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Sherzod N. Rajametov,
E.Yo. Yang*, M.Ch. Cho, H.B. Jeong

National Institute of Horticultural and Herbal Science,
Rural Development Administration
Wanju, 55365, Republic of Korea

*Correspondence: yangyang2@korea.kr (E.Y.Y.)

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Ш.Н. Ражаметов, Ёнґ Янґ*,
Мёнґ Чеол Чо, Хёо Бонґ Жеонґ

Национальный научный институт плодовоощеводства и лекарственных растений, Администрация развития сельского хозяйства, г. Вонжу, 55365, Южная Корея

*Автор для корреспонденции:
yangyang2@korea.kr (E.Y.Y.)

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Effect of leaf cold damage after chilling temperature treatment on growth and reproductive parameters of chilli pepper plants



Abstract

Relevance. Pepper is sensitive to chilling temperatures in all growth stages and low temperatures are main factors affecting plant growth, fruit growth and development and productivity. Evaluation and identification low temperature (LT) tolerant pepper genotypes at different growth stages is actual in breeding program for developing new cultivars. In present study we investigated the effect of the leaf cold damage within 25% (LCD) after chilling treatment in seedling stage on vegetative and reproductive traits of pepper accessions with different cold tolerance.

Material and methods. In this study two pepper accessions "PE-J-2" and "Neokgwang" selected as chilling tolerant and susceptible in juvenile stage (3-4 true leaf stage), were used respectively. The seedlings of the selected pepper accessions with 25% visual cold damages of leaf green part (become lightly yellowed-whited or desiccated-dried) and control non-treated (NT) were grown in a glasshouse condition (D/N 30-32/22-24°C) for 10 weeks to evaluate the effect of LCD on pepper accessions vegetative and reproductive parameters after chilling pre-treatment. In pepper plants the vegetative parameters such as plant height (PH), leaf length (LL) and width (LW), number of internodes (NI), length of main axis (LMA), plants fresh weight (PFW) and roots fresh weight (RFW), and reproductive the number of flowers (NFL) and fruits (NFR), fruit set ratio (FS), fruit length (FL) and diameter (FD), total yield per plant (TY) were measured. The experimental design of this study was completely randomized. Statistical analysis was performed using the SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA).

Results. According to the research result several accessions were identified: the accessions screened in juvenile stage as cold tolerant cannot always manifest good some agronomical traits performance at growth stages and it may range depending on the genotype specific features. The seedlings with LCD within 25% may significantly affect the vegetative and reproductive parameters of pepper plants. The phenomenon was recorded more distinctive in the correlations between some vegetative and reproductive parameters among NT and LCD plants.

Keywords: pepper, temperature, seedling, plant, root, leaf, flower, fruit set, fruit, correlation

Влияние степени повреждения листьев после обработки низкой температурой (охлаждения) на рост и репродуктивные параметры перца острого

Abstract

Актуальность. Растения перца чувствительны к пониженным температурам на всех этапах роста, и низкие температуры являются основным фактором, влияющим на рост растений, рост и развитие плодов и продуктивность. Оценка и идентификация низкотемпературных (LT) устойчивых генотипов перца на разных стадиях роста актуальна в селекционной программе для создания новых сортов. В настоящем исследовании мы исследовали влияние повреждения листа в пределах 25% (LCD) после обработки низкой температурой в стадии проростков на рост, вегетативные и репродуктивные параметры образцов перца с различной холодоустойчивостью.

Материал и методика. Использовали два образца перца «PE-J-2» и «Neokgwang», отобранные как устойчивый и чувствительный к низким температурам на ювенильной стадии развития (стадия 3-4 настоящих листьев), соответственно. Проростки выбранных образцов перца с 25% визуальными повреждениями зеленой части листа от низких температур (становятся слегка пожелтевшими-беловатыми или высушенными) и растения в контроле (NT) выращивали в условиях теплицы (день/ночь 30...32/22...24°C) в течение 10 недель, чтобы оценить влияние LCD на вегетативные и репродуктивные параметры образцов перца после предварительной обработки охлаждением. У растений перца были измерены вегетативные параметры, такие как высота растения (PH), длина листа (LL) и ширина (LW), количество междоузлий (NI), длина главной оси (LMA), свежая масса растений (PFW) и корней (RFW), а также репродуктивные признаки: количество цветков (NFL) и плодов (NFR), коэффициент завязывания плодов (FS), длина (FL) и диаметр (FD) плодов, общая продуктивность растения (TY). Эксперимент был полностью рандомизирован. Статистический анализ выполняли с использованием SAS Enterprise Guide 7.1 (SAS Institute Inc., Кэри, Северная Каролина, США).

Результаты. В результате исследования было выявлено, что отобранные образцы острого перца, холодоустойчивые на стадии проростка, не всегда могут проявлять хорошие агрономические показатели на стадиях роста, и они могут колебаться в зависимости от особенностей генотипа. Проростки с повреждением листьев в пределах 25% могут существенно повлиять на рост вегетативных и репродуктивных параметров растений перца. Выявлены более четкие корреляции между некоторыми вегетативными и репродуктивными параметрами у NT и LCD растений.

Ключевые слова: перец, температура, проросток, растения, корень, листья, цветок, завязываемость плодов, плод, корреляция

Introduction

Temperature is one of the main environmental factors affecting vegetative and reproductive growth of plants [1-3]. Low temperature stress is a major environmental factor in temperate zone and limits plant growth and productivity [4,5], and plants have different abilities to deal with low temperatures, where LT significantly exhibits reduction in photosynthesis, delays the formation of leaf, truss appearance, number of flowers, fruit set and fruit growth [2, 6-9].

Under such conditions, plants try to maintain homeostasis to acquire freezing tolerance and this involves extensive reprogramming of gene expression and metabolism [10-12], and the changes in physiological and biochemical processes under LT conditions are related to the transcript levels of a number of cold-regulated genes [13-15]. Although several hypotheses have been proposed to explain tolerance or sensitivity to chilling in tomato plants, where the physiological mechanisms responsible for cold tolerance remain unclear [16].

LT stress in plants can be divided into two categories [15]: chilling stress (0–20°C) and freezing stress (<0°C). Chilling-sensitive plants such as vegetable crops pepper and tomato may suffer from cold injury and reduce productivity within temperature 0–15°C [17,18].

The plants may develop several mechanisms to minimize potential damage during LT stress and subsequent recovery periods, where the plants response is a highly complex process that involves physiological and biochemical modifications [9,19].

However, in literature scarce information is available on the influence of the degree of cold damages on the agronomical traits at recovery among pepper exposed to LT in seedlings stage, which makes it difficult to assess cold tolerance potential of each genotype. Recently, multiple methods to screen and understand the mechanism for cold-tolerant genotypes among the *Solanaceous* crops were validated at different growth stages under low temperature stress condition [18,20-24]. Moreover, several methods to screen cold tolerant genotypes among the tomato and pepper crops have been developed in early growth stages [11,13,19,25,26].

Meanwhile, recently the findings in evaluation of the tomato genotypes heat tolerance in juvenile stage revealed that the heat tolerance rate in the seedling stage might not always be associated with reproductive parameters [27]. Therefore, in present study our target was to evaluate the effect of pre-cold treatment on pepper plants agronomical traits distinguished by tolerance rates to low temperature and identifies the any linkage of cold damage in juvenile stage with subsequent flowering and reproductive growth periods. It is a continuation of our research work which has been recently done on identification of the early screening methods of pepper (*Capsicum* L.) seedlings tolerance to low temperature [28].

Materials and Methods

Plant materials and growth condition

Twenty-one pepper accessions seedlings were sown in 31st January of 2020 in plastic trays (52 x 26 cm in size, 6 x 6 cm cells with pot volume 5 liter) containing 1:1 ratio of sand and commercial bed soil (Bio Sangto, Seoul, Korea) containing coco peat (47.2%), peat moss (35%), zeolite (7%), vermiculite (10.0%), dolomite (0.6%), humectant (0.006%) and fertilizers (0.194%). A liter of water was provided to each tray daily, and the trays were placed in a glasshouse under normal condition

(26/18 °C in day/night with relative humidity within 60-70%) in National Institute of Horticultural and Herbal Science (NIHHS). Hereinafter, the seedlings 38 days old with 3-4 true leaf stage were transferred into growth chamber on 9th March 2020.

The pepper seedlings were maintained at 5°C, light intensity 800 μmol m⁻²s⁻¹ (D/N 12/12h) and relatively humidity was within 65-70% and were treated under low temperature condition for 25 days [28]. All seedlings after low temperature treatment were transferred into normal condition as described above for recovery during 7 days. Then, accessions “PE-J-2” and “Neokgwang” were selected as cold tolerant and susceptible according to screening results of pepper seedlings on chilling tolerance among twenty-one accessions, where LCD in two accessions were 30.0 and 72.2% after chilling treatment, respectively.

Methods

The experiment was grouped as control (NT) and plants within 25% LCD among two accessions. To assay effects of LCD on agronomical traits the seedlings of both pepper were transplanted into pots on 6th April 2020 after 7 days of recovery. Soil in the pots were prepared as described above and regularly watered to avoid drought and fertilized weekly (Mulpure, Daeyu Co. Ltd., Gyeongsan, South Korea), and plants were cultivated for 10 weeks in a glasshouse condition (D/N 30-32/22-24°C, RH 40-60%).

Plant height (PH) was measured with interval 7 days for 70 days after transplanting (DAT). Days to flowering after transplanting of plants were measured from second internode flowers. Leaf length (LL) and width (LW), fruit length (FL) and diameter (FD) were measured from leaves located on 3rd internodes of plants, using 5 replicates. The reproductive parameters number of flowers (NFL) and fruits (NFR), fruit set ratio (FS), total yield per plant (TY) were counted from first to fourth internodes of each plant. Number of internodes (NI), length of main axis (LMA), TY and plants fresh weight (PFW) and roots fresh weight (RFW) were measured on 70 DAT.

Fruit set (FS, %) was calculated as follows:

$$\text{Fruit set (\%)} = \frac{\text{The number of fruits}}{\text{The number of flowers}} \times 100$$

Statistical analysis

The experimental design of this study was completely randomized. Statistical analysis was performed using the SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, North Carolina, USA), and mean values were compared with a significance level of 5% using Duncan’s multiple range test or the Student’s t-test at the p<0.05, p<0.01, and p<0.001 levels, respectively. Correlation coefficient analyses were done with EXCEL 2016 (Microsoft, WA, USA).

Results

Effect of leaf cold damage on vegetative parameters

Leaf cold damage affect differently growth rate of plant height among pepper accessions (Fig. 1). So, the biggest differences (p≤0.001) in growth rate in the percentage ratio between NT and LCD plants within first week after transplanting into pots were observed. Pepper accession “PE-J-2” showed the highest index differences in plant growth than “Neokgwang” (Fig. 1A), but the index was lower in both accessions on 59.0 and 44.0% than in NT plants, respectively.

The highest intensity growth rate was determined in both accessions plants in NT within 28 and 35 DAT, then, hereinafter the intensity growth in LCD plants of “Neokgwang”

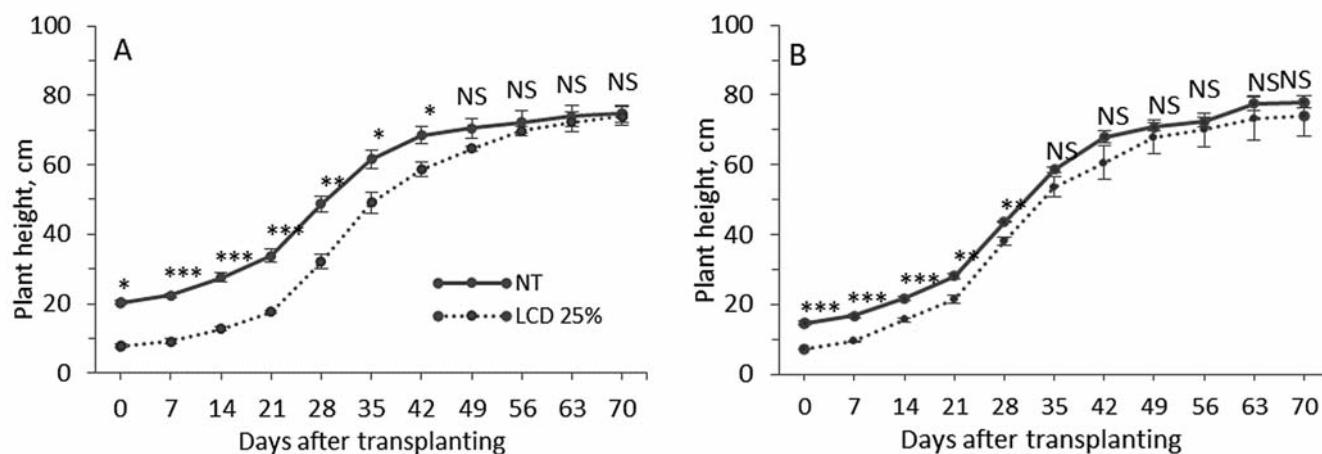


Figure 1. Effect of leaf cold damage on pepper accessions growth rate- “PE-J-2” (A) and “Neokgwang” (B). Vertical bars represent SE ($n=5$) and significant differences were evaluated with Student’s t-test ($p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$) and denoted by *, **, and ***, respectively and NS indicates not significant

increased slightly on a par with NT plants and within 35 DAT showed no differences (Fig. 1B). On 50 DAT no significant differences in growth rate between NT and LCD plants in both accessions were identified; the index reduced in “Neokgwang” and “PE-J-2” on 4.0 and 8.3%, respectively and reached almost the same value of plants in NT condition.

The effect LCD within 25% on vegetative and reproductive traits among pepper accessions was different. So, in both accessions regardless of cold tolerance degree significant increase of the PFW in LCD plants compared to NT were observed (Fig. 2A), while contrary result, decreasing of the LMA in LCD plants were identified (Fig. 2B). Only, in LCD plants of “PE-J-2” higher index of the LL than in NT plants were observed (Fig. 2C), whereas in

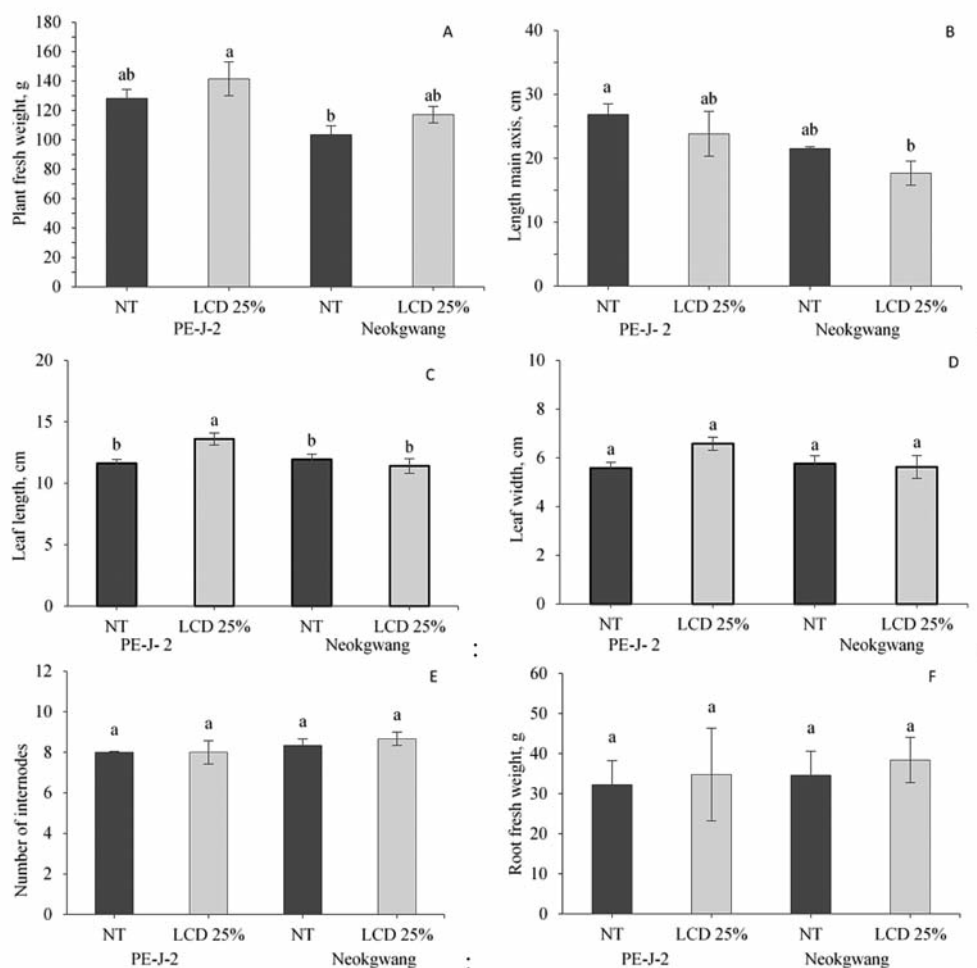


Figure 2. Effect of leaf cold damage (LCD) on pepper accessions vegetative parameters. The data indicate the means \pm SE ($n=5$). Different letters above bars indicate significant difference based on Duncan’s multiple range test ($p \leq 0.05$) among accessions plants from control (NT) and leaf cold damage (LCD) on 70 days after transplanting

measurement of the LW no identified difference between LCD and NT plants in both accessions was registered (Fig. 2D). Also, LCD 25% no critical effect on NI and RFW traits, where significant differences between NT and LCD plants in both pepper accessions were observed (Fig. 2E and F).

Effect of leaf cold damage on reproductive parameters

Days to flowering among NT and LCD plants after transplanting showed that NT plants of accessions “PE-J-2” and “Neokgwang” started to flowering within 18.3 and 25.7 DAT, respectively. Hereinafter, LCD plants started to flowering within 30.3 and 29.3 DAT, respectively, where significant difference in days were identified in “PE-J-2” about 12 days, whereas for LCD plants of “Neokgwang” needed the shortest 3.7 days to begin flowering (Table 1).

Development of flowers was not significantly ranged among NT and LCD plants of both accessions (Fig. 3A), but the quantity of fruits in “Neokgwang” was significantly increased in LCD plants compared to NT (Fig. 3B), whereas in “PE-J-2” contrary results were observed, that is decreases in LCD plants. Moreover, essentially, such a pattern manifested itself in the study of fruit set (Fig. 3C). Assay of the FL showed that LCD decreased the FL in both accessions plants compared to NT (Fig. 3D), but in the measurement of the FD no difference in values between NT and LCD plants in both accessions were found.

In evaluation of the index of the total yield in NT and LCD plants of the both accessions were found that crop yield on the 70 DAT increased in LCD plants of “Neokgwang”, whereas in “PE-J-2” it was decreased compared to NT plants (Fig. 3F).

Table 1. Flowering period among control (NT) and leaf cold damaged (LCD) pepper plants

Accessions/LCD	Flowering period	Days to flowering after transplanting	diff_days between NT and LCD plants
PE-J-2 - NT	April 24.3 ± 2.5x	18.3 ± 4.4	0.0
PE-J-2 - LCD 25%	May 6.3 ± 2.1	30.3 ± 2.1	12.0 ± 2.4
Neokgwang - NT	May 1.7 ± 0.6	25.7 ± 2.1	0.0
Neokgwang - LCD 25%	May 5.3 ± 0.6	29.3 ± 0.6	3.7 ± 1.5

xData shown represent means ± SE (n=5).

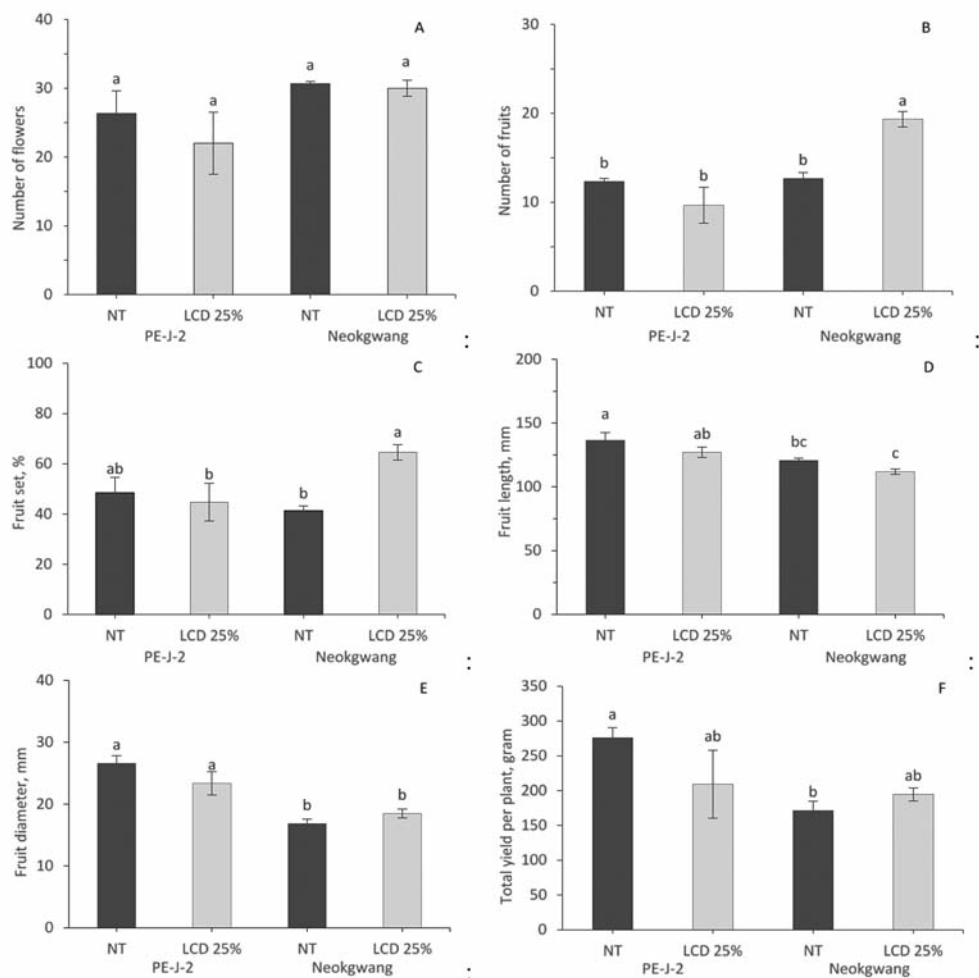


Figure 3. Effect of leaf cold damage on pepper accessions reproductive parameters. Values are means ± SE (n=5). Different letters above bars indicate significant difference based on Duncan's multiple range test (p ≤ 0.05) among accessions plants from control (NT) and leaf cold damage (LCD) on 70 days after transplanting

Table 2. Correlation coefficients (*r* values) among agronomical traits from control (A) and LCD (B) plants

Traits	PH	LMA	NI	PFW	RFW	NFL	NFR	FS	LL	LW	FL	FD	TY
PH	1.0												
LMA	-0.488	1.0											A
NI	0.539	-0.346	1.0										
PFW	0.007	0.514	-0.194	1.0									
RFW	0.052	-0.395	0.159	-0.004	1.0								
NFL	-0.236	-0.165	0.316	-0.803*	0.273	1.0							
NFR	-0.193	0.102	-0.327	-0.578	-0.734	0.249	1.0						
FS	0.175	0.164	-0.412	0.622	-0.521	-0.948**	0.066	1.0					
LL	-0.099	0.016	0.763*	-0.054	0.284	0.453	-0.441	-0.587	1.0				
LW	-0.493	0.340	0.323	-0.203	-0.429	0.386	0.280	-0.281	0.600	1.0			
FL	-0.095	0.646	-0.200	0.938**	-0.325	-0.796*	-0.324	0.702	-0.326	-0.069	1.0		
FD	-0.296	0.807*	-0.232	0.849*	-0.398	-0.623	-0.199	0.562	-0.216	-0.024	0.869*	1.0	
TY	-0.696	0.867*	-0.371	0.585	-0.365	-0.287	-0.013	0.282	0.139	0.504	0.740	0.884**	1.0
PH	1.000												
LMA	-0.145	1.000											B
NI	0.696	-0.804*	1.000										
PFW	-0.448	0.930**	-0.934**	1.000									
RFW	-0.616	-0.334	-0.116	-0.167	1.000								
NFL	-0.106	0.003	-0.109	-0.128	0.415	1.000							
NFR	-0.015	-0.255	0.168	-0.394	0.293	0.823*	1.000						
FS	-0.037	-0.272	0.201	-0.348	0.017	0.378	0.823*	1.000					
LL	-0.253	0.934**	-0.804*	0.937**	-0.151	-0.183	-0.470	-0.474	1.000				
LW	-0.140	0.862*	-0.652	0.772*	-0.127	-0.049	-0.135	-0.082	0.905**	1.000			
FL	-0.185	0.311	-0.342	0.509	-0.443	-0.790*	-0.866*	-0.550	0.468	0.192	1.000		
FD	-0.035	0.877**	-0.654	0.875**	-0.518	-0.412	-0.665	-0.559	0.572	0.264	0.703	1.000	
TY	-0.233	0.764*	-0.724	0.688	-0.290	0.437	0.262	0.187	0.517	0.503	0.033	0.500	1.000

Significant levels for correlations: * $P < 0.05$, ** $P < 0.01$. PH – plant height; LMA – length of main axis, NI – number of internodes, PFW – plants fresh weight, RFW – root fresh weight, NFL – number of flowers, NFR – number of fruits, FS – fruit set, LL – Leaf length, LW – leaf width, FL – fruit length, FD – fruit diameter, and TY – total yield per plant

Correlation between agronomical traits

The analyses of correlation coefficients among NT and LCD plants of pepper accessions (Table 2) showed that LMA had positive significant correlation with FD and TY in NT ($r=0.807^*$ and 0.867^* , respectively) and those in LCD ($r=0.877^{**}$ and 0.764^* , respectively). Also, PFW positively correlated with FD ($r=0.849^*$ and $r=0.875^{**}$, respectively), while NFL showed negative significant correlation with FL in both NT and LCD plants ($r=-0.796^*$ and $r=-0.790^*$). In NT condition, NI positively correlated with LL but in LCD these correlations showed somewhat different negative correlation coefficient ($r=0.763^*$ and $r=-0.804^*$, respectively).

Discussion

Effect of LCD within 25% in seedling stage on the subsequent growth stages of agronomical traits showed that it might be critical for recovery of pepper plants and significantly affect the development of some vegetative and reproductive parameters. It is well known that, LT significantly reduces the growth rate of plants [2,8,23,24,29], which was detected in plants with LCD in the present study, where lag behind in growth rate of PH in the percentage ratio than NT plants within 50 DAT was determined. Furthermore, a decrease of value of the differences in development was observed but the ranges depended on accession. Despite this, within 4-5 weeks equal the growth intensity in NT and LCD plants was observed, but the difference in growth rate was persistent among accessions.

LCD contributed to increase the index of PFW, but decreased the LMA in both pepper accessions, whereas the index of LMA in pepper accessions might be varied depending on the genotypes under LT condition [24]. Meanwhile, the index of LL was increased only in LCD plants of "PE-J-2", but no differences between LCD and NT plants in both accessions in measurement of the LW were recorded. There no critical effects of the LCD 25% on NI and RFW traits in both pepper accessions plants were detected.

In particular, the effect of the abiotic stress on the reproductive traits is well known in Solanaceous crops [1-3,6,26,30], and there is enough information about the effect of the cold damages in juvenile phase on the floral and fruit traits at growth stages of pepper. In fact, recently it was found that the selected pepper accessions under LT condition were primarily involved in the positive trends with the reproductive index including NFR, FL, FD, and TY traits and recommended to utilize for pepper breeding programs to develop LT tolerant cultivars [24].

The results of the analysis of LCD influence on the flowering period indicate that in cold damaged plants flowering started relatively later than plants in NT and varietal differences were observed. No significant effect of LCD on the development of the NFL was observed, whereas LCD effected the development of NFR but among accessions differences persist in the varietal

aspect, where susceptible accession "Neokgwang" distinguished with high NFR and relatively FS per plant than in NT plants.

In the agreement with our previous studies of pepper genotypes under LT condition [24], our current finding also showed that the NFL among pepper accessions showed no remarkable difference between the LCD and NT.

The effect of LCD on the agronomical traits with NT plants was more distinctive in the correlations between LMA, FD, TY, PFW and NFL, and a small correlation between FD and FL was observed. Same correlations were also found between TY in NT and that in LCD, which indicates that accessions with high yield in NT can also perform well in terms of harvest in LCD plants, which was also reported in previous research results [24,31].

Based on NFR or FS various genotypes from Solanaceae family were selected and used as predictor for abiotic stress tolerance [9,27,32,33]. However, in our study chilling susceptible accession "Neokgwang" in seedling stage showed good physiological traits performance such as high rates of NFR, FS and TY in comparison with screened one as tolerant in seedling stage "PE-J-2". It means that cold tolerance rate in seedling stage is not always associated with reproductive traits.

In addition, recently, a similar results revealed in evaluation of the physiological and biochemical parameters related to heat tolerance and to determine critical leaf heat damage levels for selecting heat-tolerant in hot pepper genotypes [34]. Leaf heat damage levels over 25% in seedling stage was critical for selecting heat-tolerant genotypes in pepper, and remarked that constant photosynthetic rate via increased transpiration rate as well as high proline content in heat stress condition confers faster recovery from heat damage of heat-tolerant cultivars in seedlings stages. In accordance with these results, further studies need to focus on elucidating the mechanism and role of the impact of physiological parameters on vegetative and reproductive traits in pepper plants with different low temperature tolerance.

Conclusion

Overall, our results imply that, the genotypes screened in juvenile stages as cold tolerant cannot always manifest good agronomical traits performance at growth stages and it may range depending on the genotype specific features. LCD within and over 25% may contribute to increase the index of PFW but might decrease the LMA and FL delaying the days to flowering regardless of the tolerance of the pepper accessions to low temperature. Moreover, agronomic traits associated in LCD plants differed by genotype specific. And, future researches on screening and selection for pepper abiotic stress tolerance should be evaluated at the vegetative and reproductive stages with consideration of reproductive parameters and molecular functions, combined with DNA- and RNA-seq.

Об авторах:

Шерзод Нигматуллаевич Ражаметов – PhD (сельское хозяйство), докторант-исследователь, Отдел овощных культур, sherzod_2004@list.ru. <https://orcid.org/0000-0001-7055-9932>
Янг Ын-Ёнг* – PhD (сельское хозяйство), Руководитель проекта, Отдел овощных культур, yangyang2@korea.kr
Чо Мён Чоел – PhD (сельское хозяйство), зав. лабораторией, Отдел овощных культур, chomc@korea.kr, <https://orcid.org/0000-0002-8321-4826>
Жеонг Хёо Бонг – старший научный сотрудник, Отдел овощных культур, bong9846@korea.kr, <https://orcid.org/0000-0003-0178-6765>

About the authors:

Sherzod Nigmatullayevich Rajametov – PhD (Agriculture), Post Doctoral Researcher, sherzod_2004@list.ru, <https://orcid.org/0000-0001-7055-9932>
Eun-Young Yang – Project Manager, yangyang2@korea.kr
Myeong-Cheoul Cho – PhD (Agriculture), Head of Laboratory, chomc@korea.kr, <https://orcid.org/0000-0002-8321-4826>
Hyo-Bong Jeong – Senior Researcher, bong9846@korea.kr, <https://orcid.org/0000-0003-0178-6765>

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