Brief Communication

Genetic manipulation of Soc1-like genes promotes photosynthesis in flowers and leaves and enhances plant tolerance to high temperature

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The rapid rise in mean global temperature as a result of global warming threatens plant productivity (Li et al., 2015). Chloroplasts and chloroplast proteins are associated with environmental stresses (Alexia et al., 2019; Hong et al., 2020). Many heat-shock proteins (HSPs) associate with chloroplast development and improve plant tolerance to heat stress at a high temperature (Shen et al., 2015; Zhong et al., 2013), whereas no gene is reported to promote chloroplast development and enhance tolerance to high temperature synchronously. The impact of high temperature on chloroplast is of particular significance since photosynthesis is often inhibited after other cell functions are impaired (Zhang et al., 2010). Thus, promoting chloroplast biogenesis and photosynthesis is a potential method to enhance heat tolerance of plants. We previously found that overexpression of SOC1 or SOC1-like genes in heat-stressed plants induces chloroplast biogenesis in petals (Wang et al., 2019). However, it is unknown whether the photosynthesis apparatus is impaired and whether the plant thermodotolerance is enhanced in transgenic plants. In our present study, the transplastomic (harbouring GFP reporter gene driven by psbA promoter of chloroplast), multigene transgenic tobacco (Fbp21 gene was introduced to the genome of the pure line of GFP transplastomic tobacco-labelling nFbp21*pGFP) and transgenic petunia-overexpressing FBP21 gene were produced by chloroplast and nuclear transformation. Additionally, the transgenic plants (Fbp21-labelling F21 and Fbp21*22-labelling F21_22 in this paper) harbouring SOC1-like genes and RNA-Seq data of petals, previously reported (Wang et al., 2019), were also integrated. Finally, a series of experiments related to RNA sequencing in leaves, biological and physiological, anatomical and phenotypic determination were undertaken.

When plants were grown at high temperature (40°C days/28°C nights), it showed that only nonphotosynthetic plastids containing plastoglobules were seen in pink petals of control tobacco plants. We observed morphologically normal chloroplasts in green petals of the SOC1-like gene transgenic tobacco plants (Figure 1a). Chloroplasts in green petals of nFbp21*pGFP transplastomic tobacco were observed to emit red and green fluorescence simultaneously at high temperature (Figure 1b). It indicates that chloroplast genes were expressing in these heat-stress-induced plastids in petals. Maximum photochemical efficiency values ($F_v/F_m$) determination also showed that photosynthesis took place in chloroplast-containing petals (Figure 1c and d). Most of the photosynthesis genes were dramatically up-regulated in chloroplast-containing green petals (Figure 1e). Immunoblot analysis also showed many photosynthesis-associated proteins were synthesized in green petals (Figure 1f).

Heat-resistant assay showed that the SOC1-like gene transgenic tobacco (F21 and F21_22) was substantially different from the control tobacco in their tolerance to prolonged extreme heat stress. For 2-week-old tobacco plants, more light yellow seedlings were seen in the wild-type tobacco than in transgenic lines after heat stress (Figure 1g). $F_v/F_m$ values in transgenic lines were also notably higher than that of wild type (Figure 1g and h). The lower electrolyte leakage (EL) (Figure 1i) and higher survival rate (Figure 1j) suggest that these 2-week-old transgenic tobacco had enhanced heat tolerance. Plants were grown at normal temperature, more chloroplasts in cells of leaves of the transgenic tobacco (including F21 or F21_22 tobacco) also observed in the nFbp21*pGFP transplastomic tobacco according to red and green fluorescence compared to GFP transplastomic tobacco (Figure 1k). These results suggest that overexpression of SOC1-like genes promote chloroplast biogenesis in transgenic leaves. When grown at high temperature, the leaf chloroplasts of the transgenic tobacco (also seen in nFbp21*pGFP) maintained normal appearance and orderly distribution and emitted more green fluorescence (Figure 1k). These observations indicate that the chloroplast genes can normally express at high temperature, whereas the chloroplasts in leaves of control tobacco became swollen, globular and irregular. It was consistent with what reported by Kwon and colleagues in GFP transplastomic tobacco (Kwon et al., 2013). The structural changes of chloroplasts and their scattered distribution in control tobacco (Figure 1k) suggest higher instability of varied cell membranes and cell damages by heat at high temperature.

The response to high temperature of 6-week-old tobacco plants was also markedly different between control and transgenic tobacco (Figure 1l). Many photosynthesis genes were dramatically up-regulated in leaves of transgenic plants growing at high temperature (Figure 1m). Immunoblot analysis showed that photosynthesis-associated proteins were accumulated in
Figure 1  Soc1-like genes promote photosynthesis and improve heat tolerance in plants. (a) Colour and anatomy of transgenic tobacco grown at higher temperature. (b) Red and green fluorescence in heat stress petals. (c-d) $F_{m}/F_{o}$ values in control and transgenic tobacco petals. (e) Heat map shows expression profiles of differently expressed genes (DEGs) associated with photosynthesis in green petals. (f) Immunoblot analyses of photosynthesis proteins (PSAD and LHCA1) in transgenic tobacco petals. (g) Phenotypes of 2-week-old transgenic tobacco suffering from 4 days of heat stress (45°C day/28°C night). (h-j) $F_{m}/F_{o}$ values (h), EL levels (i) and seedling survival rate (j)—scored to those recovered at 3 days (26°C day/22°C night)—of control and transgenic tobacco after heat stress. (k) Chloroplasts in leaves of the GFP and nFBP21* pGFP transgenic tobacco grown at normal and high temperature. (l) Phenotypes of 6-week-old transgenic tobacco after heat stress (9 h 45°C). (m) Heat map shows expression profiles of DEGs associated with photosynthesis in the leaves of transgenic tobacco plants grown at normal and high temperature. (n) Immunoblot analyses of photosynthesis proteins in tobacco leaves. (o) Leaf $F_{m}/F_{o}$ values of 6-week-old control and transgenic tobacco before and after stress. (p) Leaf $F_{m}$ values of wild type and transgenic tobacco at different times of heat stress. (q-r) Magnesium content (r) and EL levels (r) in leaves of 6-week-old control and transgenic tobacco. (s) Heat map shows expression profiles of DEGs encoding heat-shock proteins in leaves of transgenic tobacco grown at normal and high temperature. (t) Amino acid content of leaves in transgenic tobacco. (u) Phenotype of transgenic tobacco grown at high temperature. (v) Phenotypes of 2-week-old Fbp21 transgenic petunia acclimated for 5 days at high temperature (45°C/30°C). (w) Phenotypes of 6-week-old transgenic petunia before and after heat stress. (x) Light green petals in heat-treated Fbp21 transgenic petunia. Note: Error bars represent ± SE (n = 3). Asterisks indicate significant differences (*** P < 0.001, **** P < 0.0001).
transgenic plants (Figure 1n). Under continuous heat stress (45°C for 9 h), Fv/Fm values were also significantly higher in leaves of transgenic tobacco plants (Figure 1o). A time series of net photosynthetic rate (Pn) determination indicated that the leaf Pn rate of transgenic tobacco plants was higher than that of wild type (Figure 1p). Magnesium is part of the chlorophyll and essential for photosynthesis (Leonard, 1954), and higher magnesium content was also detected in leaves of F21 transgenic tobacco plants (Figure 1q). Taking together, these results suggested that SOC1-like gene transgenic tobacco plants possess enhanced photosynthetic capacity under heat stress conditions.

Leaf EL value of 6-week-old transgenic tobacco was lower than that of wild type after heat stress (Figure 1r). RNA-seq analysis showed that genes encoding heat-shock proteins were also greatly up-regulated in leaves of transgenic tobacco under heat stress (Figure 1s). GC-MS analysis showed the proline content was significantly higher in SOC1-like gene transgenic tobacco plants (Figure 1t). It is worth noticing that during budding phase, the transgenic tobacco plants were not impaired and flowered normally under heat stress (12-h light cycle for 1S days). In contrast, wild-type tobacco plants did not flower or flowered poorly under the same heat stress condition (Figure 1u).

Additionally, the transgenic petunia-overexpressing Fbp21 gene was also more heat tolerant at varied growth phases than wild-type petunia plants (Figure 1v and w). The highest survival rate (96.60%) was recorded for 2-week-old transgenic petunia after heat stress (Figure 1v). For 6-week-old petunia seedlings, none of the wild-type petunia seedlings survived after 3 days under high temperature (Figure 1w). Similar to tobacco-overexpressing SOC1-like genes, the transgenic petunia-overexpressing Fbp21 gene also produced light green petals under heat stress (Figure 1x).

In an earlier study, we reported for the first time that SOC1-like genes promote chloroplast biogenesis in heat-stressed petals (Wang et al., 2019). In the present study, we show that cell containing increased number of chloroplasts is observed more frequently in leaves of Soc1-like gene transgenic plants than in wild-type plants and that SOC1-like genes up-regulate photosynthesis and heat-shock-associated genes, improve plant photosynthesis and alleviate heat stress damage to the chloroplast. Our results demonstrated that the super plants having chloroplast-containing petals, higher chlorophyll contents, increasing photosynthesis and enhancing heat tolerance could be synchronously achieved by genetic engineering. We showed that plant flowers can perform photosynthesis to further improve carbon utilization efficiency under heat stress and that overexpression of SOC1-like genes reduce the deleterious effects of heat stress on chloroplast and enhance photosynthesis in plants. Our observation provides a novel insight into the crosstalk mechanism between high temperature, plant functional chloroplast biogenesis, plant photosynthesis and plant heat tolerance. Producing heat-tolerant plants will be of great ecological and economic significance under the increasing threat of global warming.

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Competing financial interests
The authors declare no competing financial interests.

Author contributions
G.N. designed the experiments and wrote the manuscript. G.N., M.B., H.D and S.J. supervised the research and finally reviewed the paper. H.C., R.D., W.Z., Y.R and W.W conducted the experiments. X.Y analysed the RNA-Seq data. All authors participated in data interpretation.

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