ANATOMICAL CHARACTERISTICS OF VASCULAR BUNDLES ASSOCIATED WITH HEAT TOLERANCE IN *Phragmites australis*

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Abstract. Reed plants were grown in a controlled laboratory environment. The aim of study is explain the effect of heat stress to anatomical feature of vascular system in *Phragmites australis*. The results show that high temperature increasing the diameter and density of xylem vessels. However, high temperature decreases the density of xylem vessels. There are differences in development of vascular bundle at 25 °C. It is represented as optimum degree for growing. When the temperature increased to 35 °C and 45 °C, the diameter of the metaxilem vessels and the diameter of the phloem are decreased over time until 18 weeks to the lower diameter. The diameter and vascular distances are showed an increase in the thickness of cell walls with increased thermal stress. Sclerenchyma cells are increased in number with increased exposure to heat stress. A comprehensive study of the temperature effects on plant vascular tissues and water can help us to understand plant responses to climate change. Thus improving breeding programs for climate-resistant types.

Keywords: *Phragmites australis*; stress; metaxylem; phloem; vascular bundles.

INTRODUCTION

The main components of climate change are atmospheric CO_2, air temperature, and drought [8, 12, 13]. The average global surface air temperature has already increased by 1-2 °C, and is expected to rise by another 2-3 °C by 2100 [13] with one of the warming of northern latitudes [14]. Since the industrial revolution, carbon dioxide in the atmosphere has increasing, with an existing concentration of more than 400 μmol (CO_2) mol^{-1}, and concentration of 700 μmol (CO_2) mol^{-1} of the end of the century [13, 14]. Under ambient conditions of CO_2 and O_2, photosynthesis and the export rates of assimilates in *Salvia* declined as leaf temperature were raised from 25 °C to 40 °C [25]. Genotypes or lines within the different types of their ability to respond to stress heat. For example, when 12 open-pollinated families of *Picea glauca* Moench seedlings were exposed to high temperatures of 42 °C to 50 °C for 30 min., the stability of photosystem II, as determined by chlorophyll fluorescence, decreased as temperature is increased [8]. To overcome the effects of high temperature, plants also have evolved to adapt/acclimate and survive in stressful environmental situations [3, 6, 20]. It is important to identify these heat tolerant lines so that breeding programs can be used these plants to improve thermal tolerance of new releases [15]. Many living organisms, including plants, accumulate Heat Shock Proteins (HSP) at response to non-lethal temperatures to prevent the transcription of natural proteins [8]. Small Thermal Shock Proteins (sHSP) with molecular weights of 15 to 30 kD are classed of HSP which is predominate in plant types during thermal stress [13]. It is reported that there is significant variation in thermal tolerance within these varieties. One of the most important anatomical characteristics of plants that undergo heat stress is the vascular system. Water transport through xylem is more effective than *plasmodesmata* of parenchyma [18, 16]. Xylem consists of conductive units (conifer tracheid or angiosperm vessels) are responsible for transporting water and flow it through the stem to the leaves [2, 7, 22]. Previous studies have indicated that vascular anatomy is important in plant adaptability. For example, the common oak trees (*Quercus robur* L.), which died in response to the extensive drought and these anatomical features may increasing susceptibility to drought [5]. Also, vegetation shifts due to climate change lead to more ecological drought [17] and can affect plants and their vascular system in the new environment. Often, these changes occur as a result of dehydration caused by an imbalance between water by the roots and water loss through leaf [3], with conditions extremely temperature changes that have the greatest impact on plant survival and productivity. In particular, the physiological function of vascular tissue is very weak as the survival of plants depends on their ability to conserve water supplies to the crown of plants under changing environmental conditions [15]. Therefore, the objectives of this research were to study the effects of the heat and heat stress on the anatomical trial of vascular system and adapting to reed plant (*Phragmites australis).*

MATERIALS AND METHODS

The genus *Phragmites* were selected in this study based on their response to high temperatures as described by Kim [1]. Is one of family Poaceae comprising of the most common perennial, rhizomatous, of temperate and tropical wetlands of all over the world. It has ability to mitigate the climate changes. Experimental plants were prepared from new growths brought from their natural habitat were planted in diameter of 10 cm (432 cm^3) using mixed soil (50% sand + 50% clay). All plants were grown and each treatment was applied in one in environmental growth chambers. For control stations and growth periods between pre-treatments, day and night temperatures were keeped at T1= 25 °C / day, T_2= 35 °C, with a combination of lamps capacity at 115W and T3= 45 °C, with a combination of lamps capacity at 60 W.

With light period of 500 µmol·m^{-2}·s^{-1} at 14 h and 70% R.H. (± 5%). Plants were irrigated and fertilized with 100 mgL^{-1} (NPK). The soil remained moist at all times during the experiment. Where the temperature was regulated at 25 °C for 3 hours at each day. This treatment has been identified to be most effective in preparation the heat. The plants were then transferred to two extremely temperature at 35 °C day and 30 °C night and 45 °C day and 40 °C night, for five weeks. The temperature was based on 18 °C to 25 °C, which it the optimal range for production of greenhouse. Temperatures from 35 °C to 45 °C usually occur in different climates during the mid-T1. The tissue samples and all plant measurements were taken at first, third, fifth week and at the end of the processing at 8 weeks. Leaves of each anatomical procedures can be divided into steps. For the paraffin double stain method with modification these steps: the tissue removed by sharp blade into pieces of 10×10 mm then putted in FAA (Formaline - Acetic Acid - Ethanol 10:5:85) for one hour followed by dhydration in a gradualy series of ethanol (from 100% to 50%) for 6 hours to remove the water from the tissue and then filtrated with a mixture of paraffin wax and zillion for two hours and then embedding in pure paraffin at 58 °C [1]. After that cleaning and staining with safranin solution for 3 h and washing by ethanol 70% then green solution of 30 m. Measurements were taken using camera microscope (AMCap 104, China) using an optical micrometer, which is calibrated by a phase micrometer. Temperature (T1) at 25 °C and 18 °C at night, temperature (T2) at 35 °C and 30 °C at night, and temperature (T3) at 45 °C and 40 °C at night are measured of first week (W1) and 3 weeks (W2), 5 weeks (W3), and 8 weeks (W4). Metaxylem vessels diameter showed the lowest value in W4 at T3 and the highest value in W4 at T1. There are no significant differences at all different weeks at T2. But W2, W3, and W4 at T1 and T3 there are significant differences in different weeks.

Statistical analysis

There are six units of treatment of the temperature into the growth chamber. ProcMix is adjusted using Tukey's method at SAS (SAS Institute, Cary, NC) to evaluate the significant differences between measured parameters.

RESULTS

There are two types of small and large of foliar vascular bundles in *Phragmites australis* leaf of reeds. A reed plant has two types of vascular. Both types consist of about four sectors, the xylem, phloem, sclerenchyma and bundle sheath (Fig. 1). There are significant differences in the dimensions of the bundles in different temperature and time. These differences are similar for both the small and large vascular bundle tissues, so only the characteristics of the large vascular bundles are selected for comparison (Figs. 2-4, and Table 1). There are no significant differences ($P<0.05$) at different temperatures. But there are significant differences of different weeks. Metaxylem vessels diameter showed the lowest value in W4 at T3 (21 µm) and the highest value in W4 at T1 (29.8 µm) (Table 1, Figs. 2-4). There are no significant differences ($P<0.05$) at all different weeks of T_2. But W_2, W_3, and W_4 at T1 and T3. Phloem diameter showed the lowest value in W1 at T3 (25.9 µm) and the highest value in W3 at T1 (36.2 µm) (Fig. 1 and Table 1). There are significant differences ($P<0.05$) at all temperatures in all week and the lowest value in W2 in T1 (75 µm) and (65 µm) and the highest value in W1 in T3 (88 µm) and in T3 (85.3 µm), respectively (Table 1, Figs. 2-4). There are significant differences ($P<0.05$) at all temperatures and weeks. The lowest value in T1 in W1 (171 µm) and greater leaf blade thickness is at T_3 in W_4 of 230 µm (Table 1, Figs. 2-4). The vascular distance and vascular diameter are meaned the lenth and wideth of vascular.

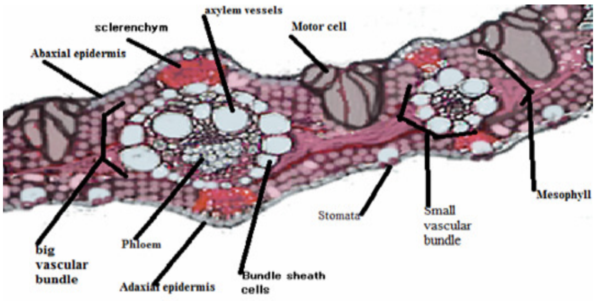

Figure 1. Leaf cross-sections of *Phragmites australis* in control treatments

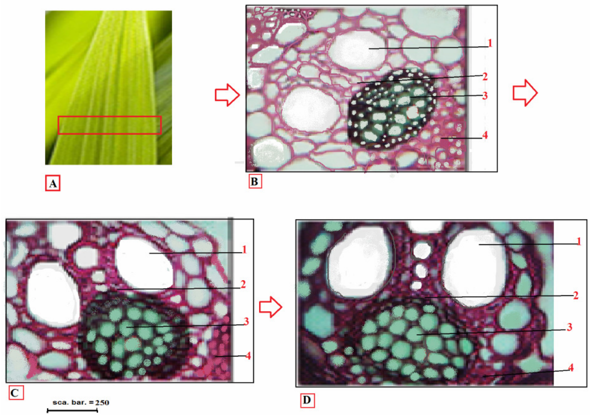

Figure 2. Cross sections of vascular bundle tissues of *Phragmites australis*. The images are shown in ×40 magnification, (1= xylem, 2- parenchyma cells, 3- phloem, 4- sclerenchyma cells) different letters (a–d) indicate variation between plants in different weeks in T1 treatments

Table 1. Variation in anatomical feature of vascular bundles in different temperature and time in *Phragmites australis* plants

Temperature	T1				T2				T3			
Time / Parameter (µm)	W1	W2	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4
Metaxylem vessels diameter	25 b	24 B	24.5 b	29.8 c	24.6 b	28.5 c	28 c	28 c	23.8 b	23 b	21 a	21 a
Phloem diameter	31.5 b	28 A	36.2 b	26.4 a	35.8 b	34.6 b	31 b	31 b	25.9 a	35 b	32 b	31 b
Vascular diameter	80.4 b	75 A	81.3 b	82.4 b	76 a	78.6 a	82 b	85 b	88 c	84 b	84 b	83 b
Vascular distance	77.3 c	65 A	67.5 a	70.3 b	75.9 c	71.5 b	79 c	81 d	85.3 e	79 c	81 d	79 c
Leaf thickness	171 a	218 B	216 b	226 e	186 a	215 b	220 b	222 b	188 a	224 b	221 c	230 d

T1- Temperature treatment 25 °C and 18 °C at night, T2- Temperature treatment 35 °C and 30 °C at night, T3- Temperature treatment 45 °C and 40 °C at night, W1- Plants in Control and first week, W2- Plants after 3 weeks, W3- Plants after 5 weeks, W4- Plants after 8 weeks

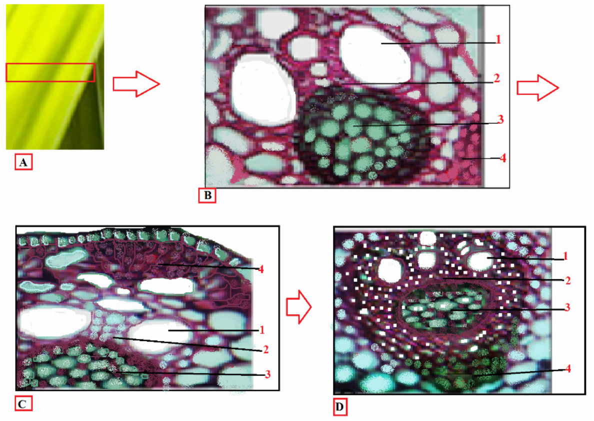

Figure 3. Cross sections of vascular bundle tissues of *Phragmites australis*. The images are shown in ×40 magnification, (1- xylem, 2- parenchyma cells, 3- phloem, 4- sclerenchyma cells) different letters (a–d) indicate variation between plants in different weeks in T2 treatments.

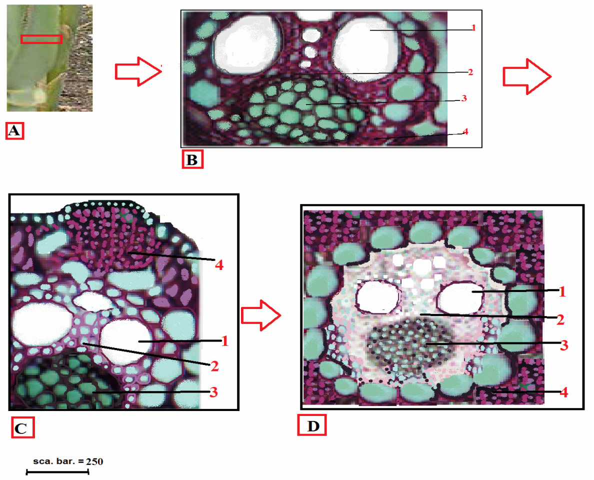

Figure 4. Cross sections of vascular bundle tissues of *Phragmites australis*. The images are shown in ×40 magnification, plate show (1- xylem, 2- parenchyma cells, 3- phloem, 4- sclerenchyma cells) different letters (a–d) indicate variation between plants in different weeks in T3 treatments.

DISCUSSION

The vascular bundle is very important due to their roles in photosynthesis and exchange of substances. The size of the vascular bundle has strong negative correlation with heat stress [10, 11, 21]. Leaf vascular bundles are differed significantly with different temperatures and weeks. T1 in W1 exhibited higher metaxylem vessels. W1 has higher vascular bundles. Bundle-sheath cell, phloem diameter, and lesser in T3 implying more water and solutes. It were exchanged and photosynthesis more efficient in *Phragmites australis* that were normally under heat stress. It indicating different mechanisms for water in the stress environments and possibly leading to their stress [24]. The vascular bundles and the distance between vascular bundles are considered important taxonomic characteristics in pine [4]. In order to predict how the plant hydraulics is respond to thermal stress, whether structural or functional ingredients, such as plant metabolism, properties of xylem tissue, vascular engineering, and leaves thickness are needed to be research the development of vascular tissues including wood tissue [9, 26]. There is no author who made similar studies. Also, these data are presented for the first time in the world in this article. This study is a novelty at a global level.

Thermal stress is increasing the thickness of the total leaves and then increasing the resistance of plants and their ability to survive. The results are indicated that the thickness and higher-frequency cultivars in the vascular system has achieved higher biomass aggregation to compensate heat loss. These plants also obtained a more rapid cover and able to maintain the health of leaves and provide potassium for reproductive development and root growth. These properties can be used to provide guidance for breeding of heat resistant varieties. The research has significance in studying the plant adaptations of thermal stress and thus knowing the limits of tolerance of plants and the possibility of increasing them for the purpose of increasing the plant's resistance to climate changes.

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