Supplementary calcium can help enhance photochemical activity in peanuts (*Arachis hypogaea*) leaves under low night temperature stress

Qiaobo Song ¹ , Qingwen Shi ¹ , Chunming Bai ² , Huixin Wang ^{1,2} , Di Wu ¹ , Qiping Dong ¹ , Xin Cheng ¹ , Yifei Liu ^{Corresp., 1} , Xiaori Han ^{Corresp., 1}

¹ College of Land and Environment, National Engineering Laboratory for Efficient Utilization of Soil and Fertilizer Resources, Key Laboratory of Protected Horticulture of Ministry of Education, Shenyang Agricultural University, Shenyang, China

² Liaoning Academy of Agricultural Sciences, Shenyang, China

Corresponding Authors: Yifei Liu, Xiaori Han Email address: liuyifei@syau.edu.cn, hanxiaori@163.com

Exogenous calcium is able to maintain photosynthesis level under low night temperature (LNT) stress. Nevertheless, the mechanism for supplementary calcium to mitigate photosynthesis barriers under LNT has not been as clear as expected so far. This study mainly covered the response rules to Ca²⁺ and Ca²⁺ inhibitors for Photosystem II (PSII) photoinhibition, photochemical activity and allocation of absorbed light in leaves of peanut seedlings under low night temperature stress and during their recovery process. As the results indicated, low night temperature stress boosted excitation energy at PSII reaction centers of peanut leaves, and inhibited electron transfer, leading to imbalanced excitation energy distribution with lower photochemical efficiency between these two photosystems. The ratio of antenna heat dissipation increased, while the ratio assigned to photochemical reaction reduced in the process of light absorption, so photosynthetic efficiency declined. Foliar spray of exogenous calcium ameliorates the imbalanced excitation energy between Photosystem II(PSII) and Photosystem I (PSI), increasing electron transfer rate (ETR) and efficiency of light energy conversion at PSII reaction centers (Fv/Fm). More light energy is used for photosynthesis, thus promoting the growth of peanut seedlings. Supplementary calcium available helped to adjust PSII activity via increasing electron transfer rate, more excitation energy was transported to PSI, and the damage of PSII reaction center caused by excess excitation energy reduced. The increase of active reaction centers has enhanced the utilization efficiency of light energy.

- **Supplementary calcium can help enhance photochemical**
- 2 activity in peanuts (Arachis hypogaea) leaves under low

3 night temperature stress

- 4 Qiaobo Song¹, Qingwen Shi¹, Chunming Bai², Huixin Wang^{1, 2}, Di Wu¹, Qiping Dong¹, Xin
- 5 Cheng¹, Yifei Liu¹, Xiaori Han¹
- ⁶ ¹College of Land and Environment, National Engineering Laboratory for Efficient Utilization of
- 7 Soil and Fertilizer Resources, Key Laboratory of Protected Horticulture of Ministry of Education,
- 8 Shenyang Agricultural University, Shenyang, China
- 9 ²Liaoning Academy of Agricultural Sciences, Shenyang, China
- 10 Corresponding Author:
- 11 Yifei Liu
- 12 liuyifei@syau.edu.cn
- 13 Xiaori Han
- 14 <u>hanxiaori@163.com</u>
- 15

16 Abstract

- 17 Exogenous calcium is able to maintain photosynthesis level under low night temperature (LNT)
- 18 stress. Nevertheless, the mechanism for supplementary calcium to mitigate photosynthesis
- 19 barriers under LNT has not been as clear as expected so far. This study mainly covered the
- 20 response rules to Ca²⁺ and Ca²⁺ inhibitors for Photosystem II (PSII) photoinhibition,
- 21 photochemical activity and allocation of absorbed light in leaves of peanut seedlings under low
- 22 night temperature stress and during their recovery process. As the results indicated, low night
- 23 temperature stress boosted excitation energy at PSII reaction centers of peanut leaves, and
- 24 inhibited electron transfer, leading to imbalanced excitation energy distribution with lower
- 25 photochemical efficiency between these two photosystems. The ratio of antenna heat dissipation
- 26 increased, while the ratio assigned to photochemical reaction reduced in the process of light
- absorption, so photosynthetic efficiency declined. Foliar spray of exogenous calcium ameliorates
- the imbalanced excitation energy between Photosystem II(PSII) and Photosystem I (PSI),
- 29 increasing electron transfer rate (ETR) and efficiency of light energy conversion at PSII reaction
- 30 centers (Fv/Fm). More light energy is used for photosynthesis, thus promoting the growth of
- 31 peanut seedlings. Supplementary calcium available helped to adjust PSII activity via increasing
- 32 electron transfer rate, more excitation energy was transported to PSI, and the damage of PSII
- 33 reaction center caused by excess excitation energy reduced. The increase of active reaction
- 34 centers has enhanced the utilization efficiency of light energy.

35 Introduction

- 36 Peanuts (Arachis hypogaea) are not only regarded as major oil crops and cash crops worldwide,
- 37 but also as major food protein source and edible vegetable oil source, so they play a decisive part
- in edible oil safety in China (Fenget al., 2018; Mukesh et al., 2011; Kong et al., 2014). Low
- 39 temperature is one of the environmental factors as restrictive to the geographic distribution and
- 40 production for peanuts. Chilling damage prevails in peanut production for temperate regions in
- 41 the whole world, particularly in the Chinese northern regions with high latitude, or in the
- 42 southwestern regions with high altitude. Peanuts under low temperature stress often experience
- 43 slow growth and seedling missing even upon recovery, so this shall severely impact peanut yield
- 44 and quality formation (Bagnall, King&Farquhar, 1988; Liu et al., 2011; Prasad et al., 2003; Qin
- 45 et al., 2011; Bhatnagarmathur et al., 2009; Kalisz et al., 2015). Low night temperature exerts its
- 46 impacts to physiological metabolism for thermophilous plants, since photosynthesis, as the basis
- 47 for plant growth and development, is one of the most sensitive processes to low night
- 48 temperature stress (Damian&Donald, 2001; Liu, Qi & Li, 2012; Qin et al., 2011; Yu et al., 2002).
- 49 Therefore, the necessity to improve photosynthetic capacity for peanuts after low night
- temperature stress is under urgent consideration in terms of both theoretical research andproduction practice.
- 52 Ca^{2+} , one of the essential elements for plants, serves not only as structure substance in cells,
- 53 but also as the second messenger to adjust the response process of plants to environmental
- changes (Ding et al., 2018; Nash, Miyao&Murata, 1985; Patrick et al., 2018), allowing plants to
- enhance their cold resistance (Liu et al., 2013; Biswal et al., 2011). As some early studies have
- 56 confirmed, calcium is most regulative to tomato photosynthate production and accumulation, and

- 57 plants with calcium applied enjoy better cold resistance and more yields (Zhang et al., 2014).
- 58 Changes of these environmental factors lead to increased activity for free Ca²⁺ in cytoplasm, and
- 59 then activity changes emerge for in vivo protein kinase as a result, hence inducing
- 60 the related gene expression, or regulating related enzymes activity (Cheng et al., 2002). The early
- 61 study by our research group has confirmed that foliar spray of exogenous calcium helped to
- 62 improve peanut cold resistance remarkably. Nevertheless, the mechanism for calcium to mitigate
- 63 low night temperature photosynthesis barriers has not been as clear as expected so far.
- 64 Chlorophyll fluorescent parameters are deemed as important indicators to assess if
- 65 photosynthetic apparatus of plants is damaged, and then these parameters are applied to reflect
- 66 the damage severity for plants under adverse stress. Both chlorophyll fluorescence in the plant
- 67 leaves and photosystems in photosynthesis are highly related with those responses, such as light
- energy absorption, transfer, dissipation and allocation, so influence from these different factors
- 69 on photosynthesis shall be represented via changes of chlorophyll fluorescent parameters
- 70 (Piñol&Simón, 2009). In this experiment, further study was carried out with the help of changes
- of chlorophyll fluorescent parameters concerning the response rules to Ca^{2+} and Ca^{2+} inhibitors
- 72 for PSII photoinhibition, photochemical activity and allocation of absorbed light in peanut
- 73 seedling leaves.
- 74 Materials & methods
- 75 Plant material, experimental design and treatment
- 76 This experiment was performed from 2016 to 2017 at the plant nutrition research center of
- 77 Chinese national technical system for peanut industry in Shenyang Agricultural University. The
- 78 experimental cultivar was "Fenghua 1" (which was a famous cultivar in the main peanuts
- 79 cultivating region). During this experiment, each sample was all under soil culture in a pot (a
- 80 plastic one for culture with bottom hole $R^{H=20*26}$ cm), one plant in each pot with uniform
- 81 watering and fertilizing to ensure consistency.
- 82 Then 15 pots of peanut seedlings in uniform style were selected and placed into the artificial
- so climate chamber (CONVIRON, Canada), with daytime temperature 25°C, and night temperature
- ⁸⁴ 20°C. Those peanut seedlings, upon 3 days for adaption, were divided into such five groups as
- 85 CK, LNT, LNT+Ca, LNT+EGTA and LNT+TFP. Ca²⁺ and its inhibitors with optimal
- 86 concentration for regulation, which were screened in early experiments, were sprayed evenly
- 87 onto these leaves by moisture sprayers two days before low night temperature stress (once
- respectively at 16:00 each day and twice in all). Treatments for each sample were presented as
- 89 the following: CK (optimal night temperature 20°C/optimal daytime temperature 25°C+distilled
- 90 water sprayed), LNT (low night temperature 10 °C/optimal daytime temperature 25°C+distilled
- 91 water sprayed), LNT+Ca (low night temperature 10 °C/optimal daytime temperature
- 92 25°C+15mmol•L⁻¹CaCl₂), LNT+EGTA (low night temperature 10 °C/optimal daytime

Peer Preprints

93	temperature 25 $^{\circ}C$ +5mmol•L ⁻¹ EGTA) and LNT+TFP (low night temperature 10 $^{\circ}C$ /optimal
94	daytime temperature $25^{\circ}C+0.5$ mmol·L ⁻¹ TFP). Group CK was defined as the normal temperature
95	control one under treatment at night temperature 20°C, while the other groups were under
96	treatment at low night temperature for 6 days. Later on, recovery treatment at optimal night
97	temperature 20 $^{\circ}$ C/optimal daytime temperature 25 $^{\circ}$ C was conducted for 6 days, 12 days in all.
98 99 100	Indicator determination and sampling were performed on treatment day 1, 3, 5, 7, 9 and 11 separately, and plant height was measured accordingly. Single plant measuring and sampling were performed and repeated for three times. Daily temperature treatment method: cooling from
101	19:00, up to night temperature at 21:00 (namely 20 $^\circ$ C and 10 $^\circ$ C), then warming up from 9:00 the
102	next morning, up to the defined temperature 25°C at 11:00 for each sample with their exposure to
103	light (9:00-21:00), illumination intensity 600 μ mol•m ⁻² •s ⁻¹ . The CO ₂ concentration inside the
104	culture room was 400 μ mol•mol ⁻¹ , humidity 60% equally for day and night.
105	Determination of chlorophyll fluorescent parameters and fluorescence in-situ imaging
106	PAM chlorophyll fluorometer of Dual-PAM-100 (Heinz Walz GmbH, Germany) was used to
107	determine chlorophyll fluorescent parameters.
108	The major parameters are listed as: Fv/Fm, efficiency of light energy conversion at PSII
109	reaction centers, for which the more this value declines, the more damage PSII will suffer (Ort,
110	Yocum&Heichel, 1996); qP, the photochemical quenching coefficient, which is reflective of
111	photosynthetic activity magnitude (Cao, 1990), PSII reaction center openness magnitude (Ruban,
112	Johnson&Duffy, 2012), together with PSII electron transport activity magnitude (Xu et al., 2008);
113	NPQ, non-photochemical quenching coefficient, which is of a self-protective mechanism for
114	photosynthetic system in a way (Ogweno et al., 2008); PSII or Y(II), actual photochemical
115	efficiency; ETR, electron transport rate, concerning the transport efficient for photosynthetic
116	quantum (Bondarava, Beyer&Kriegerliszkay, 2005); 1-qP, PSII excitation pressure,
117	representative of the ratio for PSII reaction centers in a closure state (Bilger& Schreiber, 1986);
118	α , PSI excitation energy; β , PSII excitation energy; β/α -1, unbalanced coefficient for excitation
119	energy between PSI and PSII; P, the energy for photochemical reaction which is supplied by
120	PSII antenna pigment molecules from their absorbed light energy; D, heat dissipation; E, excess
121	light energy from reaction centers.
122	IMAGING-PAM (Heinz Walz GmbH, Germany), the PAM chlorophyll fluorometer was
123	employed in our experiment to depict the fluorescence imaging pictures of peanut seedling
124	leaves under Ca ²⁺ and Ca ²⁺ inhibitors (EGTA, TFP). Then image color shades were referred to
125	determine chlorophyll fluorescence magnitude, based on which to estimate impacts from Ca ²⁺
126	(CaCl ₂) and Ca ²⁺ inhibitors (EGTA, TFP) on PSII photochemical activity of peanut leaves under

127 low night temperature and during their recovery process.

128 Statistical analysis

- 129 Excel 2007 and SPSS software (Statistical Product and Service Solutions) were adopted in the
- 130 statistical analysis to analyze experiment statistics and provide graphics.
- 131 Results
- 132 Effects of Ca²⁺ and Ca²⁺ inhibitors on peanut growth under low night temperature stress
- 133 Figure 1 and Fig. 2 showed the plant height contrast of peanut seedlings under different
- 134 treatments after six days of LNT stress. As it demonstrated, all these four groups of peanut
- 135 seedlings under LNT treatment grew more slowly than those under normal temperature (CK),
- 136 while among these four groups of peanut seedlings under LNT stress, one group of plants
- 137 sprayed with CaCl₂ grew significantly better than those with the other treatments, and the height
- 138 was close to that of peanut seedlings under normal temperature. Whereas for plants sprayed with
- 139 EGTA or TFP, they were under much severer LNT stress, particularly for peanut seedlings
- 140 sprayed with TFP, for they grew flaccidly under the severest stress.
- 141 Effects of Ca²⁺ and Ca²⁺ inhibitors on PSII photoinhibition and photochemical activity for

142 peanut leaves under low night temperature and during their recovery process

- 143 As Fig. 3 indicated, the maximum photochemical efficiency for peanut seedling leaves impacted
- 144 by LNT, Fv/Fm declined markedly during LNT stress period. Though Fv/Fm of peanut seedling
- leaves sprayed with CaCl₂ was not up to that of CK, it was still higher than that under LNT
- 146 treatment. Fv/Fm of peanut seedlings sprayed with EGTA or TFP were lower than that under
- 147 LNT treatment, but Fv/Fm of peanut seedlings sprayed with TFP declined much more obviously.
- 148 During normal night temperature recovery period, all these peanut seedlings under LNT
- 149 treatment demonstrated a rise to different extent in terms of their photosynthetic activity. Those
- plants sprayed with calcium enjoyed the best recovery, basically up to CK level; plants under
- 151 LNT treatment enjoyed an impressive rise in photosynthetic activity; plants sprayed with EGTA
- 152 or TFP enjoyed a rise in photosynthetic activity, but not so conspicuous when compared with
- 153 their initial values.
- 154 With Fv/Fm changes, Φ PSII, (the actual photochemical efficiency of PSII), and qP (the
- 155 photochemical quenching coefficient), along with ETR (electron transport rate), all displayed the
- same variation trends (as shown in Fig. 4A, 4B and 4C). Nevertheless, NPQ, non-photochemical
- 157 quenching coefficient, displayed a contrary variation trend (as shown in Fig.4D).

158 Effects of Ca²⁺ and Ca²⁺ inhibitors on allocation of absorbed light for peanut leaves under

- 159 low night temperature and during their recovery process
- 160 As indicated in Fig.5A, 5B and 5C, both PSII excitation pressure and its allocation coefficient
- 161 under LNT stress increased remarkably, while α, PSI excitation energy allocation coefficient,
- 162 decreased distinctly (as shown in Fig.5C), so excitation energy balance between PSII and PSI
- 163 was disturbed. During the whole process of LNT and normal night temperature recovery, α of
- 164 peanut seedlings under LNT treatment was lower than that of CK, with β higher than that of CK.
- As indicated in Fig.5D, such imbalanced allocation was perceived the most evidently at 5d
- 166 during this experiment. 1-qP, β and β/α -1 all declined clearly at 11 d when compared with those
- 167 at 5 d, but slightly higher than those during preliminary experiment period. Imbalance in
- 168 excitation energy allocation was mitigated greatly for these plants when CaCl₂ was sprayed, but

severer imbalance in excitation energy allocation for plants emerged when Ca²⁺ inhibitors were 169 sprayed. 170

- As indicated in Fig.6, during the whole LNT treatment and normal night temperature 171
- recovery process, P-value assumed the trend of early decrease with later increase. From 1-5 d, 172
- with the extension of LNT stress duration, P-value under LNT treatment declined constantly, 173
- obviously lower than CK level at 5d in particular. However, for plants sprayed with CaCl₂ on 174
- their leaves, P-value at 1 d was on a visible rise, higher than that of CK, and then on a fall 175
- somewhat as the stress duration extended, but such a value was still higher than that of plants 176
- under LNT treatment. For peanut seedlings spraved with EGTA or TFP, P-value dropped 177
- dramatically, especially for those spraved with TFP. During recovery process at 7-11 d, 178
- photosynthetic activity for all the plants under LNT treatments improved equally, but P-value for 179
- plants sprayed with Ca²⁺ inhibitors was under slower recovery with a lower level. Absorbed light 180
- by PSII antenna pigments in leaves was mainly consumed during photochemical reaction 181
- transduction, and then the rest was consumed in the form of heat dissipation (D) and excess light 182
- energy (E) at reaction centers. As indicated in Fig.6, CK, LNT and LNT+Ca under LNT stress 183
- did not display any regular variations in a distinctive way. However, for plants under 184
- LNT+EGTA or LNT+TFP treatment, their heat dissipation portion increased remarkably-for 185
- 186 12.1% concerning plants sprayed with EGTA at 5d under LNT treatment, and for 10.6%
- concerning plants sprayed with TFP at 5d under LNT treatment. Accordingly, the excess light 187
- energy at reaction centers for these two groups of plants increased for 7.57% and 12.09% 188
- 189 respectively.
- Effects of Ca²⁺ and Ca²⁺ inhibitors on information acquisition concerning fluorescence in-190
- 191 situ imaging for peanut leaves under low night temperature stress and during their

192 recovery process

- Figure 7 presents the contrast pictures of fluorescence in-situ imaging for each parameter of 193
- plants at 5d under LNT stress and at 5d under normal temperature recovery. As illustrated, 194
- Fv/Fm changed the most distinctively, while photosynthetic capacity for peanut seedlings 195
- sprayed with CaCl₂ on their leaves under LNT stress was basically the same with that of CK. 196
- 197 However, for plants sprayed with EGTA or TFP on their leaves, fluorescence imaging color
- 198 displayed obvious changes, especially the group sprayed with TFP, whose color was light blue.
- 199 Photosynthetic activity for this group steadily declined even during normal night temperature
- recovery, and the color turned green in the end. Y(NPQ), the quantum yield as regulatory to 200
- energy dissipation, is a critical indicator for photoprotection, while Y(NO), the quantum yield as 201 non-regulatory to energy dissipation, is a critical indicator for photodamage. As known from the 202
- figure, plants under LNT treatment were impacted by low night temperature accordingly, and
- 203
- their Y(NPQ) value rose slightly, but Y(NPQ) value for plants under LNT+Ca, LNT+EGTA or 204
- LNT+TFP treatment rose significantly, and the last two treatment groups in particular, though 205 Y(NO) showed no obvious changes at this moment. However, Y(NPQ) for leaves under LNT 206
- treatment rose somewhat upon normal night temperature recovery, and Y(NPO) for leaves under 207
- LNT+Ca treatment declined remarkably. On the contrary, Y(NO) for leaves under TFP 208
- application rose remarkably. Low night temperature led to low quantum yields of peanut leaves, 209

210 while foliar spray of calcium was able to improve quantum yield, which was reduced by Ca^{2+}

211 inhibitors in turn.

212 Discussion

213 Low night temperature stress is a common abiotic stress during crop growth and development

- 214 process. Low temperature with its wider range of influence and obvious effects proves to be a 215 fatal threat to crop high yield (Berry&Björkman, 1980). Currently, many reports home and
- fatal threat to crop high yield (Berry&Björkman, 1980). Currently, many reports home and abroad have indicated that low temperature is able to restrict chlorophyll formation by affecting
- photosynthetic apparatus activity of crop leaves, by affecting heat-sensitive ETR in PSII, PQ
- piolosymetric apparents derivity of elep feaves, by affecting flear sensitive Diff in PSI, i Q 218 pool redox state, ETR in PSI (Liu et al., 2012) or by affecting plant physical characteristics,
- stomatal movement magnitude, for instance (Mathur, Agrawal&Jajoo, 2014). Then normal
- 220 photosynthesis shall be inhibited further, which leads to restricted photosynthate accumulation,
- 221 or even serious crop failure.
- In this experiment, regulation by calcium ions to peanut seedling cold adaption was firstly
- studied, and then with focus on PSII, our study was carried out on how the calcium ions were able to mitigate photosynthetic barriers under LNT stress. As this study has confirmed, most
- 224 able to initigate photosynthetic barriers under LNT stress. As this study has committed, most 225 measurement indicators represented some specific regularity under LNT stress and during
- normal night temperature recovery process. Simple analysis for peanut seedlings heights and
- 227 Fv/Fm, the maximum photochemical efficiency, were conducted and showed that LNT stress
- forced peanut seedlings heights to be lower than that of CK, and their Fv/Fm value declined
- significantly. Thus, it was preliminarily concluded that LNT was able to affect photosynthetic
- 230 capacity of peanut seedling leaves and restrict carbohydrate accumulation, so plant growth would
- be limited. For plants under foliar spray with CaCl₂, their photosynthetic efficiency recovered
- distinctively and this help to slow down plant height declination accordingly, while heights of those peanut seedlings under foliar spray with Ca^{2+} inhibitors declined further, and then their
- photosynthetic efficiency declined at different levels, too. It was much illustrative that Ca^{2+}
- 235 played an important part in the trophic signal transduction to mitigate photosynthetic barriers for
- 236 peanut plants under LNT. Ca^{2+} inhibitors, on the contrary, inhibited peanut seedlings growth and
- 237 accelerated photosynthetic efficiency reduction further. When stress was removed, peanut
- 238 seedling leaves under different treatments displayed recovery to various extent, so it meant that
- 239 PSII was able to assume self-healing somewhat to enhance plant photosynthetic activity.
- 240 Moreover, exogenous source of calcium as sprayed was able to help PSII improve its repair
- 241 competence, allowing it to recover up to CK level. As for those under LNT did not recover up to
- CK level, it was just because PSII was damaged under LNT stress, and Ca²⁺ inhibitors
 intensified such damage further.

Under constant impacts from LNT stress, energy absorbed by PSII intended for photochemical reaction decreased gradually, which caused portion in the open state at PSII reaction centers to decrease accordingly, in spite of the same illumination intensity and the same number of electrons at electron donor side. Since the portion in the open state at PSII reaction centers fell down, PQ number under normal operation fell down as well. Electron accumulation at PQ aggravated and then restricted ETR in a further way. Moreover, the actual light energy conversion efficiency for PSII was also affected, as illustrated in Fig.4D, so with the purpose of

self-protection, PSII would release excess light energy by way of heat dissipation, namely non-251 photochemical quenching (NPQ). Calcium ions were able to help PSII apply more light energy 252 to photochemical reaction, so plants under LNT stress, when spraved with CaCl₂ displayed a 253 mild decline apparently in terms of photosynthetic activities, actual quantum yield and ETR. For 254 255 plants spraved with EGTA, though their calcium ions extracellular were chelated, which inhibited absorption of calcium ions extracellular for these peanut seedlings, a specific number of 256 calcium ions intracellular still remained there. Yet, for plants sprayed with TFP, their calcium 257 ions united with calmodulin and were out of activity, so signal transduction function for the 258 whole calcium signal system was blocked. As a result, for peanut seedlings spraved with TFP, 259 their PSII reaction center activity became weaker than that of peanut seedlings spraved with 260 261 EGTA. As indicated in Fig.4, during the whole LNT and recovery period, photosynthetic activity of plants under LNT treatment assumed the trend of early decrease with later increase, so it 262 meant that PSII reaction centers were able to assume self-healing somewhat. However, due to 263 low temperature damage, photochemical activity for peanut seedlings was not up to CK level, 264 even if recovery was available at normal night temperature. qP and NPO for plants sprayed with 265 CaCl₂ boosted visibly, an illustrative fact that calcium ions not only helped to enhance reaction 266 center activity and improve plant photosynthetic capacity, but also helped to accelerate heat 267 268 dissipation dynamics, so that plants could dissipate their excess light energy as heat in a faster and better way to mitigate photoinhibition impacts on themselves. For plants treated with EGTA 269 or TFP, since there was a lack of calcium ions, though their photochemical activity assumed an 270 uptrend under recovery at normal night temperature, such elevated values were still lower 271 ultimately as compared with those initial ones. This suggested that recovery at normal night 272 temperature was available to mitigate inhibition effects from Ca²⁺ inhibitors, but PSII reaction 273 centers were damaged irreversibly under LNT at early days. 274 1-qP, an essential parameter to evaluate if excitation energy captured by photosystem is to 275

keep the balance with the energy applied (Havaux, Strasser&Greppin, 1991). LNT stress forced 276 1-qP, β and β/α -1 to rise, α to fall down at the same time, and this meant that more light energy 277 flew from PSI to PSII, so PSII excitation pressure built up with more O_A in reduction state, 278 279 which shall hamper electron transport and reduce photosynthetic rate, inflicting damage to PSII 280 accordingly. Excitation energy distribution among photochemical reaction (P), nonphotochemical dissipation at reaction centers (E) and antenna heat dissipation (D) was affected at 281 different levels. Under LNT stress, light energy absorbed by peanut seedling leaves for 282 photochemical reaction (P) reduced remarkably, while portions used for antenna heat dissipation 283 (D) rose distinctively, with qP down and NPO up correspondingly. This indicated that light 284 energy, normally intended for photochemical reaction, had to be dissipated in the form of 285 thermal energy to tackle with the excess energy under LNT stress, since PSII was damaged and 286 some of the reaction centers closed. However, from these two groups of peanut seedling leaves 287 sprayed with Ca²⁺ inhibitors, we observed that not only D-value rose significantly, but E-value 288 rose significantly as well. This indicated that Ca²⁺ inhibitors made PSII unable to converse all the 289 excess energy, if only by way of heat dissipation. E-value increase shall boost PSII excitation 290 pressure, causing triplet chlorophyll (³Chl) to decrease and singlet chlorophyll (¹Chl) to increase. 291

- 292 Singlet chlorophyll shall transfer energy to O_2 , then producing singlet oxygen (1O_2) of strong
- 293 oxidizability (Asada, 1999), and this shall do great damage to the photosynthetic membrane
- system of chloroplast (Diao et al., 2014). P-value rose rapidly for peanut seedling leaves with
- exogenous calcium application (E-value variations were not remarkably and D-value decreased),
- which all mitigated adverse impacts from LNT on PSII and enhanced leaves photosynthetic
- 297 capacity.

298 Conclusions

- 299 Low night temperature stress decreased the photosynthetic activity of the peanut seedlings. The
- 300 excitation energy between PSII and PSI was unbalanced, and then the electron transfer rate and
- 301 the energy for photochemical reaction decreased significantly, but non-photochemical quenching
- 302 coefficient increase significantly. Supplementary calcium can help enhance photochemical
- 303 activity in peanuts leaves under low night temperature stress. Exogenous CaCl₂ protected the
- 304 PSII from LNT stress via ameliorating excitation energy distribution imbalance and increasing
- 305 the electron transfer rate of PSII, which improved the use of excitation energy and the
- 306 accumulation of photosynthate. In contrast, plants that were sprayed calcium inhibitors (EGTA,
- 307 ethylene glycol tetraacetic acid and TFP, trifluonerazine) are the opposite. This is an illustrative
- fact that as the receptor of Ca^{2+} , calmodulin (CAM) played an important role for Ca^{2+} in the
- 309 thermal signal transduction to alleviate peanut photosynthesis barriers under low night
- 310 temperature stress.

311 Acknowledgements

- 312 This work was supported by the Natural Science Foundation of China (31772391, 31301842),
- the Sheng Jing Talents Project of Shenyang City (RC170338), China Scholarship Council
- Project (CSC 201708210143), Program of Liaoning Excellent Talents in Universities
- 315 (LJQ2015100) and National Peanut Research System (CARS-14).

316 **References**

- 317 Asada K.1999. The water-water cycle in chloroplasts: scavenging of active oxygens and
- dissipation of excess photons. *Annual Review of Plant Biology* 50:601-639.
- 319 DOI:10.1146/annurev.arplant.50.1.601.
- Bagnall DJ, King RW, Farquhar GD. 1988. Temperature-dependent feedback inhibition of
 photosynthesis in peanut. *Planta* 175:348-354. DOI:10.1007/BF00396340.
- 322 Berry J, Björkman O. 1980. Photosynthetic response and adaptation to temperature in higher
- 323 plants. Annual Review of Plant Biology 31:491-543.
- 324 DOI:10.1146/annurev.pp.31.060180.002423.
- 325 Bhatnagarmathur P, Devi MJ, Vadez V, Sharma KK. 2009. Differential antioxidative responses
- in transgenic peanut bear on relationship to their superior transpiration efficiency under
- drought stress. *Journal of Plant Physiology* 166:1207-1217.
- 328 DOI:10.1016/j.jplph.2009.01.001. Epub 2009 Feb 7.
- 329 Bilger W, Schreiber U. 1986. Energy-dependent quenching of dark-level chlorophyll
- fluorescence in intact leaves. *Photosynthesis Research* 10:303-308.
- 331 DOI:10.1007/BF00118295.

Biswal B, Joshi PN, Raval MK, Biswal UC. 2011. Photosynthesis, a global sensor of 332 environmental stress in green plants: stress signalling and adaptation. Current Science 333 101:47-56. DOI://www.ias.ac.in/currsci/10jul2011/47.pdf. 334 Bondarava N, Beyer P, Kriegerliszkay A. 2005. Function of the 23 kDa extrinsic protein of 335 photosystem II as a manganese binding protein and its role in photoactivation. Biochimica et 336 Biophysica Acta-Bioenergetics 1708:63-70. DOI:10.1016/j.bbabio.2005.01.005. 337 Cao J, Govindjee. 1990. Chlorophyll a fluorescence transient as an indicator of active and 338 inactive Photosystem II in thylakoid membranes. Biochimica et Biophysica Acta-339 Bioenergetics 1015:180-188. DOI:10.1016/0005-2728(90)90018-Y. 340 Cheng SH, Willmann MR, Chen HC, Sheen J. 2002. Calcium signaling through protein kinases. 341 342 The Arabidopsis calcium-dependent protein kinase gene family. Plant Physiology 129:469-485. DOI:10.1104/pp.005645. 343 Damian JA, Donald RO. 2001. Impact of chilling temperatures on photosynthesis in warm-344 climate plants. Trends in Plant Science 6:36-42. DOI:10.1016/S1360-1385(00)01808-2. 345 Diao M, Ma L, Wang JW, Cui JX, Fu AF, Liu HY. 2014. Selenium promotes the growth and 346 photosynthesis of tomato seedlings under salt stress by enhancing chloroplast antioxidant 347 defense system. Journal of Plant Growth Regulation 33:671-682. DOI:10.1007/s00344-014-348 349 9416-2. Ding, W., Clode, P., Clements, J., Lambers, H. 2018. Sensitivity of different Lupinus species to 350 calcium under a low phosphorus supply. Plant, cell & environment. DOI:10.1111/pce.13179. 351 Feng Cui, Na Sui, Guangyou Duan, Yiyang Liu, Yan Han, Shanshan Liu, Shubo Wan, Guowei 352 Li. 2018. Identification of Metabolites and Transcripts Involved in Salt Stress and Recovery 353 354 in Peanut. Frontiers in Plant Science 9:217. DOI: 10.3389/fpls.2018.00217. 355 Havaux M, Strasser RJ, Greppin H. 1991. Effects of incident light intensity on the yield of steady-state chlorophyll fluorescence in intact leaves. An example of bioenergetic 356 homeostasis. Environmental & Experimental Botany 31:23-32. DOI:10.1016/0098-357 8472(91)90004-8. 358 Kalisz A, Sekara A, Grabowska A, Cebula S, Kunicki E. 2015. The effect of chilling stress at 359 360 transplant stage on broccoli development and yield with elements of modeling. Journal of 361 Plant Growth Regulation34:532-544. DOI:10.1007/s00344-015-9488-7. Kong J, Dong YJ, Xu LL, Liu SL, Bai XY. 2014. Effects of exogenous salicylic acid on 362 alleviating chlorosis induced by iron deficiency in peanut seedlings (Arachis hypogaea L.). 363 Journal of Plant Growth Regulation 33:715-729. DOI:10.1007/s00344-014-9418-0. 364 Liu YF, Han XR, Zhan XM, Yang JF, Wang YZ, Song QB, Chen X. 2013. Regulation of calcium 365 on peanut photosynthesis under low night temperature stress. Journal of Integrative 366 Agriculture 12:2172-2178. DOI:10.1016/S2095-3119(13)60411-6. 367 Liu YF, Li TL, Xu T, Qi MF, Xu CQ. 2011. Effect of low night temperature treatment and 368 recovery on photosynthesis and the allocation absorbed light energy in leaves. Journal of 369 Horticultural Science & Biotechnology 86:91-96. DOI:10.1080/14620316.2011.11512731. 370 Liu YF, Oi MF, Li TL. 2012. Photosynthesis, photoinhibition, and antioxidant system in tomato 371 leaves stressed by low night temperature and their subsequent recovery. Plant Science196:8-372

17. DOI:10.1016/j.plantsci.2012.07.005. 373 Mathur S, Agrawal D, Jajoo A. 2014. Photosynthesis: Response to high temperature stress. 374 Journal of Photochemistry & Photobiology B Biology 137:116-126. 375 DOI:10.1016/j.jphotobiol.2014.01.010. 376 Mukesh J, Bhuvan PP, Alice CH, Maria G. 2011. Calcium dependent protein kinase (CDPK) 377 expression during fruit development in cultivated peanut (Arachis hypogaea) under Ca²⁺-378 sufficient and -deficient growth regimens. Journal of Plant Physiology 168: 2272-2277. DOI: 379 10.1016/j.jplph.2011.07.005. 380 Nash D, Miyao M, Murata N. 1985. Heat inactivation of oxygen evolution in photosystem II 381 particles and its acceleration by chloride depletion and exogenous manganese. Biochimica et 382 383 Biophysica Acta-Bioenergetics 807:127-133. DOI:10.1016/0005-2728(85)90115-X. Ogweno JO, Song XS, Shi K, Hu WH, Mao WH, Zhou YH, Yu JQ. 2008. Brassinosteroids 384 alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency 385 and enhancing antioxidant systems in Lycopersicon esculentum. Journal of Plant Growth 386 Regulation 27:49-57. DOI:10.1007/s00344-007-9030-7. 387 Ort DR, Yocum CF, Heichel IF.1996. Oxygenic Photosynthesis: the light reactions. In: Ort DR, 388 Yocum CF, Heichel IF, ed. Oxygenic Photosynthesis: the Light Reactions. Kluwer Academic 389 390 Publishers, Netherlands, 1-9. Patrick E. Hayes, Peta L. Clode, Rafael S. Oliveira, Hans Lambers. 2018. Proteaceae from 391 phosphorus - impoverished habitats preferentially allocate phosphorus to photosynthetic 392 cells: An adaptation improving phosphorus - use efficiency. Plant Cell Environ 41:605-619. 393 394 DOI: 10.1111/pce.13124 Piñol R, Simón E. 2009. Effect of 24-epibrassinolide on chlorophyll fluorescence and 395 photosynthetic CO2 assimilation in Vicia faba plants treated with the photosynthesis-396 inhibiting herbicide terbutryn. Journal of Plant Growth Regulation 28:97-105. 397 DOI:10.1007/s00344-008-9077-0. 398 Prasad PVV, Boote KJ, Jr LHA, Thomas JMG. 2003. Super-optimal temperatures are 399 400 detrimental to peanut (Arachis hypogaea L.) reproductive processes and yield at both ambient and elevated carbon dioxide. Global Change Biology 9:1775-1787. 401 DOI:10.1046/j.1365-2486.2003.00708.x. 402 Qin H, Gu Q, Zhang JL, Sun L, Kuppu S, Zhang YZ, Burow M, Payton P, Blumwald E, Zhang 403 H. 2011. Regulated expression of an isopentenyltransferase gene (IPT) in peanut 404 significantly improves drought tolerance and increases yield under field conditions. Plant 405 Cell Physiology 52:1904-1914. DOI:10.1093/pcp/pcr125. 406 Qin LQ, Li L, Bi C, Zhang YL, Wan SB, Meng JJ, Meng QW, Li XG. 2011. Damaging 407 mechanisms of chilling-and salt stress to Arachis hypogaea L. leaves. Photosynthetica 49:37-408 42. DOI:10.1007/s11099-011-0005-3. 409 Ruban AV, Johnson MP, Duffy CDP. 2012. The photoprotective molecular switch in the 410 photosystemII antenna. Biochimica et Biophysica Acta-Bioenergetics 1817:167-181. 411

- 412 DOI:10.1016/j.bbabio.2011.04.007.
- 413 Xu ZZ, Zhou GS, Wang YL, Han GX, Li YJ. 2008. Changes in chlorophyll fluorescence in
- 414 maize plants with imposed rapid dehydration at different leaf ages. *Journal of Plant Growth* 415 *Regulation* 27:83-92. DOI:10.1007/s00344-007-9035-2.
- 416 Yu LH, Guo HL, Xu XD, Feng B. 2002. Effect of the photosynthesis inhibitor DCMU on
- 417 chlorophyll synthesis in heterotrophic cyanobacteria. *Acta Hydrobiologica Sinica* 1:102-104.
- 418 DOI://ssswxb.ihb.ac.cn/EN/Y2002/V/I1/102.
- 419 Zhang GX, Liu YF, Ni Y, Meng Z, Lu T, Li T. 2014. Exogenous calcium alleviates low night
- 420 temperature stress on the photosynthetic apparatus of tomato leaves. *PloS One* 9:1-12.
- 421 DOI:10.1371/journal.pone.0097322.

Plant height of peanut seedlings contrast under Ca²⁺ and Ca²⁺ inhibitors after low night temperature stress.

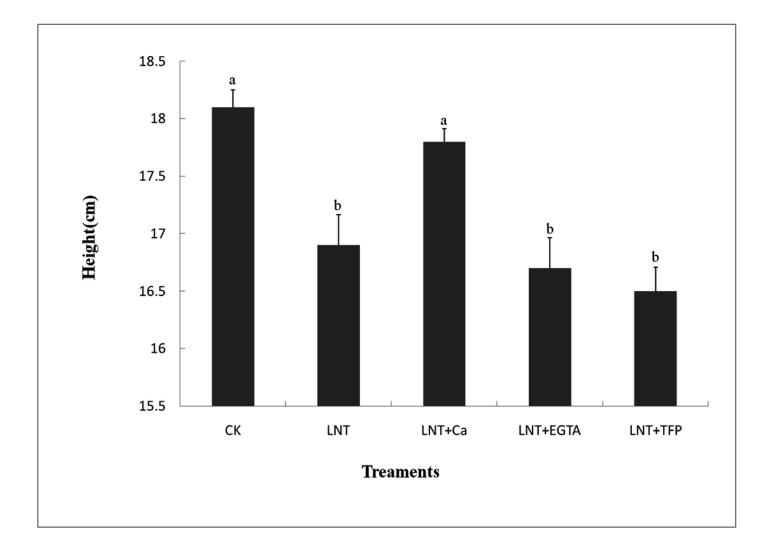
Fig 1 - Plant height of peanut seedlings contrast under Ca²⁺ and Ca²⁺ inhibitors (EGTA and TFP) after six days of low night temperature stress.

*Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.



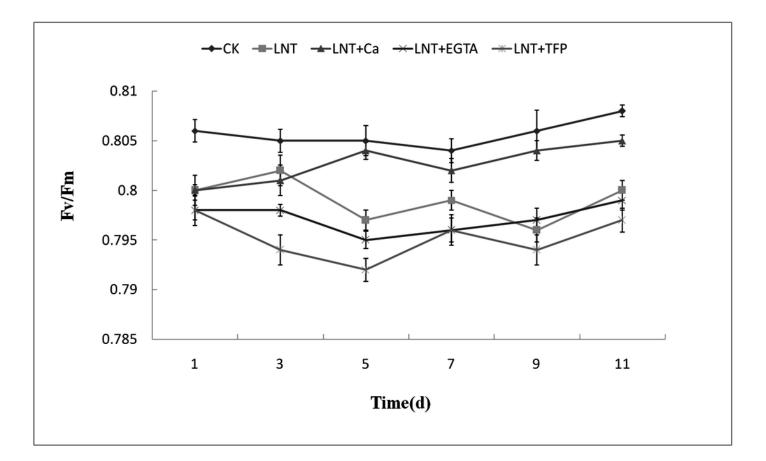
Plant height of peanut seedlings contrast under Ca²⁺ and Ca²⁺ inhibitors after low night temperature stress.

Fig 2 - Plant height of peanut seedlings contrast under Ca²⁺ and Ca²⁺ inhibitors (EGTA and TFP) after six days of low night temperature stress.



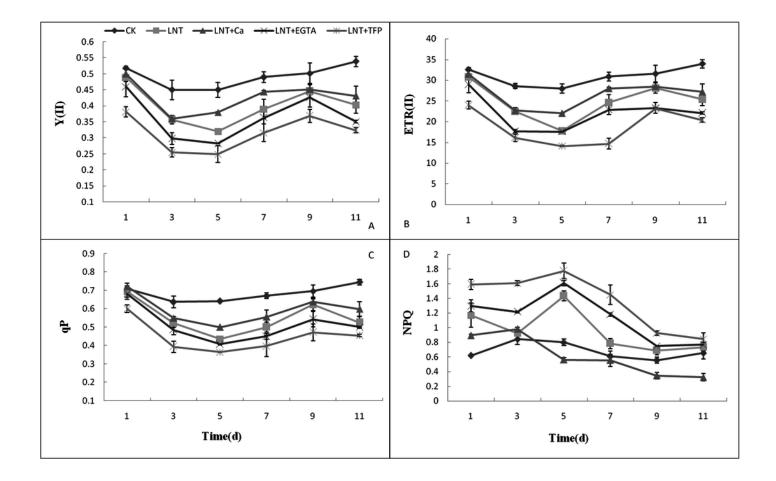
Effects of Ca²⁺ and Ca²⁺ inhibitors on Fv/Fm activity in leaves of peanut seedlings under low night temperature stress and during their recovery process.

Fig 3 - Effects of Ca²⁺ and Ca²⁺ inhibitors (EGTA and TFP) on Fv/Fm activity in leaves of peanut seedlings under low night temperature stress and during their recovery process.



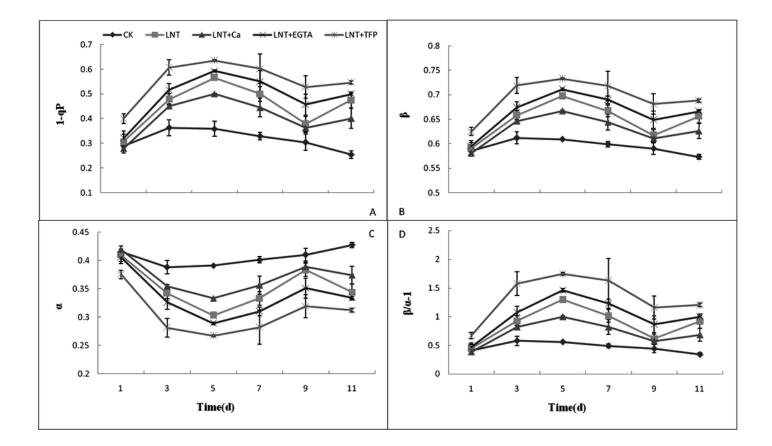
Effects of Ca^{2+} and Ca^{2+} inhibitors on ETR, φ PSII, qP, NPQ activity in leaves of peanut seedlings under LNT stress and during their recovery process.

Fig 4 - Effects of Ca²⁺ and Ca²⁺ inhibitors (EGTA and TFP) on ETR, φPSII, qP, NPQ activity in leaves of peanut seedlings under low night temperature stress and during their recovery process.



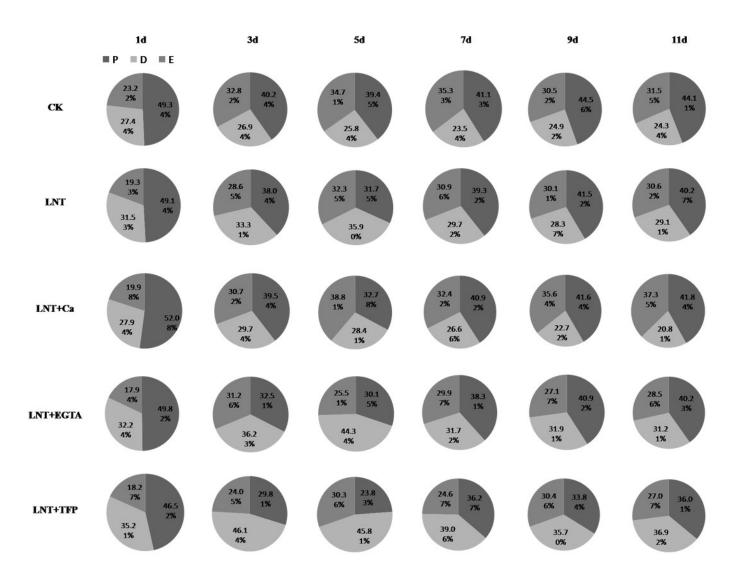
Effects of Ca²⁺ and Ca²⁺ inhibitors on1-qP, β , α and β / α -1 in leaves of peanut seedlings under LNT stress and during their recovery process.

Fig 5 - Effects of Ca²⁺ and Ca²⁺ inhibitors (EGTA and TFP) on1-qP, β , α and β / α -1 in leaves of peanut seedlings under low night temperature stress and during their recovery process.



Effects of Ca²⁺ and Ca²⁺ inhibitors on the allocation of absorbed light of peanut seedlings under LNT stress and during their recovery process.

Fig 6 - Effects of Ca²⁺ and Ca²⁺ inhibitors (EGTA and TFP) on the allocation of absorbed light of peanut seedlings under low night temperature stress and during their recovery process.



Effects of Ca²⁺ and Ca²⁺ inhibitors on fluorescence in-situ imaging pictures of Fv/Fm, Y(NPQ) and Y (NO) for peanut leaves under LNT stress and during their recovery process.

Fig 7 - Effects of Ca²⁺ and Ca²⁺ inhibitors (EGTA and TFP) on fluorescence in-situ imaging pictures of Fv/Fm, Y(NPQ) and Y (NO) for peanut leaves under low night temperature stress and during their recovery process.

