Plant adaptations to anaerobic stress caused by flooding

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Abstract. Flooding imposes a severe selection pressure on plants principally because excess water in their surroundings can deprive them of certain basic needs, notably of oxygen and of carbon dioxide and light for photosynthesis. It is one of the major abiotic influences on species' distribution and agricultural productivity world-wide. A cultivated species, *O. glaberrima* is origin from Africa that spread to floodplains area along river. Work to develop more tolerant crops or manage flood-prone environments more effectively is also included. Here, recent progress in elucidating the mechanisms determining tolerance versus intolerance to anaerobic stress caused by flooding in higher plants is discussed. This work integrates various specialized approaches ranging from morphology to physiology, and demonstrates how plant biology can be harnessed to improve stress tolerance in an important crop species. Materials and Methods: The research is conducted in various place of fields. As materials of rice and sugarcane, and chilli pepper are used in the experiments. Results and Discussion: We emphasize that *Sub1A* is not appropriate when selecting and breeding rice cultivars of *O. glaberrima* for resilience to longer-term submergence. Under these circumstances, a vigorous ethylene-mediated underwater elongation response by leaves is needed to return leaves to air-contact and full photosynthetic activity. Root aerenchyma is formed in waterlogged condition to on the genotype of sugarcane and oxygen in the air is supplied to the root system thorough aerenchyma. The supplied oxygen is used for root system respiration.

1. Introduction
Flooding imposes a severe selection pressure on plants principally because excess water in their surroundings can deprive them of certain basic needs, e.g. access to oxygen, carbon dioxide, and light for photosynthesis. It is one of the major abiotic influences on species’ distribution and agricultural productivity world-wide. A strong submergence-induced elongation is one of the most common escape mechanisms and one that helps submerged individuals regain or retain contact with the aerobic environment on which they depend [8]. However, the submergence tolerance gene, *Sub-IA*, uses a different strategy, depressing shoot elongation under short-term submergence to survive. Considering the circumstances of climate change and unstable environmental conditions in Tropical area, the ability to tolerate flooded conditions is a major constraint for sustainable agriculture in the region. Due to natural disasters caused by a heavy rain and/or change of precipitation pattern, rice production in Tropical region is often unstable though there is high demand and consumption. In recent years, the reports of flooding damage to rice plants have been increasing with the expansion of rainfed lowland rice cultivation. The strategic measures that rice plants employ to survive submergence vary according to the varying type of submergence, which is classified according to the water level and duration of the
submergence. A floating rice area is defined as the continuous presence of 1 to 6 m of water level during at least half of the rice growing period [2]. Deepwater areas are defined by the continuous presence of 0.2 to 1 m of water for longer than 1 month, and within that there is an intermediate deepwater area (20-50 cm). Shallow, submergence-prone areas are defined as areas which are submerged for short periods by flash floods [12]. However, it is difficult to use this classification system to describe the environments in rainfed environment.

Mechanism in intolerant cultivar to floodwater has shoot elongation and reorientation of leaves with hormonal regulation actively, in consequence rapid translocation of carbon to newly developing leaves during submergence resulted in plant exhausts carbohydrate [16]. On the other hand, tolerant cultivar is characterized by conservation of energy and carbohydrate and leaf acclimation by changed photosynthesis, in consequence submerged plant utilizes photosynthetic product stored before submergence effectively during submergence. These contrasting responses affect to survival and adaptation to aerobic condition at the post-submergence. Most rice plants show rapid shoot elongation in response to submergence. Shoot elongation enables rice plants to resume aerobic metabolism and photosynthetic fixation of CO₂ by raising their shoots above the water surface [14][7]. The rate of gas exchange is very slow in water, because of small diffusion coefficients for gases (oxygen, 0.21 cm² s⁻¹ in air; 2.38x10⁻⁵ cm² s⁻¹ in water) [1]. When water becomes stagnant, the concentration of oxygen becomes low at night because of the respiration of alga at night. Rice plants increase the rate of alcoholic fermentation under low oxygen environments. However, alcoholic fermentation produces only 2 molecules of ATP per glucose molecule, and is not efficient when compared with aerobic respiration, where 32 molecules of ATP are produced per glucose molecule. Therefore, rice cannot survive in a low oxygen environment for a long period, because of the shortage of carbohydrate for energy production. Furthermore, photosynthesis is limited by low irradiance when the plant is submerged. It is necessary to improve photosynthetic capacity and effective use of photosynthetic product as well to survive under water. On the other hand, most rice cultivars escape from anaerobic environments by developing a canopy above the water surface for photosynthesis. Rice plants can also survive by restoring aerobic metabolism during submergence. However, in short-term submergence, such as that caused by flash floods, rapid leaf elongation adversely affects the recovery of plants after desubmergence [19][6][10].

The wetland can be defined as the land that has a predominance of hydric soils and is inundated or saturated by surface or groundwater at a frequency and duration sufficient to support a prevalence of hydrophytic vegetation typically adapted for life in saturated soil conditions. The expansion of agricultural areas to feed a growing population has led to a major conversion of wetland into farmland in many countries, resulting in disappearance of 50% of the world’s wetlands for agriculture and urbanization in the twentieth century. However, there is still a need to increase agricultural production and crop production as well in developing regions of Asia and Africa where the vast area of wetlands is often kept unused for agriculture mainly because of traditional perception of the wetlands as wastelands, bad lands, or sources of disease. In the past, the conversion of wetlands to agriculture has been rarely done in a harmonious way with its natural environment and it is now becoming a big issue on how to restore those degraded wetlands. Extensive changes in water environment in wetlands have often led the degradation of physical and chemical properties of soils, microclimatic conditions of ecosystem that is subtly kept in balance and composition of biota. For future development of wetlands to crop production, it is very important to take into account the sustainability issue from the beginning. One way is, instead of making a considerable change in the wetland environments, to incorporate adaptation ability to the wetland into the crop that will be introduced in a particular wetland. Through this approach, agriculture and wetlands can maintain a very harmonious relationship.

2. Strategy of submergence tolerance in rice.
During complete submergence, none of plant part remains on water surface, those tunnels which facilitate gas exchange to aerial part seems useless without combination of other mechanism. In this case, some plant species consider to elongating faster in order to reclaim aerial surface contact. Because plants consider to escaping from oxygen depletion, this mechanism called as escape strategy. Other strategy, plant aims to stop their growth temporary (quiescence) and regrowth after the water subside [13]. It is interested in carbon translocation during submergence of anaerobic condition because of
limited photosynthesis. Through our investigation, a carbon translocation during complete submergence differed between genotype of quiescence and shoot elongation in rice [16]. Sub-1 expression plants translocate labelled carbon slowly form photosynthetic leaves before submergence to newly developed leaves during submergence compared to shoot elongation genotype. It may be a strategy of submergence tolerance. [11] indicated that a suppression of underwater elongation, brought about by the mutated form of Sub-1A in O. sativa, is beneficial for the endurance of complete submergence. Thus, non-shoot-elongation-cultivars during submergence show tolerance to short-term submergence, so-called flash flood for a few days or weeks. However, [15] emphasized that this trait is not appropriate when selecting and breeding cultivars of O. sativa or O. glaberrima in cultivated rice for resilience to longer term submergence. Under these circumstances, a vigorous ethylene-mediated underwater elongation response by leaves is needed to return leaves to air-contact and full photosynthetic activity for long term complete submergence. [18] demonstrated that the adverse effects are mainly caused by reduced photosynthesis capacity due to CO₂ starvation in the shoot organs during submergence. Furthermore they suggested that relationship between ethylene concentration, leaf chlorosis and leaf elongation. However partial submergence treatment to deep water rice never affects carbohydrate and sugar contents in newly developed leaves under the water compare to the control [17]. Elongation with floating ability is the most important morphological feature of deep water rice; in particular internode elongation is a more important mechanism for increasing shoot length. Internode’s elongation is closely related to the plant hormones. Submergence lowers the level O₂ in rice internodes; low O₂ levels simulate ethylene synthesis; ethylene accumulation in the submerged internodes; and the high internodal ethylene concentration increases the sensitivity of the tissue to gibberellic acid or increases the concentration of physiologically active gibberellins, thus leading to the growth responses commonly observed. Deepwater rice differ in their ability to accumulate carbohydrate content within a cultivars carbohydrate content does not correlate with total internode length or plant length [20]. I would like to focus attention on the flooding response of O. glaberrima, however, it is not clear from [5] whether shoot elongation contributed flooding tolerance in different water regime or not. To understand the physiological responses of young rice plants to short-term submergence stress so-called “flash flood” under rainfed conditions for O. glaberrima by comparison with several genotypes of O. sativa, thirty day-old seedlings were submerged completely for 10 d at 45 cm water depth at 13 d after transplanting in a lowland field [9]. O. glaberrima showed higher shoot elongation ability during submergence than any genotype of O. sativa that we tested. However, O. glaberrima lodged easily after desubmergence due to longer and rapid shoot elongation during submergence, and thus triggered a decrease in its survival rate (Fig.1). The submergence tolerant genotype of O. sativa maintained the dry matter weight of the leaf blade during submergence through the inhibition of shoot elongation by the quiescence strategy, thus a survival rate is 93%. The escape strategy for O. glaberrima is thus an effective usage of stored carbohydrate for shoot elongation in a severely photosynthesis-limited environment. However, failure to regain contact with air and the oxygen, carbon dioxide and light it supplies inevitably gives rise to serious carbohydrate depletion. Therefore, this escape strategy carries a high risk for young rice plant [11]. We suggested that O. glaberrima is susceptible to short-term submergence while it may adapt to prolonged flooding because of improved restoration of aerial photosynthesis and survival rate through shoot elongation ability. Enhancement of shoot elongation during submergence in water that is too deep to permit re-emergence by small seedlings represents a futile escape strategy that takes place at the expense of existing dry matter in circumstances where underwater photosynthetic carbon fixation is negligible. Consequently, it compromises survival or recovery growth once floodwater levels recede and plants are re-exposed to the aerial environment. From this viewpoint, shoot elongation ability to return to anaerobic growth condition in needed for long term flood survival.
3. Physio-morphological responses to waterlogging

Waterlogging is the saturation of ground with water. Waterlogging affects approximately 10% of the global land area and significantly reduces crop yield except for rice. In agricultural soils, the primary causes of waterlogging are heavy rainfall, but also inadequate soil drainage and particular soil conditions. According to trends in climate change under global warming, waterlogging risk will increase in near future. The saturation of soil pores with water reduces gas exchange with the atmosphere, so that oxygen concentration decreases rapidly. Anoxia is the first negative effect of waterlogging on plants, but also secondary effects can be detrimental to plant growth. Introduction of upland crops in lowland have been developed, such as wheat, soybeans and maize due to excess moisture injury is problematic in Japan. Using system of low cost control of ground water, FOEAS was introduced and reducing negative impact as injury of waterlogging to the production system have been obtained. On the other hand, progress will not go up in crop improvement research. Effective evaluation methods of versatile moisture resistant crops is not established enough. Growth traits in common upland crops, excess moisture injury factors and the distribution of ventilation, lignification of root, rooting ability, photosynthesis, respiration and oxidative reductivation [3]. Authors have been studied and obtained useful knowledge related waterlogging tolerance of crop like rice, sugarcane and chilli pepper.

Field tests were conducted in Tanegashima’s field for two years in 2017 and 2018 in Japan. The tested varieties used the local recommended varieties NiF8, NiTn18, Ni22. Cultivation was carried out according to the conventional method, and waterlogged treatment was performed between July 25 to August 8 in 2017 and July 18 to August 15 in 2018. Irrigated water was used as the waterlogged condition that the soil was saturated. Resistance of variety (Ni22) under waterlogging formed until the apical aerenchyma whereas aerenchyma of the root of susceptible cultivar (NiF18) is not formed (Fig. 2). Root porosity during waterlogging was larger significantly in Ni22 than in NiF18. Most likely this can involve waterlogging resistance of crops. On the other hand, submerged during the germination of plants is enormous with impact on the growth and yield, from applicant effect on the germination of rice seeds by flooding can vary depending on the breed and species clearly has [4]. And in this study, diverse field crops until the apical oxygen from the leaf sheath of air organization, even about the availability of oxygen for roots and examined to develop crop moisture evaluation approach, both on the root surface ROL barrier function were evaluated.
Chilli pepper is commonly grown by local farmers at riparian wetland during dry season in Indonesia. However, during the last decade, unpredictable distribution and intensity of rainfall have threatened chili pepper production at the riparian wetlands due to untimely water saturated rhizosphere (WSR) occurrences like waterlogging. This study was designed to evaluate morpho-physiological effects of short-term WSR exposure on chili pepper. Two Indonesian (Laris and Romario) and one Japanese (Takanotsume) chili pepper varieties were grown under waterlogged condition. Results of this study revealed that roots suffered more than aerial organs as indicated by increase in shoot/root ratio from 4.56 at pre-exposure to 7.03 after exposure. Total leaf area significantly reduced since fallen older larger leaves were replaced by newly developed smaller leaves. Relative water content (RWC) in all organs decreased, but not to detrimental level to the plants. Amongst organs, leaf was the most sensitive organ, RWC decreased from 83.6% at pre-exposure to 77.8% after exposure, but was able to rebound to 81.5% after 7 days of recovery. Both net photosynthetic and transpiration rates sharply decreased, associated with decrease in leaf stomatal conductance during WSR exposure and did not recovered after 7 days the WSR exposure was terminated (Fig. 3). Laris showing earlier recovery than the other varieties. Overall, Laris was better than the other two varieties in withstanding WSR condition however it can be concluded that chili is relatively sensitive to waterlogging.

Figure 3 Change of photosynthetic rate of genotypes in chilli. Lines of blue, red and green indicate variety of Laris, Takanotsume and Romario.

4. Conclusions
Rice (Oryza sativa L.) can grow well in paddy fields and is highly tolerant of excess water stress, from either submergence or waterlogging. A quiescence strategy of rice based on Sub1A is used for the growth controls under complete submergence like flash flood. On the other hand, O. glaberrima can elongate shoot rapidly to have aerobic photosynthesis under stagnant flooding but not tolerate to flash
flood. Rice can control waterlogging stress by forming lysigenous aerenchyma and a barrier to radial O₂ loss (ROL) in roots surface in order to supply O₂ to the root tip. These adaptive traits enable plants of O. sativa and O. glaberrima to have high tolerance to submergence or waterlogging compared with other upland crops. Even in sugarcane as an upland crop, tolerance to flooding varies between genotypes. These resources should accelerate understanding of the mechanisms involved in adaptation of upland crop to excess water stress.

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