT (Figure 3B). Next, the fruit set (FS) ratio was investigated among all tomato accessions, the values of FS were widely ranged regardless of the fruit types (Figure 3C). Interestingly, the FS of a T11 accession in cherry type was higher in LT than that in NT, suggesting that the T11 genotype could play an important role in LT (Figure 3C). The percentage of FS in T01 and T02 in wild, T04, T07, T08, T09, T10, T12, T14, T15, T16, T17, T18, T19, T20, and T21, T22 in cherry type, T27, T30, and T31 in medium fruit type, and T34 and T35 in large type in LT were dramatically decreased compared to those in NT (Figure 3C). Furthermore, the FS of T03, T05, T06, and T13 in cherry type, T23, T24, T25, T26, T28, T29, and T32 in medium fruit type were observed with no significant difference in both NT and LT conditions (Figure 3C).

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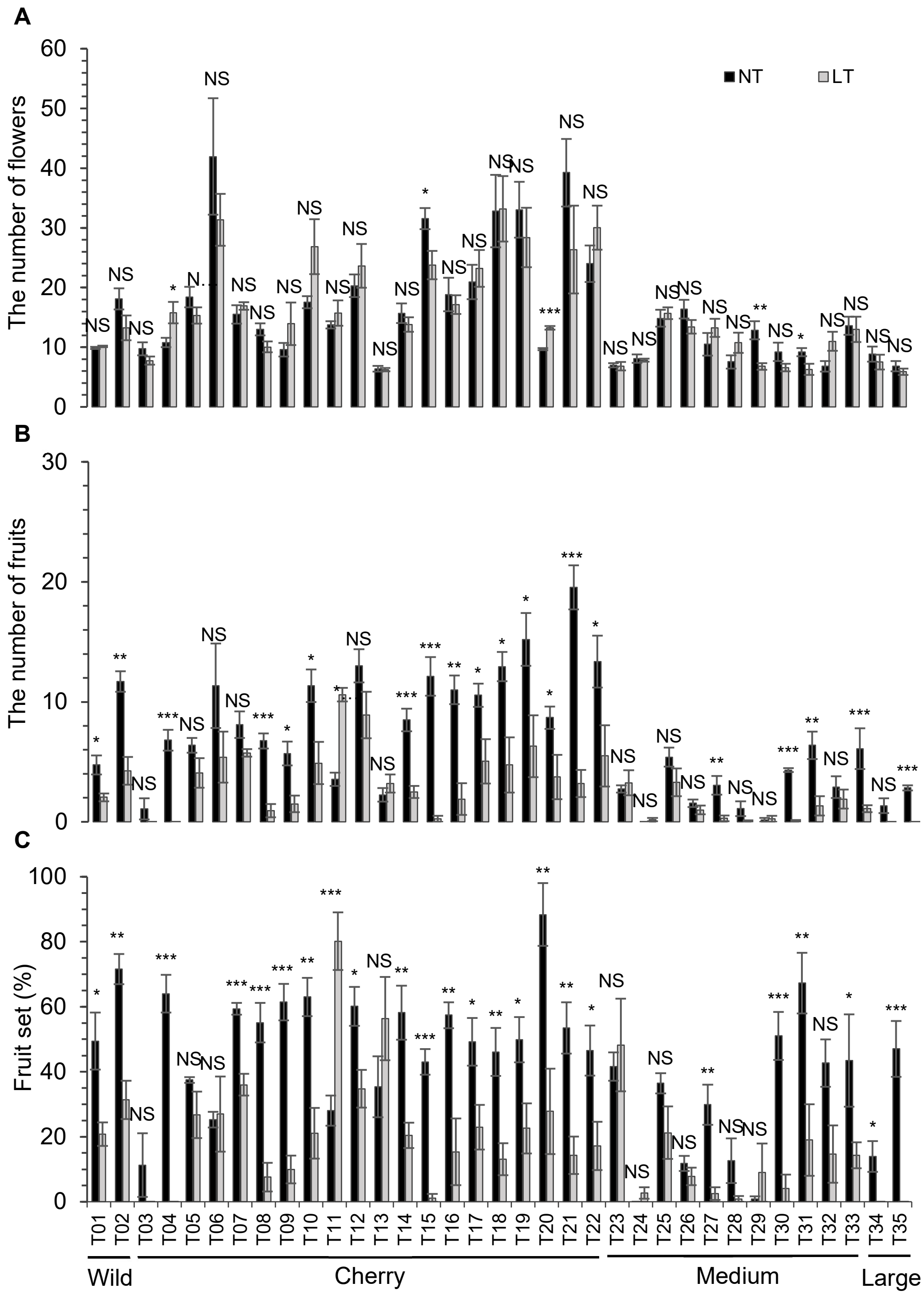

Figure 3. The analysis of reproductive traits on (A) the number of flowers, (B) the number of fruits, and (C) fruit set among 35 tomato accessions with different fruit types under LT and NT greenhouses. Average values (n = 5) are provided and significant differences were evaluated with student's *t*-test ($p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$) and denoted by *, **, and ***, respectively. NS indicates not significant and bars indicate ± standard deviations.

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2.4. The analysis of reproductive traits for fruit yield, marketable yield, and output of marketable fruit among thirty-five accessions with different fruit type in LT and NT

Next, in order to investigate the fruit marketability, the reproductive traits including fruit yield (FY), marketable yield (MY), and output marketable fruit (OMF) were determined among thirty-five tomato populations. The FY and MY were drastically reduced in T04, T05, T08, T09, T15, T16, T18, and T21 in cherry type, T23, T25, T27, T31, T33, T34, and T35 in medium type, T34 and T35 in large size under LT compared to NT, whereas TY was noticeably increased in T24 in medium size, which would be classified as cold-tolerant-accession (Figure 4A and 4B). In addition, the OMY between NT and LT showed that genotypes of T06, T07, T11, T14, T20 and T22 in cherry type and T28, T29, T30 and T32 in medium type were observed in no significant difference (Figure 4C). It was remarkable that the OMY over 60% were between LT and NT was observed in T05, T17, T19 and T20 in cherry type, T23, T25, T28, and T32 in medium size, and T34 in large size type, which exhibited that the percentage of normal fruits in LT was distinguishably higher than other accessions (Figure 4C).

To compare the difference in FS ratio between NT and LT, FS in NT was subtracted from the FS in LT. The difference in FS exhibited that T06, T11, T13, T23, T24, and T29 were positively influenced under LT condition. The positive difference over 20% were observed in T13 (21.0%) and T11 (52.1%). However, the negative difference below 50% were found in T09 (-51.5%), T20 (-60.5%) and T04 (-64.0%) (Supplemental Figure 2A). In addition to this, the difference in FY in LT was subtracted from FY in NT. The data showed that the positive difference over 0 kg was observed in T24 (0.15 kg), T02 (0.08 kg), and T11 (0.05 kg), while the negative difference below 1.0 kg were observed in T31 (-1.48 kg), T15 (-1.37 kg), T35 (-1.26 kg), T30 (-1.22 kg) and T34 (-1.09 kg) (Supplemental Figure 2B).

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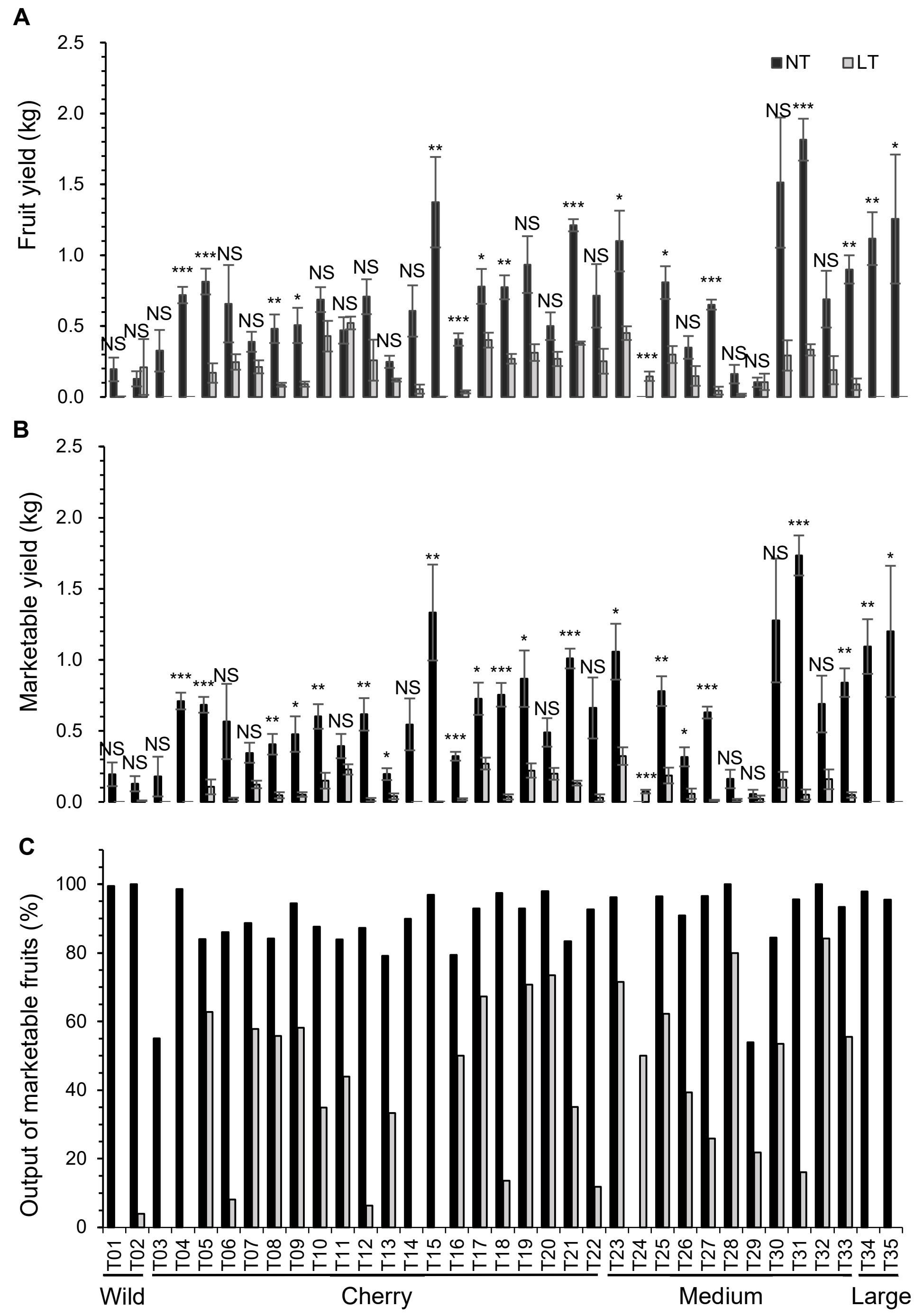

Figure 4. The analysis of reproductive traits on (A) fruit yield, (B) marketable yield, and (C) output of marketable fruit among 35 tomato accessions with different fruit types in LT and NT greenhouses. Average values (n = 5) are provided and significant differences were evaluated with student's *t*-test ($p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$) and denoted by *, **, and ***, respectively. NS indicates not significant and bars indicate ± standard deviation.

2.5. Correlation analysis with vegetative and reproductive traits in LT and NT

It is essential to select low-temperature tolerant tomato lines at the early growth stage to enhance the breeding speed. In order to understand whether the vegetative parameters including LW, LL, SD, and PH in LT and NT conditions are related to the repoductive

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parameters including FS, FY, MY, NFL, and NFR in LT and NT, the correlation coefficient was calculated among 35 tomato populations (Supplemental Table 2-4). The data showed that SD in vegetative traits in NT was positively correlated with FY (r = 0.554**) and MY (r = 0.993**) in NT (Supplemental Table 2). Also, PH in NT was positively correlated with NFL (r = 0.366*), NFR (r = 0.489**), and FS (r = 0.345*) in NT (Supplemental Table 2). In addition, PH in LT was positively correlated with FY (r = 0.365*), NFR (r = 0.470**), and FS (r = 0.352*) in LT (Supplemental Table 2). In detail, we analyzed the relavance of vegetative parameters in sub-populations including cherry type (n=20) and medium size (n=11) with reproductive parameters in both LT and NT (Supplemental Table 3). In cherry type (n=20), SD in NT was positively correlated with NFL (r = 0.448*) and NFR (r = 0.505*). LW was positively correlated with NFL (r=0.431*) in NT. In LT condition, PH was positively related to FY (r = 0.640**), MY (r=0.508*), NFR (r = 0.587**), and FS (r = 0.504*). LL was positively related to FY (r = 0.575**), MY (r = 0.485*), NFR (r=0.588**), and FS (r=0.506*) and LW was positively related to FY (r = 0.466*) and NFR (r = 0.524*) in LT. The analysis of correlation coefficient was performed in medium size tomato (n=11) (Supplemental Table 4). SD in NT was correlated to with FY (r = 0.678**), MY (r = 0.622**), and FS (r = 0.486*). FY in LT was positively correlated to SD (r = 0.514*), LL (r = 0.436*), and LW (r = 0.429*).

2.6. The relavance of physiological traits associated with fruit yield in LT and NT

Fruit yield (FY) is one of the most important physiological traits in tomato plants to determine the pro. In order to evaluate the relavance of FY with NFL, NF, and NFR in both LT and NT, the analysis of correlation coefficient was carried out. In NT condition, FY was not correlated with the NFL (r = 0.191NS) in total population (n=35) and the NFL (r = 0.152NS) in medium fruit types (n = 11), whereas FY was significantly correlated with NFL (r = 0.718**) in cherry fruit types (n = 20), indicating that the FY is influenced by the NFL in cherry fruit type in NT (Figure 5A-C). In LT condition, FY was significantly correlated with the NFL (r = 0.376*) in total population as well as the NFL (r = 0.488*) in cherry fruit types, but FY was not correlated with the NFL (r = -0.385NS) in medium fruit types (Figure 5D-F). This suggests that the FY is associated with the NFL in total population and in cherry fruit types, but not in meddle size fruit in LT. Moreover, the correlation coeffiecent was analyzed with the FS and FY in NT and LT (Figure 6A-C). The FY in NT was only correlated with FS (r = 0.955**) in medium fruit types, but not in total population and cherry fruit types. The FY was significantly correlated with FS (r = 0.623**) in total population, FS (r = 0.527**) in cherry fruit types, and FS (r = 0.794**) in medium fruit types in LT (Figure 6D-F). The correlation coeffiecent was analyzed with the NFL and the NFR in NT and LT (Supplemental Figure 3). The NFL in NT was correlated with the NFR (r = 0.817**) in total population and the NFR (r = 0.806**) in cherry fruit types, but not the NFR (r = 0.169NS) in medium fruit size (Supplemental Figure 3A-C). The NFL in LT was correlated with the NFR (r = 0.583**) in total population, but not with the NFR (r = 0.401**) in cherry fruit types and the NFR (r = 0.244NS) in medium fruit size (Supplemental Figure 3A-C).

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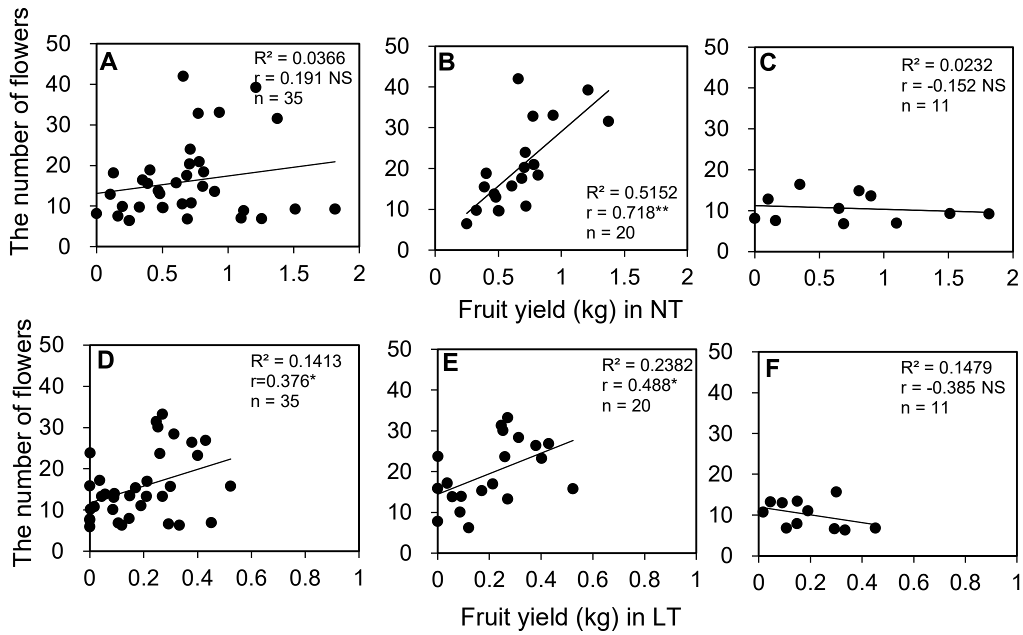

Figure 5. The scatter plots and the correlations coefficients were shown between the number of flowers and fruit yield under LT and NT greenhouses among total population (A), (D) and sub-populations with cherry (B), (E) and medium fruit types (C), (F), respectively.

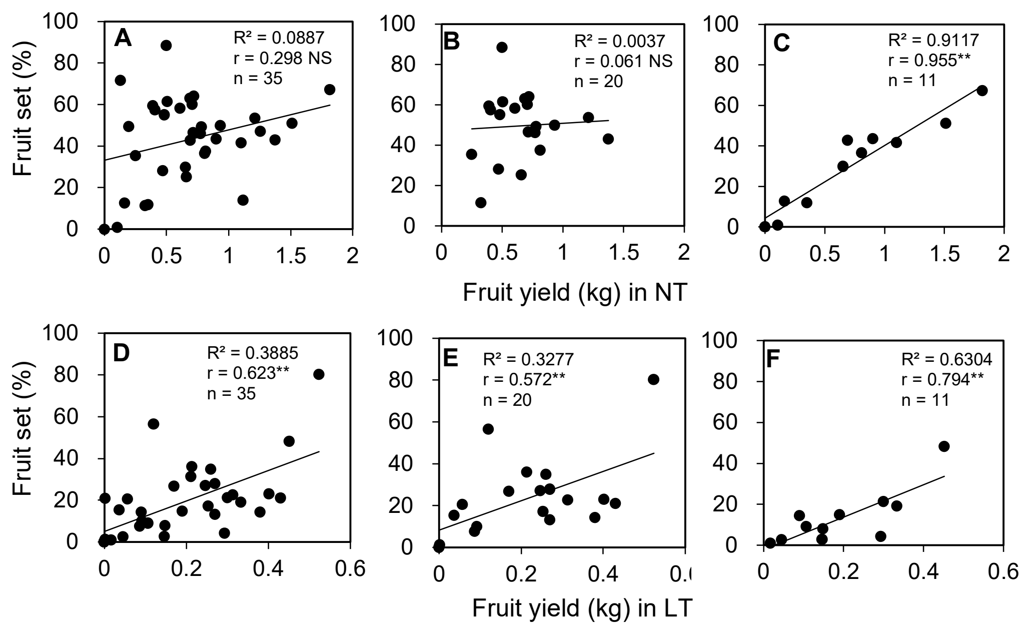

Figure 6. The scatter plots and the correlation coefficients were shown between the fruit set and fruit yield under LT and NT greenhouses among total population (A), (D) and sub-populations with cherry (B), (E) and medium fruit types (C), (F), respectively.

3. Discussion

3.1. The impact of LT on vegetative traits of tomato plants

Tomato plants have been evolved against adverse environment factors and current tomato plants harbor the several mechanisms to overcome cold stress [30-35]. Previous studies have mainly focused on the response to low temperature stress with several limited genotypes as well as a short period of the treatment during the growth stage under

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cold conditions [19,32,36-39]. In this study, we observed thirty-five accessions with vegetative and reproductive traits during entire growth stages in LT and NT under winter greenhouse. LT noticeably influenced some vegetative traits including PH, LL, and LW in tomato plants at 70 DAT compared to NT. In particular, the PH in LT condition was remarkably decreased in most accessions except for one accession, T32, which was not significant different between LT and NT (Figure 1A). The result was identical with previous researches that proved the retarded growth of tomato plants in night low temperature (LTN) condition [5,26,28,29]. However, SD of most accessions in LTN was similar to NT, except for T21 accession which showed more inhibited growth of SD in LTN (Figure 1B). Intriguingly, LL and LW in LT was inhibited in 100% of accession (2 out of 2) and 50% of accession (1 out of 2) in wild, 60% of accession (12 out of 20) and 40% of accession (6 out of 11) in cherry fruit-types, and 50% of accession (1 out of 2) and 0% of accession (0 out of 2) in large fruit types, respectively, compared to those in NT (Figure 2), implying that the LL and LW in LT was varied among all accessions regardless of fruit types.

3.2. The impact of LT on reproductive traits of tomato plants

We analyzed the reproductive parameters such as NFL, NFR, and FS in LT and NT. However, LT did not affect NFL in most accessions (Figure 3A) and NFL of two accessions was increased in LT more than NT, whereas NFL of three accessions were decreased in LT (Figure 3A), suggesting that the differences of NFL in LT might be involved in effect of genotype as previous mentioned in [18]. Furthermore, the effect of LT on NFR uncovered that 70.0% of accessions (14 out of 20) with cherry fruits and only 36.36% of accessions (4 out of 11) with medium fruits in NFR compared to NT was affected (Figure 3B). The effect of aforementioned NFR in LT was closely related to the FS in cherry fruit and medium fruit that showed 80% (16 out of 20) and 36.36% (4 out of 11) of accessions was influenced (Figure 3C). NFR and FS of T11 accession in LT was significantly higher other than accessions in both LT and NT, implying that T11 accession could be selected for the low temperature tolerant-cultivar for breeding programs in winder greenhouse condition. FY and MY were significantly affected by LT (Figure 3C). For instance, approximately 48% (17 out 35) of FY and 60% (21 out 35) of MY were reduced under LT condition. Previous studies have illustrated that FS plays a key role in determining LT tolerant-tomato cultivars [18,20-22]. Indeed, based on the parameter of FS, a wide range of tomato accessions were chosen. Recent report has demonstrated that NFR could be used as an index for FS in HT, which was positively correlated with FS in HT [23]. We also studied the relationship between FS in LT and NT (Figure 3C). Our result showed that FS of most accessions in LT was negatively decreased, except for T11 accession which exhibited the increase of FS under LT compared to that in LT. Collectively, the results showed that the NFL of most accessions was not associated with the NFR, FS, and FY. Although it need to be further confirmed with experiments such as pollen germination and pollen grain staining to measure pollen activity [6,20,22], the poor quality of flower pollens among many accessions in LT might result from the decrease in FS of the accessions [14,20,22], while the development of ovule and stigma could not be related to FS [40-42].

3.3. Correlation between physiological traits in LT condition

The result of correlation coefficient between vegetative and reproductive traits depending on fruit types exhibited that only PH in total population (n = 35) in LT was positively asscociated with reproductive NFR, FS, MY, and FY in LT, but not in the vegetative parameters of SD, LL, and LW in LT (Supplemental Table 2). The analysis was expanded in sub-population including cherry type (n = 20) and medium type (n = 11). The correlation result of PH in LT in the cherry-type was observed as similar to that of total population in LT, while the LL in LT was positively correlated with NFR, FS, MY, and FY in LT. Also, the LW in LT was positively correlated with NFR and FY in LT (Supplemental Table

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3). Importantly, the SD, LL, and LW in LT was associated with only FY in LT (Supplemental Table 4). The similar experiment was performed in HT condition and the research demonstrated that the vegetative parameters in either NT or HT was correlated with the reproductive parameters in HT with fruit types [23], also indicating that, based on the tomato fruit types and cultivars, the vegetative parameters in LT could be used for the index to early predict and select LT-tolerant tomato genotypes, which expect to display the good performance of reproductive parameters.

We classified thirty-five tomato accessions into wild, cherry, medium, and large fruit types of sub-populations as previously described [18]. Since FY trait is a key factor to determine high yields of tomato in agriculture, the correlation analysis of FY trait in LT was mainly evaluated with reproductive parameters of NFL and FS depending on fruit types. Interestingly, we identified the correlation of physiological traits with FY under LT and NT condition (Figure 5-6 and Supplemental Figure 3). The significant correlation (r = 0.488*) of NFL and FY in LT was observed in cherry fruit type, but not in medium fruit type (r = -0.385NS). Also, this significant correlation (r = 0.718**) in NT was observed in only cherry fruit type (Figure 5), implying that NFL could be used as an index for selecting LT-tolerant tomato accession in only cherry type in both LT and NT conditions. Moreover, the relationship of FS and FY was also pronounced in LT and NT condition and the correlation coefficient of FS and FY was distinct in total population (r = 0.623**) in LT, cherry type (r = 0.572**) in LT, and medium size type (r = 0.955** and r = 0.794**) in LT and NT (Figure 6), providing the implication that FS could be used as an index for selecting LT-tolerant tomato accessions in both cherry type and medium type in LT. Considering the fact that the high correlation coefficient of FS and FY in medium type and NFL and FY in cherry type in NT as well as LT, FS and NFL could be helpful to select LT-tolerant tomato cultivars just in NT condition by analyzing these indices [18, 23].

4. Materials and Methods

4.1. Plant material and growth conditions

The plant material and growth conditions were followed as previously described [18]. Thirty-five of tomato breeding lines from National Institute of Horticultural and Herbal Science (NIHHS) (Wanju, South Korea) were used in this research (Supplemental Table 1). All tomato accessions were classified into two Wild (<10 g), twenty Cherry (10-30 g), eleven Medium (31-80 g), and two Large (>81 g) depending on fruit sizes [18]. The seeds of thirty-five tomato accessions were sown on 31 August, 2020 in plastic pots containing 1:1 ratio of sand and commercial bed soil (Bio Sangto, South Korea), which were composed of coco peat (47.2%), peat moss (35%), zeolite (7%), vermiculite (10.0%), dolomite (0.6%), humectant (0.006%), and fertilizers (0.194%) and grown in a glasshouse with 26/18°C (day/night) temperature [18,43]. Tomato seedlings with 10-12 leaves and first truss were transplanted on 28 October, 2020. The seedlings were transferred into two plastic film greenhouses, where night temperature set-point was maintained at 15°C for 14 days in both greenhouses, adapting the seedlings to new environment conditions. Subsequently, night temperature set-point of each greenhouse was controlled for low temperature (LT) at 10 °C and normal treatment (NT) at 15°C, respectively. Tomato seedlings of five plants per accession were planted with a plant distance of 40 cm by 40 cm between plants [18] in both LT and NT greenhouses. The thirty-five of tomato accessions were randomly selected and planted with keeping the same arrangement of the accessions between LT and NT greenhouses. The soil was prepared in greenhouses as previously described in [18]. The temperature and the relative humidity (RH) were monitored in both LT and NT greenhouses during the periods of whole growth and development using data logger (WatchDog 1450, Spectrum Technologies Inc., Aurora, USA) (Supplemental Figure 1).

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4.2. Data collection on vegetative and reproductive growth

The vegetative parameters including plant height (PH), leaf length (LL), leaf width (LW), and plant stem diameter (SD) were measured using 70 days after transplanting (DAT) from five individual plants per accession in both greenhouses. The reproductive parameters including the number of flowers (NFL), the number of fruits (NFR), fruit set (FS, %), fruit yield (FY, kg), marketable yield (MY, %), and output of marketable yield (OMY) were evaluated by calculating from the third to six trusses of each plant. Differences in FS and TY parameters between plants grown in 10°C and 15°C greenhouses were calculated by subtracting index of FS and FY of NT from LT, respectively [18]. Fruit set (FS, %) with diameter ≥ 0.5 cm was calculated as follows [6]: Fruit set (%) = (The number of fruits / The number of flowers) x 100. In addition, fruit yield (FY) was determined by the sum of fresh weight (FW) in kg of all fruits harvested from the third to sixth trusses from five individual plants.

4.3. Data analysis

The significance of difference in vegetative parameters of PH, SD, LL, and LW, and reproductive parameters of NFL, NFR, FS, TY, MY, and OMY under LT and NT was assessed as described in the figure legends with student's *t*-test using EXCEL 2016 program (Microsoft Co. Ltd., USA). The analysis of correlation coefficients was performed among total population (n = 35) and subpopulation classified into cherry (n = 20) and medium (n = 11) using for correlation coefficient with EXCEL 2016 (Microsoft, WA, USA).

5. Conclusions

We characterized the physiological traits of thirty-five tomato accessions in the response to night low temperature, which is economically important for the tomato cultivation of winter greenhouse. Several vegetative parameters were correlated with reproductive parameters and the correlation coefficients between the physiological traits were different in cherry, medium, and large fruits types, suggesting that LT-tolerant cultivars are possibly selected during vegetative stage and the selection for LT-tolerant cultivars is required to consider different selection index relying on fruit types [18,24]. Moreover, we studied the evaluation of FS between LT and NT. Interestingly, T04, T08, T09, and T20 in cherry fruit type, T30 in medium fruit type, and T35 in large fruit type were LT-sensitive accessions, while T11 and T13 in cherry fruit and T23 and T29 in medium fruit were LT-tolerant accessions. Future researches will be required to evaluate more accessions of large fruit type under LT greenhouse condition and the identified LT-tolerant and/or -sensitive accessions will be focused on the determination of the physiological and the molecular functions including pollen development and viability [6,22], chlorophyll contents and photosynthetic parameters [12,19,39,45] total free prolines [44,45], and electrolyte conductivity [46], combined with DNA- and RNA-seq [36,37,47,48]. This will provide the deeper insights of how those genotypes are involved in the mechanism of LT-tolerant and/or -sensitive phenotypes and further help the breeders to establish more specific, accurate, and speed breeding systems relying on cultivars.

Supplementary Materials: Figure S1: Air temperature in LT and NT greenhouses during the period of tomato growth and development. Figure S2: The analysis of difference in FS and FY among 35 tomato accessions in LT and NT. Figure S3: The scatter plots and the correlations coefficients between the number of fruits (NFR) and the number of flowers (NFL) in LT and NT, Table S1: Tomato accessions for evaluation of physiological traits against low temperature in winter 2020-2021., Table S2: The correlations between vegetative and reproductive traits in total population of tomato with different fruit types in LT and NT greenhouses., Table S3: The correlations between vegetative and reproductive traits in total population of tomato with different fruit types in LT and NT greenhouses., Table S4: The correlations between vegetative and reproductive traits in sub-population cherry fruit types in LT and NT conditions.

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Abbreviations:

LT—low temperature, LTN—low temperature night, NT—normal temperature, PH—Plant heights, LL—leaf length, LW—leaf width, SD— plant stem diameter, PH—plant height, FT, NFL—the number of flowers (NFL), NFR—the number of fruits, FS—fruit set, FY—fruit yield, MY—marketable yield, OMY—output of marketable yield, HT—high temperature, DAT—days after transplanting, RH—relative humidity

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