Effects of Water Stress on Leaf Temperature and Chlorophyll Fluorescence Parameters in Cotton and Peanut

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Abstract: A greenhouse experiment was conducted to study the adaptive mechanism of cotton and peanut under water stress conditions. Five cultivars of cotton and six cultivars of peanut were grown in pots under two water levels; the control and water stress condition, where irrigation water equal to 100% and 50% of the daily transpiration, respectively, was daily applied. Peanut showed a greater increase than cotton in leaf temperature (TL) and non-photochemical quenching (NPQ) and a greater decrease in water content per unit leaf area (WCLA), chlorophyll content and maximum quantum yield of photosystem II (PSII) (Fv/Fm) in the water stress condition. On the other hand, the water stress lowered the transpiration rate, actual quantum yield of PSII (ΔF/F'm) and leaf area (LA) more in cotton than in peanut. Cotton showed greater reduction in LA along with little reduction in the root dry weight (RDW) leading to high WCLA, while peanut showed increased RDW with little reduction in LA under the water stress condition. It was concluded that photodamage and down regulation in PSII were induced by water stress, coinciding with increases in leaf temperature regulated mainly by transpiration. Peanut showed more severe photodamage in PSII than cotton under the water stress condition.

Key words: Chlorophyll fluorescence, Cotton, Leaf temperature, Peanut, Transpiration, Water stress.

Under conditions of water scarcity, plants are often subjected to a high temperature, which increases their vulnerability to light stress and consequently the photoinhibition (Carpentier, 1996). Supraoptimal leaf temperatures limit carbon dioxide fixation through limitation in activity of photosynthetic enzymes such as Rubisco (Berry and Björkman, 1980; Kohza and Edwards, 1987). When carbon dioxide fixation is limited, the rate of active oxygen formation increases in chloroplasts as excess excitation energy, which would either become manifest as oxidative damages to the plant or would result in activation of defense and repair mechanisms which could prevent such damage from occurring (Smirnoff, 1993). Plants have several mechanisms for avoiding and/or dissipating the excess excitation energy non-destructively. Some plants regulate incident solar radiation on leaf by paraheliotropic leaf movement (Begg and Torsell, 1974; Ehleringer and Forseth, 1980), production of a thicker cuticle (Chatterton et al., 1975; Sanekoa and Ogata, 1987) and pubescence on the leaf surface (Wooly, 1964; Johnson, 1975). In addition, most of the plants adapt themselves to water stress by dissipating the excess excitation energy thermally with the down regulation of photosystem II (PSII) activity to protect photosynthetic apparatus from photodamaging effect under water stress often coinciding with high leaf temperature (Tl) (Bilger and Björkman, 1990; Björkman and Demming-Adams, 1994; Inamullah and Isoda, 2005b).

Previously, it was indicated that cotton protected its photosynthetic apparatus from photodamage by keeping its Tl lower through high transpiring ability than soybean under water stress condition (Inamullah and Isoda, 2005a, b). On the other hand, soybean dissipated the excess excitation energy thermally along with the down regulation of PSII activity supported by paraheliotropic leaf movement. Since these experiments were conducted using only one cultivar of cotton and soybean, the diversity of chlorophyll fluorescence parameters in relation to Tl needs to be studied using the different cultivars and/or species. Murata (1981) reported on the CO2 assimilation rate and radiation use efficiency in various crops. However, there are few reports on the differences in the photochemical reactions in photosynthesis among species, although there are reports for individual species, such as apple (Massacci and Jones, 1990), coffee (Lima et al., 2002), potato (Jeffries, 1994) and wheat (Havaux and Lannoye, 1985). Studies on the varietal and/or crop differences in photosynthetic characteristics will help to understand not only the relationship among these characteristics and but also...
adaptive mechanisms of each species to water stress comprehensively. In this experiment, we investigated the eco-physiological adaptive mechanism of cotton and peanut cultivars under a water stress condition, especially in chlorophyll fluorescence parameters in relation to changes with $T_L$.

Materials and Methods

The experiment was conducted in the greenhouse at Faculty of Horticulture, Chiba University, Japan. Five commercial cultivars of cotton and six of peanut in China were used (Table 1). Cotton and peanut seeds were sown on 6 June 2007 in the 1/2000a Wagner pots (height 30 cm and diameter 24 cm) and 1/650a styrofoam boxes (length 45 cm, width 36 cm and height 30 cm), respectively. Two irrigation treatments were used for each crop. The pots and boxes were arranged in a randomized complete block design with four replications in a split plot arrangement. Water levels were allocated to the main plot while cultivars were used (Table 1). Cotton and peanut seeds were sown after sowing. Five and three seeds per pot were sown to ensure the crop stands for cotton and peanut, respectively. After the establishment of seedlings, all were thinned to a single plant per pot and box.

Irrigation treatment was started on 29 July, when cotton was at the square formation stage and peanut was at the flowering stage. Irrigation was applied on daily basis in the evening between 1730 to 1930. In the control and water stress conditions, the irrigation water applied was equal to 100% and 50% of the transpiration, calculated by weighing the pots/boxes with an electronic balance. Evaporation was controlled by covering the soil surface of the pots and boxes with aluminum foil to exclude the effect of evaporation. Water was applied to the pots through small L-shaped PVC pipes (diameter 10 mm) fitted in a small hole in the bottom portion in each pot very near to the base. In the case of styrofoam boxes, water was applied directly to the soil surface near the root zone by taking up the aluminum foil and replacing it again just after the irrigation. Although data were collected for several days, the data collected on 25 August 2007 (the 28th day of water treatment), which was the most suitable sunny weather condition, were used for the chlorophyll fluorescence parameters and $T_L$. During data collection, the cotton plants were in the ball formation stage and peanut in the pod filling stage.

Table 1. Cotton and peanut cultivars used in the experiment.

| Cotton         | Peanut       |
|----------------|--------------|
| Xinluzao 7    | Lhua 11      |
| Xinluzao 10   | Hua 22       |
| Xinluzao 26   | Hua 20       |
| Xinshi K-7    | Hua 16       |
| Kangli 168    | Hua 14       |
| Xinshi K-7    | Quyu 101     |

Data for $T_L$ and chlorophyll fluorescence parameters were collected on the upper fully expanded leaf. The data regarding $T_L$, air temperature, air relative humidity and global solar radiation were collected at one-minute intervals from 0600 to 1800 by a datalogger (Eko Denki Inc., Thermocore E, Japan) connected to a personal computer. $T_L$ measuring thermocouples were attached to the abaxial side of leaf/leaflet. Air relative humidity and global solar radiation were measured with a humidity sensor (CHS-UPS, TDK Electronic Co. Ltd, Japan) and global radiation sensor (Model MS-4, Eko Instruments Trading Co., Ltd, Japan), respectively.

Maximum quantum yield of PSII ($F_v/F_m$, where $F_v=F_m-F_o$) of the dark adapted leaves and actual quantum yield of PSII ($\Delta F/F_m$, where $\Delta F=F_m-F_t$) of the illuminated leaves were measured using a chlorophyll fluorometer (PAM-2000, Walz, Germany). $F_t$ represents the maximum and minimum fluorescence yield of dark-adapted leaves, respectively. $F_m$ represents the maximum fluorescence yield of an illuminated leaf via saturation pulse method, while $F_t$ represents the steady state fluorescence yield, measured at any given time. $F_t/F_m$ was determined before dawn, i.e., before the direct sunlight hit the leaves (around 0800), while data of $\Delta F/F_m$ was collected in the morning (0930–1130), noon (1230–1430) and afternoon (1500–1700). Non-photochemical quenching (NPQ) was calculated as ($F_m/F_t$–1) (Bilger and Björkman, 1990).

After the water treatment, all leaf lamina were detached from petioles and weighed to record their fresh weight, which were then dried in an oven for 48 hr at 80°C to take their dry weight. Using the fresh weight (FW) and dry weight (DW) of all the leaves, water content per unit leaf area (WCLA) was calculated according to the following formula:

$$WCLA = \frac{FW - DW}{\text{leaf area}}$$

Leaf area (LA) was measured with an automatic area meter (AAM-8, Hayashi Denko Co. Ltd., Japan). Roots were taken out from the pots/boxes and washed gently for root dry weight (RDW). Fine roots were collected by sieving the soil water mixture through a fine mesh screen and weighed after drying in an oven for 48 hr at 80°C.

The data were analyzed using statistical software StatView (SAS, 1999). The significances of differences among cultivars were determined according to Fisher’s LSD test. Correlation coefficients were also determined on the selected data using StatView.

Results

1. Transpiration

Figure 1 shows the changes with time in transpiration (transpiration per plant per day) for cotton and peanut.
cultivars. In the water stress condition, the amount of transpiration was greater in peanut than in cotton on the average throughout the water treatment period. Xinluzao 26 had the largest amount of high transpiration on the average among the cotton cultivars under the control condition. All cultivars of cotton responded almost at the same level similarly to the water stress condition, although Xinluzao 10 and Xinluzao 26 had a little more higher transpiration than Kangdi 168 which showed the least with the lowest transpiration. Qunyu 101 had the largest amount of high transpiration among the peanut cultivars on the average under the control and water stress conditions.

2. **Leaf area (LA), water content per unit leaf area (WCLA) and root dry weight (RDW)**

LA, WCLA and RDW were significantly affected by the water stress. Varieties (V), irrigation level (I) and their interactions (I×V) had also significant effects on LA, WCLA and RDW in both crops (Fig. 2). The water stress decreased LA by 63% and 45% in LA on the average for cotton and peanut, respectively. Xinluzao 26 and Qunyu 101 showed the least with the lowest transpiration. Xinluzao 26 had significantly highest WCLAs under both conditions, while the lowest values were recorded in Xinluzao 7 under both conditions. In peanut, the highest WCLAs were recorded in Qunyu 101 under both conditions. Huayu 16 and Huayu 20 had the lowest WCLAs under both conditions. Under the water stress condition, RDWs decreased in cotton by 39%, while it increased by 51% in peanut on the average. Xinluzao 26 and Qunyu 101 had the heaviest RDW under both conditions.

3. **Transpiration rate, SPAD value and leaf temperature (T<sub>L</sub>)**

On the last day of water treatment, cotton had higher transpiration rates (transpiration per unit leaf area per day) than peanut in the control, while peanut showed higher transpiration rates than cotton under the water stress condition (Fig. 3). There were significant varietal differences in transpiration rate for both treatments in cotton. Xinluzao 26 had the highest value on the average under the control condition. All cotton cultivars except Xinluzao 10 showed almost at the same values under the water stress condition. Xinluzao 26 and Qunyu 101 showed the least with the lowest transpiration. Xinluzao 26 and Qunyu 101 had significantly higher transpiration rates under the control and water stress conditions, respectively. In peanut, Xinluzao 26 had the highest WCLAs under both conditions, while the lowest values were recorded in Xinluzao 7 under both conditions. In peanut, the highest WCLAs were recorded in Qunyu 101 under both conditions. Huayu 16 and Huayu 20 had the lowest WCLAs under both conditions. Under the water stress condition, RDWs decreased in cotton by 39%, while it increased by 51% in peanut on the average. Xinluzao 26 and Qunyu 101 had the heaviest RDW under both conditions.

**Fig. 1** Transpiration in cotton and peanut cultivars under the control and water stress conditions during the treatment.

- **Control**; Applied irrigation water equal to transpiration
- **Water Stress (WS)**; Applied irrigation water equal to 50% of the transpiration

The data are represented as the mean of four replications.

C1, C2…C5 and P1, P2…P6 mean Xinluzao 7, Xinluzao 10, Xinluzao 26, Xinshi K-7, Kangdi 168 and Luhua 11, Huayu 22, Huayu 20, Huayu 16, Huayu 14, Qunyu 101, respectively.
In both crops, TL was increased significantly by the water stress. Peanut showed higher TL than cotton under both conditions. Under the water stress condition, TL increased by 3.5ºC and 4.2ºC on the average of cultivars in cotton and peanut, respectively. There were significant varietal differences in TL in both cotton and peanut. In cotton, Xinluzao 26 maintained TL lower, while Xinluzao 7 and Xinluzao 10 showed significantly higher TL than Xinluzao 26 and Kangdi 168 under the water stress condition. In peanut, Luhua 11 showed the highest increase in TL under the water stress condition.

On the average, cotton had higher SPAD values than peanut under both conditions. The average SPAD value in cotton under the water stress condition was 12.4% lower than in the control. The water stress reduced significantly SPAD values also in peanut. There was no significant difference between water stress and control in SPAD values in cotton. Among the peanut cultivars, Huayu 20 had a significantly lower SPAD value than the others in the control, while Luhua 11 and Huayu 20 showed lower SPAD values than Huayu 22 and Huayu 16 under the water stress condition.

4. Maximum quantum yield of PSII (Fv/Fm), actual quantum yield of PSII (ΔF'/Fm) and non-photochemical quenching (NPQ)

Fv/Fm was significantly affected by the water stress...
Shahenshah and Isoda —— Effects of Water Stress on Chlorophyll Fluorescence Parameters in Cotton and Peanut

had the highest ΔF/Fm value under both conditions, while Luhua 11 under the control and Huayu 20 under the water stress conditions showed the lowest ΔF/Fm value. NPQ was also significantly affected by the water stress treatment in both crops. NPQ was increased by 173% and 176% due to the water stress in cotton and peanut, respectively. In cotton, Xinluzao 26 had the lowest NPQ under both control and water stress conditions, while the highest NPQ was recorded in Kangdi 168 under the control condition and in Xinluzao 7 and Xinluzao 10 under the water stress condition. Xinluzao 26 had lower NPQ values than Kangdi 168 and Xinluzao 7 under the control and water stress conditions. In peanut, Huayu 16,

Fig. 3. Transpiration rate, leaf temperature and SPAD value of cotton and peanut cultivars under different irrigation treatments.

The water stress also affected ΔF/Fm significantly in both crops, particularly in cotton. Water stress decreased ΔF/Fm by 44.4% and 31.1% on the average in cotton and peanut, respectively. There was no significant difference in ΔF/Fm among the cotton cultivars, while significances of varietal differences were observed in peanut. Qunyu 101 had the highest ΔF/Fm value under both conditions, while Luhua 11 under the control and Huayu 20 under the water stress conditions showed the lowest ΔF/Fm value.

NPQ was also significantly affected by the water stress treatment in both crops. NPQ was increased by 173% and 176% due to the water stress in cotton and peanut, respectively. In cotton, Xinluzao 26 had the lowest NPQ under both control and water stress conditions, while the highest NPQ was recorded in Kangdi 168 under the control condition and in Xinluzao 7 and Xinluzao 10 under the water stress condition. Xinluzao 26 had lower NPQ values than Kangdi 168 and Xinluzao 7 under the control and water stress conditions.
Huayu 14 and Qunyu 101 had significantly lower NPQ under the control, while the highest NPQ was recorded in Huayu 20 under both conditions.

5. Correlations of ΔF/Fm with Fv/Fm and NPQ and correlations of Tc with Fv/Fm, ΔF/Fm and NPQ

ΔF/Fm was affected by Fv/Fm in both crops (Fig. 5), indicating some photodamage in PSII would occur in both crops, especially in peanut. Varietal differences in ΔF/Fm in peanut in the control and water stress conditions were not dependent on Fv/Fm, while this relationship was significant only in the control in cotton. A highly significant and negative correlation was observed between ΔF/Fm and NPQ in both crops under the control and water stress conditions. This suggested that the down regulation of PSII activities would decrease ΔF/Fm.

A highly significant negative correlation was observed between Tc and Fv/Fm in cotton and peanut (Fig. 6), suggesting higher photodamage in the photosynthetic apparatus of peanut due to increase in Tc. The correlation of Tc with ΔF/Fm was negative in both crops in the morning.
noon and afternoon. The correlation of $T_2$ with NPQ was positive in both crops except in the afternoon in peanut.

6. Factors related with SPAD, transpiration and WCLA

SPAD value had highly significant positive and negative correlations with $Fv/Fm$ and $T_L$ on the average, respectively, in both crops (Table 2). This suggested the damage of PSII through the decrease in chlorophyll content, which was affected by high $T_L$ under the water stress condition. The varietal differences in $Fv/Fm$, however, did not depend on SPAD value.

Transpiration rate (transpiration per unit leaf area) affected the differences in $T_2$ between the control and water stress conditions in both crops (Table 3). However, varietal differences in $T_2$ in both crops were not significantly correlated with transpiration rate in both the control and water stress conditions. RDW had significant effects on differences between treatments in both transpiration and WCLA in cotton. In peanut, negative and no significant relations of RDW with transpiration rate and WCLA, respectively, were found. The varietal differences in transpiration rate and WCLA in both crops were not correlated largely with RDW in both the control and water stress conditions except those in WCLA of cotton, suggesting that the volume of root would not affect varietal differences in transpiration and WCLA.

Discussion

Water uptake was reported to be maximized by increasing root depth and/or water loss minimized by reducing LA, stomatal control and/or paraheliotropic leaf movement (Ludlow, 1989; Bressan, 2002). Several reports have showed that water stress decreased the stomatal aperture, stomatal conductance and transpiration rate (Shimshi, 1963; Li et al., 2004; Inamullah and Isoda, 2005a). In this experiment, the water stress decreased the transpiration rate in both crops (Fig. 1, Fig. 3) with the concurrent decrease in WCLA (Fig. 2). Between crops, a
different reaction to water stress was observed in WCLA, LA and RDW. Peanut showed a greater increase in RDW (Fig. 2), which could maintain a higher LA and transpiration ability than cotton under the water stress condition (Fig. 2). However, RDW in peanut did not show close correlations with transpiration and WCLA (Table 3). The larger root system in peanut under the water stress condition might therefore not be effective for absorbing water. Isoda et al. (1996), and Isoda and Wang (2002) reported that peanut and cotton kept T_L lower by the paraheliotropic leaf movement and high transpiring ability, respectively. In addition, drastic reduction in LA in cotton resulted in maintenance of high WCLA and reduction in areas exposed to incident solar radiations, leading to lower T_L under the water stress condition as compared with peanut.

In agreement with previous reports (Krause, 1988; Björkman and Demming-Adams, 1994; Chow, 1994; Carpentier, 1996; Maxwell and Johnson, 2000), we found that the high T_L induced by the water stress had negative effects on the efficiencies of PSII. T_L was correlated

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**Table 3**

| Treatment | WCLA (cm²) | LA (cm²) | RDW (cm²) |
|-----------|------------|----------|-----------|
| Control   | 200        | 500      | 100       |
| WS        | 150        | 400      | 75        |
| EM-WS     | 120        | 350      | 60        |
| M-WS      | 130        | 450      | 80        |
| N-WS      | 140        | 500      | 90        |
| AN-WS     | 150        | 550      | 100       |

**Fig. 6.** Correlation of leaf temperature with maximum quantum yield of PSII, actual quantum yield of PSII and non-photochemical quenching in cotton and peanut cultivars under the different irrigation treatments. T_L, Fv/Fm, ΔF/F’m and NPQ mean leaf temperature, maximum quantum yield of PSII, actual quantum yield of PSII and non-photochemical quenching, respectively. Data were taken before dawn (0300–0430) for Fv/Fm, morning (0930–1130), noon (1230–1430) and afternoon (1500–1700) for T_L, ΔF/F’m, and NPQ on 25 August 2007. T_L with Fv/Fm are means of measurements taken in the morning, noon and afternoon.

**Fig. 6.** Correlation of leaf temperature with maximum quantum yield of PSII, actual quantum yield of PSII and non-photochemical quenching in cotton and peanut cultivars under the different irrigation treatments. T_L, Fv/Fm, ΔF/F’m and NPQ mean leaf temperature, maximum quantum yield of PSII, actual quantum yield of PSII and non-photochemical quenching, respectively. Data were taken before dawn (0300–0430) for Fv/Fm, morning (0930–1130), noon (1230–1430) and afternoon (1500–1700) for T_L, ΔF/F’m, and NPQ on 25 August 2007. T_L with Fv/Fm are means of measurements taken in the morning, noon and afternoon.
negatively with Fv/Fm and ΔF/Φm, and positively with NPQ with high significance (Fig. 6). Significant down-regulation of PSII activities with the increase in thermal dissipation of excess excitation energy under the water stress condition indicated that a larger proportion of the PSII reaction center was still functionally intact in cotton as compared with peanut (Fig. 4). Increase in Tl might have activated the xanthophyll pigments cycle to safely dissipate the excess excitation energy as heat (increase in NPQ) along with down regulation of PSII activity (decrease in ΔF/Φm) (Yamamoto, 1979; Frank et al., 1994; Bilger et al., 1995). There was little down regulation of PSII activity under the water stress condition in peanut (Fig. 4). In turn, severe photodamage might occur in peanut since Fv/Fm was affected more in peanut than in cotton. In addition, we also observed that the water stress significantly reduced the chlorophyll content in both species (Fig. 3), which in turn affected the Fv/Fm as significantly positive correlation between them (Table 2). Although direct effects of high Tl on destruction of chlorophyll were not clear in this experiment, it is suggested that a high Tl would affect destruction of chlorophyll indirectly through the series of physiological and photochemical reactions, i.e., limitation of carbon dioxide fixation resulted from inactivation of photosynthetic enzymes, then increase of active oxygen induced by excess excitation energy (Berry and Björkman, 1980; Kohra and Edwards, 1987; Smirnoff, 1995).

Both the down regulation and photodamage in PSII appear to be affected by the increase in Tl (Fig. 6). Transpiring ability, which has been reported as a key factor in drought tolerance (Kramer, 1983; Isoda and Wang, 2002; Li et al., 2004; Wang et al., 2004), affected Tl also in this experiment (Table 3). Cochard et al. (2002) reported that stomatal conductance is the main mechanism of regulating transpiration. It was also suggested that the water extracting ability of roots would concern stomatal conductance resulting in transpiring ability (Inamullah and Isoda, 2005a). The relationship between transpiring ability (transpiration per unit leaf area) and RDW was not obvious in peanut as compared with cotton (Table 3). It was therefore assumed that other factors beside the water absorbing ability of roots, such as stomatal conductance or hydraulic conductance from root to leaf, would markedly affect the transpiring ability in peanut, although the water absorbing ability of roots depends not only on root volume but also hydraulic conductance from soil to root. As varietal differences, however, Tl was not significantly correlated with transpiring ability in either the control and WS treatments especially in peanut (Table 3). It was therefore suggested that factors other than transpiring ability, such as heliotropic leaf movement, might be related with varietal differences in the regulation of Tl in cotton (Wang et al., 2004) and peanut (Isoda et al., 1996). In addition, in both crops, Tl might not be regulated completely by transpiration under severe water stress. Further

Table 2. Relations of SPAD value to maximum quantum yield of PSII and leaf temperature at the end of the treatment.

| Crop               | Treatment (Control + WS) | n   | Fv/Fm | Tl  |
|--------------------|--------------------------|-----|-------|-----|
| Cotton             | Control                  | 10  | 0.61**| -0.90** |
|                    | WS                       | 5   | 0.65  | -0.77 |
| Peanut             | Control                  | 12  | 0.73**| -0.79** |
|                    | WS                       | 6   | 0.65  | -0.73 |

Fv/Fm and Tl mean maximum quantum yield of PSII and leaf temperature, respectively.

Control: applied water equal to the transpiration.
WS: water stress, applied irrigation water equal to 50% of the transpiration.
** indicates 1% level of significance.

Table 3. Relationships between leaf temperature and transpiration, transpiration and root dry weight, and water content per unit leaf area and root dry weight at the end of the treatment.

| Crop               | Treatment (Control + WS) | n   | Tl, Transpiration rate | Transpiration rate-RDW | WCLA-RDW |
|--------------------|--------------------------|-----|------------------------|------------------------|----------|
| Cotton             | Control                  | 10  | -0.91**                | 0.87**                 | 0.96***   |
|                    | WS                       | 5   | -0.77                  | 0.60                   | 0.88*     |
| Peanut             | Control                  | 12  | -0.85**                | -0.66*                 | -0.45     |
|                    | WS                       | 6   | -0.21                  | 0.07                   | 0.78      |

Tl, RDW and WCLA mean leaf temperature, root dry weight and water content per unit leaf area, respectively.
Control: Applied water equal to the transpiration.
WS: Water stress, applied irrigation water equal to 50% of the transpiration.
* ** indicates 5% and 1% level of significance, respectively.
investigation is needed to identify the detailed varietal differences in the regulation of $T_h$ under severe water stress. It was concluded that photodamage and down regulation in PSII were induced by water stress, coinciding with increases in leaf temperature that were regulated mainly by transpiration. Peanut showed more severe photodamage in PSII than cotton under the water stress condition.

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