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PHYSIOLOGICAL INDEXES OF *POPULUS EUPHRATICA* LEAVES FROM DIFFERENT CANOPY POSITIONS IN THE LOWER REACHES OF TARIM RIVER

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Abstract

Populus euphratica have serious degeneration under the long-term stress in the lower reaches of Tarim River and many trees began to wither from upper canopy to lower canopy. In this paper, differences in physiological indexes of *Populus euphratica* leaves between upper canopy, middle canopy and lower canopy, which response to the external stress were studied. The results showed that the contents of water (RWC), soluble sugar (SS), free proline (Pro) and the activities of peroxidase (POD) decreased from lower canopy to upper canopy, while the content of malondialdehyde (MDA), the activities of superoxide dismutase (SOD) and the ratio of chlorophyll *a* and chlorophyll *b* (Chl a/b) increased. These results indicated that the external stress on the upper canopy was higher than the middle and lower canopy. That may be the main reason resulting in the death of the upper canopy. The contents of chlorophyll *a* (Chla), chlorophyll *b* (Chlb) and total chlorophyll (Chlt) were slightly higher in the middle canopy than in other canopies, indicating the ability of absorbing light was same in canopies. These findings from this study will contribute to the best management of *Populus euphratica.*

Introduction

Plants were often easily affected by various environmental stresses under natural conditions, such as high temperatures, drought, and salinity. Among these stresses, drought and salinity were the widespread environment problems (Zhou & Yu, 2009; Khan *et al.*, 2012), especially in the arid and semi-arid areas. These stresses limit the growth and productivity of most plant species in these areas. Understanding the plant responses to the prevailing environmental stresses is important for effective management and rehabilitation of the degenerated ecosystems. Plants have evolved a series of mechanisms to maintain turgor pressure and protect their metabolism from external stresses through the changes of physiological indexes, such as the activities of peroxidase (POD) and superoxide dismutase (SOD) and the contents of soluble sugar (SS), free proline (Pro) and chlorophyll (Kim *et al.,* 2010; Zhang *et al.,* 2010; Khan *et al.,* 2011).

Several environmental factors vary with different positions in a plant crown (Araujo *et al.,* 2008). These differences of environmental factors also bring different stresses for leaves in different canopy positions. The leaves in the different canopies have changed some physiological indexes to adjust the external stress. So, it is important to study the physiological changes for understanding the cause resulting in the wilt of plants. *Populus euphratica* plays a very important role in maintaining ecosystem function and improving environment in arid and semi-arid regions. The distribution area of *Populus euphratica* in the world is about 64.87×10^4 hm^2, and the forest in Tarim River Basin account for about 54% of the total area in the world. The rest is mainly distributed in Russia (Wang & Chen, 1995). After the Daxihaizi Reservoir was built in 1972, the downstream of Tarim River was dried up (Yu *et al.*, 2011). The depth of groundwater was lowered to 8 ~ 12m in the downstream. The decrease of the groundwater, the loss of soil water and the high salt concentration in soil lead to the degradation of *Populus euphratica* (Ye *et al.,* 2009; Chen *et al.,* 2010). Zhuang & Chen (2006) and Chen *et al*., (2004b) have studied the physiological responses of *Populus euphratica* under the drought stress, and the results demonstrated that the physiological indexes, such as MDA, SOD, ABA were more susceptible to changes of the environmental factors.

In present study, we divided the tree crown into 3 canopies, upper canopy, middle canopy and lower canopy, and investigated the difference of physiological indexes between the 3 canopies and their role in osmotic adjustment under the external stresses. The study will help in understanding the adaptation mechanisms of stresses in plants in arid and semi-arid regions.

Material and Methods

Plant materials: All *Populus euphratica* plants used in this study were about 5 ~ 7m in height, healthy and free of infections. These trees were chosen from the Alagan section in the lower reaches of Tarim River, Xinjiang Province, China. The crown of each tree was divided into three canopies, lower canopy, middle canopy and upper canopy (Fig. 1). Then, we collected 50 ~ 70 full expanded leaves from each canopy around the tree. These leaves were packed in sealed plastic bags, and then placed immediately into an cooler containing liquid nitrogen for transport to laboratory.

Measurement of water content (RWC): The leaves were cut into pieces and their fresh weights were determined (W_f). Then, these leaves were killed at 105°C for 15 min, and dried at 80°C to constant weight for recording the dry weight (W_d). The water content of leaves was determined by the following formula:

$$RWC(\%) = [{(W_f - W_d)}/{W_f}] \times 100\%$$

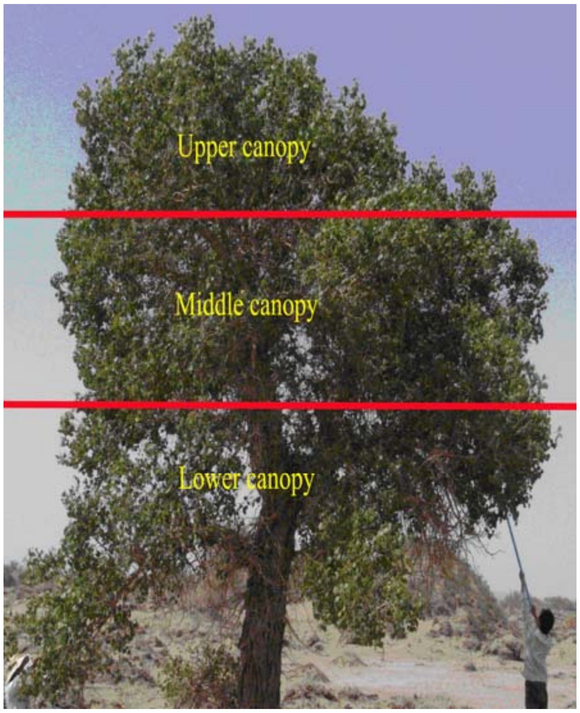

Fig. 1. The three canopy of the *Populus euphratica*.

Measurement of malondialdehyde (MDA), superoxide dismutase (SOD) and peroxidase (POD): The MDA content of leaves was measured in the thiobarbituric acid (TBA) reaction as described by Dhindsa *et al.,* (1981). Leaf samples (0.5 g of fresh leaves) were ground in 2 ml of 10% trichloroacetic acid (TCA). After 10 min of centrifugation (4000 rpm), 2 ml of the supernatant was added to 2 ml of 0.6% thiobarbituric acid (TBA). The mixture was heated at 100°C for 15 min and then cooled. The specific absorbance (at 532 nm, 600 nm and 450 nm) of the extract was detected.

Activity of SOD was determined by using the NBT photoreduction method as described by Lin *et al.,* (2010). Fresh Leaves (0.5g) were ground to a fine powder in liquid nitrogen and then homogenized in 2 ml of extraction buffer. The homogenate was centrifuged at 10000 rpm for 15 min at 4°C, and the supernatants were collected and placed in 4000 lux sunlight for reduction. The absorbance (at 560 nm) of the extract was detected by using a UV-265 ultraviolet- visible spectrometer.

Activity of POD was determined based by the method of Zhuang & Chen (2006). Fresh Leaves (0.5g) were ground and then homogenized in 2ml of extraction buffer. The homogenate was centrifuged at 4000 rpm for 15 min. 2 mL mixture of 50 mL Phosphate buffered saline (PBS, pH 6.0), 28μL methyl catechol and 19μL 30% H_2O_2 was mixed with 1 mL enzyme liquid, and the activity was determined immediately by using a UV-265 ultraviolet-visible spectrometer at 470 nm at 1 min intervals.

Extraction and estimation of chlorophyll: Leaf tissues (0.2 g) were homogenized in chilled 80% (v/v) acetone overnight. The absorbance of the acetone extracts was measured at 663 and 645 mm. Chlorophyll*a* (Chla), chlorophyll*b* (Chlb), total chlorophyll (Chlt), were calculated according to the formula:

$$C_a(\mathrm{mg{\cdot}L^{-1}}) = 12.72\ A_{663} - 2.69\ A_{645}$$
$$C_b(\mathrm{mg{\cdot}L^{-1}}) = 22.88\ A_{645} - 4.68\ A_{663}$$
$$C_T(\mathrm{mg{\cdot}L^{-1}}) = 20.21\ A_{645} + 8.02\ A_{663}$$

In which, A_{645} and A_{663} represent the absorption under the 663 and 645 mm. C_a, C_b and C_T represent the concentration of Chla, Chlb and Chlt. The contents of the Chla, Chlb and Chlt were calculated as follows:

$$Chl = C \times V/W$$

where, C represents the concentration of chlorophyll (mg/L), V represents the volume of the extracts (mL), W represents the weight of the sample (g).

Measurement of soluble sugar content (SS): Soluble sugars were determined by the anthracenone colorimetric method. 0.3g of fresh leaves were homogenized and oven-dried at 80°C, leached for 30 min in 100°C hot water after the addition of 6 mL 80% ethanol, and then centrifuged for 5 min; this procedure was repeated twice. Three extracts were made up to the required volume with 80% ethanol. Tubes containing 1 mL extract were placed in a water bath to boil off the ethanol, and 1.5 mL distilled water and 5 ml H_2SO_4 was added. The mixture was agitated until the sugar was dissolved. Optical density was determined at 630 nm by using a UV-265 ultraviolet-visible spectrometer (Najafi *et al.,* 2010).

Measurement of free proline content (Pro): Free proline content was determined based on the method of Bates *et al.,* (1973). 0.5 g of fresh leaves were homogenized in 5 ml 3% aqueous sulphosalicylic acid and placed in a boiling water bath for 10 min. After cooling, the liquid was centrifuged at 13000 rpm for 5 min. The supernatant was used for estimation of free proline content. The reaction mixture consisted of 2 ml supernatant, 2 ml acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100°C for 40 min. After termination of reaction in bath, the reaction mixture was extracted with 4 ml of toluene. Color matching was carried out at 520 nm by using a UV-265 ultraviolet spectrophotometer.

Statistical analysis: All measurements were performed at least three times. The values are expressed as the means of the three independent experiments and their standard errors. Statistical analysis was carried out with the SPSS statistical computer package (SPSS 13.0 for windows, Release 13.0, 1 Sep 2004, USA).

Results

The RWC content of *Populus euphratica* leaves in different canopy positions: Water plays an important role for the survival of plants, normal physiological activity can be done under the optimum water conditions. The RWC can reflect the water status of plant tissue. As shown in Fig. 2, our results confirmed that the RWC content of *Populus euphratica* leaves significantly decreased with the increasing of leaves position from lower layer to upper layer.

The MDA content of *Populus euphratica* leaves in different canopy positions: The MDA content of leaves was monitored in lower, middle and upper canopy positions (Fig. 3). A slight decrease of MDA content of leaves from upper canopy to lower canopy were observed in the lower reaches of Tarim River. The MDA content at the upper canopy, middle canopy and lower canopy was 0.0496, 0.0428, 0.0386 µmol/g, respectively.

The Pro and SS content of *Populus euphratica* leaves in different canopy positions: The contents of soluble sugars (SS) in *Populus euphratica* leaves generally increased from the upper canopy to lower canopy (Fig. 4). It is noticed that the SS content of *Populus euphratica* leaves at the middle and lower canopy was significantly higher than that at the upper canopy. However, the difference of SS content at the middle and lower canopy was small. The Pro content of leaves had the same change trend as the SS content (Fig. 5). It increased with the decreasing of leaves position.

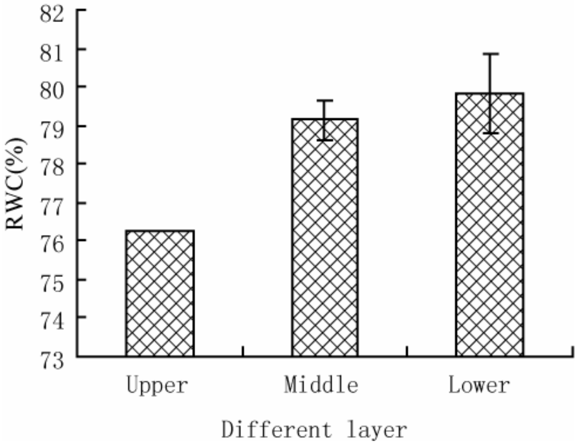

Fig. 2. The RWC content of leaves in different canopy positions.

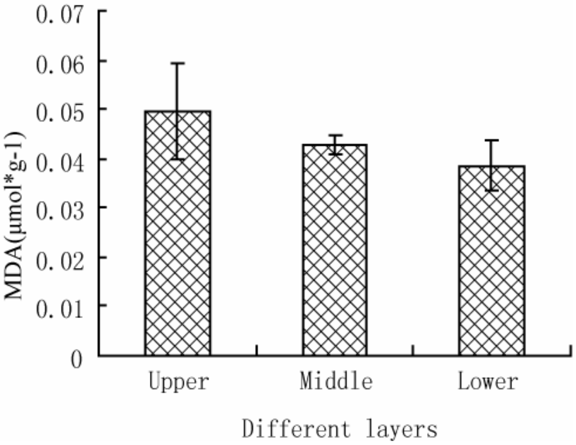

Fig. 3. The MDA content of leaves in different canopy positions.

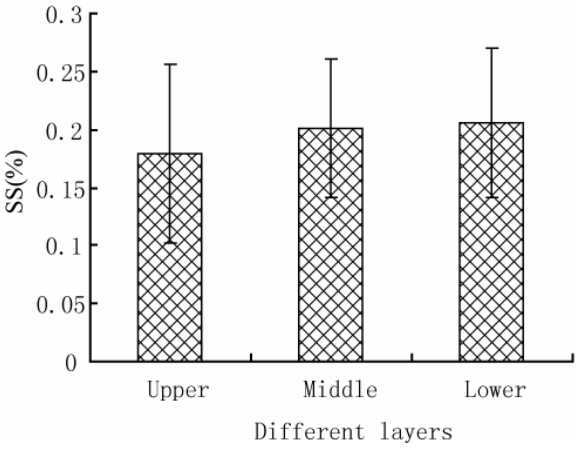

Fig. 4. The SS content of leaves at different canopies.

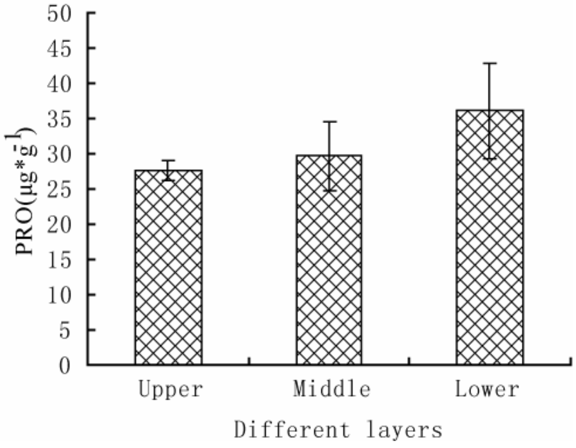

Fig. 5. The Pro content of leaves at different canopies.

The Chl content and Chla/b ratio of *Populus euphratica* leaves in different canopy positions: The contents of Chlt, Chla, Chlb and Chla/b ratio of *Populus euphratica* leaves in different canopy positions are presented in Fig. 6 and 7. As shown in Fig. 6, the contents of Chlt, Chla and Chlb in *Populus euphratica* leaves at the middle canopy were all higher than that at the upper and lower canopies, and the contents of Chlt and Chla in leaves were all significantly higher than that of Chlb at the 3 canopies. It is noticeable that, unlike the changes of Chlt, Chla and Chlb content at different canopies, the ratio of Chla/b was marked decreased from the upper canopy to lower canopy (Fig. 7). The ratio of Chla/b at the upper, middle and lower canopy was 4.28, 4.23 and 3.96, respectively.

The POD and SOD activities of *Populus euphratica* leaf in different canopy positions: The change trend of POD and SOD activities of *Populus euphratica* leaves in different canopy positions were founded in Fig. 8 and Fig. 9. From the upper canopy to lower canopy, the activities of POD in *Populus euphratica* leaves significantly increased, especially from the middle canopy to the lower canopy, which increased 25.57 u/g min (Fig. 8). In contrast, the activities of SOD in *Populus euphratica* leaves significantly decreased from the upper canopy to the lower canopy with the leaf position decreasing (Fig. 9).

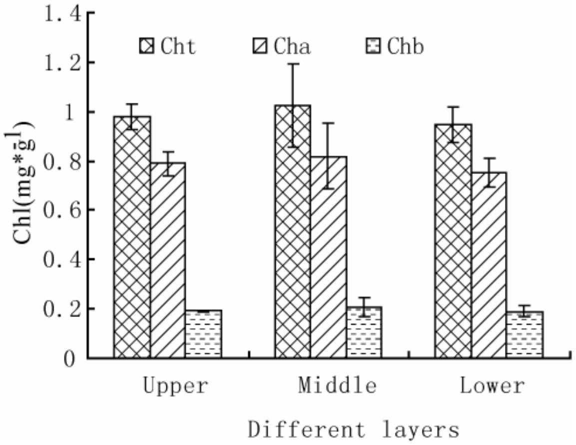

Fig. 6. The Chlt, Chla and Chlb content of leaves at different canopies.

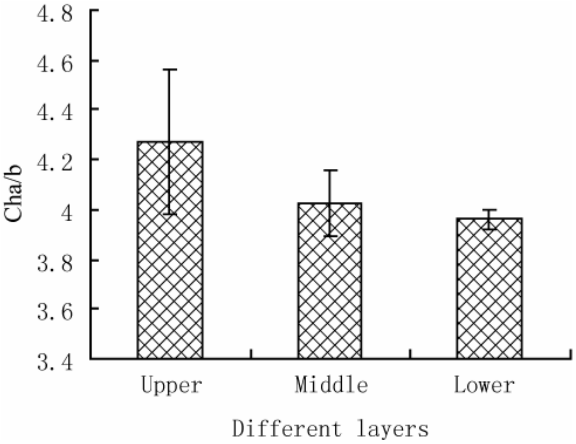

Fig. 7. The ratio of leaves Chla/b at different canopies.

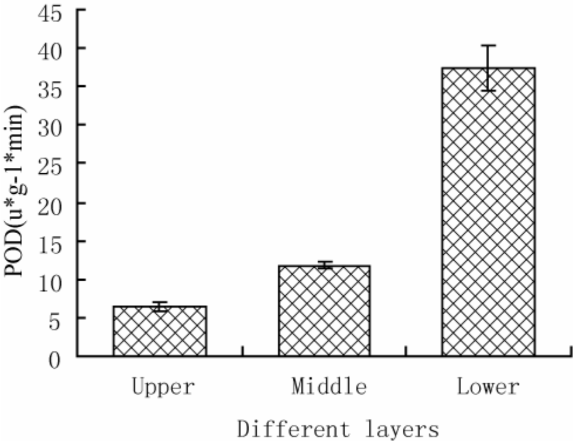

Fig. 8. The POD activities of leaves at different canopies.

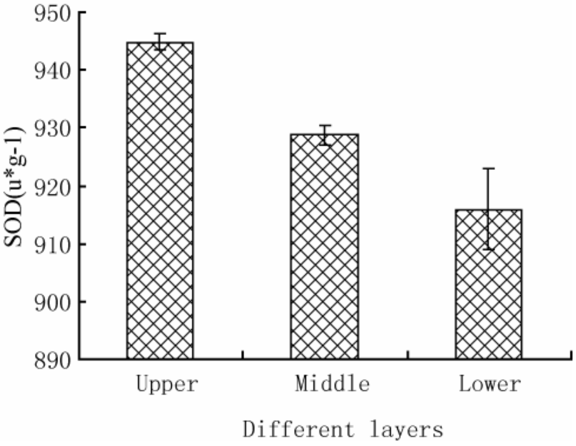

Fig. 9. The SOD activities of leaves at different canopies.

Discussion

Drought is one of the most important environmental factors limiting growth and yield of plants (Chaves *et al.,* 2003). During the past several decades, groundwater depth in the lower reaches of Tarim River had decreased greatly, with severe impacts on the survival and development of the local vegetation (Xu *et al.,* 2007). Plant is capable of sensing external stress when the environment changed and through self-regulation system to occur the adaptive responses of physiological and morphological. The physiological indexes of *Populus euphratica* leaves also underwent many changes to improve the viability under extreme drought stress. Water was the main component of cell protoplasm. The leaf water content reflected the physiological conditions of plants under stress. As is shown in Fig. 2, the RWC content of *Populus euphratica* leaves at the upper canopy was significant lower than that at the middle and lower canopy. It appeared that, the lower and middle canopy leaves had priority to get more water for using in the dry environment.

As the main product of peroxidation, MDA has a strong toxic effect, the accumulation of MDA in the plant cells reflects the movement of free radical and the damage conditions of cell. It had been used to measure the response of plants to stress (Sudhakar *et al.,* 2001; Michel & Kaufmann, 1973). Chen *et al.,* (2004a) studied the relationships between the MDA content of *Populus euphratica* leaves and different groundwater levels. They reported that the MDA content of *Populus euphratica* leaves was significantly accumulated along with aggravation of drought stress and decrease of groundwater levels. From the change trend of MDA content of *Populus euphratica* leaves (Fig. 3), our results indicated that the external stress which did their work on the upper canopy was significantly higher than it on the middle and lower canopy. The cause could possible be due to some changes in the peroxidation or the decrease of water content from lower canopy to upper canopy.

Free proline (Pro) and soluable sugars (SS) can play an important role in the osmotic adjustment of the plant under stress. The free proline, as a compatible osmolyte, can help lower the osmotic potential in cell (Chen *et al.,* 2003; Ru *et al.,* 2009). Plants increased the free proline levels by proline biosynthesis under different stresses, such as hydrolysis of proteins and oxidative degradation process (Dungey & Davies, 1982; Eva *et al.,* 2010; Rayapati *et al.,* 1991). In our experiment, free proline was accumulated with the decrease of leaves position, indicating an adaptive mechanism operating under long-term stress. It has been found that other organic compounds, such as soluble sugars, also increased when plants were subjected to stresses and the increase of soluble sugar was proportional to osmotic adjustment (Sanchez *et al.,* 1998). So, we can assume that the increase of soluble sugar content in *Populus euphratica* leaves was also an osmotic adjustment. The contents of Pro and SS were lowest at the upper canopy (Figs. 4 & 5), indicating that the leaves at the upper canopy was already in degradation.

The contents of photosynthetic pigment in leaves were an important indicator for photosynthetic capacity. The change in chlorophyll contents has been used to evaluate the influence of environmental stress on plant growth and yield. In our study, the Chla, Chlb and Chlt content of leaves was highest at the middle canopy, but the difference between the three canopies was slightness. The result indicated that the light harvesting complex of thylakoid membranes was not altered significantly under the stress. Our result agreed with those of Xiao *et al.,* (2008), who reported that the chlorophyll almost unchanged under the progressive drought stress. The reasons that they had slight differences between the three canopies, could possibly be due to their slow synthesis or fast breakdown. In addition, the ratio of Chla/b significantly increased from the lower canopy to upper canopy. These may be closely related to some changes in the lipid or protein ratio of chlorophyll.

Reactive Oxygen Species (ROS), including superoxide radicals (O_2^-) and hydrogen peroxide (H_2O_2), which are generated during the normal cellular metabolism, can cause oxidative stress to cell (Foyer＆ Noctor, 2005). Plants subjected to stress often enhances the oxidative damage (Ru *et al.,* 2009). The balance between ROS and antioxidant ability was important for plants to overcome the various stresses (Xiao *et al*., 2008). Mechanisms of sustaining the balance existed in all plants, such as superoxide dismutase (SOD), peroxidase (POD) and so on (Reddy *et al.,* 2004). SOD is known to convert O_2^- into H_2O_2, representing an important protective mechanism against possible stress induced ROS production. POD not only catalyzes the breakdown of H_2O_2, but also may participate in the oxidation of phenolics in leaves under external stress (Ru *et al.,* 2009). In our study, the activities of SOD continually decreased from the upper canopy to the lower canopy and POD activities significantly increased from the upper canopy to lower canopy, indicating that they may have stronger ability of scavenging ROS and avoiding damage of oxidative stress. The difference in POD and SOD activities may be closely related to the H_2O_2 level in leaves.

Different plant species have evolved different mechanisms of resistance. *Populus euphratica* in the lower reaches of Tarim River had serious degenerated, and some upper canopies of *Populus euphratica* already withered away. All these results indicated that the physiological indexes for different canopies of *Populus euphratica* are different. These differences show that the stress on the upper canopy is higher than that on the middle and lower canopy. These differences of physiological indexes in stress responses also provide some useful explanations for the wilt of upper canopy.

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