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# Morphological, anatomical and photosynthetic consequences of artificial allopolyploidization in *Cucumis*

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**Abstract** Studies have shown that polyploidy has pronounced effects on plants in multiple aspects, including genome structure, gene expression, metabolism and so on, which finally induce changes in phenotype. Many of these changes occurred immediately after polyploidization and retained or lost later. Therefore, it is meaningful for crop breeders to gain knowledge of these changes when it is relatively stable. In this study, we investigated the phenotypes of a highly self-pollinated synthesized allotetraploid in *Cucumis*, named with *Cucumis* × *hytivus* J. F. Chen & J. H. Kirkbr. (*C.* ×*hytivus* for short). Results showed that although many phenotypes of *C.* ×*hytivus* were intermediate between its parents, total leaf area and cell size exhibited parent-of-origin and dosage effect, respectively. Additionally, *C.* ×*hytivus* exhibited divergent biomass allocation strategy compared to the parents, developing more leaves. Beside the commonalities across different polyploid systems, the combination of hybridity and genome duplication in allopolyploids may lead to a diverse possibility of phenotypic changes. In spite of the reduced light absorption by less photosynthetic pigments in young leaves, allopolyploidy caused limited adverse effect on the photosynthesis of *C.* ×*hytivus*, which may benefit from the increased leaf thickness and potentially facilitated the survival and speciation of this novel species. The present study offers novel insights into the varied phenotypic effects of polyploidy and is a valuable reference for the crop improvement through polyploidization.

**Key words:** Allopolyploidy · Anatomy · *Cucumis* · Morphology · Photosynthesis

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## Introduction

The significance of polyploidy in plant evolution has been widely acknowledged, all the angiosperms are or used to be polyploids (Jiao et al. 2011; Van de Peer

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et al. 2017; Zhang et al. 2019). Polyploids are usually divided into autopolyploids and allopolyploids depending on whether the genome contains two or more identical or distinct subgenomes. Allopolyploids are considered showing greater range of changes than autopolyploids, since they contain hybridity in addition to genome duplication (Sarilar et al. 2013). The process of allopolyploidization also plays an essential role in plant speciation and is an important strategy for crop improvement (Lashermes et al. 2014; Zhang et al. 2019). The merger of divergent genomes into one cell could result in radical changes to the genome, which was known as 'genomic shock' (McClintock 1984). Besides, gene expression (Ha et al. 2009a; Yoo et al. 2014; Jung et al. 2015) and small RNAs (Ha et al. 2009b; Jiao et al. 2018) are extensively affected in allopolyploids as well. These genomic or transcriptomic chanbges may finally yield phenotypic variation, which are of particular interest for breeders. With the increasing reported synthetic and natural polyploid systems, the phenotypic consequences of polyploidy showed a wide variety, such as larger cells and organs due to increased ploidy level (Miller et al. 2012), intermediate or transgressive (non-additive) traits resulted from hybridity (Bassene et al. 2009; Ni et al. 2009; Chen 2010).

Photosynthesis, the most important biological process in this planet, is a prime example of how polyploids (or allopolyploids) differ phenotypically from their diploid progenitors (Coate and Doyle 2013). Photosynthesis is often profoundly affected by allopolyploidization as it is a comprehensive mechanism driven by multiple gene networks distributed over both nuclear and chloroplast genomes. Previous studies have shown that photosynthetic rates per leaf area in polyploid exhibited variation, ranging from lower, similar to higher than their diploids (Ježilová et al. 2014). It is difficult to generalize the effect of polyploid on photosynthesis in plants, especially in allopolyploids that combined the effect of hybridization and genome duplication.

Crop improvement through polyploidy has been widely used in many crop species. Cucumber (*Cucumis sativus* L., 2n = 2x = 14) is an economically important vegetable crop in many countries, including the main producing and consuming country China. Meanwhile, cucumber is also a model crop and the first sequenced vegetable crop. However, the genetic improvement of cucumber has been limited due to the narrow genetic base of cultivated cucumber and lack of available wide germplasm (Dijkhuizen et al. 1996; Huang et al. 2009). Very few species can be crossed with cucumber, *C. hystrix* Chakrav. (2n = 2x = 24) is one of these. Through interspecific hybridization and genome duplication, a newly synthesized allotetraploid was obtained, named with *Cucumis* × *hytivus* J. F. Chen & J. H. Kirkbr. (2n = 4x = 38) (*C.* ×*hytivus* for short hereafter) (Chen and Kirkbride 2000). *C.* ×*hytivus* is not only a novel species that can be used directly for breeding but also a bridge for transferring useful genes from wide species into cucumber. Previous studies reported extensive genomic and epigenetic changes were detected in *C.* ×*hytivus* (Chen et al. 2007; Chen and Chen 2008). Phenotypic variations (e.g., flowering time, fruit shape and leaf maturation) were also observed (Chen et al. 2002; Chen et al. 2003; Yu et al. 2018). These results suggested that *C.* ×*hytivus* is an ideal model system to study the allopolyploidization for its clear genetic relationship with its diploid parents, rather than natural allopolyploids that are not only the immediate consequence of allopolyploidization but also shaped by natural selection over long timeframes (Ježilová et al. 2014). The aim of this study is to investigate the morphological, anatomical and photosynthetic effects of allopolyploidy in *C.* ×*hytivus*, offering practical valuable information for the utilization of this novel species and new insights into the understanding of polyploidization in plant evolution and speciation.

## Materials and methods

### Plant materials

The self-cross plants ($S_{14}$) of a synthesized allotetraploid, *C.* ×*hytivus* and inbred lines of the parents, *C. hystrix* Chakr. and *C. sativus* 'BeijingJietou' were used. Seeds were sown in plastic pots (11 cm diameter, 0.5 L) filled with a peat-based potting mix (Pindstrup 2, Pindstrup Mosebrug A/S, Ryomgaard, Denmark) in early spring. Plants were grown randomly in a greenhouse with the following climate set points after germination: 25/20 °C day/night, ambient $CO_2$, relative humidity 60–70%. Irrigation was given daily with a nutrient solution (N:P:K ratio of 160:35:190, pH 5.8, electric conductivity 1.8) at 9 am for 15 min. In the following measurements, young

and mature leaf are referred the first unfolded leaf and the first fully developed leaf counting from the top of plant. Three replications were conducted in each measurement.

### Leaf number, leaf area and biomass determination

After 30 days of growth, the leaf number of each plant was counted. Each plant was separated into three parts, leaf, stem and root. The leaf area was measured by a LI-3100C area meter (LI-COR, Lincoln, NE, USA). Subsequently, the soil was washed from the roots and afterwards each part of the plants was weighted with water on the surface removed. Then, each part was dried in an oven at 80 °C 24 h for dry weight determination. The biomass allocation of each species was evaluated by leaf mass fraction, stem mass fraction and root mass fraction calculated according to Poorter et al. (2012). Specific leaf area (SLA) was calculated as SLA = total leaf area/leaf dry mass.

### Seed size and weight measurements

Average seed weight was determined by weighting mature dry seeds in batches of 100. The weights of four batches were measured for each species using an analytical balance. Sizes of seeds were analyzed by measuring the length and width of 10 randomly selected mature dry seeds from each species. The mean value of seeds weight and size of each species was calculated accordingly. The measurements were performed on previous harvested seeds of each species.

### Leaf spectral measurements

The spectral measurements were taken 29 days after germination. Young and mature leaves from each species were measured. The intact leaf samples were clamped into a transparent acrylic holder and placed into the integrating sphere for absorptance measurements. The sphere was coated with several layers of eastman white reflectance paint, which has a barium sulfate base. The radiance of the sphere wall was determined with a photomultiplier photometer (Gamma Scientific, Model 2020), and the spectral absorptance calculated from the difference in readings. The bandwidth was constant at 20 nm. The following parameters were calculated for each wavelength. The reflectance (R), transmission (T) and absorption (A) is measured as the fractions of the total incoming light, so $R + T + A = 1$. We measure R and T and calculate A. For both parameters two measurements have been made. First a reference measurement was done (IrR and IrT for reflectance and transmission, respectively), to establish how much light the lamp supplies into the sphere. Afterwards the sample (IsR and IsT for reflectance and transmission, respectively) was measured. The following equations were used.

$$\text{Reflectance} = \text{IsR}/\text{IrR}$$
$$\text{Transmission} = \text{IsT}/\text{IrT}$$
$$\text{Absorption} = 1 - R - T.$$

### Gas exchange measurements

Gas exchange measurements were measured 29 days after germination. Measurements were taken in the morning between 9:00 and 12:00. An IRGA system (CIRAS-2; PP-systems, Amesbury, MA, USA) was used for gas exchange measurements with a leaf cuvette of 2.5 $cm^2$ (leaf area) with a LED light unit. The light response curves of the young and mature leaves of each species were measured with $CO_2$ level at 400 ppm and leaf temperature at 25 °C. The cuvette flow was set to 200 $cm^3$ $min^{-1}$. To prevent photoinhibition, the measurements were initiated at 300 µmol $m^{-2}$ $s^{-1}$, entering darkness and subsequently returning to the initial light level in a stepwise fashion, and continuing in steps up to 1500 µmol $m^{-2}$ $s^{-1}$ with ten light levels (0, 50, 75, 100, 150, 200, 300, 500, 900 and 1500 µmol $m^{-2}$ $s^{-1}$) all together with steady-state net photosynthetic rate ($P_n$) and stomatal conductance ($g_s$). Curve fits were performed by using Photosynthesis Work Bench (Li-Cor, Lincoln, NE, USA) to calculate the gas exchange parameters, including light compensation point (LCP), light saturating point (LSP), dark respiration ($R_d$), apparent quantum efficiency ($\alpha$) and net photosynthesis rate at saturate light ($P_{sat}$).

### Chlorophyll fluorescence

Chlorophyll fluorescence was measured 29 days after germination as described in Yu et al. (2015). Measurements were taken on the young and mature leaves of each species, measuring the same part of the leaves

as used for gas exchange measurements by using a MINI-PAM (Walz, Effeltrich, Germany). The maximum quantum efficiency of PSII ($F_v/F_m$) was measured on leaves that were dark-adapted for 30 min with dark leaf clips DLC-8 (Walz, Effeltrich, Germany). The quantum yield of PSII, $\Phi_{PSII}$ were measured at non-saturating moderate PPFD of 200 μmol $m^{-2}$ $s^{-1}$. A halogen lamp (Schott KL 1500, Göttingen, Germany) with manual light control was used for the actinic light source. The light sources were fitted near to the leaf clip holder so the required light level was achieved without heat transmission to the leaf. A thermo and micro quantum sensor on the leaf clip holder recorded the leaf temperature and the incident PPFD.

### Anatomical variables

Anatomical observation was conducted 30 days after germination before harvest. The length, width and density (i.e. number per unit leaf area) of stomata and leaf thickness were assessed on the young and mature leaves of the three species. Stomatal measurements were conducted by using the silicon rubber impression (elite HD+, Zhermack, BadiaPolesine, Italy) method as described in Zhou et al. (2015). Imprints were conducted 2 h following the onset of the light period. This time is required for plants exposed to prolonged darkness (i.e. night-time period) to reach steady-state operating stomatal conductance (Drake et al. 2013). Measurements were carried out on the abaxial (lower) leaf surface since the other side (upper) is too hairy to get good quality pictures of stomata. Stomatal length and width were determined on 30 randomly selected stomata per leaf, and density was counted on 10 non-overlapping rectangular fields of view per leaf, by using magnifications of 19.2 × and 9.6 ×, respectively. Stomatal length and width were determined from transverse sections using ImageJ 1.47v (NIH, USA).

Sections of leaf blades held in fresh carrot pith were cut around the middle region and immersed in formalin:aceticacid:ethanol (FAA; 5:5: 90, v/v) for 24 h. The FAA was then replaced by 70% ethanol until analysis. The samples were dehydrated with an ethanol series and embedded in Technovit 7100 resin (Kulzer, Wehrheim, Germany) according to the manufacturer's instructions. Embedded samples were sliced into 20–30 μm sections, placed onto slide glass and stained with toluidine blue. Stained cross sections were observed under a microscope (SZX161; Olympus, Shinjuku, Japan or Axiostar; Carl Zeiss, Oberkochen, Germany) equipped with a CCD camera (DP73; Olympus). Dimensions of upper and lower epidermis, and of palisade mesophyll; and cuticle and leaf thickness were investigated. Leaf thickness were determined from transverse sections using ImageJ 1.47v (NIH, USA).

### Statistical analyses

One-way and two-way analysis of variance (ANOVA) were performed to reveal the differences between species and the differences between the species and leaf developmental stages, respectively. The analyses were performed using the software R (i3862.15.0, www.r-project.org/). Mean separations was done using the Duncan Multiple Range Test of $P < 0.05$.

## Results

### Plant growth

*C.* ×*hytivus* and its parents showed significant divergence in both whole plant morphology and leaf morphology (Yu et al. 2015) (Fig. 1). *C.* ×*hytivus* had significantly more leaves than the parents (Fig. 2a). However, the total leaf area per plant is similar between *C.* ×*hytivus* and *C. sativus* (Fig. 2b), suggesting a parent biased effect. Phenotypic variation of delayed leaf maturation was also reported (Yu et al. 2018). As shown in our earlier study, due to the additive effect of hybridity, many traits of *C.* ×*hytivus* was intermediate between its parents, including plant size, height and shoot dry weight (Yu et al. 2015). In fact, both the fresh weight and dry weight per plant of *C.* ×*hytivus* were intermediate between the parents (Fig. 2c, d). To determine the biomass allocation of the three species, we quantified their biomass allocation patterns to leaves, stems and roots of seedlings. All the three species shared same biomass allocation with the most in leaf, second in stem and third in root. However, biomass allocation strategy differed between the three species. Compared to the *C. hystrix* (17.42%) and *C.* ×*hytivus* (18.60%), *C. sativus* allocated more in stem (27.76%). *C.* ×*hytivus* allocated more in leaf (73.18%) than its parents (68.84%

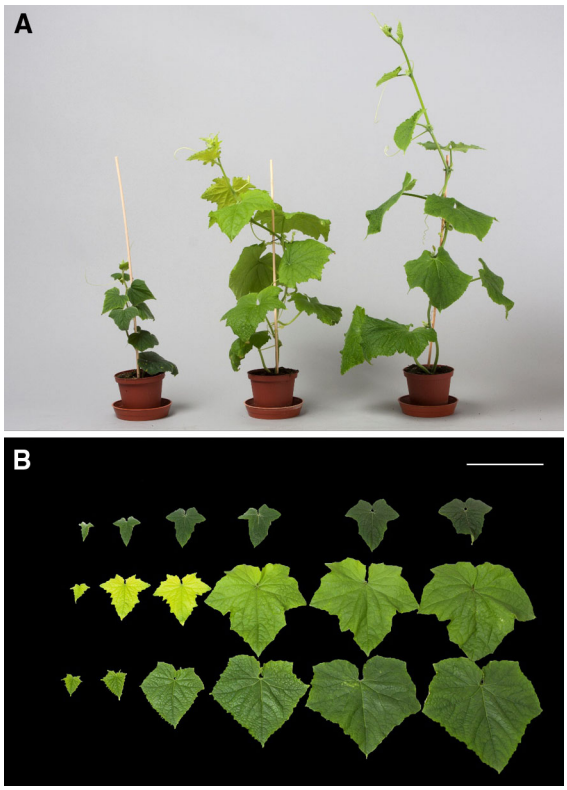


**Fig. 1** Plant and leaf morphology of the three species photographed 30 days after germination. **a**, *C. hystrix* (left), *C.* ×*hytivus* (middle) and *C. sativus* (right). **b**, (from 1st top leaf to 6th leaf): *C. hystrix* (upper), *C.* ×*hytivus* (middle) and *C. sativus* (lower), scale bar indicates 10 cm.

and 66.93%) and *C. hystrix* developed more biomass in root (13.73%) than *C. sativus* (5.32%) and *C.* ×*hytivus* (8.23%), which can be also found in the fresh weight fraction. No significant difference between the three species was observed in either specific leaf area (SLA) (Table 1).

## Seed size and weight

To determine the effect of allopolyploidization on seed size and weight, we measured the seed length and width, and seed weight of *C.* ×*hytivus* and its diploid parents. The seed of *C.* ×*hytivus* has both intermediate size and weight between its diploid parents (Fig. 3).

## Reflection, transmittance and absorption spectra

The spectral reflectance, transmittance, and absorptance for both young and mature leaves of *C. hystrix*, *C.* ×*hytivus* and *C. sativus* were shown in Fig. 4. *C. hystrix* and *C. sativus* displayed almost same light reflectance, transmittance and absorption spectra both in young and mature leaves. However, *C.* ×*hytivus* showed much less absorption in the range between 500 and 670 nm, which reached the lowest at 551 nm (green light), and slight less absorption between 690 and 800 nm (red light) than *C. hystrix* and *C. sativus*. Correspondingly, more reflectance and transmission were observed in *C.* ×*hytivus* along with the difference of absorption spectra. The difference of reflectance, transmittance and absorption spectra between *C.* ×*hytivus* and its parents were narrowed in the mature leaf in comparison to the young leaf.

## Photosynthesis and chlorophyll fluorescence

In general, leafage affects all the photosynthetic parameters in the investigated three species, except for apparent quantum efficiency (α) (Table 2). Significant species difference was detected in the LCP, α, $R_d$ and $P_{sat}$. Young leaf of *C. hystrix* exhibited significantly lower LCP and $P_{sat}$ than the other two species. The young leaf of *C.* ×*hytivus* exhibited significantly lower α compared to that of its parents and had significantly lower $R_d$ than the young leaf of *C. sativus*, which was not detected in mature leaves.

Chlorophyll fluorescence measurements showed that young leaf of *C.* ×*hytivus* exhibited significantly lower $F_v/F_m$ than mature leaf, which was not found in the parents. No species difference was observed in $\Phi_{PSII}$, whereas young leaves was significantly lower than mature leaves regardless of species (Fig. 5).

## Leaf cross section and stomatal density and size

The cross section of both young and mature leaves from *C. hystrix*, *C.* ×*hytivus* and *C. sativus* are shown in Fig. 6. It was clearly observed a distinct layer of long palisade parenchyma cells in the upper part of the mesophyll and more irregularly shaped and arranged spongy parenchyma cells in the lower part of the

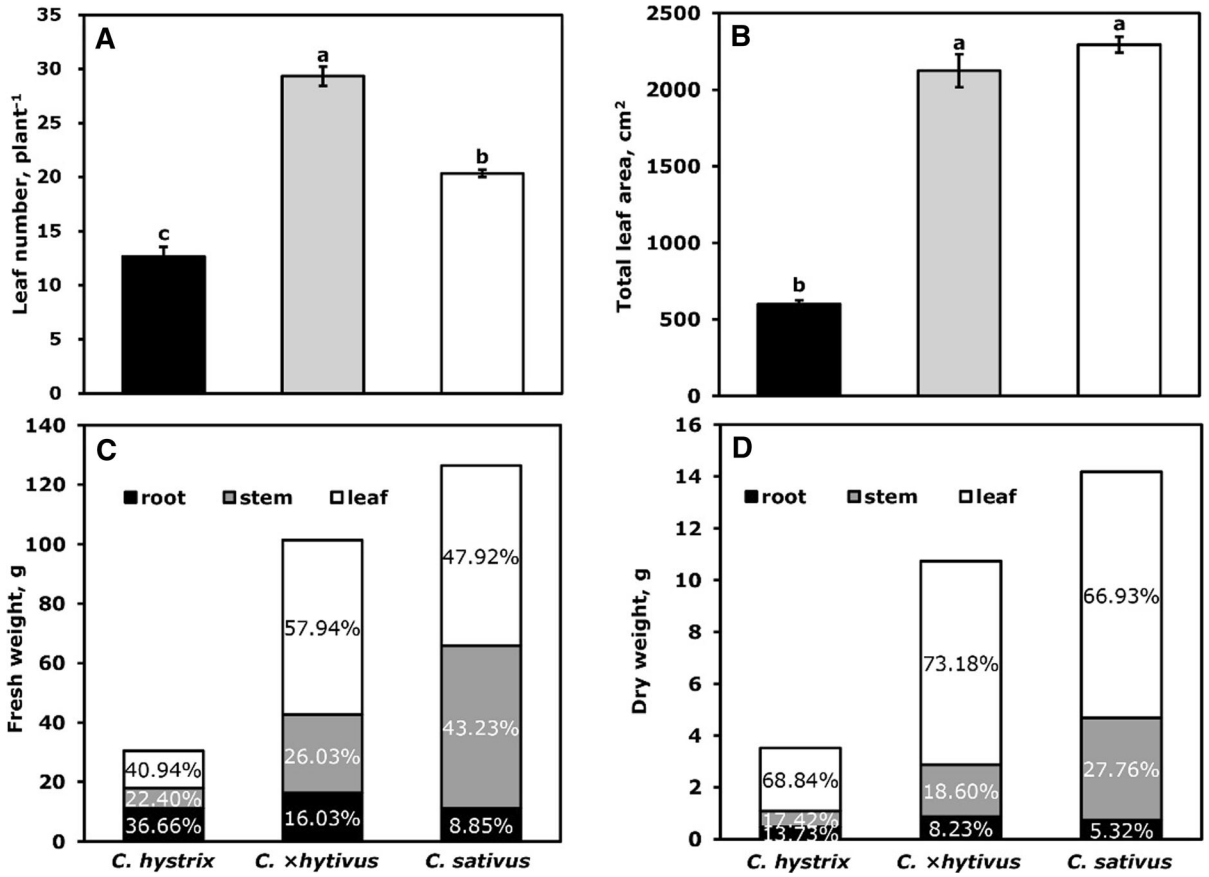


**Fig. 2** **a**, Leaf number per plant, **b**, total leaf area and plant biomass partition (**c**, fresh weight; **d**, dry weight) of the three species are shown: *C. hystrix*, *C. ×hytivus* and *C. sativus*. The data are mean values ± SD where n = 3. Assignment of different letters above the bars in A and B show a significant difference between species at 0.05 ($P < 0.05$). Percentages in the bars of C and D stand for the biomass allocation of leaf mass fraction, stem mass fraction and root mass fraction.

mesophyll in all the leaves. The parenchyma cells in young leaves appeared to be more compact than those in the mature leaves. In all the three species, mature leaves tended to have significantly thicker leaf and larger cells than young leaves (Table 1 and Fig. 6). Both species and leafage effect on leaf thickness was detected, as well as a significant interaction effect of species and leafage (Table 1). *C. ×hytivus* had significantly thicker leaf than its parents both in young and mature leaf, whereas *C. sativus* had thicker mature leaf than *C. hystrix* (Table 1 and Fig. 6). In addition to that, the parenchyma cells were looser in *C. ×hytivus* than the parents.

To determine the effects of allopolyploidization on stomatal size and density, stomatal imprints were taken of *C. ×hytivus* and its diploid parents. Significant species and leafage difference were observed in the stomatal density, stomatal length and width of the three species. Interaction between species and leafage had significant effect on the stomatal density of the three species (Table 1). In young leaves, stomata were significantly less in *C. sativus* compared to *C. hystrix* and *C. ×hytivus*, whereas *C. sativus* had the most stomata in mature leaf, followed by *C. hystrix*, least in *C. ×hytivus*. The stomata in *C. ×hytivus* were significantly larger than those in *C. hystrix* and *C. sativus* regardless of leafage.

**Table 1** Leaf thickness and stomatal analysis of young and mature leaves of three species: *C. hystrix*, *C. ×hytivus*, *C. sativus*. Stomatal density, stomatal length and stomatal width are shown. Values represent means ± SD (n = 3). Assignment of different letters within column show significant difference at 0.05 ($P < 0.05$)

| Species | Leaf stage | Specific leaf area (SLA) ($cm^2\ g^{-1}\ plant^{-1}$) | Leaf thickness (μm) | Stomatal density ($mm^{-2}$) | Stomatal length (μm) | Stomatal width (μm) |
|---|---|---|---|---|---|---|
| *C. hystrix* | Young | $248 \pm 10^{a}$ | $71 \pm 2^{e}$ | $819 \pm 25^{ab}$ | $11 \pm 0.1^{c}$ | $15 \pm 0.3^{d}$ |
| | Mature | | $114 \pm 3^{c}$ | $723 \pm 51^{bc}$ | $11 \pm 0.1^{c}$ | $16 \pm 0.2^{d}$ |
| *C. ×hytivus* | Young | $275 \pm 19^{a}$ | $96 \pm 2^{d}$ | $764 \pm 49^{b}$ | $15 \pm 0.1^{b}$ | $21 \pm 0.5^{b}$ |
| | Mature | | $149 \pm 1^{a}$ | $343 \pm 48^{d}$ | $16 \pm 0.6^{a}$ | $25 \pm 1.0^{a}$ |
| *C. sativus* | Young | $242 \pm 11^{a}$ | $69 \pm 1^{e}$ | $631 \pm 37^{c}$ | $11 \pm 0.1^{c}$ | $16 \pm 0.1^{d}$ |
| | Mature | | $122 \pm 2^{b}$ | $871 \pm 62^{a}$ | $12 \pm 0.1^{c}$ | $18 \pm 0.1^{c}$ |
| Species effect | | NS | ** | ** | ** | ** |
| Leafage effect | | | ** | ** | * | ** |
| Species × leafage effect | | | * | ** | NS | NS |

Species and/or leafage effect (NS, non-significant; *$P < 0.05$; **$P < 0.01$).

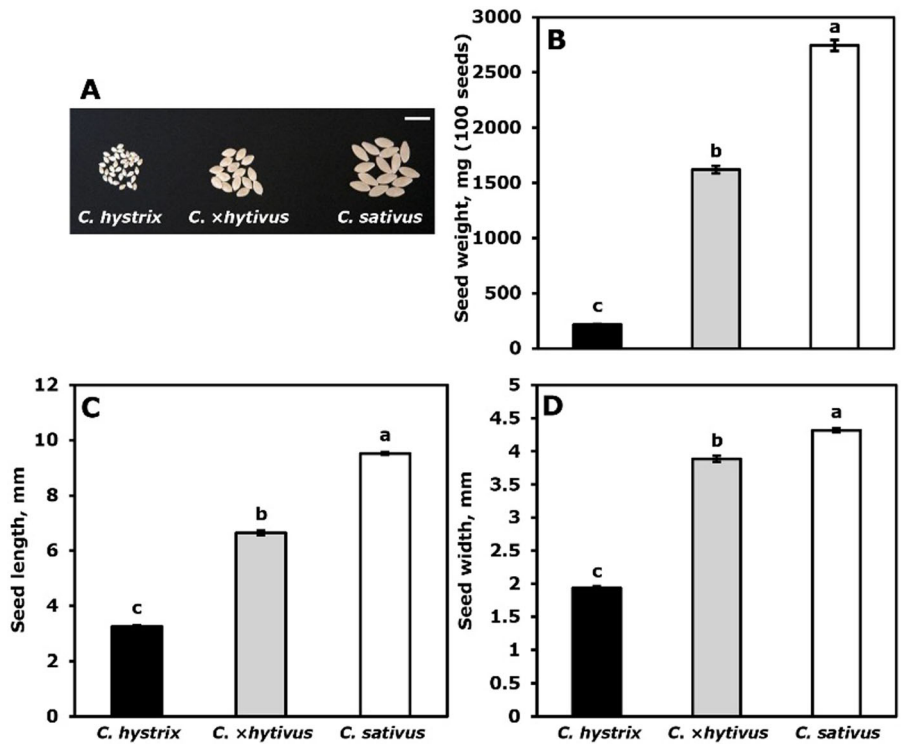


**Fig. 3** Seed weight and size (length and width) of the three species. **a**, seeds of *C. hystrix*, *C. ×hytivus* and *C. sativus* (bar = 1 cm). **b**, seed weight per 100 seeds (n = 4). **c**, seed length (n = 4). **d**, seed width (n = 4). Different letters above the bars in B, C and D show a significant difference between species at 0.05 ($P < 0.05$).

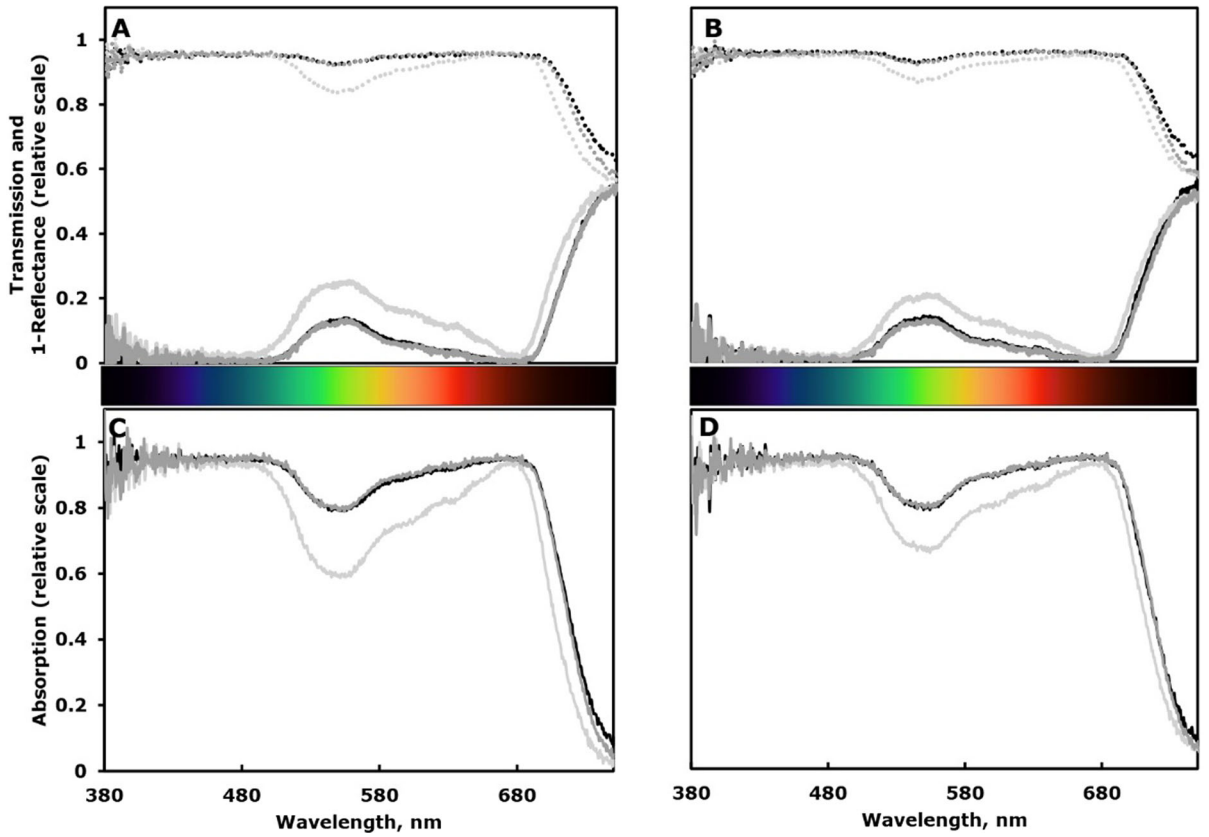


**Fig. 4** Spectra of leaf in three species (upper two: spectra of transmittance and reflectance; lower two: absorption spectra; middle: color bar of visible light spectrum): *C. hystrix* (Black), *C.* ×*hytivus* (light grey) and *C. sativus* (dark grey). By using 1-reflectance instead of reflectance, the reflectance was in the top of the figure ('upper side of the leaf') and transmission in the bottom of the figure ('lower side of the leaf'). **a** and **c**, young leaf; **b** and **d**, mature leaf. The color bar corresponds to the color of light at different wavelength.

**Table 2** Photosynthetic characteristics of young and mature leaves of three species: *C. hystrix*, *C.* ×*hytivus*, *C. sativus*. Light compensation point (LCP), light saturating point (LSP), dark respiration ($R_d$), apparent quantum efficiency (α) and net photosynthesis rate at saturate light ($P_{sat}$) are shown. Values represent means ± SD (n = 3). Assignment of different letters within column show significant difference at 0.05 ($P < 0.05$)

| Species | Leaf age | LCP ($\mu mol\ m^{-2}\ s^{-1}$) | LSP ($\mu mol\ m^{-2}\ s^{-1}$) | α ($mol\ CO_2\ mol^{-1} \cdot s^{-1}$) | $R_d$ ($\mu mol\ m^{-2}\ s^{-1}$) | $P_{sat}$ ($\mu mol\ m^{-2}\ s^{-1}$) |
|---|---|---|---|---|---|---|
| *C. hystrix* | Young | $48.9 \pm 2.02^{b}$ | $765.8 \pm 85.20^{c}$ | $0.080 \pm 0.0116^{a}$ | $2.83 \pm 0.109^{bc}$ | $8.6 \pm 0.95^{d}$ |
| | Mature | $19.9 \pm 3.62^{c}$ | $1034.5 \pm 70.02^{abc}$ | $0.063 \pm 0.0046^{ab}$ | $1.22 \pm 0.192^{a}$ | $15.3 \pm 1.26^{b}$ |
| *C.* ×*hytivus* | Young | $66.9 \pm 5.86^{a}$ | $845.7 \pm 88.26^{c}$ | $0.038 \pm 0.0024^{c}$ | $2.48 \pm 0.262^{b}$ | $11.3 \pm 1.36^{cd}$ |
| | Mature | $27.9 \pm 1.05^{c}$ | $1253.9 \pm 178.95^{ab}$ | $0.047 \pm 0.0064^{bc}$ | $1.33 \pm 0.249^{a}$ | $19.4 \pm 0.81^{a}$ |
| *C. sativus* | Young | $67.0 \pm 5.47^{a}$ | $1012.4 \pm 145.73^{bc}$ | $0.060 \pm 0.0047^{ab}$ | $3.50 \pm 0.257^{c}$ | $12.4 \pm 0.92^{bc}$ |
| | Mature | $28.0 \pm 2.00^{c}$ | $1380.0 \pm 120.68^{a}$ | $0.060 \pm 0.0013^{b}$ | $1.66 \pm 0.102^{a}$ | $20.1 \pm 1.09^{a}$ |
| Species effect | | ** | NS | ** | ** | ** |
| Leafage effect | | ** | ** | NS | ** | ** |
| Species × leafage effect | | NS | NS | NS | NS | NS |

Species and/or leafage effect (NS, non-significant; $*P < 0.05$; $**P < 0.01$).

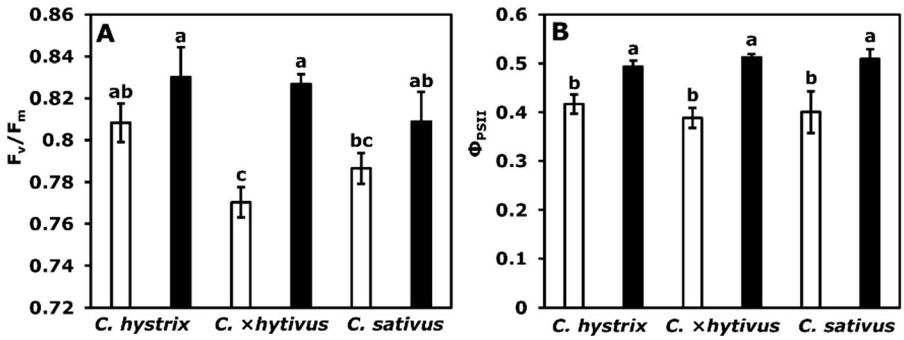


**Fig. 5** **a**, Maximal photochemical efficiency of PSII ($F_v/F_m$) and **b**, quantum efficiency of PSII ($\Phi_{PSII}$) of three species are shown. White and black bars refer to young and mature leaves, respectively. Vertical bars represent the mean ± SD (n = 3). Assignment of different letters above the bars show significant difference at 0.05 ($P < 0.05$).

## Discussion

### The morphological effects of polyploidy are both species and trait dependent

The hypothesis of genomic shock by McClintock (1984) proposes that a burst of genomic changes occurs immediately after the allopolyploidization. Efforts have been made to examine the impact of allopolyploidy on genome by using early generations of allopolyploid (Chen et al. 2007; Zhuang and Chen 2009; Zhuang et al. 2009; Sarilar et al. 2013). However, the genome of allopolyploid is not stable until later generation. Indeed, existing allopolyploids (e.g., wheat, coffee, cotton, oat and canola) are all formed more than thousands of years ago and well adapted for a very long time (Osborn et al. 2003). Therefore, it is practically meaningful to investigate the phenotypic difference caused by the retained genomic or epigenetic changes after the fast burst of genomic shock in the stable generation of allopolyploid. In this study, we reported the high self-pollinated generation ($S_{14}$) of a newly synthesized allotetraploid in *Cucumis*, *C.* ×*hytivus*, which exhibited phenotypically different from its diploid parents. As reported in our earlier analysis, many traits of *C.* ×*hytivus* were intermediate between its parents (Yu et al. 2015), which is also supported in this study. The commonly observed growth vigor of allopolyploid was not observed in *C.* ×*hytivus*. However, *C.* ×*hytivus* had the most leaf number, which is counted both from main stem and branches. Considering the similar total leaf area with *C. sativus*, it was suggested that *C.* ×*hytivus* distributed more branches with small young leaves, indicating a different growth habit.

The difference of growth strategy can be also seen from the varied biomass allocation patterns. Plants has leaf to fix carbon, stem to provide mechanical support and a hydraulic pathway, and root for absorption of water and nutrients and anchorage (Poorter et al. 2012). According to Wilson (1988) and Poorter et al. (2012), the shoot / root ratio is positively related to plant size in herbaceous plant, which is supported by the resulted present within the three species (Fig. 2). It was also believed that the pattern of biomass allocation is strongly associated with species-specific habitat characteristics (Villar et al. 1998). Species from areas with high annual rainfall typically was found to show less biomass allocation to roots and more biomass allocation to stems and leaves (Villar et al. 1998). Being opposite, *C. hystrix*, originated from a tropical forest in Xishuangbanna, southwest China, 21°36′42″–58″N, 101°3′26″–47″E (Chen et al. 1994), with average annual rainfall of 1200 to 2500 mm (Cao et al. 2006), which is much higher than other regions in China, showed relatively more biomass allocation to roots (Fig. 2). It could be probably explained by the high density of plant and living in low-rainfall habitats can benefit from having a larger root system to compete for water and nutrient. Moreover, as suggested by Poorter et al. (2012), lower SMF in *C. hystrix* can be the characteristic of being a typical climbing species, which is mechanical parasite that do not need large allocation to stems for self-supporting. Conversely, in habitats where water and nutrient availability is high, species that allocate more biomass to the shoot are more likely to be successful

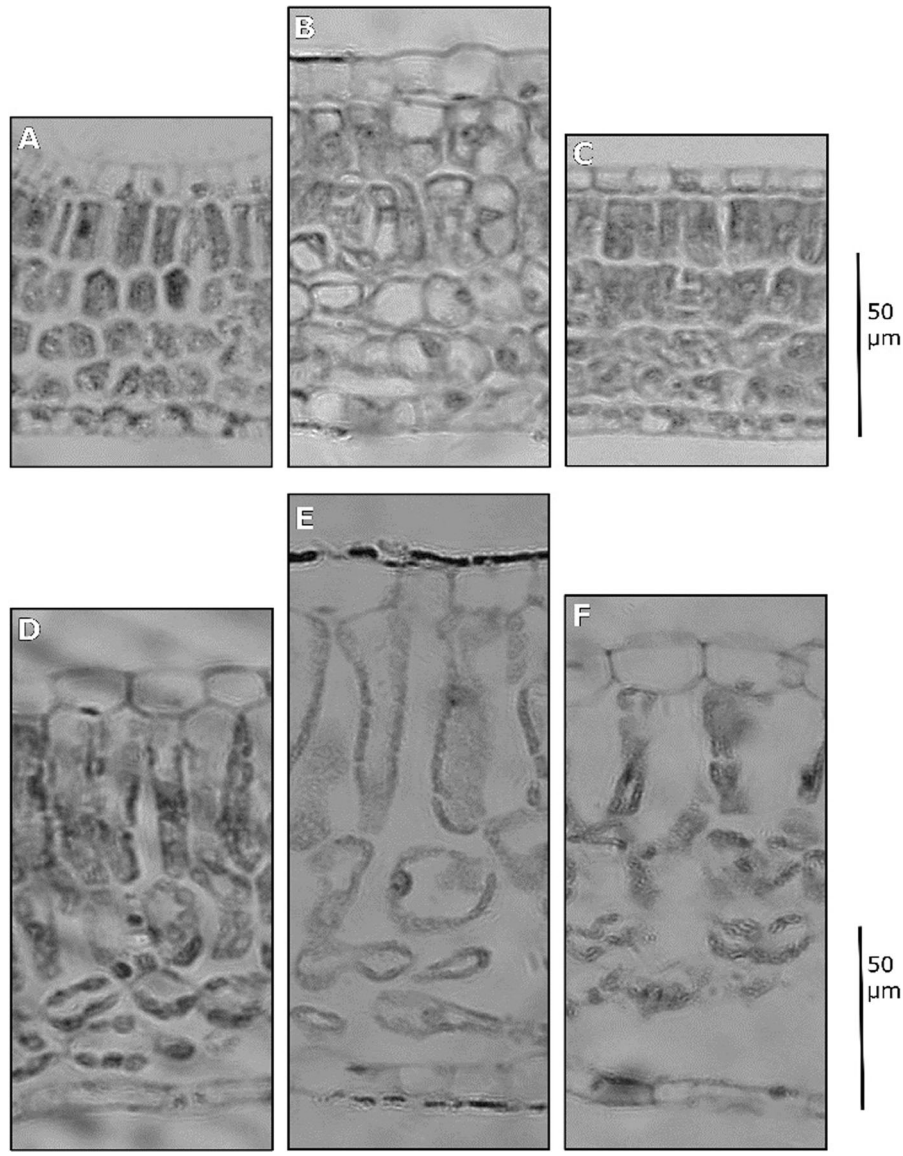


**Fig. 6** Transverse sections of young (upper three) and mature (lower three) leaves of *C. hystrix* (a, d), *C.* ×*hytivus* (b, e) and *C. sativus* (c, f). Depth of sections is 20–30 μm. Bars represent 50 μm.

(Poorter et al. 2012). A greater allocation to the stem and leaves might be indispensable in such circumstances, especially stronger investment in stem could be helpful in the competition of light, which is the case of *C. sativus*, a cultivated cucumber that have been selected for commercial higher productivity demand. In comparison to the distinct biomass allocation patterns of parents, C. ×*hytivus* allocated comparatively the most biomass in leaf (73.18%), significantly more than its parents (68.84% and 66.93%). According to our previous study, C. ×*hytivus* exhibited delayed leaf maturation with initial chlorophyll deficiency (Yu et al. 2018). Therefore, developing more leaves may be a compensation strategy to maintain a positive carbon budget.

Seed size and weight are reported strongly affected by the ploidy levels, whereas the effects of hybridity on seed size and weight are not obvious (Bretagnolle et al. 1995; Miller et al. 2012). And a significant parent-of-origin effect was proposed on seed weight in reciprocal diploid hybrids of *Arabidopsis thaliana* (Miller et al. 2012). In contrast to those research, seed size and weight of *C.* ×*hytivus* were intermediate between its diploid parents, indicating that the

polyploidy effect on seed morphology is species dependent.

Polyploids tends to have larger cells with relatively lower density (Masterson, 1994; Miller et al. 2012). In most cases, stomatal size is positively correlated with ploidy levels, and stomatal density is negatively correlated with genome dosage (Miller et al. 2012). Consistently, larger mesophyll cells, both palisade and spongy cells, and larger stomatal size with lower density was observed in allotetraploid, *C.* ×*hytivus*, compared with its diploid parents, *C. hystrix* and *C. sativus*. That supports the notion of estimating polyploid plant frequency using stomatal size in fossil samples (Masterson, 1994).

### Polyploidy had limited influence on photosynthesis despite reduced light absorption

Chlorophyll fluorescence is a commonly used technique in plant physiology that provides a non-invasive assessment of the photochemistry (Murchie and Lawson 2013). $F_v/F_m$ reflects the maximum quantum efficiency of the PSII and is a sensitive indicator of plant photosynthetic performance, and $\Phi_{PSII}$ is the proportion of absorbed energy used in photochemistry (Maxwell and Johnson 2000). Except for the lower $F_v/F_m$ in young leaves of *C.* ×*hytivus*, the three species showed similar $F_v/F_m$ and $\Phi_{PSII}$. In addition to that, the young leaves of *C.* ×*hytivus* were also shown lower $\alpha$, suggesting lower photosynthetic capacity under low light, as indicated in our previous low light experiments (Yu et al. 2015). However, *C.* ×*hytivus* had identical photochemical capacity as its parents under normal light conditions. In other words, allopolyploidization did not lead to obvious difference in the photosynthesis of *C.* ×*hytivus* with the exception for the reduced photosynthesis efficiency under low light in young leaf, which could be owing to the poorly developed chloroplast membrane system (Yu et al. 2018).

Plants start the photosynthesis with absorbing the light through pigments, mainly chlorophylls. Subsequently, the energy absorbed will be converted photochemically into stored energy. A lack of Chl pigmentation can reduce the absorption of light, which inhibit the photosynthesis and finally can affect the growth of the plant. As shown by Gates et al. (1965), the absorption spectrum of a pale leaf lacking chlorophyll in *Hedera helix* displayed drastically less absorption than that of a normal green leaf. Dissimilarly, the less chlorophylls and carotenoids of *C.* ×*hytivus* did not cause significant change to the action spectrum of photosynthesis, though a less absorption in the green and orange region was observed (Fig. 4). It can be probably explained by the increased leaf thickness in *C.* ×*hytivus* (Table 1 and Fig. 6), which leads to increased multiple scattering and optical path length through the leaf that compensates for the less chlorophylls and carotenoids as compared to thinner leaves in its diploid parents (Vogelmann 1993). Along with the aforementioned limited effect of photochemistry, it can reinforce our earlier conclusion that allopolyploidy did not cause significant adverse impact upon the photosynthesis of *C.* ×*hytivus*, which eventually helped this novel species to be naturally established (Yu et al. 2015).

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

## Reference

Bassene JB, Froelicher Y, Dhuique-Mayer C, Mouhaya W, Ferrer RM, Ancillo G, Morillon R, Navarro L, Ollitrault P (2009) Non-additive phenotypic and transcriptomic inheritance in a citrus allotetraploid somatic hybrid between *C. reticulata* and *C. limon*: the case of pulp carotenoid biosynthesis pathway. Plant Cell Rep 28:1689

Bretagnolle F, Thompson JD, Lumaret R (1995) The influence of seed size variation on seed germination and seedling vigour in diploid and tetraploid *dactylis glomerata* L. Ann Bot 76:607–615

Cao M, Zou X, Warren M, Zhu H (2006) Tropical forests of xishuangbanna, China. Biotropica 38:306–309

Chen L, Chen J (2008) Changes of cytosine methylation induced by wide hybridization and allopolyploidy in *Cucumis*. Genome 51:789–799

Chen JF, Kirkbride JH (2000) A new synthetic species of *Cucumis* (Cucurbitaceae) from interspecific hybridization and chromosome doubling. Brittonia 52:315–319

Chen J, Zhang SL, Zhang X (1994) The Xishuangbanna gourd (*Cucumis sativus* var. xishuangbannesis Qi et Yuan), a traditionally cultivated plant of the Hanai People, Xishuangbanna, Yunnan, China. Cucurbit Genet Coop Rep 17:18–20

Chen JF, Staub J, Adelberg J, Lewis S, Kunkle B (2002) Synthesis and preliminary characterization of a new species (amphidiploid) in *Cucumis*. Euphytica 123:315–322

Chen J, Staub J, Qian C, Jiang J, Luo X, Zhuang F (2003) Reproduction and cytogenetic characterization of interspecific hybrids derived from *Cucumis hystrix* Chakr. × *Cucumis sativus* L. Theor Appl Genet 106:688–695

Chen L, Lou Q, Zhuang Y, Chen J, Zhang X, Wolukau JN (2007) Cytological diploidization and rapid genome changes of the newly synthesized allotetraploids *Cucumis* × *hytivus*. Planta 225:603–614

Chen ZJ (2010) Molecular mechanisms of polyploidy and hybrid vigor. Trends Plant Sci 15:57–71

Coate JE, Doyle JJ (2013) Genomics and transcriptomics of photosynthesis in polyploids. In: Chen ZJ, Birchler JA (eds) Polyploid and hybrid genomics. Wiley, Oxford, pp 153–169

Dijkhuizen A, Kennard WC, Havey MJ, Staub JE (1996) RFLP variation and genetic relationships in cultivated cucumber. Euphytica 90:79–87

Drake PL, Froend RH, Franks PJ (2013) Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. J Exp Bot 64:495–505

Gates DM, Keegan HJ, Schleter JC, Weidner VR (1965) Spectral properties of plants. Appl Optic 4:11–20

Ha M, Kim ED, Chen ZJ (2009a) Duplicate genes increase expression diversity in closely related species and allopolyploids. Proc Natl Acad Sci USA 106(7):2295–2300

Ha M, Lu J, Tian L, Ramachandran V, Kasschau KD, Chapman EJ, Carrington JC, Chen X, Wang XJ, Chen ZJ (2009) Small RNAs serve as a genetic buffer against genomic shock in Arabidopsis interspecific hybrids and allopolyploids. Proc Natl Acad Sci USA 106(42):17835–17840

Huang S, Li R, Zhang Z et al (2009) The genome of the cucumber, Cucumis sativus L. Nat Genet 41:1275–1281

Ježilová E, Nožková-Hlaváčková V, Duchoslav M (2014) Photosynthetic characteristics of three ploidy levels of Allium oleraceum L. (Amaryllidaceae) differing in ecological amplitude. Plant Spec Biol 30:212–224

Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS, Soltis DE, Clifton SW, Schlarbaum SE, Schuster SC, Ma H, Leebens-Mack J, dePamphilis CW (2011) Ancestral polyploidy in seed plants and angiosperms. Nature 473:97–100

Jiao W, Yuan J, Jiang S, Liu Y, Wang L, Liu M, Zheng D, Ye W, Wang X, Chen ZJ (2018) Asymmetrical changes of gene expression, small RNAs and chromatin in two resynthesized wheat allotetraploids. Plant J 93:828–842

Jung Y, Kawaura K, Kishii M, Sakuma S, Ogihara Y (2015) Comparison of genome-wide gene expression patterns in the seedlings of nascent allohexaploid wheats produced by two combinations of hybrids. Genes Genet Syst 90(2):79–88

Lashermes P, Combes MC, Hueber Y, Severac D, Dereeper A (2014) Genome rearrangements derived from homoeologous recombination following allopolyploidy speciation in coffee. Plant J 78(4):674–685

Masterson J (1994) Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science 264:421–424

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence-a practical guide. J Exp Bot 51:659–668

McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792–801

Miller M, Zhang C, Chen ZJ (2012) Ploidy and hybridity effects on growth vigor and gene expression in *Arabidopsis thaliana* hybrids and their parents. G3 (Bethesda) 2: 505–513

Murchie E, Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. J Exp Bot 64:3983–3998

Ni Z, Kim ED, Ha M, Lackey E, Liu J, Zhang Y, Sun Q, Chen ZJ (2009) Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. Nature 457:327–331

Osborn TC, Pires JC, Birchler JA, Auger DL, Chen ZJ, Lee HS, Comai L, Madlung A, Doerge RW, Colot V, Martienssen RA (2003) Understanding mechanisms of novel gene expression in polyploids. Trends Genet 19:141–147

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol 193:30–50

Sarilar V, Palacios PM, Rousselet A, Ridel C, Falque M, Eber F, Chèvre AM, Joets J, Brabant P, Alix K (2013) Allopolyploidy has a moderate impact on restructuring at three contrasting transposable element insertion sites in resynthesized *Brassica napus* allotetraploids. New Phytol 198:593–604

Van de Peer Y, Mizrachi E, Marchal K (2017) The evolutionary significance of polyploidy. Nat Rev Genet 18:411–424

Villar R, Veneklaas EJ, Jordano P, Lambers H (1998) Relative growth rate and biomass allocation in 20 *Aegilops* (Poaceae) species. New Phytol 140:425–437

Vogelmann TC (1993) Plant tissue optics. Ann Rew Plant Biol 44:231–251

Wilson JB (1988) A review of evidence on the control of shoot: root ratio, in relation to models. Ann Bot 61:433–449

Yoo MJ, Liu X, Pires JC, Soltis PS, Soltis DE (2014) Nonadditive gene expression in polyploids. Ann Rev Genet 48:485–517

Yu X, Hyldgaard B, Rosenqvist E, Ottosen CO, Chen J (2015) Interspecific hybridization in *Cucumis* leads to the divergence of phenotypes in response to low light and extended photoperiods. Front Plant Sci 6:802

Yu X, Wang X, Hyldgaard B, Zhu Z, Zhou R, Kjær KH, Ouzounis T, Lou Q, Li J, Cai Q, Rosenqvist E, Ottosen CO, Chen J (2018) Allopolyploidization in *Cucumis* contributes to delayed leaf maturation with repression of redundant homoeologous genes. Plant J 94:393–404

Zhang K, Wang X, Cheng F (2019) Plant polyploidy: origin, evolution, and its influence on crop domestication. Hort Plant J 5:231–239

Zhou R, Yu X, Kjær KH, Rosenqvist E, Ottosen CO, Wu Z (2015) Screening and validation of tomato genotypes under heat stress using $F_v/F_m$ to reveal the physiological mechanism of heat tolerance. Environ Exp Bot 118:1–11

Zhuang Y, Chen JF (2009) Changes of gene expression in early generations of the synthetic allotetraploid *Cucumis* × *hytivus* Chen et Kirkbride. Genet Res Crop Evol 56:1071–1076

Zhuang Y, Chen JF, Jahn M (2009) Expression and sequence variation of the cucumber *Por* gene in the synthesized allotetraploid *Cucumis* × *hytivus*. Mol Biol Rep 36:1725–1731