学 位 論 文 の 要 旨

論文題目 **Physiological Responses of Rice to Saline-Alkaline Stress**
(塩アルカリストレスに対するイネの生理的応答)

広島大学大学院統合生命科学研究科
Bioresource プログラム
学生番号 D192378
氏 名 Rowland Maganizo Kamanga

Salt stress is a major abiotic stress globally, affecting over 800 million ha of land. Depending on soil pH, salt stress can be categorized into neutral salt stress, popularly known as “salinity stress” and saline-alkaline stress, also known as “alkali” stress. Over the years, salinity stress has been extensively studied, hence physiological and molecular mechanisms underpinning plants’ responses to salinity stress have been thoroughly elucidated. Contrarily, relatively less is known regarding how saline-alkalinity confronts plant growth. Meanwhile, physiological mechanisms regulating plants’ resistance under saline-alkaline stress remain hugely conjectural. Rice (*Oryza sativa* L.) represents a crop of prominent worldwide food value in addition to being a key model research crop, yet known for its reputation of extreme sensitivity to salt stress. This study was aimed at unravelling comparative physiological mechanisms in which saline-alkalinity confronts growth and physiology of rice plants. Furthermore, the study explored the potential of acclimation to different stress components in developing cross-tolerance to saline alkaline stress.

In order to evaluate comparative physiological responses of rice to saline-alkalinity, 21 day old seedlings of rice cultivar Hinohikari were subjected to control conditions (0 mM Na^+, pH 5.5) neutral salinity stress (50 mM Na^+, pH 5.5), moderate and severe alkalinity (0 mM Na^+, pH 7.5 and 8.5 respectively) and moderate and severe saline-alkalinity (50 mM Na^+, pH 7.5 and 8.5 respectively) for 16 days. Time course changes in growth were monitored every 4 days. The study revealed that saline-alkaline stress was the most growth limiting, with growth nearly ceasing on the fourth day of stress imposition, whereas under salinity stress growth decreases were only apparent from the 12th day.

Furthermore, at the same growth media pH, sole alkalinity was less detrimental relative to saline-alkalinity, but more lethal than salinity stress. Therefore, effects of rhizospheric Na^+ are aggravated by growth media pH, rendering saline-alkalinity more harmful on growth than sole salinity and sole alkalinity.

Next, comparative physiology underlying saline-alkaline damage on rice seedlings was investigated. Plant grown under both alkaline and saline-alkaline conditions was characterized by chlorosis, which was confirmed to have resulted from Fe deficiency, particularly in younger leaves owing to immobile nature of Fe. In older leaves, alkaline stressed plants exhibited healthier leaves, whereas saline-alkaline stressed plants were severely chlorotic and nearly dying off. This chlorosis was ascribed to leaf blade Na^+ toxicity, which was inordinately high in saline-alkaline stressed plants, resulting into premature senescence of older leaves. Additionally, saline-alkaline stress incited K^+ deficiency predominantly in roots, due to tremendous root K^+ leakage, thus, while resistance to saline-alkaline stress may be complex and multigenetic, ability to regulate root K^+ loss may be a valuable trait. Furthermore, it was observed that under saline-alkaline stress, leaf blade membrane integrity was substantially compromised, due to toxic accumulation of Na^+ in leaf blades, and resultant oxidative stress as shown by high accumulation of H_2O_2 in leaf blades. Hence, it was concluded that at the used experimental conditions, rice plants are more sensitive to high growth media pH, and this sensitivity is aggravated under mixed saline-alkaline conditions. Primarily, hyperaccumulation of Na^+ in leaf blades, accelerated root K^+ deficiency due to root K^+ leakage, Fe deficiency in younger leaves, and oxidative stress mediated membrane damage were crucial causes of severity under saline-alkalinity.

Premised on the these findings, the study further explored the potential of acclimation in developing cross-tolerance to saline-alkalinity. Rice plants were pre-treated (PT) with three concentrations of each of NaCl, $NaHCO_2$, and H_2O_2 for one week, then subjected to 50 mM Na^+ at pH 8.25 for 14 days and investigated growth and physiological responses relative to non-pretreated (NPT) plants. Acclimation to 10 mM NaCl (salinity) and 10 μM H_2O_2 (oxidative stress) were the most effective, hence were the focus for further investigation. These PTs developed cross-tolerance to saline-alkaline stress, in addition to promoting quicker recovery and resumption of growth upon conclusion of stress. Physiologically, both PTs noticeably maintained a lower growth medium pH, employing a growth medium acidification strategy which was not further investigated. The lower growth medium pH also facilitated iron acquisition, whose uptake is else constrained under high pH. On ionic relations, NaCl pretreated plants accumulated much lower Na^+ in leaf blades by enhancing root Na^+ efflux. In H_2O_2 pretreated plants, despite higher root Na^+ efflux, leaf blade Na^+ accumulation was not reduced. However, experiments on Na^+ uptake in excised leaf blades showed lower accumulation of Na^+. These results may suggest that the high leaf blade Na^+ accumulation in H_2O_2 pretreated plants emanated from processes relating to either uptake by roots and/ or resultant transport to the shoots. That notwithstanding, deleterious effects of Na^+ were ameliorated and leaf photosynthetic competency was preserved by both H_2O_2 and NaCl PTs. Additionally, both PTs were unable to lower saline-alkaline – triggered root K^+ leakage, but efficiently reduced oxidative stress – triggered membrane damage and lipid peroxidation, and improved cell viability by constituting a complete shield of antioxidant enzymes SOD, CAT, APX and GR, establishing an ultimate equilibrium between ROS generation and scavenging.

These findings propose a possible crosstalk between salinity stress, oxidative stress and saline-alkaline stress, such that exposure to salinity stress or oxidative stress develops cross-tolerance to saline-alkaline stress. This is an important finding particularly in light of a myriad of abiotic stresses, offering a prospect for breeding plants resistant to a range of abiotic stresses. Besides, upon elimination of stress and resumption of normal growth conditions, pretreated plants, particularly those pretreated with H_2O_2 quickly resumed growth and physiologically recovered, a crucial attribute especially under recurrent abiotic stresses with some prolonged inter stress recovery periods. In order to provide a fairly broad picture of the mechanism of cross-tolerance, this study recommends a further exploration of molecular and perhaps epigenetic marks underpinning cross-tolerance, and an investigation of overlapping signaling elements between salinity, oxidative and saline-alkaline stress.