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A NEW METHOD TO IDENTIFY THE COLONIZATION LEVEL OF RIPARIAN VEGETATION SPECIES

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Abstract. Riparian vegetation is constantly subjected to environmental stresses and reactive oxygen species are generated in the plant tissue due to the stress. The study examined the effect of elevation on riparian vegetation along the Hii River in Japan by quantifying the environmental stresses, using foliar hydrogen peroxide (H_2O_2) concentration. In the riparian zone, soil moisture uniquely decreased with the elevation from the ordinary water level. Leaf samples of four common riparian species, *Phragmites australis*, *Phragmites karka*, *Juglans mandshurica*, and *Salix pierotii*, were used for the investigation. Leaf samples were collected at different elevations along the river. The results indicated that foliar H_2O_2 concentrations of *P. australis*, *P. karka*, *and S. pierotii* decrease with increasing soil moisture, indicating low levels of stress due to higher availability of soil moisture. However, *J. mandshurica* showed the opposite trend. Biomass and chlorophyll data indicated the threshold H_2O_2 concentration for colonization. The colonization levels of individual species could be determined by combining the studied parameters. Our study revealed that H_2O_2 is a very efficient and reliable index for quantifying environmental stress and the colonization level for vegetation management.

Keywords: riparian vegetation, environmental stress, hydrogen peroxide, soil moisture, colonization level

1. INTRODUCTION

Maintaining and improving biodiversity in potential riparian areas and the surrounding environment have been areas of focus lately (Gurnel et al. 2016). It is well reported that the riparian areas are core habitats for a wide range of semiaquatic and terrestrial species and therefore they are regarded as the ‘biodiversity corridors’ (Nallaperuma and Asaeda 2019; Corbacho et al. 2003). Each and every riparian species have its own ecological function and significance in the riparian zone. Despite the importance of riparian vegetation, dense trees obstruct flood flow, increase the upstream water level and causes bank collapse during floods. Many species of riparian vegetation are hydrochory in nature and seeds or other propagules disperse in shorelines during floods (Tockner et al. 2000; Bunn and Arthington 2002; Asaeda et al. 2011a). Thus, flood water plays a key role in this process of the recruitment and colonization of riparian vegetation (Mahoney and Rood 1998; Sanjaya and Asaeda 2017).

The available moisture along a riparian zone is a critical factor for plant occurrence (Ozinga et al. 2005), as water acts as a different type of support agent by initiating germination and determining plant establishment and growth. In a riparian zone, floods are a source of water to the ecosystem (Leyer 2005; Richardson and Pysek 2012), though water availability varies along a river gradient depending on the distance and elevation from the channel (Vanden Broeck et al. 2004). In general, water availability strongly affects the growth patterns of vegetation (Mulholland and Hill 1997). However, both hydrophilic and hydrophobic species live along riparian zones, and it is difficult to determine the most suitable location and conditions for each species. Thus, in vegetation community management, there is always a need to identify species-specific, suitable locations. Regardless, very limited information is currently available.

Plants suffer from oxidative stress when exposed to harsh environments, which results in an enormous amount of reactive oxygen species (ROS) in cells (Sharma et al. 2012). Under normal conditions, a balance exists in the activities of reactive oxygen species and antioxidants generated as a defense mechanism. However, under stressful conditions, antioxidant defenses become insufficient, resulting in oxidative stress that often leads to cell death (Jana and Choudhuri 1982; Fleury et al. 2002; Asaeda and Rashid 2017; Asaeda et al. 2020). Among the different types of ROS, superoxide anion (O_2^-) and hydroxyl radicals (OH^-) are the dominant species generated under stress and are routinely converted into relatively stable hydrogen peroxide (H_2O_2), via either superoxide dismutase (SOD) or nonenzymatic systems. Thus, the concentration of H_2O_2 has a strong potential to provide information regarding the environmental stress imposed on plants (Asaeda et al. 2018; Asaeda et al. 2020).

The objective of the current study is to understand the species-specific preferable condition for colonization along the riparian zone by quantifying environmental stress using hydrogen peroxide concentrations.

1. MATERIALS AND METHODS

2.1 Study site and species

Riparian vegetation is highly affected by sediment flows during floods, particularly gravel deposition (Asaeda and Sanjaya 2017; Sanjaya and Asaeda 2017). Thus, the most typical sand-bed river in Japan, the Hii River, was selected for field observations in connection with the study objective. Field observations were conducted along the Hii River in western Japan. The Hii River, originating in the Chugoku Mountain range, flows into Shinji Lake and Naka Lake downstream before draining into the Sea of Japan. The river extends approximately 153 km and covers approximately 2017 km^2 of the total basin area. Eighty percent of the basin is covered with forest, and 20% of the downstream area is covered with rice fields and residential areas. In contrast to most Japanese rivers, this river is characterized by sandy channels including riparian zones, except for the upstream reaches, because of a large discharge of sand due to iron mining many years ago. Sediment particle size was D_{50}=0.1-0.5mm, and relatively uniform regardless of the distance from the water. Although the riparian zone of this river was once moderately vegetated (Sanjaya and Asaeda 2017), vegetation coverage has increased enormously, particularly in recent years, and is dominated by two herbs, *P. australis* and *P. karka,* and two tree species, *Salix* spp. and *J. mandshurica*. Therefore, these four species were observed in this study. The vegetation coverage has become one of the most important issues in the management of this river (Izumo River Management Office, Shimane, Japan). Observations were conducted at 13.8 km to 15.6 km from the river mouth and along one tributary that flows into Shinji Lake (35º 21’48.6” N 132º 47’19.8” E). Riparian vegetation in this area is located at elevations depending on species-specific needs, and the vegetation is often colonized in the layer along the slope of the riparian zone (Asaeda et al. 2018).

2.2 Plant and soil sampling

Observation and sample collection were done at the right bank of a 1.2 km stretch (13.9 km to 15.1 km from the mouth) of the river. On 13 October 2016, all *Salix* spp. and *J. mandshurica* trees taller than 1 m were marked and the location and elevation from the normal water level were measured. Leaves were collected from selected. Several leaves from each tree were collected for H_2O_2 and antioxidant enzyme assays, and determination of photosynthetic pigments. For herbaceous species, leaves were collected from quadrats (50 m × 50 m) set across the elevation gradient with a distance of approximately 5 m. For setting the quadrats, spots with typical homogenous stands of *P. australis* and *P. karka* were selected. The collected leaf samples were sealed in plastic bags and then transported in an icebox filled with dry ice (~ −70 °C) to the laboratory, where the samples were frozen at −80 °C until analysis. The locations of trees and quadrats were recorded using GPS (Garmin eTrex, Garmin Ltd. Olathe, Kansas, United States), and aerial photos along the river line were taken using a drone (DJI Spreading Wing S900, DJI Japan Corporation, Tokyo, Japan). For biomass estimation, aboveground shoots of *P. australis* and *P. karka* from 1 m × 1 m area within the quadrats were harvested. Belowground biomass was excavated until all biomass was retrieved. Sediment up to a depth of 30 cm from the ground surface was sampled at each location and then tightly sealed in plastic bags for transport to the laboratory.

2.3 H_2O_2 assays of plant leaves

Immediately after bringing to the laboratory, the fresh plant leaves were frozen dried with liquid nitrogen and ground (~500 mg) with ice cold pH 6.0, 50 Mm phosphate buffer. Polyvinylpyrrolidone (PVP) was added for the extraction to mask the effect of phenolic compounds in the plant materials. The extraction was centrifuged at 5000 rpm at 4^0 C for 15 minutes and the supernatant was separated. The concentration of H_2O_2 was determined by pre-prepared standard curve for known concentration series. The reaction mixture contained 750 µL enzyme extract and 2.5 mL of 1%$TiSO_4$ in 20% H_2SO_4 (v/v) and the mixture was centrifuged at 5000 rpm for 15 minutes at 20 °C temperature. The intensity of yellow color developed through the reaction was measured spectrophotometrically (UV mini 1210, Shimadzu, Japan) at the wavelength of 410 nm Jana and Choudhuri (1982). The results are presented in µmol/g FW.

2.4 Herb biomass determination

In the laboratory, the aboveground and belowground parts of the herbaceous species were through washed with running tap water and air-dried. Then they were dried for three days (or more) at 70 °C in an electric oven until no change in weight was recorded, and the dry weight was obtained. The biomass was calculated in g/m^2.

2.5 Soil analyses

The moisture contents of the soil samples were determined using weight loss method. For this, the soil sample was weighed initially and subjected to drying at 105 ºC in an electric oven until a constant weight over time was recorded. Soil moisture content was estimated by the difference between initial and finals weights of the sample. Total nitrogen (TN) in the sediment (oven dried) was analyzed by CHN elemental analyzer (Yanako CHN coder MT-5 and Auto sampler MTA-3, manufactured by Yanako CO., Ltd., Japan). Total phosphorus (TP) in the sediment (oven dried) was determined by digesting the samples with HNO_3-$HCLO_4$, and the molybdenum blue colorimetric method was applied (Murphy and Riley 1962; APHA 1998).

2.6 Statistical analysis

Raw data were checked for normal distribution with the one-sample Kolmogorov-Smirnov test as well as for homogeneity of the variances with the Levene's test. All data were subjected to one-way analysis of variance (one-way ANOVA), followed by Turkey’s multiple comparison test to evaluate mean differences at a 0.05 significance level ($p < 0.05$). Pearson’s correlations were evaluated for the chlorophyll *a*/*b* ratio, antioxidative enzyme activity, soil nutrient concentrations, and total biomass (aboveground and belowground). All statistical analyses were performed using IBM SPSS version 25.

3 RESULTS

3.1 Soil moisture distribution

Figure 1 shows the distribution of soil moisture content as a function of the elevation from the ordinary water level. Soil moisture did not differ between observed years, and gradually decreased with increasing elevation away from the channel.

The relationship between soil moisture content and site elevation follows exponential functions:

$$\text{Soil moisture (\%)} = 45\exp(-0.265 \ast \text{elevation (m)})\ (r=0.972,\ p<0.01) \qquad (1)$$

The figure also shows the elevation range of each species. The colonization elevations were *P. australis*: 0.3-2.0 m, *P. karka*: 1.0-3.0 m; *S. pierotii*: 1.0-3.0 m; and *J. mandshurica*: 2.5-4.0 m.

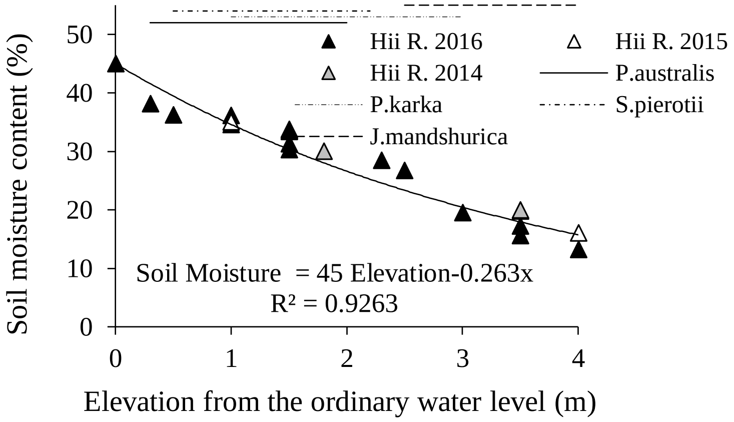

Figure 1. Soil moisture distribution with respect to the ordinal water level

3.2 H_2O_2 concentration related to edaphic condition

The H_2O_2 concentration gradually decreased with increasing soil moisture content for all of the examined species, except for *J. mandshurica* (Pearson’s correlation; *P. australis*: R = 0.90, $p < 0.01$; *P. karka*: R = 0.69, $p < 0.05$; and *S. pierotii*: R = 0.94, $p < 0.01$) (Figure 2). Its concentration in *J. mandshurica* decreased with increasing soil TN concentration (Pearson’s correlation; R = 0.84, $p < 0.01$), and there was no significant trend for the other species (Pearson’s correlation; *P. australis*: R = 0.41, $p < 0.01$; *P. karka*: R = 0.19, $p < 0.05$; and *S. pierotii*: R = 0.35, $p < 0.01$). The ratio of soil TN and TP was between 3 and 5.

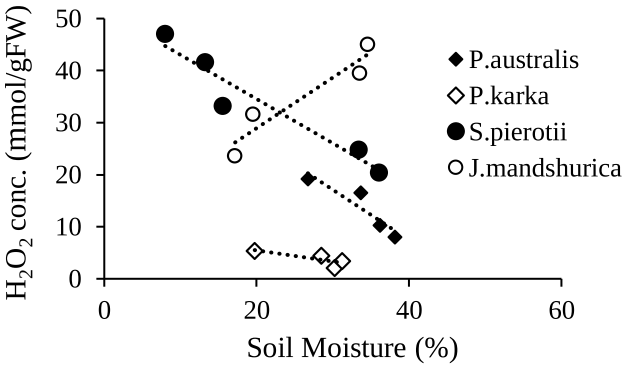

Figure 2. H_2O_2 concentration of leaf tissues with respect to soil moisture

3.3 Plant distribution

Above ground biomass of both *P. australis* and *P. karka* decreased significantly with increasing H_2O_2 concentration (Pearson's correlation; *P. australis*: R = 0.99, $p < 0.01$ and *P. karka*: R = 0.93, $p < 0.01$) (Figure 4). However, the below ground biomass of both species did not exhibit a difference with the H_2O_2 concentration.

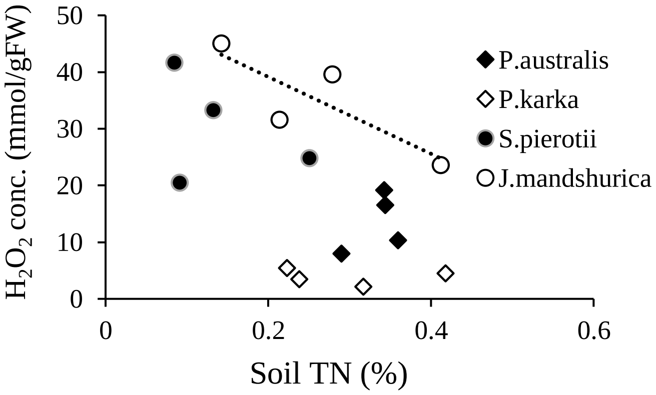

Figure 3. H_2O_2 concentration of leaf tissues with respect to soil TN contents

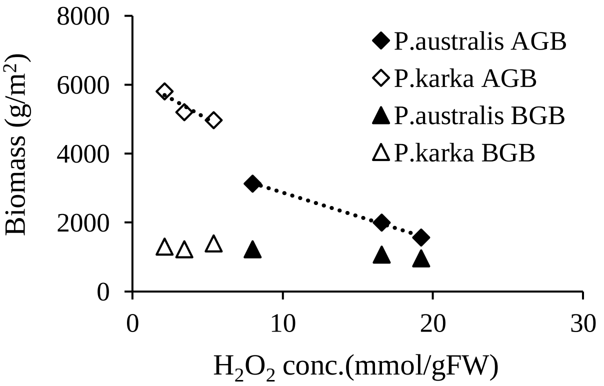

Figure 4. Biomass of *P.australis* and *P.karka* with respect to H_2O_2 concentration

Figure 5 indicates the ratio of chlorophyl and b, which indicates preferable condition. The ratio was sufficiently high until 50μmol/gFW of H_2O_2 concentration.

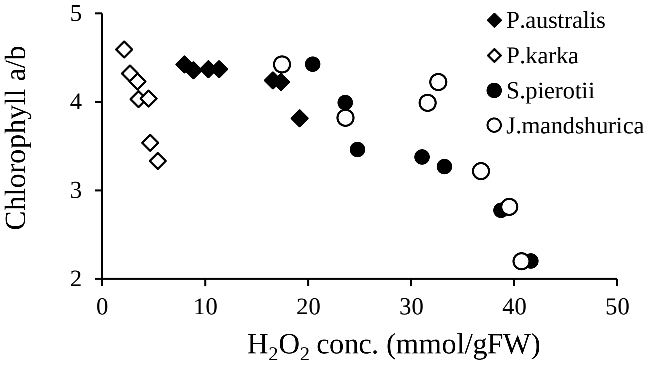

Figure 5. The ratio of chlorophyl a and b with respect to H_2O_2 concentration

4 DISCUSSION

Riparian vegetation can be found at species-specific elevations, and colonization often occurs in the layer along the slope of the riparian zone (Gregory et al. 1991; Naiman and Décamps 1997). Riparian vegetation is highly dependent on flood flows (Asaeda et al. 2011b). At the same time, however, seeds are spread downstream by flood water (Nilsson et al. 2010; Hernandez-Leal et al. 2019), followed by colonization at sites with favorable conditions (Rashid et al. 2013; Herberg et al. 2017; Rashid et al. 2019).

The effect of elevation on plant growth is not yet completely understood. The internal ROS generation with elevation is a contemporary frame of reference. Chlorophyll *a* is the main pigment responsible for the photochemical reaction, and chl *b* supports chl *a* by supplying more light energy. Under slight darkness, when chl *a* performs poorly, chl *b* has a major role in supplying more light energy to chl *a* to maintain efficient photosynthesis. Thus, chl *b* is more subjected to stress, suggesting a lower amount of light-harvesting proteins associated with the reaction center complex. However, stomata close associated with low-moisture conditions, resulting in a carbon dioxide deficit and a surplus of light energy. This process increases oxidative stress and reduces the photosynthesis rate. Thus, the ratio of chl *a* and *b* is directly related to the oxidative stress generated by a surplus of ROS. For the species treated here, chl *a* and *b* ratio keeps high values with H_2O_2 concentration of lower than 20μmol/g for *P.australis*, and 35μmol/g for *S.pierrotii*, and *J.mandshurica*. These values are considered as the threshold H_2O_2 concentration for the possible colonization. Figure 2 shows that higher than 30% soil moisture is required for *P.australis* and 20% for *S.pierotii*, and less than 20% soil moisture for *J.mandshurica* to satisfy the condition. Following Figure 1, colonization level is given as lower than 2m for *P.australi*s and 3m for *S.pierotti* and higher than 2.5m for *J.mandshurica* from the ordinary water level, which agrees well with the observation.

Vegetation management requires monitoring of plants. Common approaches are adaptive management to understand the condition of plants, where the growth and prosperity or shrinkage of stands are monitored for a long period. It is extremely time consuming and costly. The current approach used in the study quantifies the environmental stress in relatively no time, which involves a very simple methodology of analysis there by helping to quantify the environmental stress associated with different species and to understand the level of risk associated with each species and manage them accordingly (Asaeda et al. 2020). This method also helps to restore the species that are on a verge of extinction by examining the favorable location of colonization quantifying the environmental stress in a short period.

5 CONCLUSIONS

The results of the current study show the effect of elevation gradient and moisture variation on the abundance of different species along a riparian zone. Species-specific zones of colonization or recruitment were clearly revealed using hydrogen peroxide as an environmental stress quantification index.

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