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論文の内容の要旨

Mangrove forests are known to provide a number of important ecosystem services that contribute to human well-being. However, despite of its functions and values, they are one of the valuable habitats that have suffered widespread habitat loss due to increasing population density and continuing economic development in the coastal areas. The alarming rate of mangrove decline has encouraged the government and multi-lateral sectors to undertake rehabilitation initiatives as a tool to restore damaged habitats. The rehabilitation programs gained more momentum after the 2004 Asian tsunami and the 2013 Typhoon Haiyan in the Philippines as a Bioshield along coastlines. The *Rhizophora* species were commonly selected for rehabilitations due to the year-round seedling availability and it can be planted directly without requiring a nursery culture. However, the results of mangrove restoration programs include stories of mixed successes and failures. Several programs were unsuccessful as they fail to match environmental conditions to species-specific preference and resulted high mortality of the plantation. In cases where high survivals were achieved, only single mangrove species was planted, creating a monospecific plantation.

Being at the interface between the land and sea, the establishment and survival of mangrove seedlings are under the continuous influence of different abiotic drivers, particularly the salinity fluctuation, elevation gradient and tidal inundation cycles. The salinity tolerances of mangroves have been the focus of research for several decades. However, differences in findings remain unresolved. Some reported that low salinity provided the best condition for growth while others proved that some species achieved optimum growth at higher salinity. Tidal inundation is another factor that influences the mangrove community and considered to have a key role in mangrove ecology and seedling establishment and species distribution. In extreme conditions, these environmental stressors disrupt the physiological homeostasis of mangroves and consequently cause the induction of reactive oxygen species (ROS) resulting in oxidative stress and eventual mangrove mortality when conditions remain unfavorable. ROS are highly oxygen derivatives that are

formed as a by-product of metabolism. However, during times of stressful conditions, ROS are excessively generated resulting damage in cell structures. As a response to counter the adverse effects of the increases in ROS, mangroves have developed natural defense systems including the activation of antioxidant enzymes (AOX) to scavenge the ROS and a range of species-specific morphological, physiological and ecological mechanisms. . Understanding the ecology and adaptability of mangroves to the interplay of abiotic stressors requires an interdisciplinary knowledge of botany, physiology, geography and molecular and genetic sciences. However, the species-specific ecophysiology is not yet well established in the paradigm of mangrove ecology. This study assessed and compared the natural and planted mangrove forest structure and determined the levels of biochemical stress and ecophysiology of *R. stylosa* to salinity, elevation and inundation in greenhouse microcosm and field conditions. Understanding the species ecophysiology could serve as a tool in determining the species niche-width preference, vital information for selecting optimum conditions and suitable areas for science-based mangrove rehabilitation programs.

The field stud was conducted in Olango and Banacon Islands, in the central part of Philippines where *R. stylosa* plantations were established over 60 years ago. An aerial survey was conducted with a drone during the first sampling to identify the distribution of the planted and natural mangrove areas and served as a basis in subsequent sampling. Transect line method was employed traversing perpendicular to the shoreline and the mangrove forest structure and ecological indices (density, diversity, girth size, height) were assessed. In the subsequent field works, the leaves of *R. stylosa* were collected from natural and planted forests in the part of the upper canopy directly exposed to sunlight. For every sampled tree, the light intensity and the pore water salinity was measured in situ and the elevation gradient was estimated based on the mean water level at neap tide. The collected samples were immediately frozen with dry ice inside a thermal box and transported to the laboratory. On the other hand, for microcosm experiments, mature propagules of *R. stylosa* were collected in Olango Island and two experimental set up were conducted. In the first setup, the propagules were individually planted in seed bags and cultured in aquarium tanks filled with different salinity treatments: low (LS) – 0 ppt, moderate (MS) – 20 ppt and high salinity (HS) – 35 ppt. The seedlings were arranged on the top of a platform and irrigated with low water (LW, 3-5 cm), mid-water (MW, 10-13 cm) and high water (HW, 30-33 cm). The developments of the first leaves were monitored, and the average height, biomass and leaf tissues were measured and sampled at 5 and 10 months. In another setup, the seedlings were cultured at the low-water level in three different salinity treatments. After 15 months, the seedlings were subjected to inundation hydroperiod simulating the tidal cycle as semi-diurnal inundation (SDI), diurnal inundation (DI) and permanent submersion (PS) for one week. These microcosms simulated emerged and inundated conditions, mimicking intertidal inundation that seedlings would experience. Leaf samples from field and experiments were analyzed for hydrogen peroxide (H_2O_2), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD), pigments, carotenoids, Fv/Fm and proline. Statistical analyses were done using XLSTAT Premium.

The mangrove forest structure (density, species diversity, average height, girth size) showed a significant difference between the natural and planted forests. The planted forest had lower structural complexity than the reference natural forest. Even 60 years after the forest was created in Banacon Island, it still lacked the understory of young cohorts. While a forest has been created, it does not mimic a natural forest. Therefore, mangrove restoration programs should consider planting several species and maintain sufficient spacing for growth in order to reproduce in the rehabilitated areas some of the key ecosystem characteristics of natural mangrove forests and to achieve the best outcome and

functionality of the restored habitat. Results of the biochemical analyses showed that the H_2O_2 and AOX activities for samples collected at the higher elevation areas that rarely inundated have significantly higher values compared with the samples collected at the inundated areas. On the other hand, the Chlorophyll a and b of samples at the inundated areas showed an increasing with the salinity, while those in higher elevation and rarely inundated areas showed a decreasing trend. The long-term negative effects of high H_2O_2 at the plant and community levels were manifested in the reduction of growth rate in plants cultured in the greenhouse and the reduction of height in the 30-year-old *R. stylosa* plantation.

The results of the greenhouse experiment showed that salinity significantly influenced the early growth and biomass production of *R. stylosa*. Adaptive morphological plasticity was exhibited in the early establishment and growth of the greenhouse-cultured *R. stylosa* seedlings. At 5 months, the highest average height and growth rate was observed in seedlings cultured at low salinity, whereas at 10 months, the highest average height was observed in moderate salinity. This implies a temporal shift in favorable conditions between the 5 and 10-month culture periods. Another morphological plasticity was exhibited in the biomass partitioning between the 5 and 10-month culture periods. Comparing the AGB and BGB allocations, at 5 months, those cultured in moderate and high salinity had higher allocations for BGB, while those in low salinity had higher allocations for AGB, as shown by the root-to-shoot ratio. However, at 10 months, there was a shift in biomass allocations. The seedlings cultured in moderate and high salinity had higher allocations for AGB, while those in low salinity had higher allocations for BGB. The observed morphological plasticity of *R. stylosa* seedlings cultured in saline conditions helped them cope with the stressful environment by changing their root to shoot ratio in response to the stressors. Additionally, physiological adaptation was exhibited in the proline accumulation in the leaves between the 5- and 10-month culture periods. The proline content of the leaves showed a significant increase for seedlings cultured in the moderate and high salinity at 10 months compared with at 5 months. The increase in proline accumulation signifies physiological adaptations to saline conditions, which is consistent with the observed changes in growth and biomass production. Results of the biochemical analyses of leaf tissues showed that levels of ROS and AOX activities were significantly lower in the emerged condition than those in an inundated condition. Periodic inundation (SDI and DI) imposed a higher order of stress as indicated by ROS levels compared with the effect of salinity, while prolonged inundation (PS) caused sublethal damage as manifested by the chlorosis of the leaves in MS and HS treatments. The chlorotic leaves developed relatively faster in HS than in MS, appearing after four and five days respectively; whereas in LS, chlorosis was not observed even at the end of the experimental period. The pigments (Chl a and b), carotenoids and Fv/Fm showed a significant reduction relative to the inundation hydroperiod in all salinity treatments, and the PS showed the highest reduction. Inundation and salinity both significantly influenced the reduction, but the inundation was the most influential factor. Another physiological plasticity was observed in the temporal variation of pigments and carotenoids between the 5- and 10-month periods. At 5 months, the temporal variation showed a negative correlation with salinity and a positive correlation at 10 months. The seedlings cultured in low salinity had a significant reduction at 10 months, whereas those in moderate and high salinity treatments showed a significant increment. The increment in saline conditions supports the observed higher RGR at 10 months of the culture period. Higher pigments translate into higher photosynthetic capacity and energy production and therefore a faster growth rate.

Extrapolating ecophysiology of R. stylosa, this species had low tolerance to inundation stress (high ROS and AOX, reduced pigments and Fv/Fm). Translating this low tolerance to field conditions, in the frequently inundated areas (i.e. seafront mangroves fringes) that are subjected to longer inundation at spring tides, this species may suffer from oxidative stress, stunted growth and consequently low survival.

論文の審査結果の要旨

マングローブの生態系サービスが重要視される中、様々な国でマングローブ植林が行われている。通常に、植林には、胎生種子が適度な大きさであることから、植林後の生存率が高く、植え易いことから *Rhizophora* 属の種が用いられることが多い。しかし、それでも植える場所によって植林後の生存率には大きな開きがあり、それに従って、生長後のマングローブ林も大きく異なる。フィリピン、セブ島沖の島では、島自体が珊瑚礁で出来上がっており、植林後のマングローブ林の生存率も極めて高い。そのため、植林によって、単一種の極めて密な群落が作られ、他の種の進入も遮られることから、多様性も極めて低くなるため、改善が求められている。こうした背景の下、本論文は、植林場所を決定する際に重要になる、植林後のマングローブの初期生長に伴ういくつかの特性について調べたものである。

本研究は、温室実験とフィリピンでのマングローブ林の野外調査で進められ、それらの結果を同一の指標を用いて整理している。

温室において、所定の条件で、*Rhizophora stylosa* の胎生種子からの生長実験を行い、生長速度と最初の葉をつけるまでの日数、最大光量子効率、活性酸素のひとつである過酸化水素濃度を用いて、環境ストレスの強度を評価、さらに、抗酸化作用として、アスコルビン酸ペルオコシダーゼ、カタラーゼ、グアイヤコルペルオキシダーゼ及びプロリン濃度を測定、ストレス耐性のしくみを考察している。主たる結果は以下のようなものである。

1）塩分耐性及び感水深の影響について

実験では、塩分濃度は 0PPT、20PPT、35PPT の 3 種類で、冠水深は、3-5cm、13-15cm、33-35cm で変化させた合計 9 種類の条件で行っている。その結果、最初の葉の出るまでの日数は、冠水深はほとんど影響しないものの、塩分濃度が低い程早く葉が出るという結果になっている。また、平均樹高、地上部、地下部のバイオマスは、5 ヵ月後、10 ヵ月後共に、塩分濃度が低い程高い値となっている。ただし、地下部と地上部の比は、5 ヵ月後には高塩分濃度のケースの方が高かったのに対し、10 ヶ月後には低塩分濃度の方で高い値となっている。細胞内の浸透圧を調整して水分の吸収を助ける働きを持つプロリン濃度は、当塩分濃度程高くなっている。最大量子効率は大きく変わらないものの、過酸化水素濃度は低塩分濃度で低い値となっており、塩分はストレスとして働いていることが示されている。

これらを総合すると、5 ヶ月を経過した時点では低塩分の方が生長にとって適しているものの、10 ヶ月を経過すると、塩分濃度が多少高い方が適していることが示されている。生長に伴う変化とともに、徐々に塩分に順応していく過程の示された結果となっている。

2）冠水頻度に対する特性について

干潮帯に生育するマングローブ林は、一日のうちで 1 回もしくは 2 回冠水する。そのため、実験では、全体の冠水時間は同じでも、1 日に 3 時間ずつ 2 回冠水するもの、1 日に 1 回、6 時間冠水するもの及び常に冠水する条件で、数ヶ月にわたって生長実験を行っている。

その結果、3 時間 2 回の冠水よりも 6 時間、1 回冠水する方が、過酸化水素濃度は高くなっている。ただし、常時冠水した場合には低い値となっている。ここで、常時冠水した場合には、葉が枯れかかった状態になっていたことから、低い過酸化水素濃度は生理活性の低下した状況にあったことがわかる。これより、冠水期間が長くなることは、マングローブのストレスが高くなること、生長にとっては好ましくないことが示唆されている。

抗酸化活性は、過酸化水素濃度とほぼ相関する関係にあり、冠水期間が長くなることでストレスが増加す

るものの、抗酸化作用を増加させることに適応していることがわかる。しかし、クロロフィル量は、高塩分濃度の場合に比べ、長期間冠水した時の方がより大きく減少している。

さらに、冠水期間の差による過酸化水素濃度の変化と塩分濃度の違いによる差を比較すると、冠水は塩分濃度以上に大きなストレスになっていることが示されている。

温室における実験結果は野外観測の結果と比較されている。

平均海水面からの比高と過酸化水素濃度との関係を、温室実験と現地観測の結果で比較すると、概ね、比高の増加とともに過酸化水素濃度は増加する傾向にあった。比高が低いと冠水期間は長くなるものの十分な露出期間が確保されると、比高が上昇することによって土壌水分濃度が低下することは、ストレスを増加させることなどが示されている。

本論文の結果は、単に研究にとどまらず、セブ市沖の島で行われている植林事業に活かされている。こうした実践に向けた取り組みも本研究の成果の重要性を示すものとなっている。

このように、本論文は、実際の植林事業から明らかになってきた問題を、実験及び観測で明らかにしたものであり、結果も実際の事業に還元されているということで評価される内容である。