Analysis of the Effects of Different Environmental Conditions on Root Elongation and Leaf Stomatal Dynamics in Crops

異なる環境条件が作物の根の伸長と葉の気孔動態に及ぼす影響の解析

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要約

地球規模の気候変動は，異常気温，干ばつ，洪水などのリスクを増大させている．作物の根は，土壌に含まれる水分や養分の吸収，呼吸など代謝経路においても重要な器官である．特に天水地域では水ストレスの問題が発生し易いことから，栽培作物種の根の動態に着目して機能を解析することは作物学上有益である．土壌中の根の分布は，様々な土壌水分状態に対する作物の応答を示している．例えば，湛水や乾燥による土壌水分の変化に対して，根系で受けたストレスは葉の気孔の開閉に反応し，いわゆる気孔コンダクタンスを制御する．この様に，作物の地下部と地上部の反応を関連づけて解析することは，植物体全体の環境応答を体系的に理解することにつながる．そこで，本研究は，光合成回路の異なるC3・C4作物を供試して，環境条件が根の伸長および葉の気孔動態に及ぼす影響を比較・検討し，作物の環境応答に係わる重要形質について明らかにすることを目的に行ったものである．

第一章では，湿潤土壌，乾燥土壌，及び湛水土壌に対する根の反応性を明らかにするために，トウモロコシ（*Zea mays* L.），ソルガム（*Sorghum bicolor* Moench.），ミレット（*Echinochloa utilis* Ohwi.）およびイネ（*Oryza sativa* L.）の遺伝子型と環境型の交互作用を解析した．その結果，トウモロコシおよびソルガムでは，根は湛水土壌では浅い土壌層に多く伸長し，乾燥土壌では深い土壌層まで伸長する可塑性を示した．イネおよびミレットの根の分布は土壌水分処理区間で有意な差異は認められなかった．地上部・地下部のバイオマスの残差分散と回帰係数の比較から，トウモロコシは根，茎葉ともに環境応答が大きく，イネは小さかった．ミレッ

トは茎葉に比べ根の残差分散が大きく，ソルガムは根の残差分散が小さいことから，作物間で地上部と地下部の環境応答が異なっていた．第二章では，第一章で根の動態が顕著に異なったトウモロコシとイネを供試して，湛水条件における根の伸長の差異について，根内部の酸素濃度の変化からその特徴を比較・検討した．その結果，根の酸素濃度はトウモロコシがイネに比べて有意に低いことが明らかになった．この根の低酸素量が，気孔コンダクタンスの低下と乾物重の減少に影響を及ぼしていることを示唆した．第三章では，作物の生存戦略の変異を明らかにする目的で，土壌水分と大気環境条件が気孔コンダクタンスと地上部バイオマスに及ぼす影響を気温の異なる生育期間で比較・検討した．その結果，供試作物ごとに，土壌水分含量，葉面積，及び気孔コンダクタンスと地上部バイオマスは有意な関係性を示した．特に気孔コンダクタンスは，高温条件下のトウモロコシ，ソルガムでは，乾燥から湿潤土壌条件で，イネでは湿潤から湛水土壌条件で大きくなった．第四章では，グロースチャンバー内で，気温と土壌水分を組み合わせた 6 つの環境条件で作物を育て，それぞれの作物の環境要因に対する気孔の反応とバイオマス生産の関係を比較・検討した．その結果，トウモロコシとソルガムでは，湛水土壌では，温度変化によらず，気孔コンダクタンス，光合成速度，及び蒸散速度が減少し，地上部のバイオマスも減少したことから，土壌中の低酸素が生育を制限していると推察した．一方で，乾燥土壌では，低温区において気孔コンダクタンス，光合成速度，及び蒸散速度が低下した． これは，C4 作物の光合成速度が低温で緩慢であるという特徴に関係していると考えた．結論として，環境変化に対する作物の根の可塑性の違いは，土壌条

件の嫌気的環境から好気的環境への変化に応答する根の生存戦略であると言える．乾燥土壌においては，根の水分吸収が必要であり，そのためには深層での根長密度の増加が重要である．また，高温条件下では，積極的に気孔を開き蒸散速度を高めて葉温冷却を行う機能が認められ，気孔コンダクタンスは最適光合成速度の維持に葉温調節の面から貢献した．土壌水分と気温の組み合わせから，トウモロコシとソルガムの成長は，気温よりも土壌水分条件により大きく影響を受けていた．これは，根で受けた水ストレス情報の葉への伝達によって気孔の開閉が影響を受けていると考えられることから，土壌水分の変動において，光合成速度を維持するには，気孔コンダクタンスの機能を強化する能力が重要であると結論付けた．

Summary

Global climate change is increasing the risk of extreme temperatures, droughts, and floods. Plant roots are important organs for absorption of water and nutrients from the soil, respiration, and other metabolic processes, and understanding their dynamics is critical for improving crop production, especially in non-irrigated areas where a-biotic stresses are a problem. For example, root distribution in the soil indicates crop response to various soil moisture conditions. Therefore, comparing and studying the effects of changes in soil moisture from waterlogging to drying on root elongation is important for understanding plant survival strategies. At the same time, examining the behavior of stomatal conductance, which controls leaf transpiration and photosynthesis and is closely related to root dynamics, will lead to a systematic understanding of the environmental response of the entire plant body. Therefore, this study was conducted to compare the effects of environmental conditions on root elongation and leaf stomatal conductance in different C_3 and C_4 crops and to identify important traits related to environmental responses in crops.

In Chapter 2, the interaction between genotype and environment was analyzed in maize (*Zea may* L.), sorghum (*Sorghum bicolor* Moench), millet (*Echinochloa utilis* Ohwi), and rice (*Oryza sativa* L.) to determine root responsiveness to moderate, dry, and waterlogged soils. Results showed that in maize and sorghum, roots showed plasticity, extending more into shallow soil layers in waterlogged soils and into deeper soil layers in dry soils. Rice root distribution did not differ significantly among soil moisture treatments. Comparison of residual variances and regression coefficients showed that maize had a greater environmental response in both roots and shoots, while rice had a smaller response. Millet had a larger residual variance for roots than for shoots, and sorghum had a smaller residual variance for roots, indicating that some species had different environmental responses above and below ground. Next, in Chapter 3, we compared and examined the characteristics of the differences in root elongation under waterlogged conditions from changes in oxygen concentrations in the inner root zone, using maize and rice, which showed markedly different root dynamics in Chapter 2. The results showed that the oxygen concentration in the roots was significantly lower in maize than in rice during waterlogging. The results suggest that this root hypoxia affects the reduction of stomatal conductance and dry matter weight in leaves. In Chapter 4, the effects of soil moisture and atmospheric environmental conditions on stomatal conductance and aboveground biomass were compared and examined with the aim of identifying variations in crop survival strategies. The experiment was repeated twice. The results showed significant relationships among soil moisture content, leaf area, and stomatal conductance and above-ground biomass for each of the prototypes. In particular, stomatal conductance was greater under dry to moderate soil conditions for maize and sorghum under high temperature conditions and moderate to waterlogged soil conditions for rice. Then next, crops were grown in a growth chamber under six environmental conditions combining atmospheric temperature and soil moisture, and the relationship between stomatal response to environmental factors and biomass production in each crop was compared and investigated. The results showed that stomatal conductance, photosynthetic rate, and transpiration rate of maize and sorghum decreased in waterlogged soils, as well as aboveground biomass, regardless of temperature change, suggesting that hypoxia in the soil limited their growth. On the other hand, stomatal conductance, photosynthetic rate, and transpiration rate decreased in the dry soil at lower temperatures. We hypothesized that this was related to the characteristic of the photosynthetic rate of C_4 crops, which is slow at low temperatures.

In conclusion, the differences in crop root plasticity in response to environmental changes could be a survival strategy of the roots in response to changes in soil conditions from anaerobic to aerobic environments. In arid soils, root water uptake is important, and for this reason, an increase in root length density in the deeper layers is important. Under high

temperature conditions, stomatal conductance contributes to the maintenance of optimal photosynthetic rate in terms of leaf temperature regulation, as it was found to function to actively open stomata to increase transpiration rate and cool leaf temperature. The combination of soil moisture and atmospheric temperatures indicated that maize and sorghum growth was more affected by soil moisture conditions than by atmospheric temperatures. This is because stomatal opening and closing may be altered by the transmission of information about water stress received by the roots, and we conclude that the ability to maintain stomatal conductance function is important for maintaining photosynthesis in fluctuating soil moisture conditions.

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CHAPTER 1

General Introduction

1. Background.

According to the United Nations (Nations, 2019), the world's population is expected to grow by more than 2 billion by 2050. The distribution of new growth will be about 50% in sub-Sahara African and 30% in South and Southeast Asia. The main crop production of these regions is rainfed, especially among cereal crops. Climate change and weather disasters are major causes of reductions in agricultural productivity (Berg et al., 2013; Giorgi et al., 2019), especially rainfed agriculture where the rain fall is the water source for crops. Climate change is expected to drive higher average temperatures. Head wave, drought and heavy rain are likely to occur more often and for greater duration. The exact extent of the combination of these factors for any given location remains highly uncertain (IPCC, 2014a).

Rainfed agriculture will continue to play a dominant role in providing food and generating livelihoods, particularly in poor countries (Rockström et al., 2010). Rice as C_3 plants and maize, sorghum, and millet as C_4 plants are major cereal crops which have been growing in many regions under variability of environment, and they are highly sharing contribution to food security.

Under rainfed agriculture, there are various types of abiotic stresses including drought, flood, salinity extreme temperatures, chemical toxicity and oxidative stress that plants encounter between seedling to harvesting stages. By 2025, the world farmers would have to produce about 3.0 billion tons cereal to feed the earth's population of nearly 8.0 billion, this means that worldwide, average cereal yield of 4 t ha^{-1} is to be achieve and sustained (Pareek et al., 2009).

Root is an important plant's organs, and its functions are wide varieties of processes including water and nutrient uptake, anchoring and mechanical support. Root architecture is often defined as spatial configuration of root system including arrangement of different roots in soil space, structures such as root type (primary root, lateral root, adventitious root) (Lynch, 1995b). It is the most important constrain of reduction of crop yield. Root architecture is affected by various factors such as soil temperature, moisture, nutrients and soil pH (Bao et al., 2014; Robbins and Dinneny, 2015). Several root characters such as morphological plasticity (Forde, 2009) and root tip diameter (Haling et al., 2013) allow the plants to adapt and respond to various environment factors, and might be quite useful for improving water use efficiency in crop species (Fenta et al., 2014). Therefore, it is very important to understand the root architecture regulating mechanisms for crop improvement.

Drought is mainly caused by low rainfall and soil water loss through evaporation and transpiration related to high-temperature results in decreasing soil moisture. Among various environmental stresses, drought is one of the serious stresses which has a significant negative impact on crop yield. It affects physiology and morphology such as leaf growth, stomatal conductance, photosynthesis rate, water use efficiency (WUE) (Negin and Moshelion, 2016). Hence, drought reduces the growth of aboveground biomass, yield loss of 50% due to the reduction of relative water content (Bouman and Tuong, 2001). Reduction in rice grain yield in intermittent and terminal drought tend to decline filled grain percentage harvest index and panicle number (Xangsayasane et al., 2014). When the water stress occurring in the root zone, root send a signal to restrict on the leaf surface to defense against water by stomatal closure (Kartika et al., 2020). Phenotyping root is one of drought management tools as root are more prone to drought conditions and play a significant role in the plant's survival by extracting resource from deeper soil layers to carry on several metabolic functions in the plant's body and its phenotyping helps to understand different root traits (Wasaya et al., 2018). Roots have been evolved to responsive and extremely adaptative to the local environment, and their morphology, growth, and physiology are closely related with plant genotype and growth medium properties. For example, elongation rate and number of lateral roots can be decreased by high soil water content or soil density, and this can also be associated with shoot growth reduction (Bengough et al., 2011). Plant's ability to extract water from deeper soil layer has great relevance in balancing water relation as well as carbon assimilation.

Flooding is another weather disaster after a drought. Heavy rainfall pattern causes increase flood event in many rainfed agriculture regions. Low oxygen diffusion in the soil is well known under waterlogged soil (Sanderson and Armstrong, 1978). Root suffers oxygen deficiency intend reduction respiration (Araki et al., 2012). Waterlogging cause reduction of photosynthesis, which directly affects growth and grain yield of maize (Tian et al., 2019). However, rice may have no or less effect by waterlogging compared to other upland crop species. To overcome oxygen deficiency, the plant develops new adventitious root associated with aerenchyma to enhance transportation external oxygen to root tip (Yamauchi et al., 2018b). The efficiency of external oxygen transport in the root indicates by aerenchyma and radial oxygen loss barrier (Yamauchi et al., 2019).

Drought and flooding are not only governed root morphology and physiology, but these are also directly affected on gas exchange, especially stomata aperture. Dynamic stomatal movement acting in response to environmental cues and internal signals in an attempt to optimize trade-off between photosynthesis and maintaining plant water status. Transpiration plays key role as evaporate cooling of leaf tissue (Hetherington and Woodward, 2003; McAusland et al., 2016). Due to current climate change, plants face variability in CO_2, water, and temperature variabilities. The effect of the combination of soil water and temperature stresses on crop's biomass production may be greater than single stress (Vile et al., 2012).

2. Problem Statement

Under rainfed condition, individual of flooding and drought, and the combination of flooding and drought with temperature variability evens are unexpectable prediction during the cropping period. The root is a first organ which directly affects by deficit or excess soil water. Under water stress, crops modified root system architecture along with type of stress. For example, under drought conditions, plants increase water uptake ability by root modification including plasticity, distribution, angle, and anatomic aerenchyma, while the root aerenchyma formation is crucial under flooding conditions. Currently, climate change related to variability of water and temperature stresses might be highly affect crop growth, especially cereal crops grown under rainfed condition. Cereal crops are common grown under rainfed condition in many regions of worldwide. The effects of individual or combination factor may lead to failure due to crop's adaptability. The comparation among the root response among the cereal crop genotypes from aerobic to anaerobic conditions is still unanswered. Moreover, the relationship between root and shoot of crop grown under combination of soil water status and temperatures needs to be clear among these crops. For example, limiting of root development under unfavorable soil water status and temperature may cause stomatal closure, which would be directly caused reduction of photosynthesis and transpiration.

This study was focus on leading cereal crops including rice as a C_3, maize, millet and sorghum as C_4. These crops had different characteristics on photosynthesis and root system and ecological cultivation. Rice is a semi-aquatic plant; it adapts well under waterlogging, but maize, millet, and sorghum are upland crop; they may not be well adapted to waterlogging conditions. The different adaptability among these crops is important to clarify the mechanism to enhance breeding program. Therefore, this study would provide fundament knowledge of understanding adaptability among these major cereal crops to avoid crop failure and secure food demand for growing world population.

6. Novelties

This study offers the following novelties:

1. Root distribution determines crop adaptation under various soil moisture statuses.
2. Stomatal conductance controls photosynthesis and water loss, and maintaining ability of stomatal conductance under the combination of soil moisture and temperature stress of each crop indicates as its adaptation ability.

7. General Objectives

This study aims:

1. to assess crops adaptation under changing environments, such as various soil moisture contents from anaerobic to aerobic conditions through analysis of root distribution as a part of root architecture.
2. to identify the relationship between root and leaf under 1) various soil moisture statuses interaction with different atmospheric environments, and 2) the combination of soil water statuses and temperatures through analysis of stomatal conductance behavior and shoot biomass.

8. Research Scope

This research was focus on the effect of various soil water statuses and the combination of soil water status and temperature variability on growth of leading four cereal crops including maize, millet, rice, and sorghum through analysis of root architecture and gas exchange. The experimental design of each experiment was simulated rainfed environmental condition. The research aims to find out the mechanism of adaptability among these crops. There were three activities, they were carried out under green house and growth chamber as below:

1. Root response to soil water status via interaction of crop genotypes and environment.
2. The effect of various soil moisture content and atmospheric environment on stomatal conductance and shoot biomass
3. Combinational variation temperature and soil water response of stomata and biomass production in maize, millet, sorghum and rice.

CHAPTER 2

Root Response to Soil Water Status via Interaction of Crop Genotypes and Environment

1. Introduction

According to the United Nations (Nations, 2019), the world's population is expected to grow by more than 2 billion by 2050. The distribution of new growth will be about 50% in sub-Sahara Africa and 30% in South and Southeast Asia. The main crop production of these regions is rainfed, especially among cereal crops. Rainfed agriculture will continue to play a dominant role in providing food and generating livelihoods, particularly in poor countries (Rockström et al., 2010). Climate change and weather disasters are major causes of reductions in agricultural productivity (Berg et al., 2013; Giorgi et al., 2019). The impact of natural disasters on rainfed agriculture in terms of total damage and loss across all sectors was 17% and 83% by flooding and drought, respectively (FAO, 2018).

The plant root system is an important part that absorbs water and nutrients from the soil for sustainable crop production, particularly in rainfed ecosystems that suffer from the increasing frequency of erratic rainfall patterns due to climate change. The optimization of root architecture for resource capture is vital for enabling the next Green Revolution (Den Herder et al., 2010; Lynch, 2007). Root distribution is included as root architecture, including root length and root mass, as a function of several factors, such as depth in the soil, distance from the stem, and others (Lynch, 1995a). Thus, scientists have been looking for optimum root development that may help plants use water more efficiently, better withstand drought conditions, and survive flood conditions (Comas et al., 2013; Suralta et al., 2010; Vadez, 2014; Wasson et al., 2012; Weaver et al., 1922; Yamauchi et al., 2018a). The growth and development of roots are under genetic control, but the genotypic performance of root distribution is highly influenced by abiotic stress (Fry et al., 2018; Grossman and Rice, 2012; McMichael and Quisenberry, 1993). Root distribution relates to deeper rooting traits in cereal crops, which can allow them to absorb water from further underground under drought stress (Gregory, 2008; Hund et al., 2009; Lynch and Wojciechowski, 2015; Uga et al., 2013). Dense and shallow root traits might better capture nutrients, such as phosphorus, that accumulate at the surface (Li et al., 2020; Liao et al., 2001).

The agronomic significance of understanding the regulation of lateral root development is now widely accepted because of its role in anchorage, soil resource acquisition, and establishment of the plant microbial community (Orman-Ligeza et al., 2013). Plant root distribution at the subsoil layer has the potential to increase water accessibility, particularly under water-limited conditions. Maize and rice root systems comprise different types of roots; for example, rice consists of crown root and lateral root, but maize consists more of nodal root number traits and lateral root such as primary root, crown root, seminal root, and lateral root (Coudert et al., 2010). However, recognition of lateral roots as a distinct category is a prerequisite for gaining fundamental knowledge about root development in these species. This is because root development is difficult to observe, quantify, and interpret. Roots grow in soil, an opaque medium from which they cannot be extricated or readily observed without introducing artifacts and destroying the original root architecture, precluding subsequent analysis of the same individual. Root systems themselves are complex structures, typically composed of thousands of individual root axes that vary developmentally, physiologically, and morphologically.

Plant phenotypes are sensitive to genotype and the environment and are influenced by the interaction between the two. By analyzing the genotype-environmental interaction using the observed phenotype, it is possible to infer the robustness of that genotype in the environment. Understanding how genetic and environmental factors influence complex traits, such as shoot and root growth, is a challenging issue in the field of plant sciences. The phenotypic expression of an individual is determined by both genotypic and environmental effects (Falconer and Mackay, 1996). To account for genotypic and environmental interaction effects, breeders evaluate genotypes in varying environmental conditions to identify those with a stable performance. The selection of suitable crop genotypes that can be adapted to poor environmental conditions has become important in rainfed conditions. By contrast, determining genetic variations among crop genotypes and comparing them to the variations caused by environmental differences is important in evaluating the environmental performance of the crop. The manner in which the resources are partitioned to the roots and the resulting belowground morphology are important in determining the ability of a plant to capture resources and its subsequent productivity (MacMillan et al., 2006). Both partitioning and morphology of roots are affected by the environment (López-Bucio et al., 2003; Malamy, 2005), and a plant can change its rooting behavior to maximize resource capture (Ho et al., 2004). Therefore, it is reasonable to measure the relative proportions of genetic and environmental interactions in root phenotyping.

Root development across environmental factors among the crop genotypes varies. In maize, our enhanced knowledge of root development regulation has already led to measurable gains in the ability of maize to exploit soil resources, with corresponding enhancements in shoot and yield attributes. Genotypic variation in the angles of the root segments of the first and second flushes of nodal roots and mean diameter of nodal roots could be considered a suitable target for large-scale screening of root architecture in breeding (Singh et al., 2010). Rice is a semiaquatic plant that is well adapted to flooding, ranging from partial to prolonged. Rice stems develop an adventitious root primordial at each node that slowly matures but emerges only when the plant is flooded, leading to the formation of a whole new secondary root system upon flooding (Lin and Sauter, 2018; Yamauchi et al., 2018a). In the root system of sorghum, severe deficit irrigation increases the angle of crown roots, root mass, root length density, and fine root surface area as compared with full irrigation (Magalhães et al., 2016). In sorghum, relationships were derived by which total root mass could be estimated from 0–10 cm root mass (r = 0.94, $p < 0.001$) or 0–20 cm root mass (r = 0.99, $p < 001$). Total root length is also a good predictor of total root mass (r = 0.799, $p < 0.001$), and 0–10 cm root length is equally satisfactory (r = 0.802, $p < 0.01$) (Myers, 1980). The role of the nodal root of sorghum in plant growth responds to variation in soil moisture, as does that of millet. Pearl millet is better adapted to hot semiarid conditions, and the common millet is better adapted to environments ranging from temperate to tropical. This study aimed to identify crop roots' response based on root distribution for nodal and lateral roots separately under various soil conditions, including dry and waterlogged and to assess crop adaptation under changing environments, such as various soil moisture contents, by using maize, millet, rice, and sorghum.

2. Materials and Methods

2.1. Site and Materials

The experiment was carried out in a greenhouse from 24 October 2019 to 25 November 2019. Four crop species including maize (*Zea mays* L. cv. Honey Bantam) is susceptible waterlogging, sorghum (*Sorghum bicolor* Moench. cv. High grain sorghum) is high adaptability to drought, but susceptible to waterlogging, millet (*Echinochloa utilis* Ohwi. cv. Kumamoto local) is Japanese common miller which can grow well in lowland and upland, and rice (interspecific progeny cv. NERICA1) is African' upland rice, but it grows well in lowland; were used in this study.

2.2. Experimental Design and Water Treatments

The seed germination of all crops was done as following processing. Seeds of each crop were placed in a petri dish containing filter paper moisturized with distilled water and left to germinate at 28 °C in an incubator under dark condition for 2–3 days. Then, the germinated seeds of each crop were sown in a seedling tray (size: 59 cm × 30 cm, containing 128 holes). The rice seeds were sown about five days earlier than other crops to adjust the same leaf age (2.5 leaves) at the transplanting time. Before transplanting, all root boxes were watered abundantly for three hours and allowed to drain excess water overnight to reach field capacity. The next day, 30 uniform seedlings of each crop were selected and transplanted one seedling per root box. After transplanting, the seedlings of all crops were allowed to grow for 10 days with adequate watering before treatment.

A root box was made from a waterproof wooden plate to prevent water absorption by the root box. The size (L × H × W: 40 cm × 40 cm × 3 cm) and design of the root box was modified from previous studies (Kano-Nakata et al., 2013; Kano-Nakata et al., 2019; KONO et al., 1987). Air-dried sandy loam soil was mixed with sand at a 4:1 ratio to provide adequate water permeability of the soil in the root box during the experiment. Then, about 5.6 kg soil at 10% (*w*/*w*) of moisture content was well mixed with 12 g balanced compound of N, P, and K fertilizer (8–8–8). To make sure that the soil was homogeneously packed bulk density (1.2 g cm^{-3}), the soil (5.6 kg box^{-1}) was equally spread into eight layers (about 0.7 kg of each portion). Then, each layer of soil was carefully filled to achieve about 5 cm of a soil layer. At 10 days after transplanting, the treatments as were started. Five of 30 root boxes of each crop were selected for water treatment, and each root box represented a replication. The treatments included three water statuses and five replications. The treatments consisted of moderate soil moisture (MSM) as a control, gradual soil drying (GSD), and continuous soil waterlogging (CSW). Under MSM, the soil moisture content was kept at field capacity by daily irrigation with approximately 200 mL of tap water onto the soil surface. This water amount was adequate for plant growth requirement, and confirmed by soil moisture sensors that it was equally spread throughout the root boxes. At the bottom of the root box, there was a hole to allow draining of any excess water under MSM. Under GSD, irrigation was withheld from the root box until the end of the experiment. Under CSW, the root boxes were submerged in a container with controlled water level at 2~3 cm over the soil surface until the end of the experiment. The example of soil moisture treatment was shown in Figure 2-1. The mean day and night temperatures, and humidity were 27.2 °C, 14.8 °C and 47.6%, 77.9%, respectively, during the experiment, and the mean of solar radiation from October to November 2019 was in the range of 13.5–11.9 MJ m^{-2} (Kagoshima, Japan Meteorological Agency).

2.3. Measurements

Soil moisture content was monitored at 20 cm depth from the soil surface using a soil moisture sensor (5TE), and data were recorded using a Datalogger Em50 Series (Decagon Devices Inc., Pullman USA) with a 60 min interval between measurements. At 25 days after treatment (35 days after transplanting), the shoot was cut and the leaf and stem were separated. Leaf area was measured using an automatic area meter (AAM-9, Hayashi Denko Co., Ltd., Tokyo, Japan). After leaf area measurement, the gathered leaves and stems were oven-dried at 80 °C to a constant weight before determining shoot dry weight. One of five root boxes of each treatment and crop was chosen as a representative for two-dimension root images. The processing of root image, the selected root boxes of each treatment and crop were opened at one side and placed on a pinboard. After that, the root box was removed and gently washed to remove the soil without root damage, and then the roots were photographed (Nikon 3500 digital camera). After photographing, the root was cut for further root analysis, as explained below. The rest of the root boxes were opened on one side, and the root samples (included soil) were divided using a knife into four layers (0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm) and three columns equally (left side, middle and right side) in the root box before the roots were washed. The root samples were kept in tap water and preserved in the freezer (–5 °C) for two weeks before root scanning. Root samples from each soil layer were scanned at 6400 dpi (EPSON XT-X830, Epson American Inc., Los Alamitos, CA 90720, USA), and the scanned images were analyzed using an image analysis system (WinRHIZO, Regent Instruments Inc., Quebec, Canada) with a pixel threshold value range between approximately 165 and 175 to assess the total root length, lateral root length, surface area, and volume, while the nodal root length was different between total root length and lateral root length (Figure 2-2). The root classification of lateral roots was categorized by a root diameter of less than 0.2 mm in rice (Gu et al., 2017; Sandhu et al., 2016; Yamauchi et al., 1987), but in the maize, millet and sorghum, the root diameter classification was modified (Passot et al., 2016; Singh et al., 2010; Yamauchi et al., 1987). In these crops, a root diameter of less than 0.35 mm was classified as a lateral root. After root analysis, root samples were oven-dried at 80 °C to a constant weight using the same process as with the leaf samples to determine root dry weight. We calculated the root parameters, such as total root length density in each soil layer, total root length density distribution in each soil column and layer such as sides (left and right sides of soil column) and middle, and specific root length density distribution in each soil layer. We also assessed the interaction effect of crop genotype × soil water status (G × E) using Finlay–Wilkinson regression analysis (Finlay, 1963) as a measure of the adaptability of crop genotype to environment (water status) and environment to crop genotype. In general, the term "genotype × environment (G × E) interaction effect" referred both to the modification of genetic risk factors by environmental risk and protective factors and to the role of specific genetic risk factors in determining individual differences in vulnerability to environmental risk factors. In this study, crop phenotypes are sensitive to genotype and the environment. The phenotype is influenced not only by the genotype and the environment, but also by the interaction between the genotype and the environment. By analyzing the interaction between G × E using the observed phenotypes, it becomes possible to infer the robustness of that genotype to the environment. The environmental index in this study showed the average value of aboveground or underground biomass of different crop species under three different soil moisture conditions. Between different crop genotypes, we analyzed the response of biomass to soil water status, so it is also important of regression coefficient of regression line of G × E to understand phenotype under different environments.

2.4. Statistical Analysis

The experiment was arranged in a randomized block design with five replications. One-way and two-way analysis of variance (ANOVA) was conducted to determine the effects of soil moisture on individual crops (maize, millet, rice, and sorghum) and interaction of crops genotype and soil moisture status, respectively. Tukey’s honest significant different test was used to compare the mean value at the 5% probability in IBM SPSS v. 26. (IBM Corp. Released in 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY, USA: IBM Corp.).

Figure 2-1. Plants growth condition at one week after imposed soil moisture stress. The (A) is plants grown under moderate soil moisture (MSM) and gradual soil drying (GSD), and (B) is plants grown under continuous soil waterlogging (CSW).

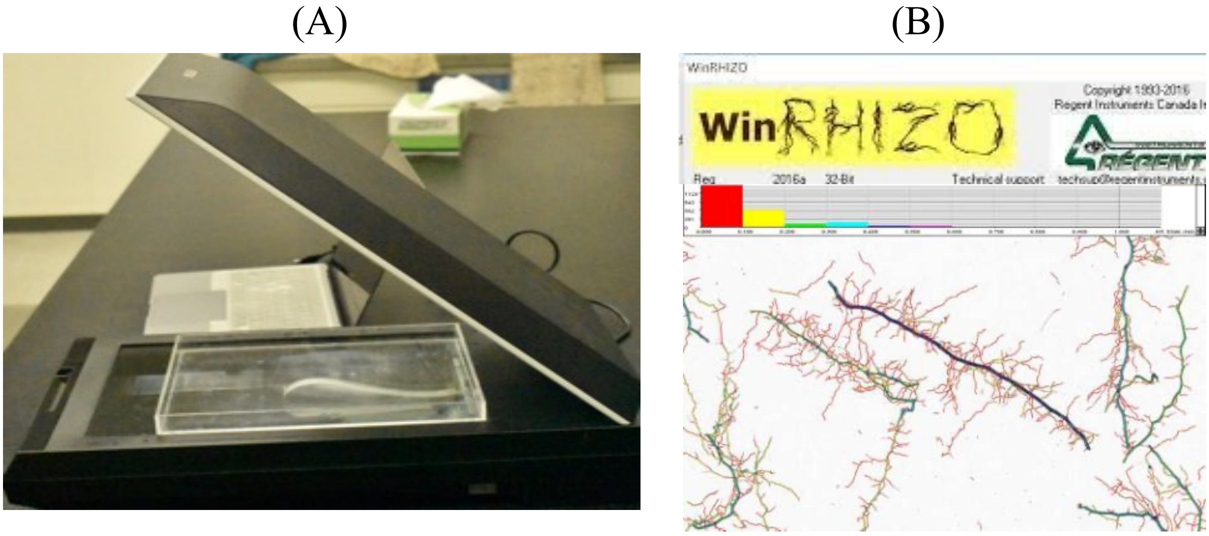

Figure 2-2. Root scanning (A) and root analysis by using winRhizo software (B).

3. Results

3.1. Soil Moisture Content Distribution

Figure 2-3 shows the volumetric soil moisture content of MSM and GSD. The soil moisture content was controlled at field capacity before the start of the treatment. After treatment, the average of soil moisture content under MSM was controlled at field capacity (~21%), while the soil moisture content under GSD in maize, millet, rice, and sorghum was dynamically decreased by withholding irrigation for 25 days. On the other hand, the volumetric soil moisture content under CSW showed very little change when the plots were submerged.

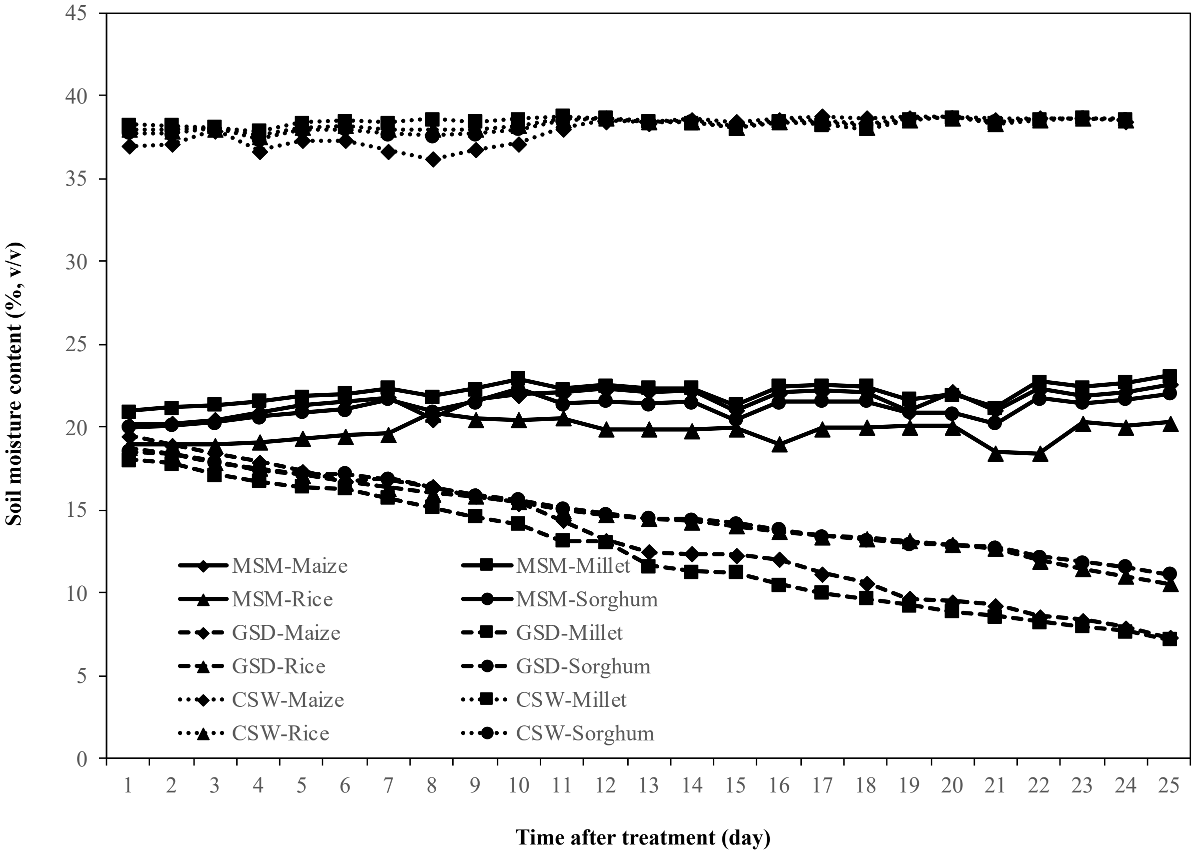

Figure 2-3. Soil moisture dynamics at a depth of 20 cm from the top of the root box during the experiment. The value shown is an average of readings from maize (◆), millet (■), rice (▲), and sorghum (●) under moderate soil moisture content (MSM) (straight line), gradual soil drying (GSD) (stitch line), and continuous soil waterlogging (CSW) (dotted line).

3.2. Shoot and Root Growth under Different Soil Moisture Statuses

Table 2-1 shows the effect of soil moisture treatment on shoot and root traits. The effect of soil moisture treatment on shoot and root traits was dependent on the crop genotypes. There was a significant effect of soil moisture treatment on shoot dry weight and leaf area in maize, millet, and sorghum, but no effect was found in rice. For shoot development, GSD significantly increased shoot dry weight and leaf area in maize, millet, and sorghum compared to CSW. In particular, CSW decreased the shoot dry weight and leaf area of maize (68% and 66% of shoot dry weight and leaf area, respectively) and sorghum (60% and 69% of shoot dry weight and leaf area, respectively) compared to MSM, but CSW did not show a significant difference in shoot dry weight and leaf area compared with MSM in millet. There was a significant effect of soil moisture treatment in all crops on all root traits, with the exception of rice, in which root dry weight and root volume did not show significant differences among the soil moisture treatments. GSD significantly increased lateral root length in all crops compared to CSW; in particular, maize and millet had a higher lateral root length under GSD than under MSM and CSW.

Table 2-1. Average of shoot biomass, leaf area (LA), root biomass, nodal root length (NRL), lateral root length (LRL), total root length (TRL), surface area (SA), and volume (VL) across gradual soil drying (GSD), moderate soil moisture (MSM), and continuous soil waterlogging (CSW) at 25 days after treatment.

Treatment	**Shoot Traits**		**Root Traits**					
	Shoot biomass (mg plant^{-1})	**LA (cm^2 plant^{-1})**	**Root biomass (mg plant^{-1})**	**NRL (m plant^{-1})**	**LRL (m plant^{-1})**	**TRL (m plant^{-1})**	**SA (cm^2 plant^{-1})**	**VL (cm^3 plant^{-1})**
Maize								
GSD	653.2 ± 64.0 a	1257.5 ± 58.1 a	117.7 ± 19.0 a	36.4 ± 6.7 a	128.9 ± 11.8 a	165.3 ± 14.2 a	1788.3 ± 189.3 a	18.1 ± 2.7 a
MSM	513.9 ± 101.8 b	1034.4 ± 88.1 b	97.6 ± 12.3 a	31.4 ± 4.9 a	94.1 ± 15.7 b	125.5 ± 17.0 b	1460.8 ± 187.3 b	14.0 ± 2.5 b
CSW	163.0 ± 30.4 c	351.9 ± 50.8 c	32.0 ± 5.3 b	12.2 ± 1.2 b	27.9 ± 3.2 c	40.1 ± 2.7 c	523.5 ± 26.9 c	5.5 ± 0.5 c
Millet								
GSD	487.0 ± 59.8 a	721.3 ± 115.5 a	88.5 ± 16.6 a	30.6 ± 5.5 a	156.9 ± 30.0 a	182.4 ± 33.3 a	1662.4 ± 242.2 a	11.9 ± 0.8 a
MSM	282.8 ± 44.3 b	491.0 ± 39.3 b	62.2 ± 8.9 b	16.8 ± 3.3 b	117.0 ± 33.3 b	138.8 ± 26.5 b	1119.1 ± 218.3 b	7.9 ± 1.4 b
CSW	271.1 ± 92.8 b	407.7 ± 95.5 b	38.1 ± 12.3 c	11.2 ± 5.1 b	70.2 ± 15.8 b	85.8 ± 19.3 c	690.0 ± 182.4 c	4.0 ± 0.6 c
Rice								
GSD	58.2 ± 21.6 a	93.2 ± 23.1 a	20.8 ± 8.0 a	11.6 ± 4.5 ab	27.3 ± 11.0 a	38.9 ± 12.6 a	352.8 ± 119.9 ab	2.7 ± 0.9 a
MSM	50.8 ± 11.5 a	89.6 ± 24.2 a	26.1 ± 3.9 a	12.9 ± 2.2 a	35.9 ± 3.4 a	48.8 ± 5.6 a	421.0 ± 60.8 a	3.0 ± 0.5 a
CSW	58.9 ± 20.6 a	98.8 ± 30.0 a	16.9 ± 4.2 a	7.2 ± 1.6 b	12.7 ± 3.0 b	19.9 ± 4.2 b	229.9 ± 65.6 b	2.2 ± 0.8 a
Sorghum								
GSD	271.5 ± 52.4 a	586.3 ± 122.6 a	50.5 ± 9.9 a	11.6 ± 1.8 a	86.6 ± 14.4 a	102.8 ± 22.0 a	942.1 ± 119.3 a	6.2 ± 1.1 a
MSM	252.8 ± 43.2 a	537.5 ± 107.9 a	55.7 ± 10.5 a	12.9 ± 1.8 a	85.0 ± 14.2 a	96.4 ± 17.9 a	880.0 ± 120.0 a	6.1 ± 1.0 a
CSW	89.4 ± 12.2 b	167.6 ± 24.5 b	26.7 ± 5.4 b	7.3 ± 1.5 b	21.9 ± 5.2 b	29.3 ± 6.5 b	349.0 ± 70.6 b	3.6 ± 0.9 b
Crop × soil moisture	***	***	***	***	***	***	***	***

The average value is shown as ± standard deviation. Columns with similar letters within a column for each crop species did not significantly differ according to Tukey's test at the 0.05 probability level. *** and ns indicate statistical significance at $p < 0.001$ and no significance, respectively.

3.3. Root distribution in Different Soil Layers and Soil Moisture Statuses

Figure 2-4 shows representative 2D images of roots of maize, millet, rice, and sorghum grown in root boxes under MSM, GSD, and CSW at 25 days after treatment. Each crop produced greater fine root than primary root. The details of root growth development are explained in Table 2-1, and Figures 2-5 and 2-6. The total root length density distribution showed different patterns subject to soil moisture status and crop species (Figure 2-5). In maize, a significant effect of soil moisture treatment was found in the 10–40 cm soil layer ($p < 0.001$) GSD significantly increased total root length density distribution compared to MSM and CSW in the 10–30 cm soil layer. However, there was no significant difference in total root length density distribution between the GSD and MSM in the 30–40 cm soil layer. Whereas, CSW showed an extremely decreased total root length density distribution in the 10–40 cm soil layer compared to MSM and GSD. The effect of soil moisture treatment on the total root length density distribution of millet was similar to that of maize under GSD, but the total root length density distribution under CSW was not significantly decreased in the 10–30 cm soil layer compared to that in MSM. In sorghum, there was a significant effect of soil moisture treatment on the total root length density distribution in the 10–30 cm ($p < 0.001$) and 30–40 cm ($p < 0.05$) soil layers; total root length density distribution was greatly decreased by CSW in the 10–40 cm soil layer compared to MSM and GSD, but a comparison between MSM and GSD did not show a significantly different total root length density distribution in all soil layers. In rice, a significant effect of soil moisture treatment was found for all soil layers ($p < 0.05$), and the total root length distribution was significantly decreased by CSW in all soil layers compared to MSM, while the GSD was not significantly different in terms of total root length density distribution compared to MSM and CSW.

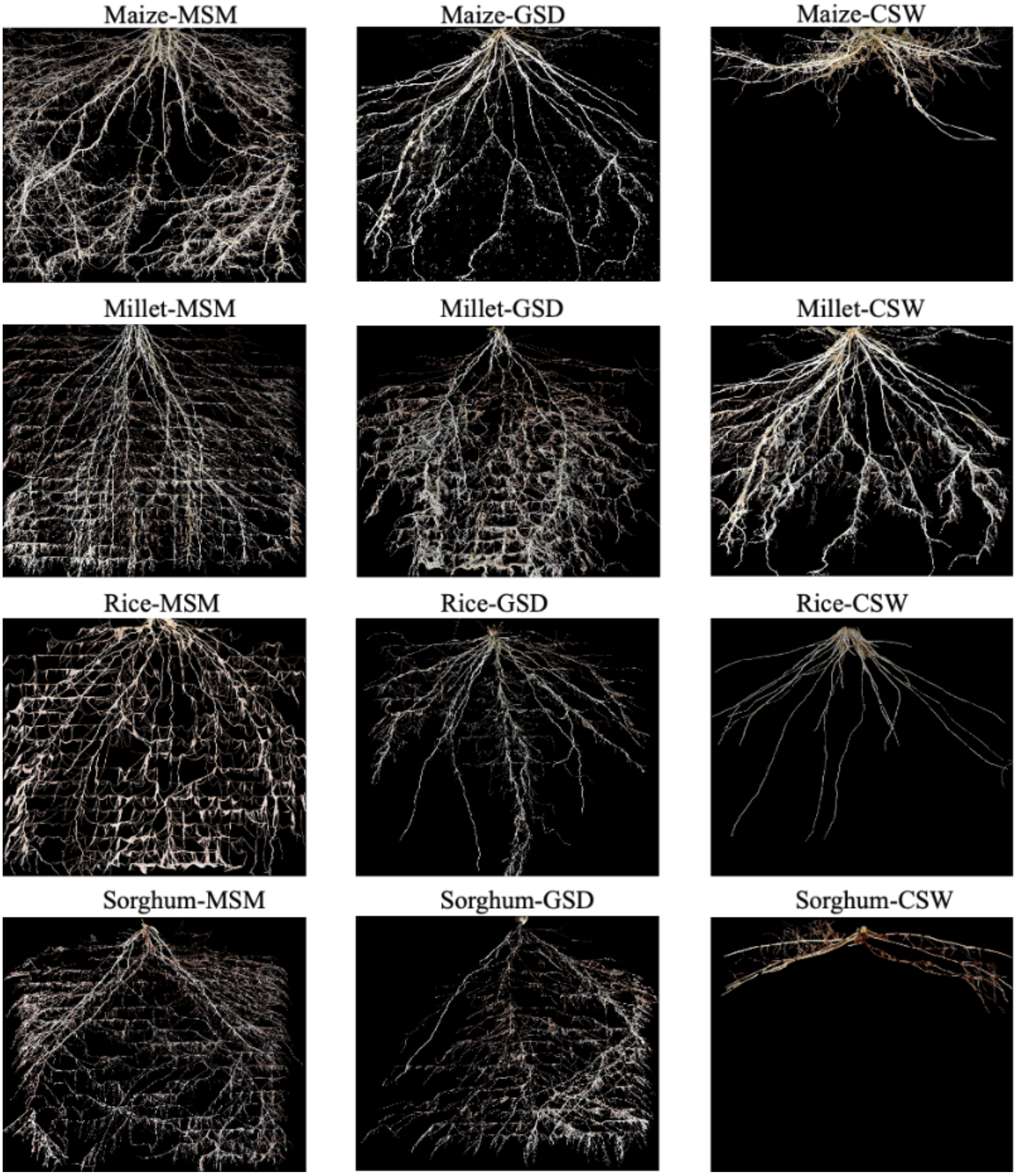

Figure 2-4. Representative two-dimensional root images of maize, millet, rice, and sorghum grown under moderate soil moistures (MSM), gradual drying soil (GSD), and continuous waterlogging (CSW) for 25 days after treatment.

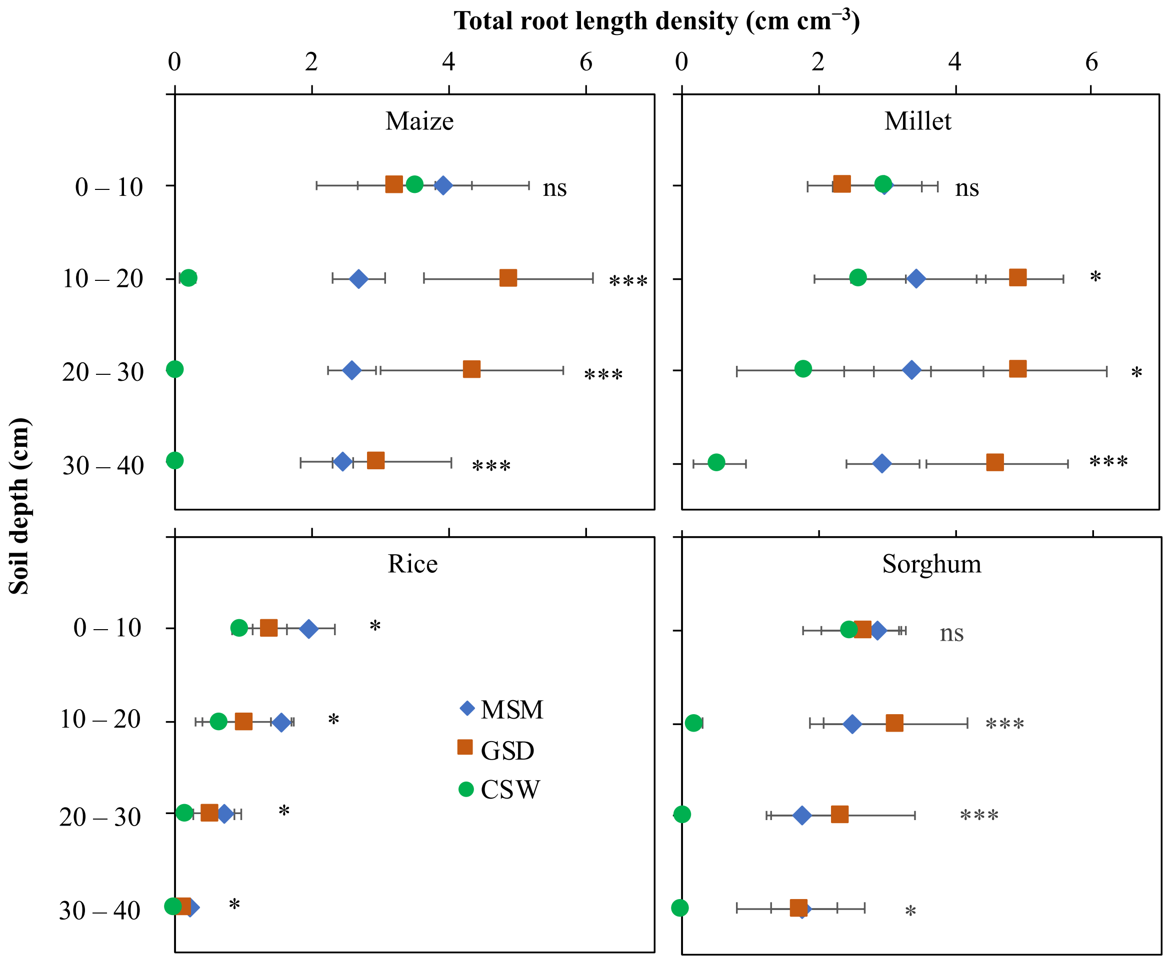

Figure 2-5. Effect of moderate soil moisture (MSM (◆)), gradual soil drying (GSD (■)) and continuous soil waterlogging (CSW (●)) on the total root length density distribution in different soil layers in maize, millet, rice, and sorghum. *, ***, and ns indicate statistical significance at p <0.05, p <0.001, and nonsignificance, respectively. Bar values indicate means ± standard deviation.

Figure 2-6 demonstrates the vertical total root length density distribution in different soil columns (sides and middle). The sides related to shallower root angle, and the middle related to steeper root angle, especially in the 0–20 cm soil layer. In maize, root density of the sides was higher than the middle in MSM, but those of GSD were higher in the middle than sides in the 0–40 cm soil layer. Under CSW, as we explained above in Figure 2-5, most of the root distribution was not able to elongate in subsoil layer (20–40 cm soil layer); total root length density of maize was significant in the middle, higher than in the sides ($p < 0.001$). In millet, total root length density distribution in comparison between MSM and GSD showed a similar trend, with the middle showing a higher total root length density distribution than the sides in the 0–40 cm soil layer. Under CSW, the total root length density distribution in the 0–10 cm soil layer was significantly ($p < 0.001$) higher in the middle than in the sides, but in the 10–40 cm soil layer, had no significant difference between the middle and the sides. In rice, total root length density was significantly higher in the middle than in the sides for MSM in the 0–10 cm and 20–40 cm soil layers ($p < 0.05$), GSD in the 0–10 cm ($p < 0.05$) soil layer, and CSW in the 0–10 cm soil layer ($p < 0.001$). In sorghum, in the 0–10 cm soil layer, it showed a significantly higher total root length density in the middle than in the sides for MSM ($p < 0.001$), GSD ($p < 0.05$) and CSW ($p < 0.001$). However, other soil layers were not significantly different between the middle and the sides for all treatments, except in the 10–20 soil layer for MSM ($p < 0.05$).

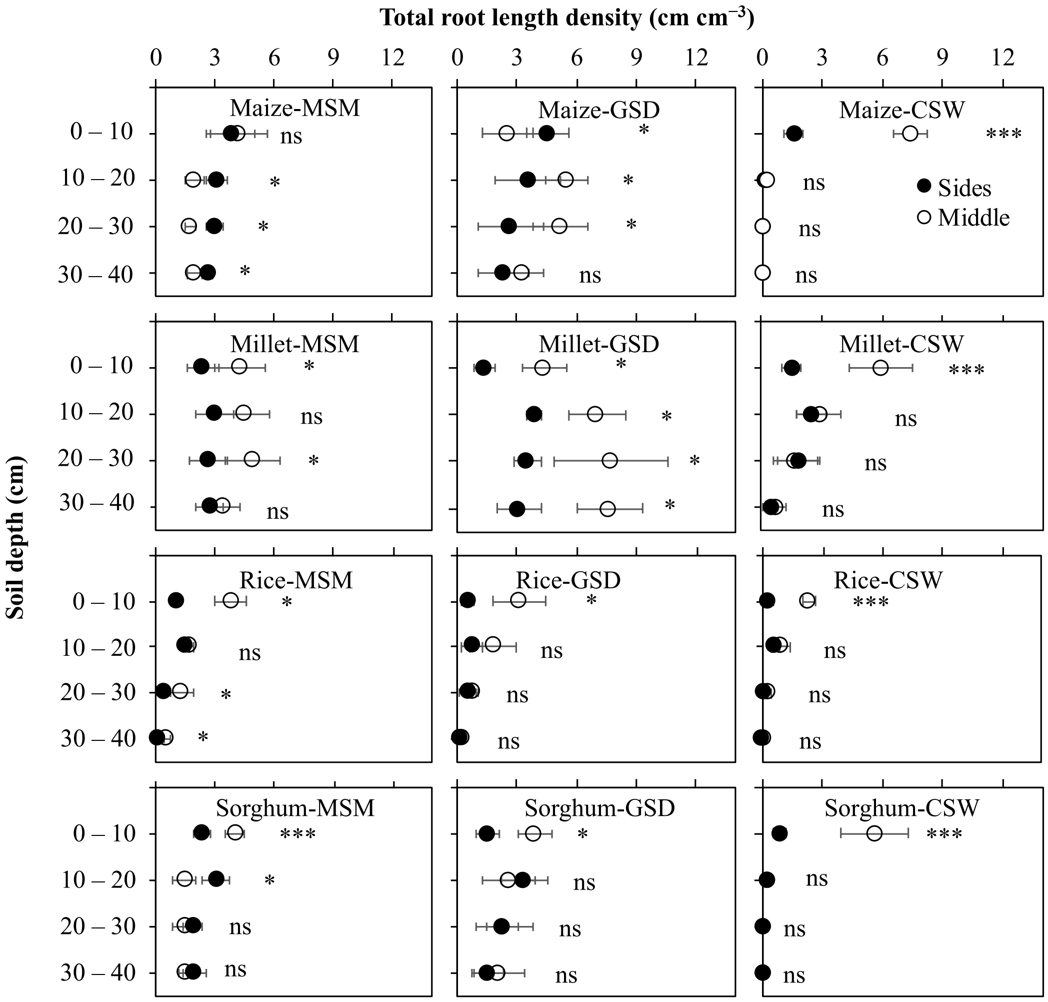

Figure 2-6. Effect of moderate soil moisture (MSM), gradual soil drying (GSD) and continuous soil waterlogging (CSW) on total root length density distribution in different soil columns in maize, millet, rice, and sorghum. Middle (○) denotes the value of total root length density distribution in the middle of the soil column, and Sides (●) signifies the average value of total root length density distribution in the left side and right side of the soil columns. *, ***, and ns indicate statistical significance at $p < 0.05$, $p < 0.001$, and nonsignificance, respectively. Bar values indicate means ± standard deviation.

The effect of soil moisture content on specific root length differed depending on the crop genotypes and soil layer (Figure 2-7). In maize, a significant effect of soil moisture treatment was found in the 20–40 cm soil layer, whereas CSW reduced specific root length in the 20–30 cm soil layer compared with MSM and GSD. In rice and millet, there were no significant effects of soil moisture treatment on specific root length in any soil layer, except in rice grown under CSW, which showed a significantly reduced specific root length in the 0–10 cm soil layer compared with both MSM and GSD. In sorghum, a significant effect of water treatment in the 10–40 cm soil layer under CSW decreased specific root length compared with MSM but not under GSD compared with MSM.

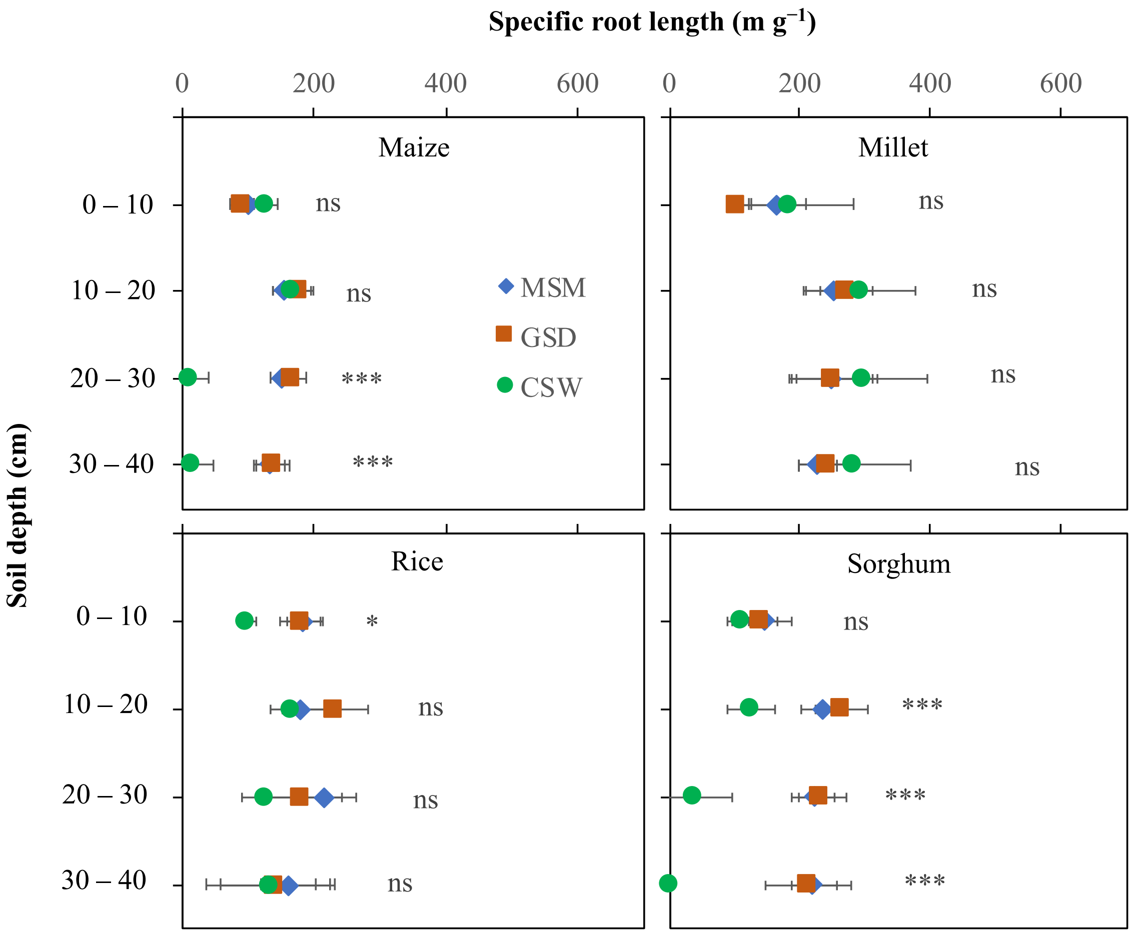

Figure 2-7. Specific root length distribution in different soil layers of maize, millet, rice, and sorghum grown under moderate soil moisture (MSM (◆)), gradual soil drying (GSD (■)) and continuous soil waterlogging (CSW (●)). *, ***, and ns indicate statistical significance at $p < 0.05$, $p < 0.001$, and nonsignificance, respectively.

3.4. Analysis of Interaction between Biomass Production of Crops and Soil Moisture Environment

Figure 2-8 shows the crop genotype × environment interaction for shoot dry weight and root dry weight. Crops showed different responses to changes in the environmental index. The relationship between the environmental index and shoot dry weight of specific crop genotype showed that the difference of shoot dry weight between crop genotype at the lowest environmental index (1.46) was smaller than that of the highest environmental index (3.51) (a). According to the simple regression equation of interaction between the environmental index and crop genotype based on shoot dry weight, the regression coefficients (a) showed positive values for maize (2.42), sorghum (0.90), and millet (0.72). However, rice showed a negative regression coefficient (−0.04). The interaction between environment and crop genotype based on root dry weight demonstrated similar tendency with shoot dry weight among the crop genotypes with respect to the environmental index. The regression coefficient of the simple regression equation for the interaction was 2.13 for maize, 1.15 for millet, 0.64 for sorghum, and 0.09 for rice. The average of shoot dry weight across crop genotypes indicated a lowest value at 0.50 g plant^{-1} and highest value at 6.53 g plant^{-1}. According to the simple regression equation, CSW had the highest environmental index. The regression coefficient between the average shoot dry weight and root dry weight across crop genotypes and environmental indices was lower in GSD than in CSW, which showed the highest regression coefficient.

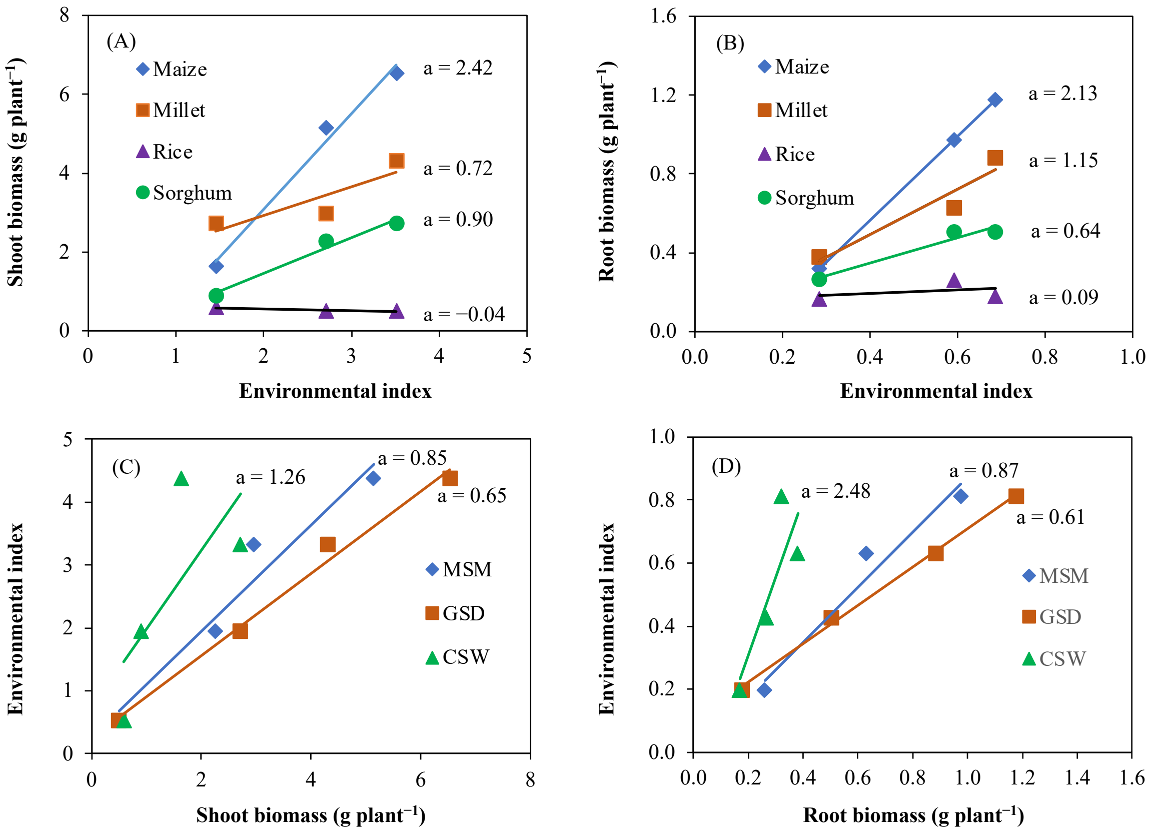

Figure 2-8. Interaction between the crop genotype and environment. Shoot biomass (A) and root biomass (B) of each crop (maize (◆), millet (■), rice (▲), and sorghum (●)) responses to the environment, and environment (moderate soil moisture (MSM (◆)), gradual soil drying (GSD (■)), and continuous soil waterlogging (CSW (▲)) effect on shoot biomass (C) and root biomass (D). Environmental index is mean value of SDW or RDW of different crop species under three different soil moisture statuses. a means regression coefficient of response.

Based on crop response to the environment (Figure 2-8A, B), we calculated the residual variance to plot against the regression coefficient for estimating the ability of crop adaptation to the environments. Figure 2-9 illustrates each crop adaptation to the environments. The performance of each crop showed a similar trend of response for shoot dry weight and root dry weight. Maize displayed the highest regression coefficient 2.42 and 2.13 for shoot dry weight and root dry, respectively, and residual variance 10.99 and 9.08 for shoot dry weight and root dry weight, respectively. On the other hand, rice has the lowest regression coefficient −0.04 and 0.09 for shoot dry weight and root dry weight, respectively, and residual variance 0.00 and 0.01 for shoot dry weight and root dry weight, respectively. Regression coefficient and residual variance of millet and sorghum were close to rice on shoot dry weight and root dry weight compared to maize.

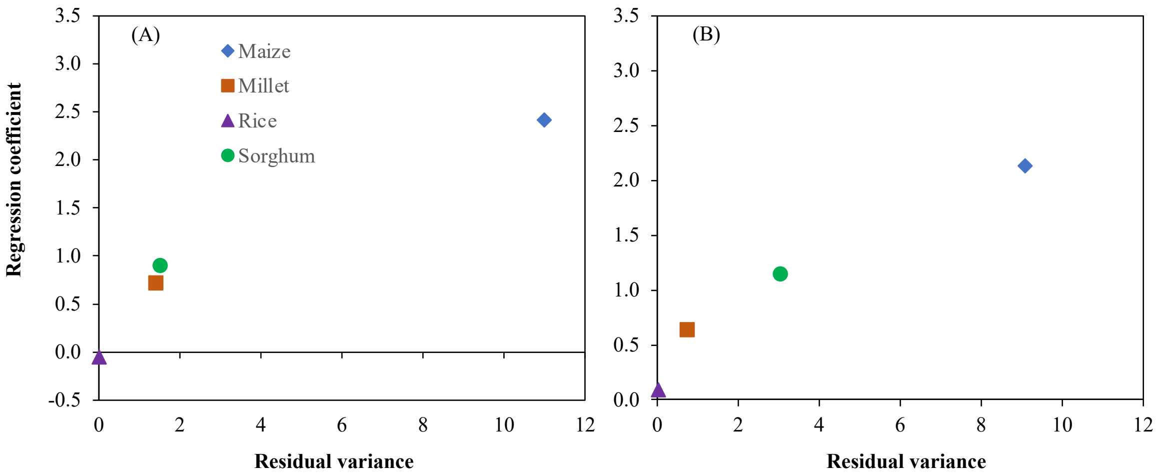

Figure 2-9. Interaction between the residual variance and regression coefficient of shoot biomass (A) and root biomass (B) for evaluating crops: maize (◆), millet (■), rice (▲), and sorghum (●) adapted to environment (soil moisture).

4. Discussion

4.1. Soil Moisture Status Affects Root Morphology and Distribution

In this study, we evaluated the root distribution of major cereal crops under water stress conditions at an early growth stage. The response of root distribution to different soil moisture treatments was distinctively different among the four cereal crops. Based on the root distribution at the subsoil layer, maize, millet, and sorghum had more extensive root growth for GSD compared to rice under dry soil condition (Figure 2-5). On the other hand, under waterlogged conditions, rice and millet exhibited more vigorous root elongation compared to maize and sorghum in the root length distribution. The root distribution at the deeper soil layer is important for maintaining or promoting better shoot growth under dry soil conditions. Maize and millet had a higher SDW under GSD than under MSM and CSW in this study. Root distribution in the deep soil layer is beneficial to absorb water from further underground under drought conditions (Gregory, 2008; Hund et al., 2009; Lynch and Wojciechowski, 2015; Uga et al., 2013). The poor extensive root growth under CSW in maize and sorghum resulted in less leaf expansion and shoot growth. The limitation of root distribution in the subsoil layer under waterlogging is caused by anoxic conditions (Biemelt et al., 1998; Vartapetian and Jackson, 1997), because waterlogged soil supplies insufficient oxygen to the submerged root which prohibit root respiration and elongation. It is well known that rice grows well under anaerobic conditions. Its root induces aerenchyma and a tight barrier to radial oxygen loss (ROL) along the root during waterlogging for transport of oxygen to the root tip (Yamauchi et al., 2019; Yamauchi et al., 2018a). Similarly, Japanese barnyard millet can induce thick cortical sclerenchyma in the nodal root which is better for enhance oxygen transport from the leaf to root tip (Galamay et al., 1991). Maize and sorghum are also able to form aerenchyma in new adventitious roots just as rice does when the root zone is flooded, but maize has smaller aerenchyma and larger living cells than rice, respectively, within the root as compared to rice, which causes an insufficient oxygen diffusion rate from the aerenchyma to the root tip (Yamauchi et al., 2019). Such aerenchyma formation in maize might be the same as that in sorghum. Sorghum (*Sorghum bicolor* Moench. cv. high grain sorghum) is susceptible to waterlogging, even though its root traits can induce aerenchyma (Promkhambut et al., 2011). Our study did not generate data on physiological traits, such as aerenchyma formation, and ROL barrier oxygen loss in the root might be the key to crop adaptation under waterlogging. Thus, further study is required to evaluate the genotypic difference in aerenchyma formation and ROL barriers among these four crops.

4.2. Effect of Soil Moisture Status on Shoot and Root Biomass Production

The genotype by environment interaction was analyzed in terms of shoot and root biomass (Figure 2-8). The four crops exhibited a distinct difference in response to water status. The root and shoot growth of maize, millet, and sorghum strongly responded to favorable water status environments, while rice did not show a correlation of root and shoot growth between specific environments (Figure 2-8A, B). This indicates that rice is well adapted across soil moisture conditions from dry to waterlogged. In other words, the reaction of rice to moisture is insensitive in this study. The wide adaptability specific to rice is in good agreement with a previous study, which reported that short-term GSD and decrease in soil moisture content (from 16% to 6%) had no or little effect on the shoot growth of rice (Kartika et al., 2020). The root dry weight of millet was decreased by CSW, but its shoot dry weight did not decrease compared to MSM (Table 2-1). However, shoot dry weight and root dry weight increased under GSD, and shoot dry weight and root dry weight adequately responded to a favorable soil moisture condition with a higher environmental index. Japanese barnyard millet can induce cortical sclerenchyma in the nodal root (Galamay et al., 1991); this enhances water use efficiency for maintaining or increasing the aboveground parts (Zegada-Lizarazu and Iijima, 2005).

However, shoot growth of the Japanese barnyard cultivar decreases when it is grown under severe drought and compacted soil conditions due to the limitations of root elongation with regard to uptake of water in deep soil (Galamay et al., 1991; Zegada-Lizarazu and Iijima, 2005). Our study showed that roots increased at deeper soil layers under GSD. The correlation between environmental index and average of shoot dry weight and root dry weight showed that the different responses to soil moisture treatments under GSD promoted higher shoot dry weight and root dry weight in the favorable soil moisture condition with a higher environmental index than in the unfavorable soil moisture condition with a lower environmental index in CSW (Figure 2-8C, D). Consequences of the phenotypic variation depend largely on the environment. This interaction between the crop genotype and environment can be used to analyze the stability of genotypes in cereal crops under various soil water statuses. Genetic and environmental interactions occur when the effect of the environmental exposure on a certain outcome is strongly influenced or is contingent upon the genotype (Kang, 1997). In this study, the interaction between the crop genotype and environment was identified and analyzed using the regression coefficient and regression residual variance (Figures 2-8 and 2-9). Among the four crop genotypes, it was discerned that rice showed almost constant biomass productivity, which was mostly unaffected by the macroenvironmental factors evaluated in this study. In addition, sorghum and millet also showed similar results because there was a lower regression coefficient and regression residual variance. By contrast, it was inferred that maize reacted variably to macro and specific environment and that variability in the environmental factors affected the variability in this genotype. As a result, GSD was the favorable environment for extending roots and shoots; however, CSW showed weak growth in maize. On the other hand, the growth response of rice across water status was poor. This means that rice can be adapted to wide soil moisture conditions ranging from dry to waterlogged.

4.3. Horizontal and Vertical Root Architecture Distribution across Soil Moisture Status

Genotypic differences were identified not only for root distribution in different soil columns, as mentioned above, but also for root distribution in different soil layers. The direction of root elongation in the soil column can be estimated by the root angle (Ali et al., 2015; Borrell et al., 2014). The root distribution to the sides of soil columns indicates shallow root angle, but middle soil column indicates steeper root angle. In the case of rice, vertical root elongation was related to a high degree of root angle-associated deep rooting, which is controlled by genes and governs the modification of root plasticity by the environment (Kano-Nakata et al., 2013; Uga et al., 2013). Under GSD, maize, millet, and sorghum promoted more horizontal root distribution at deeper soil layers. However, the root growth development in the vertical soil column showed different directions of root distribution at various soil layers; for example, maize showed more root development at the sides compared to middle of the soil column in all soil layers, but millet had higher root development in the middle than at the sides (Figure 2-6). These results mean the vertical root growth development is important for evaluating root response to soil drying conditions. Waterlogging limited the root distribution in different soil layers and root elongation to deeper soil layers in maize and sorghum (Figure 2-5). Besides the root distribution in maize and sorghum, most of the root developed only in the middle of the soil column, close to the stem base. By observation, some new emerging roots were produced, and some roots escaped above the soil surface. Similarly, other studies have found that oxygen is the limiting factor for maize and sorghum grown under CSW conditions (Colmer and Voesenek, 2009). Some cereal crop species form aerenchyma to transport oxygen diffusion from the shoot to the root tip and induce ROL as a barrier against oxygen loss in the root under flooded soil. Rice and maize form aerenchyma in the root under flooded soil, but rice can induce a tight type of ROL in the outer cell layer of the roots (Colmer, 2003; Yamauchi et al., 2013). It is suggested that soil moisture status affects the root architecture of crop genotype in cereals. In particular, in this study, the roots of maize and sorghum tended to increase their surface area to promote oxygen uptake from the air under waterlogged conditions with plasticity compared with the roots of rice and millet. Crop genotypes that showed high productivity regardless of whether the environment was good or bad have general adaptability but low regression coefficient based on the analysis of the environmental index. In that respect, the ability of rice to adapt to a wide range of moisture conditions is particularly beneficial for growth under rainfed conditions. By contrast, other crops, such as maize, are variable to unique environmental factors, which was clarified from the analysis of environmental impacts.

5. Conclusions

Root distribution associated with crop plasticity was influenced by soil water conditions in contrasting crop genotypes. Maize and sorghum depicted large changes in root distribution by promoting root plasticity in shallow and deep soil layers under anaerobic and aerobic conditions, respectively, while the root distribution of rice and millet was largely unaffected by soil moisture conditions. Root distribution was correlated with crop response and adaptation to changing soil water. The response of shoot dry weight and root dry weight under changing soil moisture environments from aerobic to anaerobic conditions showed a similar tendency, but it depended on the crop gynotypes. Rice demonstrated a wider response and adaptation to a wide range of soil moisture conditions, which is beneficial for growth under rainfed conditions, unlike maize, which was adapted to unique soil moisture factors.

CHATER 3

The Effect of Oxygen Concentration on Root distribution and Oxygen Profile in the Root

1. Introduction

Flooding is overcome the porous soil cause deficient oxygen for plant roots. The rate of oxygen diffusion in the flooded soil is about 10^4-fold slower than in the air. Thus, the oxygen transfer in submerge plant roots is limited and inhibited the aerobic respiration. Moreover, nutrients uptake is also limited by flooded roots. During the oxygen deficiency, both wetland and non-wetland plants develop aerenchyma formation in their root in order to increase the supply of external oxygen to root tissues to restore aerobic respiration(Colmer, 2003; Evans, 2003). In wetland and non wetland including rice and maize are constitutively forming lysigenous aerenchyma under waterlogging (Rajhi et al., 2011; Shiono et al., 2011), but maize normally does not form the aerenchyma under aerobic condition (Rajhi et al., 2011). In chapter 2, maize grown under mild-dry and waterlogging condition, showed higher root plasticity than rice by promoted root elongation into deeper soil layer and shallow distribution in upper soil layer under mild-dry and waterlogging respectively. In this issue, deficient oxygen concentration in soil might be caused root oxygen consumption. Unfortunately, chapter 2 data did not obtain oxygen concentration in the root and aerenchyma formation, and there is no qualitative data having report yet. Therefore, the aims of this chapter to examine the characteristics of difference in root elongation and oxygen profile in the root under waterlogging conditions from changing oxygen concentration by comparing between maize and rice. The hypothesis was that 1) oxygen concentration in the root cortex of rice grown under waterlogging will be higher than maize, and 2) shallow root might be caused by low oxygen in waterlogged soil.

2. Materials and Methods

2.1. Planting Materials and Growth Conditions

Maize and rice seeds were surface−sterilized with 10% (V/V) hydrogen peroxide for 2 minutes and then rinsed thoroughly with deionized water (Naredo et al., 1998). Seeds of maize and rice were germinated on filter paper in Petri dishes with deionized water in an incubator at 28°C in dark condition. Before treatment, the germinated seed of each specie was sown in paper pouch (W × H: 12.5 cm × 16.0 cm) and sponge (W × L × H: 2.0 cm × 2.0 cm × 2.0 cm) with moisturized half-strength nutrient solutions. Each specie was grown in a different growth chamber; maize was at 29/24 °C (day/night) for 7 days (2.5 leaf age) before treatment, and rice was 27/22 °C (day/night) for 10 days (2 leaf age) before treatment. The light flux density (MPPFD) of 930 µmol m^{-1} s^{-1} with 12/12 h and humidity of 50/80% (day;12 h/night; 12h) for all crops were set for all crops through this experiment. The composition of nutrient solution for maize and rice was descripted by Yamuchi et al. (Yamauchi et al., 2019). After 7−day−old and 10−day−old seedlings of maize and rice respectively, were transfer to 11−L pots (4 pouches and 3 sponge of each crop per pot). All pots contained agar (0.50%, v/v) and full−strength nutrient solutions. The treatments were varied oxygen concentration into three level including aerated (A; 8 mg O_2 L^{-1}), semi-aerated (SA; 2.5 mg O_2 L^{-1}), and deoxygenated (DO; dissolved oxygen (< 1 mg O_2 L^{-1})) prior to use by flushing with nitrogen gas. The number of plants in each treatment of maize and rice was seven plants (four in paper pouch and three plants in sponge), and each plant was represented a replication.

2.2 Shoot and Root Morphology Measurements

The shoot biomass, root biomass, and root length of maize and rice were obtained from paper pouch plants. At final sampling, the shoot and root parts were separate. The pouch was horizontally divided into two layers (0–8 cm and 8–16 cm). Root samples from each layer were scanned at 6400 dpi (EPSON XT-X830, Epson American Inc., Los Alamitos, CA 90720 USA). The scanned images were analyzed using an image analysis system (WinRHIZO, Regent Instruments Inc., Quebec, Canada) with a pixel threshold value range between approximately 165 and 175 to assess the total root length. The root and shoot biomasses of maize and rice were oven-drying at 80 °C to a constant before determining weight.

2.3. Oxygen microsensor

The Unisense oxygen microsensor (tip diameter ≈ 25 µm) is a miniaturized Clark-type oxygen sensor with a guard cathode designed for research applications. The sensor was equipped with UniAmp Single Chanel. The zero-oxygen calibration, the sensor tip was placed in 2 g ascorbate in 100 ml deionized (DI) H2O in 0.1 N NaOH solution. The well aeration calibration was conducted after aeration water (vigorous air bubbling for 5 minutes) had stopped movement. The demonstration of oxygen measurement shows in Figure 3-1.

2.4. Root Anatomical and Oxygen Measurements

The oxygen concentration and aerenchyma formation of each treatment in maize and rice were determined from sponge plants. An adventitious root per plant was selected from rice (120–130 mm long) and maize (100.8–150.9 mm long) for 14 days after treatment. The oxygen concentration was measured in the transparent container (W × L × H: 19 cm × 24 cm × 29 cm) containing 9–L of water. The composition of nutrient solution and oxygen of each treatment was the same with growth medium for rice and maize. An intact plant of maize and rice was placed in the prepared containers with the help by the rack for holding submerged part of root and stem. The sponge (junction where connection between stem and root) was submerged and attached with rack by rubber band to prevent the plants from moving. The small methane stick was placed on the measured root to prevent the root from moving and floating during the measurement. The Oxygen measurement was taken at three positions: 20 mm below the root-shoot junction ('basal region'), median ('intermediate region'), and 20 mm behind the root apex ('apical region'). The target area of oxygen measurement was started from cortex until stele, then average value was used to compare oxygen status among the treatments.

Root aerenchyma was performed from the same root which use for determining oxygen concentration. Three positions were assessed: 20±5 mm below the root-shoot junction ('basal region'), median ±5 mm ('intermediate region'), and 20±5 mm behind the root apex ('apical region'). The root cross-sections were hand-made using a razor blade, mounted in the water, and photographed using a light microscope (ASONE MP38T, Japan). Areas were measured with ImageJ software (Ver. 1.53K, USA).

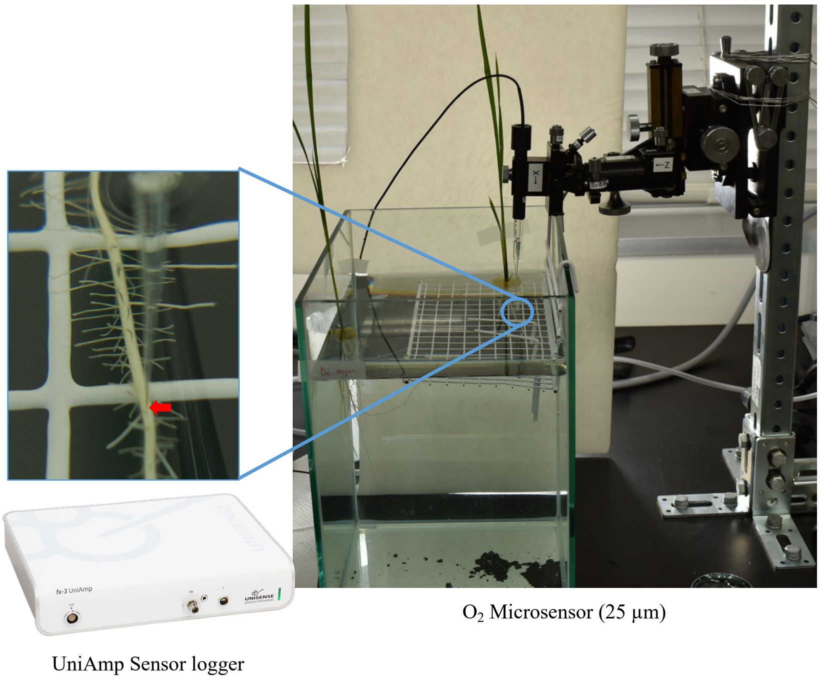

Figure 3-1. Demonstration of oxygen concentration measures in rice root. The oxygen microsensor (25 µm) connects with a UniAmp sensor logger.

2.5. Statistical Analysis

Mean of oxygen concentration and aerenchyma (% cross-section area, cortex to stele ratio (CSR), aerenchyma to cortex ratio (ACR)), root length distribution at upper and lower layers, shoot biomass. The statistic comparation among the treatment of each crop specie was analysis same as chapter 1.

3. Results

3.1. Oxygen concentration profile and aerenchyma formation

The oxygen concentration in adventitious root at three regions (base, median, and apex) shows in Figure 2. In maize, the oxygen content at base, median and apex regions was significant lower in DO than A (Figure 3-2A), while the oxygen content under A and SA did not differ significantly. The oxygen content at base, median and apex in rice was slightly lower in SA, DO than A, but there was no statistically significant difference among the treatments (Figure 3-2B).

The aerenchyma formation in maize genetically did not find under A, but rice can form the aerenchyma under all aeration conditions. In SA and DO, aerenchyma formation area of maize was high at base and median; whereas, fewer aerenchyma formation area in apex was observed (Figure 3-2C). The aerenchyma formation of rice was higher in SA and DO than A at all observed regions (Figure 3-2D). Among the different root regions of rice, the apex region showed lower oxygen content than median and base regions. At the root apex region, rice had higher ability to form aerenchyma than maize, in particularly under DO. Among the treatments, increasing cortex to stele ratio (CSR) and aerenchyma to cortex ratio (ACR) in both crops species were found under low to severe oxygen concentrations (Figure 3-3A and B). In comparison of CSR between maize and rice showed that rice had higher than maize under all treatments.

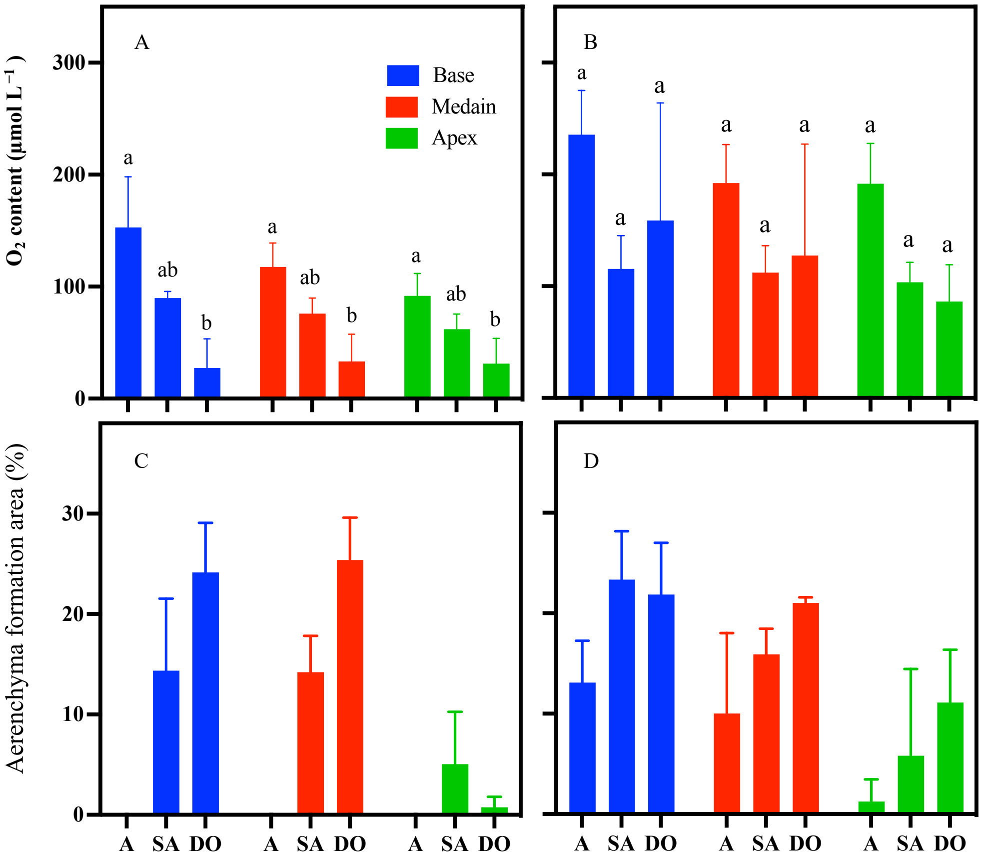

Figure 3-2. The oxygen content and aerenchyma formation in maize (A, C) and rice (B, C) grown under aerated (A), semi-aerated (SA) and deoxygenate (DO). The similar letters within a root base, median and apex for each crop specie did not significantly differ according to Tukey's test at the 0.05 probability level.

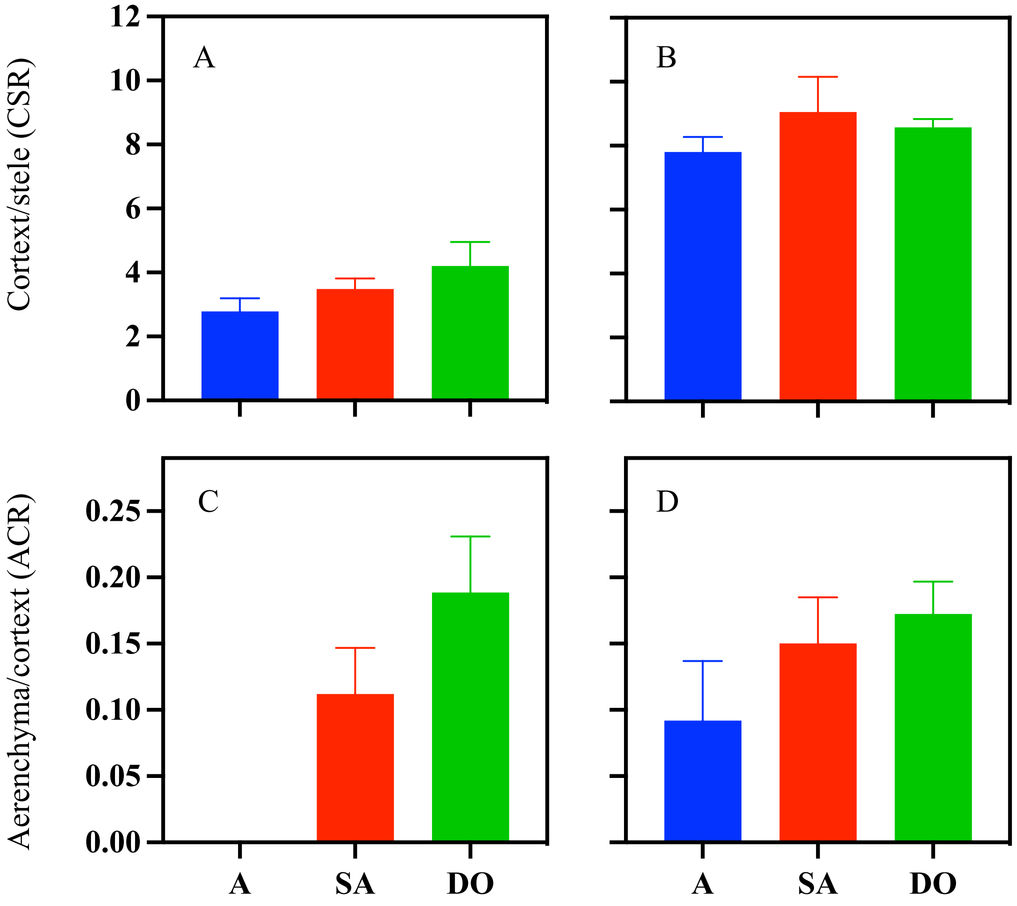

Figure 3-3. The cortex to stele ratio (ACR) and aerenchyma to cortex ratio (ACR) in maize (A, C) and rice (B, C) grown under aerated (A), semi-aerated (SA) and Deoxygenate (DO). The data was average value of base, median, and apex parts.

3.3. Root distribution, plant length and shoot biomass.

The root distribution of maize and rice was governed by oxygen concentration in the solution, in particularly under severe low oxygen treatment (DO) (Figure 3-5A and B). The total root length of maize and rice at upper layer (0–8) did not significantly decrease, but total root length of both crop species at lower layer was significant decreased under DO compared to A.

The plant length and shoot biomass of maize was significant declining according to decreasing oxygen levels (SA and DO) in the solution (Figure 3-6A and C). However, decreasing oxygen levels in solution did not significantly affect on plant length and shoot biomass in rice (Figure 3-6B and D).

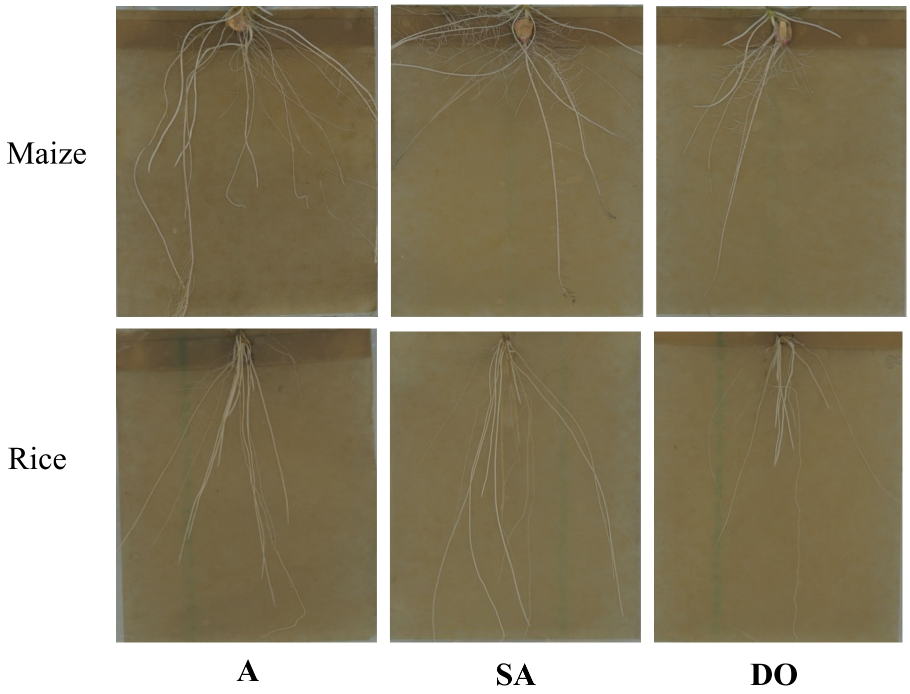

Figure 3-4. The image of root distribution of maize (upper) and rice (lower). The roots grown under three level of oxygen content in solution: aerated (A), semi-aerated (SA), and deoxygenated (DO) were photographed at 14 days after treatment.

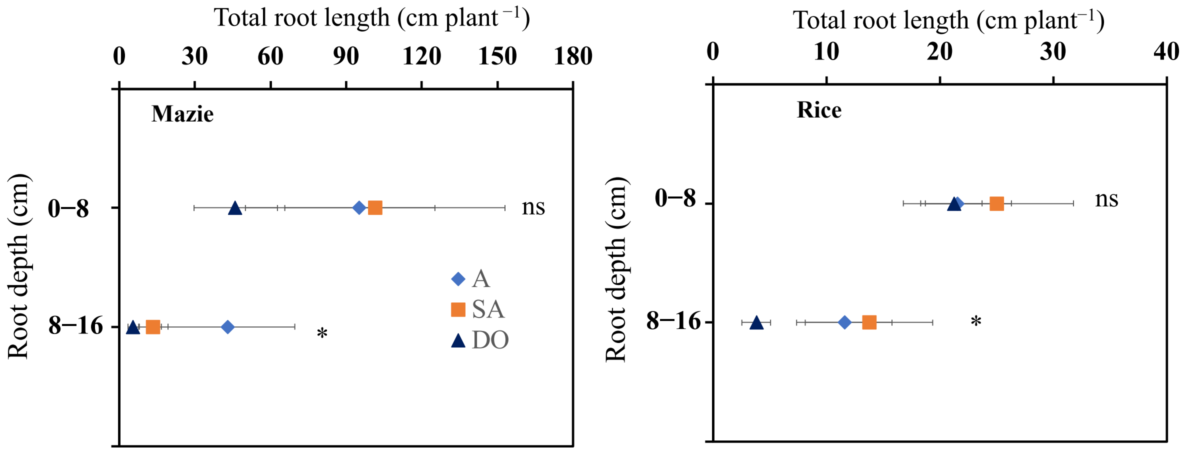

Figure 3-5. Total root length distribution at upper layer (0-8 cm) and lower layer (8-16 cm) of maize (left) and rice (right) grown under under three level of oxygen content in solution: aerated (A), semi-aerated (SA), and deoxygenated (DO). * and ns indicate statistical significance at $p < 0.05$ and no significance, respectively

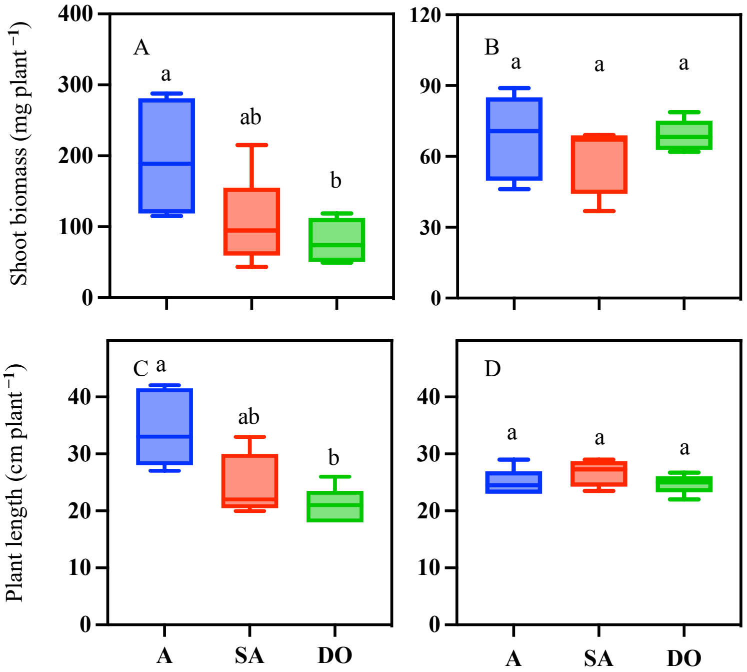

Figure 3-6. The effect of aerated (A), semi-aerated (SA) and Deoxygenate (DO) on shoot biomass and plant length of maize (A, C) and rice (B, D). The similar letters within a root base,

median and apex for each crop species did not significantly differ according to Tukey's test at the 0.05 probability level.

4. Discussion

Waterlogging stress has adverse effects not only on vegetative growth, but also on reproductive growth, eventually leading to yield loss or even complete harvest failure (Herzog et al., 2016; Khanthavong et al., 2021; Khanthavong et al., 2022; Tian et al., 2019). Waterlogging removes air from soil pores, resulting oxygen diffusion rate in the water is only 1/10,000 of that in air. Consequently, oxygen availability in waterlogged soil greatly restricted root respiration, decreased root activity, and energy shortage (Armstrong, 1979; Pan et al., 2021; van Veen et al., 2014).

Most plants are sensitive to waterlogging including maize, as the diffusion rate of oxygen in the roots of plants decrease significantly during waterlogging. Thus, it causes low oxygen concentration in root cortex of sensitive species of maize and chickpeas (Colmer et al., 2020; Pedersen et al., 2021). Data in Figure 3-2 support the hypothesis that oxygen concentration in the root cortex of rice grown under waterlogging will be higher than maize. Under waterlogging conditions, the formation of aerenchyma of plants is an anatomical adaptation (Armstrong, 1979; Colmer, 2003; Evans, 2003). It is well known to enhance the internal diffusion of atmospheric and photosynthetic oxygen from the shoot part to the flooded roots, allowing the roots to maintain aerobic respiration (Armstrong, 1979). Maize and rice form lysigenous aerenchyma (program cell death) in cortex (Drew et al., 1981; Jackson, 1985). Rice is semi-wetland specie, well grow in paddy, it can form aerenchyma in aerobic and anaerobic conditions unlike-maize which can form aerenchyma only anaerobic condition (Abiko et al., 2012; Yamauchi et al., 2019). In Figure 3-2C and D, aerenchyma formation of maize and rice under SA and DO was higher at base and median regions than apex, especially at root apex of maize was fewer aerenchyma formation than rice. On the other hand, the significant lower oxygen content in root under DO at all regions than under A was obtain in maize, whereas, oxygen content in rice root at all regions did not significant difference among the treatments. The varied oxygen content in the root governed various oxygen content in the solution related to varied shoot biomass of maize. While shoot biomass of rice did not differ comparing among the treatment. Based on aerenchyma formation and root oxygen profile can extract two evidences as following: (1) few aerenchyma formation at root apex region under DO in maize causes root hypoxia which it would highly restrict oxygen diffusion, respiration and activity, (2) low root oxygen profile under DO might caused by no or untight barrier to radial oxygen loss (ROL) in the root which indicated high oxygen loss at base region. In stagnant deoxygenated solution, Z. Nicaraguans was superior to maize in transporting oxygen from shoot base to root tip due to formation of large aerenchyma and stronger barrier to ROL in adventitious roots (Abiko et al., 2012). The large aerenchyma formation and tight barrier to ROL in rice is also well document under hypoxia (Armstrong, 1979; Yamauchi et al., 2013). Aerenchyma formation under aerated of rice is beneficial to maintain aerobic root activity when the root is flooded. While maize takes fully development of aerenchyma formation about 2.5 day (Gunawardena et al., 2001); it meaned that root activity was shutdown during the plant development of aerenchyma formation.

Maize and rice trended increasing CSR under SA and DO; however, rice was generally higher CSR than maize under all treatments (Figure 3-3). CSR indicated as useful root trait contributing to tolerance of crops to waterlogging (Yamauchi et al., 2019). This is because cortex is the tissue aerenchyma formation occur (Bary, 1884). Increasing CSR of maize and rice under hypoxia led to increase ACR (Figure 3-3). This suggested that it was genetically of adaptation of wetland and upland species. Large CRS in rice under DO, was associated large aerenchyma formation in whole root ratio, increased root oxygen diffusion related to better growth compared to maize. Indeed, it is evidence why rice can grow well in paddy.

The root distribution of maize and rice was also governed by oxygen concentration in solution (Figure 3-5). Total root of maize and rice was restricted elongation at lower layer (8–16 cm) under DO. The results supporting the hypothesis that shallow root will cause by low oxygen in the solution. Our previous study showed that rice grown in the root box under waterlogging could elongate root to deeper soil layers. Limiting root elongation of rice might cause by severe oxygen concentration of DO (<1 mg L^{-1}) compared to flooded soil. Under DO, it was not only limiting root elongation of rice, but maize as well. In nature of flooded soil, the oxygen diffusion soil surface is higher than deeper soil layers. The shallow root distribution of rice under severe oxygen deficiency (DO) did not affect on shoot biomass and plant length, but shoot biomass and plant length of maize were poor growth under this condition (Figure 3-6). Therefore, the shallow root distribution of rice indicates as avoidant mechanism to severe oxygen deficiency, whereas maize suggests as survival mechanism to severe oxygen deficiency.

5. Conclusion

Shallow root distribution was governed by severe oxygen deficiency, and it indicated as avoidant and survival mechanism of wetland and upland, respectively. Maize was lower oxygen content in the base part downward to apical part of root than rice, which caused poor growth of shoot of maize under waterlogging conditions. The ability of oxygen transportation from shoot to apical root was adaptation strategy of wetland specie (Rice), and it can be determined by oxygen measurement. Further research should focus on relationship between to aerenchyma formation and barrier to ROL and oxygen content in the root.

CHAPTER 4

Combinational Variation Temperature and Soil Water Response of Stomata and Biomass Production in Maize, Millet, Sorghum and Rice

1. Introduction

Global climate change increases variability in temperature, drought, and flooding (Beillouin et al., 2020; Pickson et al., 2020; Yang et al., 2020). Rice (*Oryza Sativa* L.) as C_3, and maize (*Zea mays.* L.), millet (*Panicum miliaceum* L.), and sorghum (*Sorghum bicolor* (L.) Moench) as C_4 are cereal crops grown under variable climates and rainfed environments in Asia, America, and Africa, sharing a high contribution to global food security (FAO, 2021). Climate change and weather disasters are major causes of reduction in agricultural productivity (Berg et al., 2013; Giorgi et al., 2019).

Under rainfed conditions in tropical and subtropical regions, these crops experience diverse individual and successive combined environmental stresses attributed to climate change such as drought, flooding, and temperature variability that directly affect their morphology and physiology, leading to crop failure. The effect of individual environmental stress such as water stress and temperature on crop production is well documented (Lamaoui et al., 2018; Matsuura et al., 2016; Maulana and Tesso, 2013; Wang et al., 2018). This study focusses on to assess crops response to combined soil water status and temperature in relation to morphological and gas exchange parameters (i.e., photosynthesis and stomatal behavior).

Stomata are the gatekeepers of gas exchange and the primary determinants of CO_2 assimilation. Stomata conductance (*gs*) response to soil water and temperature stresses is basic information for photosynthesis transpiration, and it has increasingly been a concern under global warming. The positive correlation between *gs* and photosynthesis (*A*) has been reported in the laboratory (Wong et al., 1979) and a positive correlation between *gs* and yield has also been reported in field conditions (Fischer et al., 1998). Alternatively, stomatal closure is caused by water stress and temperature (Blatt, 2000). Additionally, stomatal closure can directly influence CO_2 absorption (photosynthesis rate) and transpiration rate (Ouyang et al., 2017; Wong et al., 1979).

Rice, an original lowland C_3 crop, is resilient, and due to its crucial root anatomy to cope with soil waterlogging (Yamauchi et al., 2019; Yamauchi et al., 2021b) has been introduced to waterlogging and upland conditions (Sakagami and Kawano, 2011). In contrast, maize, millet, and sorghum are better adapted to upland conditions due to their water absorption ability that is related to their deep root system (Zegada-Lizarazu and Iijima, 2005). Nevertheless, the response of crops to soil water status depends on crop genotypes and varieties (Galamay et al., 1991; Khanthavong et al., 2021).

More than one-third of the world's irrigated area suffers due to waterlogging. Continuous flood conditions lead to lack oxygen in the soil, restricting respiration of growing roots, living organisms, and changing soil chemical property (Parent et al., 2008). The response of crops to waterlogging depends on varieties. Most of the upland crop species are sensitive to waterlogging conditions compared to wetland crop species such as rice due to their inability transport oxygen from the leaves to root tips for sustaining the root respiration and gas exchange. In condition, waterlogged soil cause reduction of *gs* and *A* in sorghum (Promkhambut et al., 2010; Zhang et al., 2019a), maize (Tian et al., 2019), and millet (Matsuura et al., 2016). It means that these crops may be able survival in waterlogging(Matsuura et al., 2016; Matsuura et al., 2021; Pardales Jr et al., 1991; Tian et al., 2019).

Drought occurs when the soil moisture is continuously low, where water extraction by root and water transport within the plant is reduced. To overcome drought stress, plants respond by increasing the water extraction and use efficiency of the root, and simultaneously reduce transpiration (water loss)) (Pareek et al., 2009) by closing stomata as well as maintaining turgor

(Farooq et al., 2009). Crops have different water requirement for growth and productivity. Rice and maize had higher water requirement than millet and sorghum (Hadebe et al., 2017). The ability to maintain photosynthesis during drought is indicative of the potential to sustain productivity under water deficit. The stomatal response to drought conditions depends on crop genotypes (Lipiec et al., 2013; Munns et al., 2010). Sorghum exhibits the ability to maintain stomatal opening and photosynthesis at low water potentials, as well as the ability for osmotic adjustment (Ludlow et al., 1990). In rice, the photosynthetic rate declines dramatically during soil drought, mainly due to the decrease in the *gs* (Ludlow et al., 1990). Stomatal limitation on photosynthesis depends on the level of drought (Farooq et al., 2009).

Extreme temperature directly impacts on the production of cereal crops. The optimal temperature range of C_3 plants (28–30 °C) is lower than C_4 plants (26–35 °C) such as maize, millet, and sorghum (Sánchez et al., 2014; Sood et al., 2015). C_3 and C_4 plant species show various responses to *gs*, *A*, and transpiration (*E*) under temperature stress (Crafts-Brandner and Salvucci, 2002; Osborne et al., 2008; Zhang et al., 2019b). Increase in global temperature can directly affect stomatal behavior and reduce yield in major crops (Lamaoui et al., 2018; Zhao et al., 2017). The increase in mean global temperature has been predicted under climate change (IPCC, 2014b). Increasing of temperature is closely associated with increased vapor pressure deficit (VPD). The key response of crops to variation of VPD is by regulation of transpiration through *gs* (Lawson and Blatt, 2014). On the other hand, low temperature is another influence on stomatal aperture of crops. Cool conditions affect stomatal closure in *Phaseolus vulgaris* and maize (Wolfe, 1991). Low temperature causes a reduction in the plant’s capacity for calcium uptake by guard cells due to stomatal closure (Hussain et al., 2018; Wilkinson et al., 2001). Calcium acts as an intracellular secondary messenger, regulating ion transport activity plasma and vacuolar membranes in guard cell turgor (Assmann and Shimazaki, 1999; Wilkinson et al., 2001).

Previously, our study showed that rice and millet have better root distribution under waterlogging than in dry conditions compared with maize and sorghum, whose root distribution was limited under waterlogging, leading to poor growth of aboveground biomass (Khanthavong et al., 2021). However, this study was conducted in a specific environment only. A combination of factors such as the variable temperatures, drought, and waterlogging occur during crop production, especially under rainfed agriculture. The effect of combination of factors on crop failure may be higher than an individual factor.

Many studies have reported the effect of combinations of water stress and temperature variability on the growth and productivity of crops (Bin et al., 2010; Craufurd and Peacock, 1993; Fahad et al., 2017; Heyne and Brunson, 1940; Rizhsky et al., 2004). However, knowledge on the effect of various soil water status and temperature variabilities such as soil waterlogging, dry conditions, and their interactions with low and high temperatures on stomatal response among crop genotypes are scant. Hence, we hypothesized that the response of shoot biomass and *gs* behavior of different crop genotypes to combinations of soil water stress and temperatures have an effect on crop genotypes due to their variable adaptability of *gs*. Therefore, we identified the variation in stomatal traits and the impact of *gs* behavior under various soil water status and temperatures on rice, maize, millet, and sorghum.

2. Materials and Methods

2.1. Seedling Preparation

Four crop species: (1) maize (*Zea mays* L. cv. Honey Bantam), (2) sorghum (*Sorghum bicolor* Moench. cv. High grain sorghum; prone to waterlogged soil but adaptable to dry soil), (3) millet (*Echinochloa utilis* Ohwi. cv. Kumamoto local), and (4) rice (interspecific progeny cv. NERICA1), as adaptable to saturated and dry soil conditions (Khanthavong et al., 2021), were used. Each crop's seed was placed in a Petri dish containing filter paper moistened with distilled water.

2.2. Experiment 1: The Effect of Various Soil Moisture Content and Atmospheric Environment on Stomatal Conductance and Shoot biomass of Four Crops

This experiment was conducted in screen house (without atmospheric environment controlling), Kagoshima University (31.5699° N, 130.5443° E), Japan, and repeated twice (Exp. 1A and 1B). Exp. 1A and 1B were carried out in early to mid-summer (24 August–9 September 2020) and late summer to early autumn (23 October–11 November 2020).

2.2.1. Experimental Site

The seedlings, leaf age number was 2.5 for rice and 3 for other crops were grown on a concrete container (360cm L × 110 cm W × 35–91 cm D) filled with a mixture of loamy soil and river sand (1:3 *v*/*v*). The container was divided into nine plots representative of different top sequence positions. The lowest to highest top sequence positions ranged from 30 to 90 cm, and the difference between each plot (top sequence position) was 6.5 cm.

2.2.2. Treatment

Each plot was divided into three replications measuring 36.6 × 41.0 cm. Two seedlings per crop were randomly transplanted in each replication with plant interval and between row spacing at 10.0 × 13.3 cm. Rice plants were transplanted as a guard row along the borders. Daily irrigation was applied in the morning and evening to maintain adequate soil moisture prior to initiate treatments.

The water treatments started at 10 days after transplanting. The treatment was ended 17 days after treatment (DAT). Water was added to the container, allowing the lowest end to be flooded and water level maintained at 2–3 cm above the soil surface. Another soil surface of eight treatments was close to or above the water level (Goto et al., 2021). Nine water treatment regimes were controlled in each treatment, categorized into three soil moisture statuses: dry, moderate, and wet. Three positions (sub-soil moisture status), namely, high, middle, and low, were contained in each soil moisture status. Details of the treatment and plot layout are shown in Table 4-1 and Figure 4-1, respectively.

Table 4-1. Explanation of soil moisture statuses (treatments) in experiment 1.

No.	Abbreviation	Soil water statuses (Treatments)
1	WL	Low position of sub-wet soil conditions (Waterlogging)
2	WM	Middle position of sub-wet soil conditions
3	WH	High position of sub-wet soil conditions
4	ML	Low position of sub-moderate soil conditions
5	MM	Middle position of sub-moderate soil conditions
6	MH	High position of sub-moderate soil conditions
7	DL	Low position of sub-dry soil conditions
8	DM	Middle position of sub-dry soil conditions
9	DH	High position of sub-dry soil conditions (Severe dry soil)

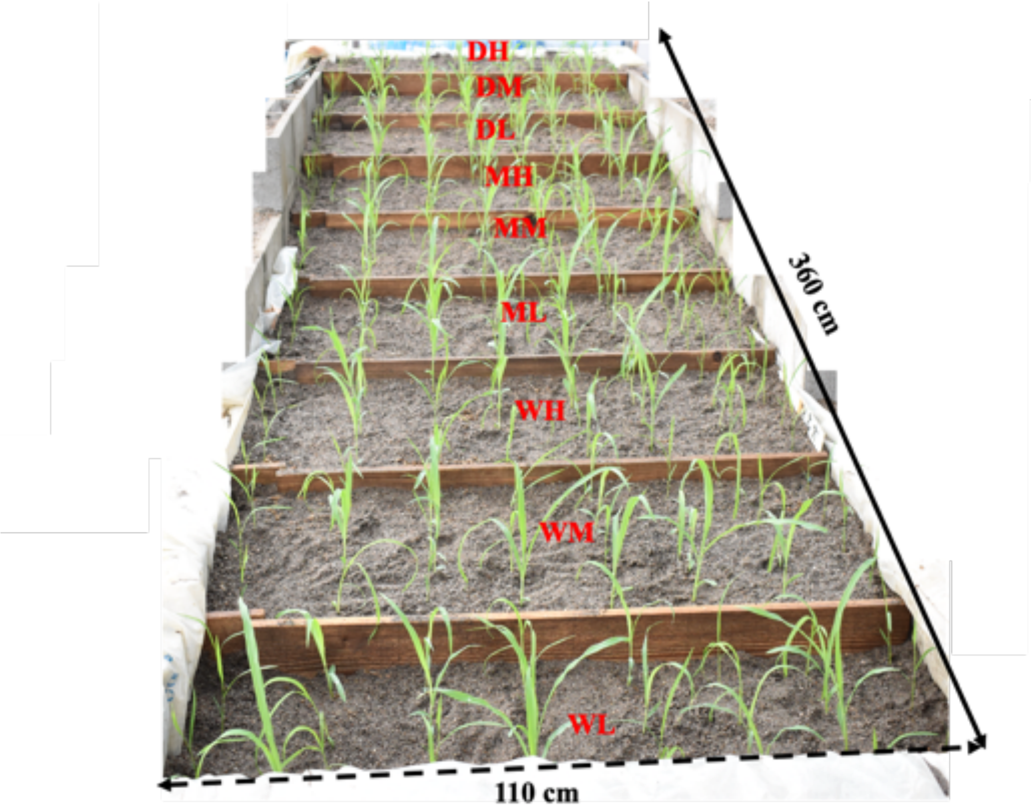

Figure 4-1. Plot layout of experiment 1 at two days after transplanting.

2.2.3. Soil moisture Content, Leaf Area, Shoot Biomass, and *gs*

A soil moisture sensor (5TE) placed at a depth of 15 cm was used to measure the soil moisture status of each plot (total nine plots). Data were recorded using a Datalogger Em50 Series (Decagon Devices Inc., Pullman, WA, USA) with a 60 min interval between each measurement through the experiment. The *gs* was measured by using a porometer (AP4, Delta-T Devices, Cambridge, UK) between 9:00 a.m. and 12:00 p.m. at 16 DAT from the second youngest fully expanded leaf. The sampled shoot biomass and leaf area (LA) were conducted at 17 DAT by cutting the shoot and separating the leaves and stems. Then, the gathered leaves and stems were oven-dried at 80 °C to a constant weight before determining shoot dry weight. An automatic area meter (AAM-9, Hayashi Denko Co., Ltd., Tokyo, Japan) was used to measure LA.

2.3. Experiment 2: The Effect of the Combination of Soil Water Statuses and Temperatures on Gas Exchange and Shoot biomass of Four Crops

This experiment confirmed the crop response to a combination of water stress and temperatures, referred to as experiment 1. This experiment was conducted at Kagoshima University, Japan, in December 2021.

This experiment hypothesized that the response of shoot biomass of different crop genotypes to combinations of soil water stress and temperatures have an effect on crop genotypes due to their variable adaptability of *gs*.

2.3.1. Experimental Site and Growing Media Preparation

The plants were grown with maximum photosynthetic photon in controlled environment chambers (Biotron NK system, model LPH-411PFQDT-SP; Nippon Medical and Chemical Instruments Co., Ltd., Osaka, Japan) with a flux density (MPPFD) of 930 µmol m^{-2} s^{-1}. The air temperature was set to 32/22 °C (day/night) with a relative humidity of 50/80% (day/night) and a light/dark regime of 12/12 h before treatment. A pot (42 cm × 28 cm × 21 cm) was filled with 7 kg of mixed soil containing 30% (*v*/*v*) soil, 30% vermiculite, and 10% peat moss. After compound fertilizer with concentration of 1.3 g of each N-P-K (8-8-8; N-P-K) per pot was mixed with the soil, the soil pH was an average of 5.65. Then, the container was watered abundantly for three hours before excess water was drained overnight to obtain the soil field capacity. After the soil field capacity was set, each container was weighed to obtain the initial weight. The measurement of soil moisture was the same method as experiment 1.

2.3.2. Method and Treatment

The experimental treatments consisted of six combinations of soil moisture and temperature, i.e., (1) combination of moderate soil moisture and low temperature (moderate soil moisture (MSM)/24/15 °C); (2) combination of moderate soil moisture and high temperature (MSM/34/25 °C); (3) combination of gradual soil drying and low temperature (gradual soil drying (GSD)/24/15 °C); (4) combination of gradual soil drying and high temperature (GSD/34/25 °C); (5) combination of continuous soil waterlogging and low temperature (continuous soil waterlogging (CSW)/24/15 °C); and (6) combination of continuous soil waterlogging and high temperature (CSW/34/25 °C). Each treatment was replicated four times. Ten days old with 3 leaves age for maize, millet, sorghum, and 2.5 leaves age for rice, two seedlings (each representative replication) per pot were randomly transplanted with plant interval and between row spacing at 10.0 cm × 13.3 cm.

To maintain adequate soil moisture content before treatments, the watering was irrigated every evening, and the amount of daily watering was estimated by water loss on the day of watering. The containers were weighed every day between 4:00 to 5:00 p.m. to calculate water loss under MSM and GSD. Under MSM conditions, the pot was refilled by water to compensate for the water loss and maintain the soil field capacity. Under GSD conditions, a maximum of 200 g of water loss per day was fixed; if the water loss over 200 g per day was filled with an equal amount of water lost, the soil was gradually dried for low- and high-temperature treatments. Lastly, the flooded water level was set at 2–3 cm above the soil surface for CSW. The duration of treatment was 17 DAT.

2.3.3. Shoot Biomass, leaf Area, *A*, *gs*, and *E*

Three plants from each treatment were selected from each growth chamber to determine the *A*, *gs*, and *E*. Using a portable gas exchange measurement system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) equipped with the standard leaf chamber (chamber area of 6 cm^2) (Figure 3-2), gas exchange parameters were measured on the attached second youngest fully expanded leaf at 0, 4, 8, 12, and 17 DAT from 10:00 a.m. to 2:00 p.m. The measurement settings included a light intensity of 830 mol m^{-2} s^{-2}, an ambient CO_2 concentration of 420 mol mol^{-1}, and a block temperature of 27 °C for 0 days of all treatments: 19 °C for treatment of any soil moisture status under low temp treatments, and 29 °C for treatment of any soil moisture status under high temperature. The humidity was set to alter close *E*. The LA and shoot biomass measurement was conducted at 17 DAT (27 days old plant) according to the same procedure as experiment 1.

Figure 4-2. Gas exchange measurement by using a portable gas exchange measurement system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) equipped with the standard leaf chamber (chamber area of 6 cm2).

2.4. Data Analysis

All parameters were transformed using standardization to compare the shoot biomass, LA, *A*, *gs*, *E*, and *WUE* among the crops. Then, a two-way analysis of variance (ANOVA) was used for both experiment 1 and 2 to compare the crop response to treatments using Graph Pad Prism 9.0 (GraphPad Software, San Diego, CA, USA; https://www.graphpad. Com). The linear or non-linear (polynomial) correlation line was used, which was decided by coefficient. Pearson's correlation was conducted to test the significant correlation of linear or non-linear correlation. A multiple linear regression was used with single and combination parameters among soil moisture status, temperature, *A*, *gs*, *E*, and *WUE* to evaluate which factors influenced shoot biomass. Turkey's test was used to determine statistical differences among the treatments.

3. Results

3.1. Experiment 1

3.1.1. Soil Moisture Content and Atmospheric Environment

The change in soil moisture content, air temperature, relative humidity, and vapor pressure deficit (VPD) during the treatment period are summarized in Figure 4-3. The trend of soil moisture for each treatment in Exp. 1A and 1B was similar, where waterlogging (WL) and dry soil (DH) had the highest (38.8% and 43.7% for Exp. 1A and 1B, respectively) and lowest moisture contents (7.6% and 10.6% for Exp. 1A and 1B, respectively), respectively (Figure 4-3A, D). Soil moisture content with severe dry soil treatment (DH) gradually declined from 16.0% to 7.6% for Exp. 1A and 15.1% to 10.6% for Exp. 1B during the treatment period. The temperature in Exp. 1A was higher than in Exp. 1B with the average temperature of day/night being 34/25 °C and 24/15 °C in Exp. 1A and 1B, respectively (Figure 4-3B). The vapor pressure deficit, relative humidity, and solar irradiance were not significantly different between Exp. 1A and 1B, but their fluctuations were different between Exp. 1A and 1B (Figure 4-3C, E, F).

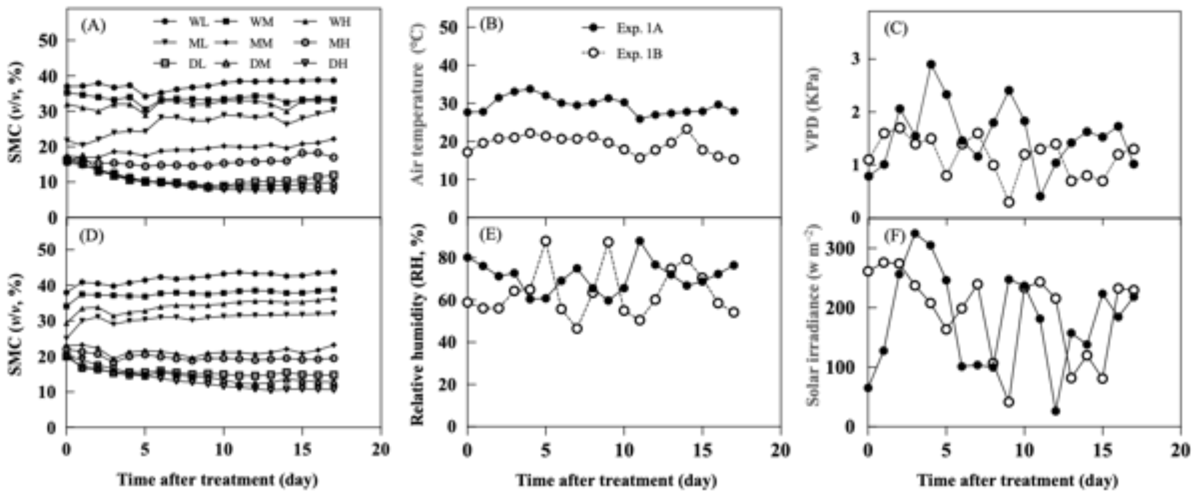

Figure 4-3. Soil moisture content (SMC) in experiment 1A (A) and 1B (D), air temperature (B), vapor pressure deficit (VPD) (C), relative humidity (E), and solar irradiance (F) during the treatment of experiments 1A and 1B.

3.1.2. The Correlation between Soil Moisture Status and Shoot Biomass, LA, and *gs*

A linear and nonlinear correlation that depended on crop and experiment existed between soil moisture status and shoot biomass, LA, and *gs* in comparison between Exp. 1A and 1B (Figure 4-4). There were significant nonlinear correlations between soil moisture status and shoot biomass, LA, and *gs* for both Exp. 1A and 1B (Figure 4-4A, E, I) in maize. Furthermore, the correlation between soil moisture status and shoot biomass, LA, and *gs* was observed as a nonlinear correlation on shoot biomass, LA, and *gs* in Exp. 1A in sorghum, whereas, in Exp. 1B, it was a linear correlation on shoot biomass, LA, and *gs* (Figure 4-4B, F, J). In millet, the nonlinear and linear correlation between soil moisture status and shoot biomass, LA, and *gs* was observed in Exp. 1A and 1B, respectively. The correlation was significant between soil moisture status and shoot biomass for both Exp. 1A and 1B, LA for Exp. 1A, and *gs* for Exp. 1A, but Exp. 1B showed no significant correlation between soil moisture status and LA (Figure 4-4C, G, K). Additionally, a nonlinear correlation between soil moisture status and shoot biomass LA, and *gs* was found in Exp. 1A and 1B in rice, whereas the excluded correlation between soil moisture status and shoot biomass in Exp. 1A showed a negative linear correlation. A significant correlation was found between soil moisture status and shoot biomass, LA, and *gs* for Exp. 1A and 1B (Figure 4-4D, H, L). The distance of correlation lines between soil moisture status and shoot biomass, LA, and *gs* showed that maize and rice had fewer distance correlation lines between Exp. 1A and 1B than sorghum and millet (Figure 4-4).

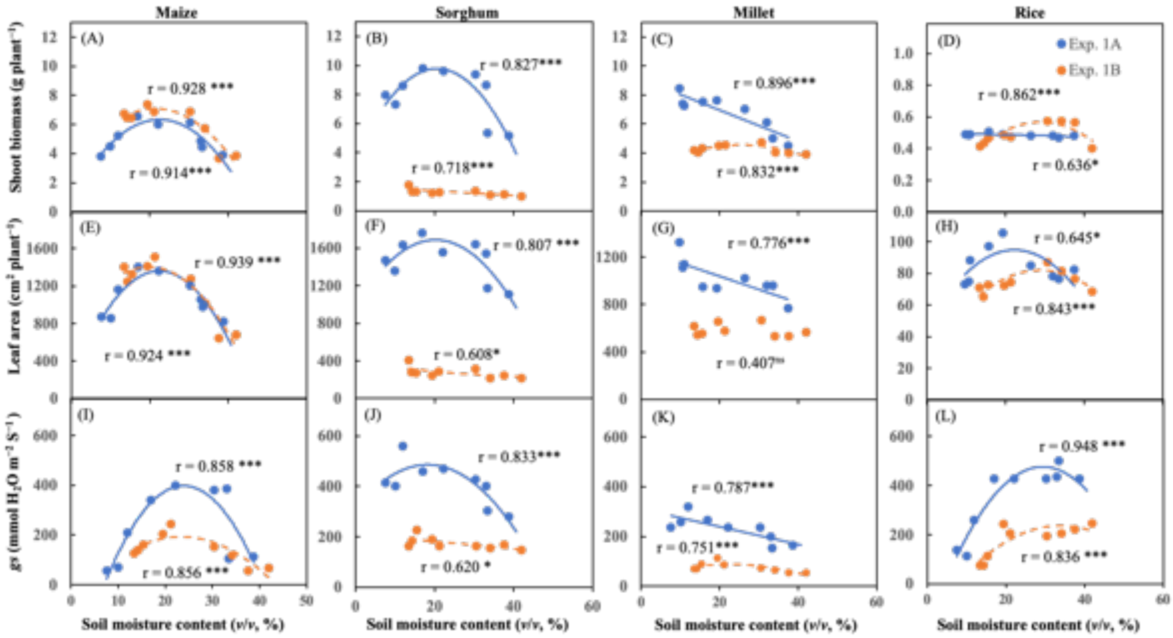

Figure 4-4. Correlations between soil moisture content and shoot biomass (A–D), leaf area(E–H), and stomatal conductance (gs; I–L) in maize (A, E, I), sorghum (B, F, J), millet (C, G, K), and rice (D, H, L). *, ***, and ns indicate Pearson statistical significance at $p < 0.05$, $p < 0.001$, and non-significance, respectively ($n = 9$). Linear or nonlinear (polynomial) correlation line is decided by coefficient.

3.2. Experiment 2

3.2.1. Response of Shoot Biomass, LA, and Gas Exchange to a Combination of Soil Moisture Status and Temperature

The volumetric soil moisture content of moderate soil moisture (MSM), gradual soil drying (GSD), and continuous soil waterlogging (CSW) combined with low or high temperature is shown in Figure 4-5. There was less difference between the soil moisture status combinations with low or high temperatures. It is because the soil moisture content was controlled at field capacity before the start of the treatment. After treatment, the average soil moisture content under MSM/24/15 °C or 34/25 °C was maintained at field capacity. In contrast, the soil moisture content under GSD/24/15 °C or 34/25 °C was gradually reduced by withholding irrigation for 17 days. Alternatively, when the pots were submerged, the volumetric soil moisture content under CSW/24/15 °C or 34/25 °C depicted very little change.

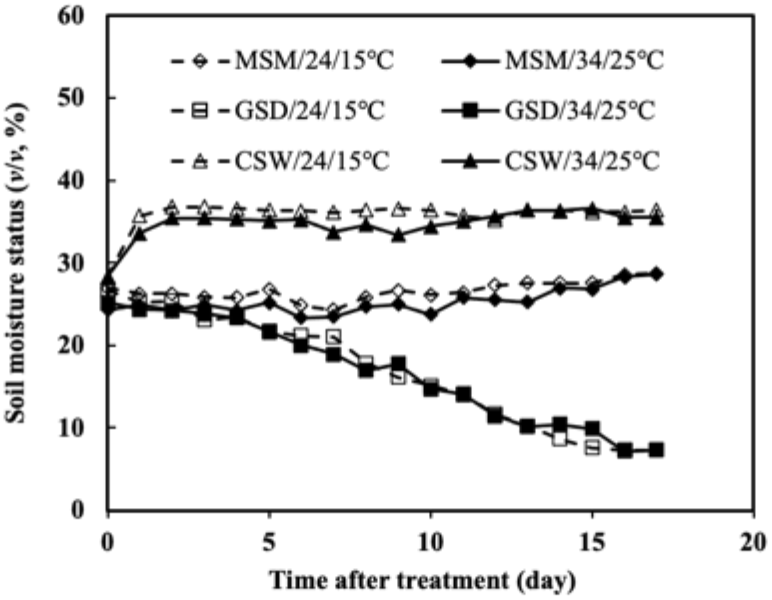

Figure 4-5. Soil moisture content (SMC) during the treatment period of experiment 2.

The effect of the combination between soil moisture status and the temperature varied significantly depending on crop genotypes ($p < 0.001$) for shoot biomass, LA, *A*, *gs, E,* and water use efficiency (*WUE*) (Table 4-2). Similarly, treatments on shoot biomass showed significant effects, *A*, *gs*, *E*, and *WUE* ($p < 0.001$) for all crops. In contrast, there was no significant effect within the crops on all parameters.

All crops showed a negative response on shoot biomass and LA under MSM with low temperature, except LA of maize showed a positive response under this condition. Maize and sorghum had better shoot biomass and LA growth under GSD/34/25 °C compared to millet and rice; maize and rice showed positive response on shoot biomass under GSD/24/15 °C, but not sorghum and millet. Under GSD/24/15 °C, the LA of all crops had a negative response. Moreover, each crop showed a similar response on shoot biomass and LA under CSW/24/15 °C and 34/25 °C. Maize and sorghum had an adverse reaction to CSW, whether 24/15 °C or 34/25 °C. Alternatively, the effect of CSW on the shoot biomass and LA of millet and rice were negatively affected by low temperature (24/15 °C).

Under various combined factors, as presented in Figure 4-4, there were variations in gas exchange among the crops. The *A* was a positive response in all crops grown under MSM/24/15 °C or 34/25 °C (Figure 4-4C). Under GSD/24/15 °C, a negative impact existed in maize and millet, and that of rice was under GSD/34/25 °C. In comparison, the negative effect of GSD on *A* of sorghum was found at low and high temperatures. Under CSW, maize and sorghum had a negative response on *A* at low and high temperatures, whereas the effect of CSW in millet and rice was found in low temperatures (Figure 4-6C). Low temperature harmed *E* of all crops grown under different soil moisture status (Figure 4-6D). Under MSM, *gs* of all crops had a positive response in high temperatures, but they showed a negative impact at low temperatures except maize. There was a high negative impact on *gs* in maize, sorghum, and millet under GSD/24/15 °C. Furthermore, GSD showed a negative response on *gs* at low and high temperatures in rice. Under CSW, *gs* and *A* of maize and sorghum demonstrated a negative response at low and high temperatures, but rice and millet showed positive response at high temperature (Figure 4-6E). Figure 4-6F shows that low temperature promoted a positive response of *WUE* under numerous soil moisture statuses, but high temperature negatively impacted *WUE* in all crops.

Table 4-2. The effect of the combination of environmental factors (soil moisture status and temperature) on shoot biomass, leaf area, A, gs, E, and WUE of crops (maize, sorghum, millet, and rice), and interaction between crops and environment in experiment 2.

Source of variation	**Shoot biomass**	**Leaf area**	***A***	***gs***	***E***	***WUE***
Crops	ns	ns	ns	ns	ns	ns
Soil water status	***	***	***	***	***	***
Crops × soil water status	***	***	***	***	***	***

*** and ns indicate statistical significance of ANOVA at $p < 0.001$ and non-significance, respectively.

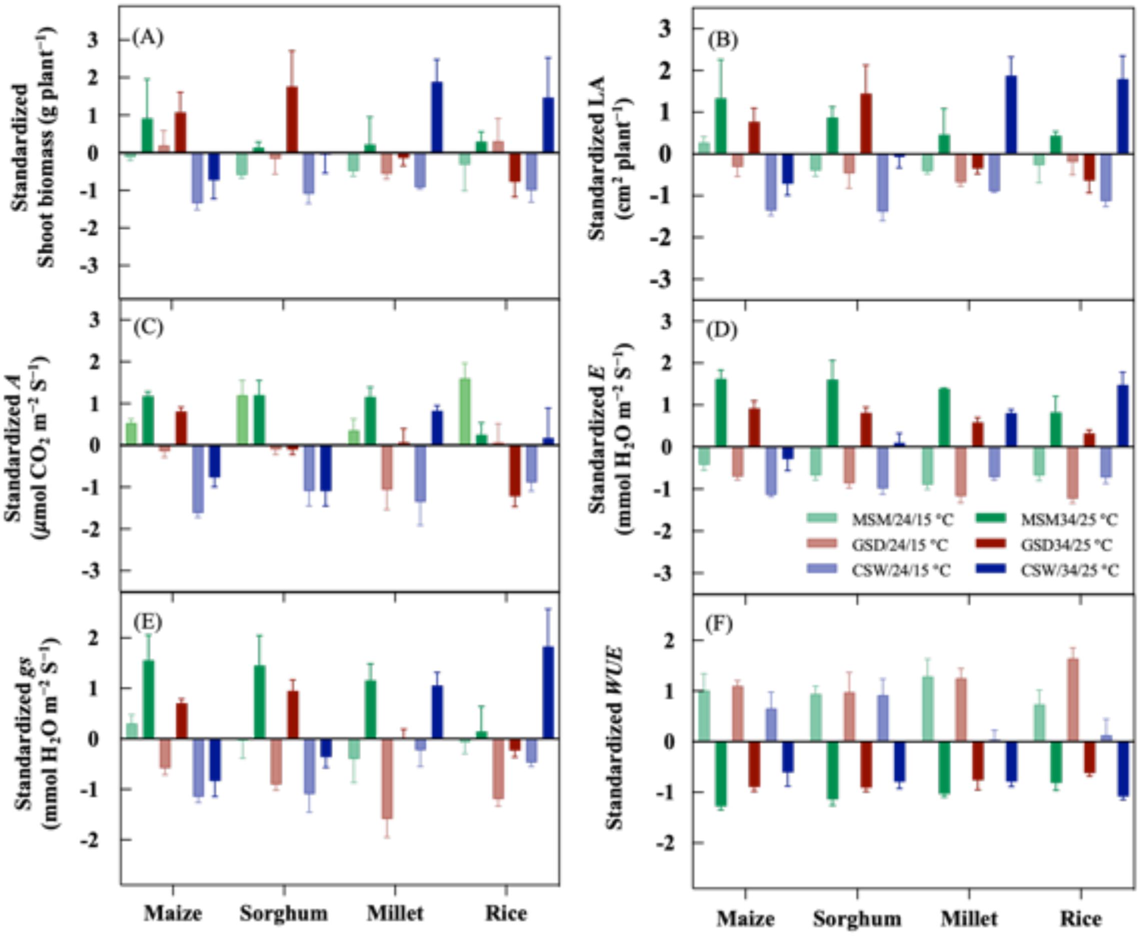

Figure 4-6. Response of stomata conductance (gs), photosynthesis (A), transpirtion rate (E), and water use efficiancy (WUE) of maize, sorghum, millet, and rice to the combination of soil moisture contents and temperatures by standardization data. The shoot biomass and LA were at 17 days after treatment, while gas exchange (A, gs, E, and WUE) was the average of all measurements after treatment. Bars indicate mean standard deviation. Standardization was used for transformation of data.

3.2.2. Changing of Gas Exchange

The effect of the combination between soil moisture status and temperature treatments on gas exchange is shown in Figure 4-7. There was a significant effect of combination treatments on *A*, *gs*, *E*, and *WUE* at 4, 8, 12, and 17 days after treatment (DAT) ($p < 0.05$) in maize, except for *gs* at 4 DAT that showed no significant difference among the treatments. With low temperature, each soil moisture status had lower *A*, *gs*, and *E* of maize than high temperature. *A*, *gs*, and *E* of maize significantly decreased in low temperature at 4, 8, and 12 DAT under GSD, but declined *A*, *gs*, and *E* were delayed at 17 DAT (Figure 4-7A, E, I, M) compared to MSM with high temperature (MSM/34/25 °C). Under CSW, *A*, *gs*, and *E* of maize significantly decreased at initial (4 DAT) after imposed soil waterlogging in low and high temperatures compared to MSM/34/25 °C and GSD/34/25 °C; *WUE* of the maize was influenced by low temperature combined with all soil water statuses, particularly MSM and GSD, compared to high temperature. Additionally, a significant effect of treatments on gas exchange was found at 4, 8, 12, and 17 DAT in sorghum. Under different combinations of various soil water status and temperature, the change in gas exchange compared to sorghum and maize was similar. The combination of soil water status and low temperature decreased *A*, *gs*, and *E*, but it increased *WUE*. After the low-temperature imposition, *A*, *gs*, and *E* of sorghum under different soil moisture levels decreased at the inceptive stage. Its *A*, *gs*, and *E* under MSM and GSD recovered at 8 DAT, but not under CSW. The *A*, *gs*, and *E* of sorghum at low and high temperatures gradually declined along with soil moisture status (Figure 4-7B, F, J, M) under GSD. Nevertheless, the *A*, *gs*, and *E* under GSD combined with low temperature (GSD/24/15 °C) was lower than high temperature (GSD/34/25 °C), and GSD/24/15 °C was not significantly different compared to CSW combined with low and high temperatures. Under CSW, the *A*, *gs*, and *E* was significantly decreased at 4 DAT both in low or high temperature (CSW/24/15 °C or CSW/34/25 °C) (Figure 4-7B, F, J, N).

There were significant effects of the combination treatments on the change of gas exchange (Figure 4-7C, G, K, O) in millet. MSM and GSD combined with low temperature showed lower at *A*, *gs*, and *E* than high temperature at 4, 8, and 12 DAT, but CSW/24/15 °C did not decrease *gs* of millet at 4 DAT compared to the treatment before. Under MSM/34/25 °C, the *A*, *gs*, and *E* did not change at all measured times, but it was reduced under MSM/24/15 °C, specifically on *E*. There was a similar reduction of gas exchange of millet under GSD/24/15 °C with MSM/34/25 °C at 4 DAT. In contrast, *A*, *gs*, and *E* under GSD/34/25 °C were delayed to record a significant decrease at 17 DAT. The impact of CSW in *A*, *gs*, and *E* depended on temperature. CSW/34/25 °C showed no significant difference on *A*, *gs*, and *E* of millet compared to MSM/34/25 °C, whereas under CSW/24/15 °C, *A* and *E* were reduced for maize at 4 DAT and *gs* at 8 DAT. Millet responded similar to maize and sorghum, where *WUE* increased under all soil water status combined with low temperature (Figure 4-7O). The effect of treatments on *gs*, *E*, and *WUE* were noticed at all measured times in rice, but *A* was seen at 12 and 17 DAT (Figure 4-7D, H, L, P). Rice showed the highest *gs* and *E* at 4 DAT under CSW/34/25 °C, but not for *A*. The highest *A* was seen under MSM/24/15 °C from 8 DAT. However, CSW/24/15 °C had lower *A*, *gs*, and *E* than CSW/34/25 °C, and CSW/34/25 °C had greater *gs* from 4 DAT than other treatments. Under GSD combined with low and high temperatures, *A* was shown to be alternative to *gs* and *E*, where it was higher under GSD/24/15 °C than under GSD/34/25 °C at 17 DAT. Although under GSD/34/25 °C, *gs* and *E* was higher at 4, 8, and 12 DAT than GSD/24/15 °C, at 17 DAT, there was no significant difference in *gs* and *E* between GSD/24/15 °C and GSD/34/25 °C.

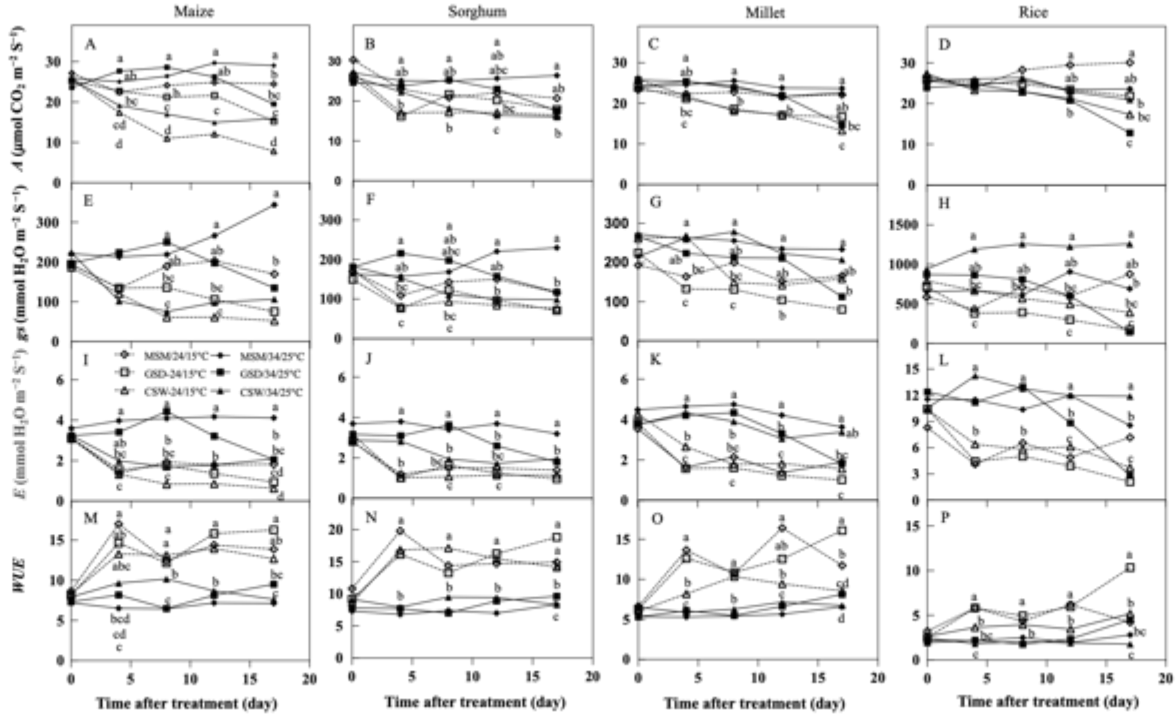

Figure 4-7. The effect of combination of soil moisture status and temperature on changing of photosynthesis rate (A; A–D), stomatal conductance (gs; E–H), transpiration rate (E; I–L), and water use efficiency (WUE; M–P) in maize (A, E, I, M), sorghum (B, F, J, N), common millet (C, G, K, O), and rice (D, H, L, P) during the course of experiment 2. Each day of measurement with similar letters did not significantly differ according to Tukey's test at the 0.05 probability level.

3.2.3. Correlation between *gs*, *A*, and *E* and Influence of Atmospheric Environment and *gs* on Shoot Biomass

Figure 4-8 presents the correlation between *gs* and shoot biomass, *gs* and *A*, and *gs* and *E* across the combination between soil moisture status and temperature treatments, which was positively significant for all crops (Figure 4-8). Maize had the highest coefficient, followed by millet, sorghum, and rice between *gs* and shoot biomass (Figure 4-8A–D). Maize had the highest correlation coefficient between *gs* and *A*, followed by sorghum, millet, and rice (Figure 4-8E–H). In contrast, a high correlation coefficient between *gs* and *E* was found in maize, followed by sorghum and rice (Figure 4-8I–L).

Multiple linear regression analysis was used to identify which environmental factors and physiological traits influenced shoot biomass across a combination of various soil water statuses and temperatures, *gs*, *A*, and *WUE*. Our results showed that soil moisture content, temperature, and *gs* were suitable parameters to generate a formula that highly contributes to multiple crops. Soil moisture content and temperature influenced *gs* of all crops. The result of multiple linear correlation showed that sorghum had the highest adjustment (Adj) of R squared (Adj. $R^2 = 0.759$, $p < 0.001$), followed by maize (Adj. $R^2 = 0.658$, $p < 0.001$), millet (Adj. $R^2 = 0.492$, $p = 0.006$), and rice (Adj. $R^2 = 0.262$, $p < 0.066$) (Table 4-3). On the basis of β-value, rice and maize were less affected by temperature and soil moisture content compared to sorghum and millet; temperature especially had a higher influence on shoot biomass of sorghum and millet than maize and rice (Table 4-3).

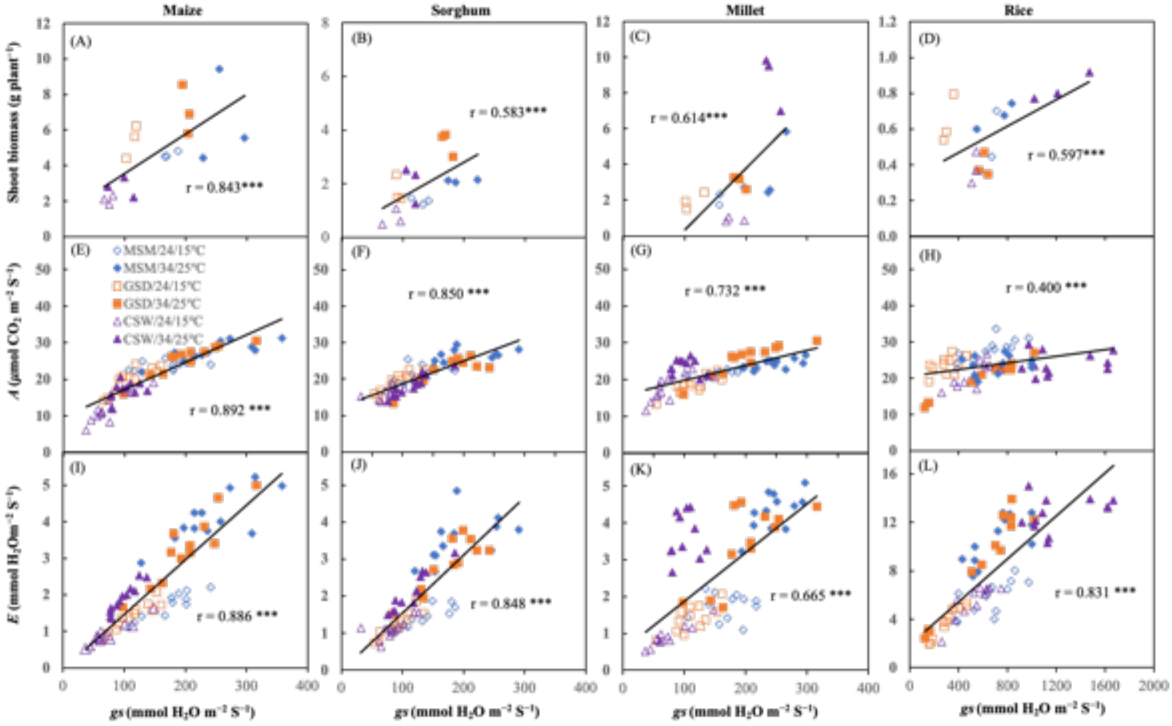

Figure 4-8. Correlation between stomatal conductance (gs), and shoot biomass (A–D), photosynthesis rate rate (A; E–H), and transpiration rate (E; I–L) in maize (A, E, I), sorghum (B, F, J), millet (C, G, K)), and rice (D, H, L). *** indicates Pearson statistical significance at $p < 0.001$ (n = 18 for shoot biomass and n = 72 for A and E).

Table 4-3. The multiple linear regression for shoot biomass (g plant^{-1}) based on parameters of temperature (Temp), soil moisture content (SMC), and stomatal conductance (*gs*) under three soil moisture regimes (MSM, GSD, CSW) and two temperatures (24/15 °C and 34/25) ($n = 18$).

Maize						Sorghum					Millet					Rice				
Equation	**Variation**	**β**	***t*-Value**	**Adjusted R²**	***p*-Value**	**Equation**	**β**	***t*-value**	**Adjusted R²**	***p*-Value**	**Equation**	**β**	***t*-Value**	**Adjusted R²**	***p*-Value**	**Equation**	**β**	***t*-Value**	**Adjusted R²**	***p*-Value**
(1)	Intercept	5.71	2.906	0.658	0.000	(2)	0.947	1.351	0.759	0.000	(3)	−9.832	−3.072	0.492	0.006	(4)	0.556	1.794	0.262	0.066
	Temp	0.071	0.983				0.163	4.875				0.455	2.514				−0.006	−0.567		
	SMC	−0.171	−3.271				−0.085	−4.602				0.208	2.126				−0.008	−0.896		
	gs	0.012	1.877				−0.005	−1.293				−0.016	−0.750				0.001	2.231		

As shown in Figure 4-9, there were significant linear relationships between measured shoot biomass and predicted shoot biomass in all crops. The highest R square value ($R^2 = 0.802$, $p < 0.001$) was found for sorghum, followed by maize ($R^2 = 0.718$, $p < 0.001$), common millet ($R^2 = 0.582$, $p < 0.001$), and rice ($R^2 = 0.382$, $p < 0.05$). Rice had lowest R square value; however, it had highest slope (*a*) (*a* = 0.835), followed by sorghum (*a* = 0.815), maize (*a* = 0.815) and common millet (*a* = 0.584).

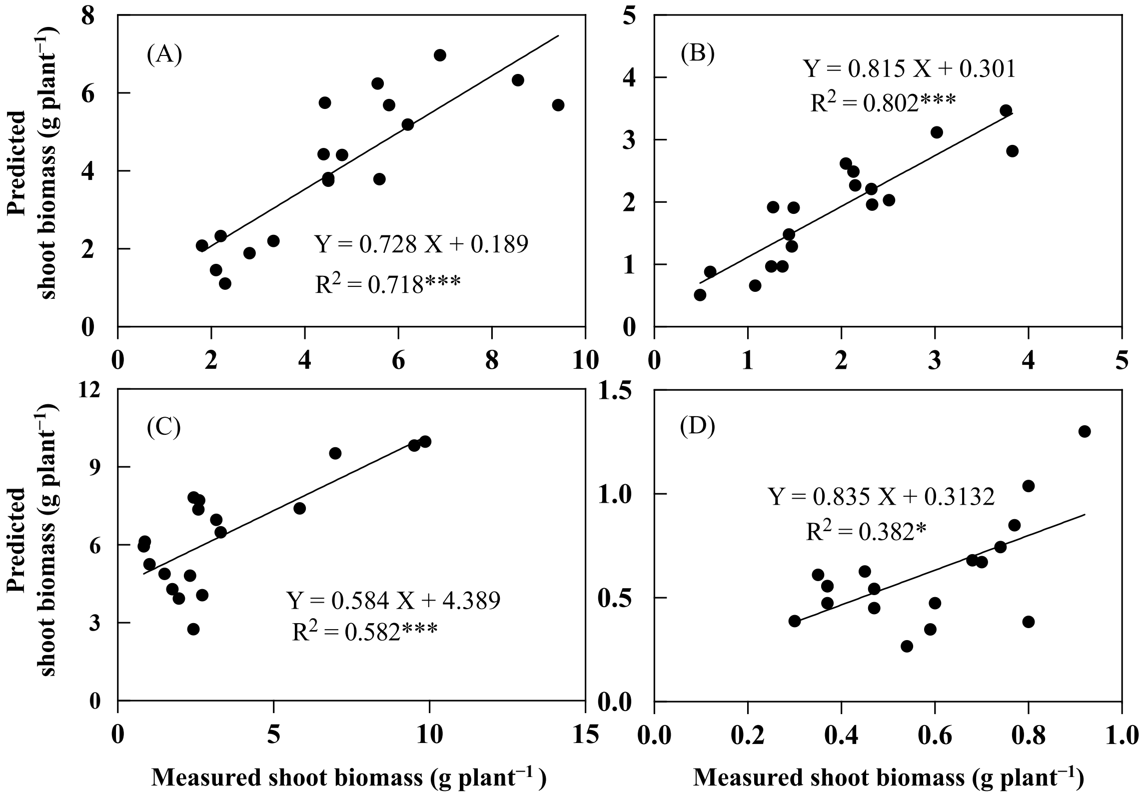

Figure 4-9. Correlation between measured shoot biomass and predicted shoot biomass in maize (A), sorghum (B), common millet (C) and rice (D) (n = 18). * and *** statistical significance at $p < 0.05$ and $p < 0.001$, respectively.

4. Discussion

4.1. The *gs* Responses to Soil Moisture Status and Environmental Influence on Biomass Production

Our study highlighted the interaction between crop genotypes and combination of soil moisture status and environment through *gs* and shoot biomass. Stomatal aperture is influenced by a number of environmental factors including water variability, leaf temperature, and CO_2. The dynamic of stomatal movement acting in response to environmental charge and internals in an attempt is to optimize the trade-off between *A* and to maintain plant water status (transpiration rate) (Becklin et al., 2021). Close positive correlation among *gs*, *A*, and plant growth have been found under the control environments and field experiments (Fischer et al., 1998; Orzechowska et al., 2021; Wong et al., 1979). Plant mechanism response to water stress includes conservative, where the plants close the stomata are faster, and non-conservative, where the plants close the stomata slower under drought conditions (Caine et al., 2019). Our study emphasized on non-conservative mechanism.

The correlation between soil moisture status and shoot biomass and *gs* was a similar tendency (Figure 4-4). Under wet soil conditions, the shoot biomass of maize and sorghum declined (Exp. 1A and 1B). Additionally, the *gs* of these two crops were limited by wet soil conditions, especially waterlogging in Exp. 1A and 1B (Figure 4-4). A similar response of shoot biomass and *gs* of maize and sorghum was noticed under waterlogging interaction with low and high temperatures. It showed that maize and sorghum were sensitive to soil waterlogging and were temperature-independent (Figure 4-6). This finding was confirmed in previous reports (Abiko et al., 2012; Promkhambut et al., 2010; Ren et al., 2014). Waterlogging extremely limited root length density at the deep soil layer and shoot biomass of maize and sorghum (Khanthavong et al., 2021; Promkhambut et al., 2011) due to their roots suffering from low oxygen diffusion in the soil (Neira et al., 2015; Sanderson and Armstrong, 1978; Yamauchi et al., 2021a).

Moreover, shoot biomass of millet showed a negative response to waterlogging in both Exp. 1A and 1B. Still, its impact on shoot biomass under combination of CSW and high temperature was the opposite in experiment 2. The temperature was similar to CSW interaction with a high temperature in experiment 2 (Figure 4-6A). Barnyard millet adapted well to waterlogging (Galamay et al., 1991; Khanthavong et al., 2021) ; but not withstanding in this study because low temperature caused a reduction of shoot biomass under waterlogging in Exp. 1B and low temperature (CSW/24/15 °C) in experiment 2 (Figure 4-6A). Under the screen house, the fluctuation of light intensity influenced *gs*, *A,* and biomass production (Kimura et al., 2020). It was reported that under optimum temperature, rice is well adapted to waterlogging (Sakagami and Kawano, 2011). However, sub-optimum temperature (<20 °C) affected reduction of shoot biomass and relative growth in rice compared to optimum temperature (Nagai and Makino, 2009). Similarly, the combination of waterlogging and low temperature caused a reduction of shoot biomass and *gs* of rice compared to a higher temperature (Figures 4-4 and 4-6A). The shoot biomass and *gs* of crops response to dry soil conditions or combination of gradual soil drying and low or high temperature were computed among crops and within the treatment in experiment 1 and 2 (Figures 4-4 and 4-6). The correlation trend between soil moisture status and shoot biomass and *gs* in Exp. 1A and 1B or response of shoot biomass and *gs* under combination of gradual soil drying and high or low temperature (Experiment 2) of each crop were similar (Figures 4-4 and 4-6A, E). These results imply that *gs* were influenced shoot biomass under gradual soil drying. Generally, crops respond to water deficit by reducing water loss and maintaining turgor by stomatal closure (Farooq et al., 2009). Nevertheless, our results in experiment 2 indicated that the effect of gradual soil drying on the reduction of *gs* was primarily caused by low temperature for all crops, and their corresponding shoot biomass except for rice. Stomatal closure under drought and cold stress conditions was affected by water stress as a hydraulic activity in roots reduction (Farooq et al., 2009; Wolfe, 1991). Exp. 1A had a considerable higher temperature than Exp. 1B; however, the impact of gradual soil drying on shoot biomass of maize, sorghum, and rice in this study could not be explained by temperature as the results showed in experiment 2 (Figure 4-6A). The *gs* of all crops under combination of gradual soil drying and low temperature was significantly reduced than in high temperature, and rice showed a positive response as its shoot biomass was promoted by *A* (Figure 3-6C). In these conditions, the alternative response between *gs* and *A* of rice (C_3) under the combination of gradual soil drying and low temperature suggested that their correlation is sometimes not positive. Furthermore, rice, a C_3 crop, had a lower optimum temperature, and it had better CO_2 assimilation than C_4 crops such as maize, sorghum, and millet (Sage and Kubien, 2007). Cold-adapted plants displayed an increase in *A* with below the optimum thermal temperature and a reduction in *A* with above the thermal optimum (Hikosaka et al., 2006; Huner et al., 1986; Sage and Kubien, 2007; Yamasaki et al., 2002). In maize, sorghum, and millet, a combination of gradual soil drying and high temperature remarkably promoted the shoot biomass, *A*, and *gs* (Figure 4-6A, C, E), but shoot biomass, *A*, and *gs* of rice decreased under a combination of gradual soil drying and high temperature. Day by day, the stomata react to changing water and temperature variables (Becklin et al., 2021); therefore, managing the responsiveness of *gs* offers breeders the potential to manage the interaction *gs* and *A*, which would impact yield (Lawson et al., 2014).

4.2. A Plant's Ability to Maintain Gas Exchange for Maintaining the Biomass Production

Stomatal conductance (*gs*), *A*, and *E* under water and temperature variability for all crops were significantly correlated ($p < 0.001$) (Figure 4-8), but in rice, the coefficient correlation between *gs* and *A* was low (Figure 4-8D). Reactive *gs* and *A* of rice (C_3) was indeed different from maize, sorghum, and millet (C_4), measured at the same environmental factor (Niu et al., 2005; Sage and Kubien, 2007). The changing of the gas exchange clarified the effect of soil moisture status and temperature viability in experiment 2 (Figure 4-7). A reduction was caused by declining *gs* to prevent desiccation (Dreyer et al., 1991; Qaderi et al., 2012; Souza et al., 2004). Under water deficit, the leaf gradually increases water potential with depletion of soil moisture content (Rodríguez-Gamir et al., 2019). Plants increase ABA hormone concentration in their leaf, which governs close *gs* and inhibition *A* (Barnabás et al., 2008). Alternately, leaf water potential is not remarkedly different under soil waterlogging (Dreyer et al., 1991). It relates to limiting root respiration due to hypoxia and reducing *gs* at the early growth stage compared to water deficit (Bansal and Srivastava, 2015; Hayashi et al., 2013).

Similarly, *gs* of maize and sorghum under combination of waterlogging and low or high temperature was declined earlier after imposed soil waterlogging compared to combination of moderated soil moisture and high temperature and gradual soil drying and high temperature. Alternatively, the *gs* of millet under combination of waterlogging and low temperature, and gradual soil drying and low temperature were also reduced earlier than the higher temperature at the same soil moisture status. This evidence suggested that the delay of *gs* leads to maintained *A* and consequently shoot biomass under water stress and temperature variability. In contrast, multi-water stress and low temperature had a higher impact on reducing *gs*, *A*, and consequently shoot biomass of maize, sorghum, and millet compared to the combination of water stresses and at higher temperature. Therefore, to consider how crops cope with the water and temperature variability of current global climate change, the ability to maintain *gs* should be a crucial parameter.

4.3. The Influence of Soil Moisture Content, Air Temperature and *gs* on Shoot Biomass of Each Crop

According to multiple linear regression, sorghum had the highest adjusted R^2, followed by maize and millet, whereas rice had considerately lowest value (Table 4-3). The developed crop growth models have been variable, but their effectiveness is only a specific environment and crop, and excludes the gas exchange parameter (Goto et al., 2021). Global climate change and water and temperature stress events are predicted to increase with greater frequency or duration (IPCC, 2014b). Thus, our crop growth model is useful for estimating multiple crops such as sorghum, maize, and millet, but not rice, under a wide range of soil water statuses and atmospheric environments. This model may therefore be considered for application in further research for estimating the influencing of soil moisture, temperature, and *gs* on growth of crops.

5. Conclusions

Different crops responded differently to different soil moisture, temperature, and these two stresses in combination. The degrees of decreased stomatal conductance and biomass accumulation were highest when crops were exposed to combined stress. However, the effect of these stresses varied among the crop genotypes. For the combination of various soil water status and temperature variation, rice, and maize were less effective on biomass production compared to millet and sorghum. Biomass accumulation of all crop genotypes was reduced by all treatments compared to optimal growing condition (i.e., moderate temperature in the presence of adequate temperature). Maize and sorghum under waterlogging conditions reduced shoot biomass, presumably due to the decreased stomatal conductance and photosynthesis, which was temperature independent, whereas, the reduction of rice and millet was also due to decreased stomatal conductance; which was temperature dependent. All crops indicated temperature-dependent stomatal conductance (at GSD/34/25 °C), where the *gs* of rice was lowest under high temperature. Thus, our results suggest that an ability to sustain *gs* is essential for photo assimilation and maintaining leaf temperature through evapotranspiration for biomass production, a mechanism of crop avoidance to combine variable soil water status and temperature.

CHAPTER 5

General discussion

Root distribution is included as root architecture (Lynch, 1995a). The plant root distribution through the different soil layers indicates root water uptake from the soil for sustainable growth development and productivity (Coelho and Or, 1999). It is important in regulating soil water use and thereby improving endurance of plants to seasonal droughts for sustainable agricultural productivity (Yu et al., 2007). Under drought, root distribution to deeper soil layer had advantage in water uptake from further underground (Gregory, 2008; Hund et al., 2009; Lynch and Wojciechowski, 2015; Uga et al., 2013). The finding in experiment 1, root distribution associated with crop plasticity was influenced by soil water conditions in contrasting crop genotypes. Root development across environmental factors among the crop genotypes varies due to characteristic of their root system (Lin and Sauter, 2018; Magalhães et al., 2016; Yamauchi et al., 2018a). Maize and sorghum showed large changes in root distribution by promoting the root plasticity in shallow and deep soil layers under waterlogging and drying, respectively. Deeper root distribution of maize and sorghum promote the shood growth compared to shallow root distribution. This means that deep root density distribution of maize and sorghum under soil drying enhance water uptake under water deficit condition. Root length density distribution at deeper soil layers indicates a mechanism of crop adaptation to water deficit condition. On the other hand, millet and rice grown under waterlogging conditions had better shoot growth compared to maize and sorghum, which correlated with higher root distribution to deeper soil layers. Waterlogged soil causing reduction diffusion of oxygen in the soil are well document (Pucciariello and Perata, 2021; Sanderson and Armstrong, 1978), which directly affected root respiration, especially for maize and sorghum (Colmer, 2003; Yamauchi et al., 2013). Therefore, shallow root distribution in maize and sorghum under continuous soil waterlogging conditions might cause by deficit oxygen in the soil. However, this study did not include the effect of oxygen on changing of root distribution. The study on the effect of oxygen and ability of oxygen transport from leave to root among these crops is unanswered. Based on G × E interaction to evaluate crop response to anaerobic to aerobic conditions, rice demonstrated a wider adaptation to wide range soil moisture condition due to lesser shoot biomass and root changing compared to other crops (Experiment 1). However, results in experiment 2 shows that the response of rice to various soil water status was temperature dependent. It means that rice also suffers to unfavorable climate under rainfed conditions.

Crops grown under rainfed condition did not suffer with water stress only, but the combination of water stress and variability temperatures as well. The finding of experiment 3 shows that the biomass accumulation of all crop genotypes was reduced by all treatments compared to optimal growing condition. The decreased biomass accumulation of all crop genotypes related to decreased stomatal conductance, and the highest decrease was observed when crops were exposed to combined stress. This indicates that the negative effect of combination soil water stress and unfavorable temperature on crop failure are higher than water stress only. Maize and sorghum under waterlogging conditions reduced stomatal conductance causing low photosynthesis and shoot biomass, which was temperature independent. Root length density (Experiment1) of maize and sorghum was limited by waterlogging condition, and shoot biomass and *gs* were also limited by the combination of waterlogging and temperatures (Experiment3). Waterlogging relates to limiting root respiration due to hypoxia (Bansal and Srivastava, 2015; Hayashi et al., 2013), which causes reducing root elongation and *gs*. Generally, root and shoot are connected, they communicate through sending hormones (ABA or ethylene) as a signal for leaf morphological and stomatal closure (Müller, 2021; Steuer et al., 1988). Waterlogging was also the cause of decreased shoot biomass and stomatal conductance; it was temperature dependent. In this condition, temperature affects root growth change of millet and rice. However, the mechanism of root response to temperature under waterlogging in millet and rice are still unclear. Under soil drying conditions, all crops indicated temperature-dependent *gs* which were higher under high temperature than low temperature, where the *gs* of rice was lowest under high temperature. Under soil drying, root dept of plants enhance water uptake for maintain stomatal open as the same time cool down the leaf by transpiration. Increase global temperature by 2 to 4 °C by end of this century would affect crops production, especially rice, which will be caused of food insecurity and unable to feed the expecting global population increase.

CHAPTER 6

General Conclusions

This study evaluated crop roots' response based on root distribution under various soil condition, including dry and waterlogged, to assess crops adaptation under changing environments, such as various soil moisture contents and to identify the impact of various soil water statuses interaction with atmospheric environment, and the combination of soil water statuses and temperature on stomatal conductance behavior and biomass. The effect of soil water status on root distribution varied with crop genotypes. Root distribution in different soil layers across different soil water statuses indicates crop adaptation ability. Maize and sorghum indicate poor root growth under waterlogging because low oxygen restricted roots respiration, which caused reduction of stomatal conductance, photosynthesis and shoot biomass; they were temperature independent. Improving anatomic root traits would be important to cope anaerobic condition. Millet and rice had better root growth, stomatal conductance and shoot biomass under waterlogging, however, those responses were negative under low temperature with the same soil water condition. Under soil drying, all crops had better root distribution association with root plasticity to deeper soil layers. Deep root distribution overcomes soil water deficiency by sucking water at lower soil layer to maintain stomatal open, photosynthesis and cool down leaf through transpiration. However, stomatal conductance of rice had negative response under the combination of soil drying and high temperature compared to other crops. Increase global temperature 2 °C to 4 °C by end of this century combination with frequency of drought even, rice will forewarningly be risker than other crops. Under water stress or the combination of water stress and temperatures, ability to sustain stomatal conductance correlate with root distribution and plasticity is essential to maintain stomatal conductance for CO_2 assimilation, transpiration, and biomass production, a mechanism of crop avoidance.

CHAPTER 7

References

Abiko, T., Kotula, L., Shiono, K., Malik, A. I., Colmer, T. D., and Nakazono, M. (2012). Enhanced formation of aerenchyma and induction of a barrier to radial oxygen loss in adventitious roots of Zea nicaraguensis contribute to its waterlogging tolerance as compared with maize (Zea mays ssp. mays). *Plant, Cell & Environment* **35**, 1618-1630.

Ali, M. L., Luetchens, J., Nascimento, J., Shaver, T. M., Kruger, G. R., and Lorenz, A. J. (2015). Genetic variation in seminal and nodal root angle and their association with grain yield of maize under water-stressed field conditions. *Plant and Soil* **397**, 213-225.

Araki, H., Hossain, M. A., and Takahashi, T. (2012). Waterlogging and hypoxia have permanent effects on wheat root growth and respiration. *Journal of Agronomy and Crop Science* **198**, 264-275.

Armstrong, W. (1979). Aeration in higher plants. *In* "Advances in botanical research", Vol. 7, pp. 225-332. Elsevier.

Assmann, S. M., and Shimazaki, K.-i. (1999). The multisensory guard cell. Stomatal responses to blue light and abscisic acid. *Plant Physiology* **119**, 809-816.

Bansal, R., and Srivastava, J. (2015). Effect of waterlogging on photosynthetic and biochemical parameters in pigeonpea. *Russian Journal of Plant Physiology* **62**, 322-327.

Bao, Y., Aggarwal, P., Robbins, N. E., Sturrock, C. J., Thompson, M. C., Tan, H. Q., Tham, C., Duan, L., Rodriguez, P. L., and Vernoux, T. (2014). Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proceedings of the National Academy of Sciences* **111**, 9319-9324.

Barnabás, B., Jäger, K., and Fehér, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant, cell & environment* **31**, 11-38.

Bary, A. (1884). "Comparative anatomy of the vegetative organs of the phanerogams and ferns," Clarendon Press.

Becklin, K. M., Ward, J. K., and Way, D. A. (2021). "Photosynthesis, Respiration, and Climate Change," Springer.

Beillouin, D., Schauberger, B., Bastos, A., Ciais, P., and Makowski, D. (2020). Impact of extreme weather conditions on European crop production in 2018. *Philosophical Transactions of the Royal Society B* **375**, 20190510.

Bengough, A. G., McKenzie, B., Hallett, P., and Valentine, T. (2011). Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. *Journal of experimental botany* **62**, 59-68.

Berg, A., de Noblet-Ducoudré, N., Sultan, B., Lengaigne, M., and Guimberteau, M. (2013). Projections of climate change impacts on potential C4 crop productivity over tropical regions. *Agricultural and Forest Meteorology* **170**, 89-102.

Biemelt, S., Keetman, U., and Albrecht, G. (1998). Re-aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. *Plant Physiology* **116**, 651-658.

Bin, T., Xu, S.-Z., Zou, X.-L., Zheng, Y.-L., and Qiu, F.-Z. (2010). Changes of antioxidative enzymes and lipid peroxidation in leaves and roots of waterlogging-tolerant and waterlogging-sensitive maize genotypes at seedling stage. *Agricultural Sciences in China* **9**, 651-661.

Blatt, M. R. (2000). Cellular signaling and volume control in stomatal movements in plants. *Annual review of cell and developmental biology* **16**, 221-241.

Borrell, A. K., Mullet, J. E., George-Jaeggli, B., van Oosterom, E. J., Hammer, G. L., Klein, P. E., and Jordan, D. R. (2014). Drought adaptation of stay-green sorghum is associated with canopy development, leaf anatomy, root growth, and water uptake. *Journal of experimental botany* **65**, 6251-6263.

Bouman, B., and Tuong, T. P. (2001). Field water management to save water and increase its productivity in irrigated lowland rice. *Agricultural water management* **49**, 11-30.

Caine, R. S., Yin, X., Sloan, J., Harrison, E. L., Mohammed, U., Fulton, T., Biswal, A. K., Dionora, J., Chater, C. C., and Coe, R. A. (2019). Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions. *New Phytologist* **221**, 371-384.

Coelho, E. F., and Or, D. (1999). Root distribution and water uptake patterns of corn under surface and subsurface drip irrigation. *Plant and Soil* **206**, 123-136.

Colmer, T. (2003). Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell & Environment* **26**, 17-36.

Colmer, T., and Voesenek, L. (2009). Flooding tolerance: suites of plant traits in variable environments. *Functional Plant Biology* **36**, 665-681.

Colmer, T. D., Winkel, A., Kotula, L., Armstrong, W., Revsbech, N. P., and Pedersen, O. (2020). Root O2 consumption, CO2 production and tissue concentration profiles in chickpea, as influenced by environmental hypoxia. *New Phytologist* **226**, 373-384.

Comas, L., Becker, S., Cruz, V. M. V., Byrne, P. F., and Dierig, D. A. (2013). Root traits contributing to plant productivity under drought. *Frontiers in plant science* **4**, 442.

Coudert, Y., Périn, C., Courtois, B., Khong, N. G., and Gantet, P. (2010). Genetic control of root development in rice, the model cereal. *Trends in plant science* **15**, 219-226.

Crafts-Brandner, S. J., and Salvucci, M. E. (2002). Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. *Plant physiology* **129**, 1773-1780.

Craufurd, P., and Peacock, J. (1993). Effect of heat and drought stress on sorghum (Sorghum bicolor). II. Grain yield. *Experimental Agriculture* **29**, 77-86.

Den Herder, G., Van Isterdael, G., Beeckman, T., and De Smet, I. (2010). The roots of a new green revolution. *Trends in plant science* **15**, 600-607.

Drew, M. C., Jackson, M. B., Giffard, S. C., and Campbell, R. (1981). Inhibition by silver ions of gas space (aerenchyma) formation in adventitious roots of Zea mays L. subjected to exogenous ethylene or to oxygen deficiency. *Planta* **153**, 217-224.

Dreyer, E., Colin-Belgrand, M., and Biron, P. (1991). Photosynthesis and shoot water status of seedlings from different oak species submitted to waterlogging. *In* "Annales des sciences forestières", Vol. 48, pp. 205-214. EDP Sciences.

Evans, D. E. (2003). Aerenchyma formation. *New phytologist* **161**, 35-49.

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., and Saud, S. (2017). Crop production under drought and heat stress: plant responses and management options. *Frontiers in plant science*, 1147.

Falconer, D., and Mackay, T. (1996). Introduction to Quantitative Genetics 4th Edition, Harlow. Addison Wesley Longman, Ltd.

FAO (2018). The impact of disasters and crises on agriculture and food security. *Report*.

FAO (2021). FAOSTAT Statistic Database. *[Rome] : Food and Agriculture Organization of the United Nations*.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., and Basra, S. (2009). Plant drought stress: effects, mechanisms and management. *In* "Sustainable agriculture", pp. 153-188. Springer.

Fenta, B. A., Beebe, S. E., Kunert, K. J., Burridge, J. D., Barlow, K. M., Lynch, J. P., and Foyer, C. H. (2014). Field phenotyping of soybean roots for drought stress tolerance. *Agronomy* **4**, 418-435.

Finlay (1963). The analysis of adaptation in a plant-breeding programme. *Aust J Agric Res.* **14**, 742-754.

Fischer, R., Rees, D., Sayre, K., Lu, Z. M., Condon, A., and Saavedra, A. L. (1998). Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop science* **38**, 1467-1475.

Forde, B. G. (2009). Is it good noise? The role of developmental instability in the shaping of a root system. *Journal of experimental botany* **60**, 3989-4002.

Fry, E. L., Evans, A. L., Sturrock, C. J., Bullock, J. M., and Bardgett, R. D. (2018). Root architecture governs plasticity in response to drought. *Plant Soil* **433**, 189-200.

Galamay, T. O., Kono, Y., Yamauchi, A., and Shimizu, M. (1991). Comparative root anatomy of seminal and nodal root axes of summer cereals with special reference to the development of hypodermis and cortical sclerenchyma. *Japanese Journal of Crop Science* **60**, 184-190.

Giorgi, F., Raffaele, F., and Coppola, E. (2019). The response of precipitation characteristics to global warming from climate projections. *Earth System Dynamics* **10**, 73-89.

Goto, K., Yabuta, S., Ssenyonga, P., Tamaru, S., and Sakagami, J.-I. (2021). Response of leaf water potential, stomatal conductance and chlorophyll content under different levels of soil water, air vapor pressure deficit and solar radiation in chili pepper (Capsicum chinense). *Scientia Horticulturae* **281**, 109943.

Gregory, P. J. (2008). "Plant roots: growth, activity and interactions with the soil," John Wiley & Sons.

Grossman, J. D., and Rice, K. J. (2012). Evolution of root plasticity responses to variation in soil nutrient distribution and concentration. *Evolutionary Applications* **5**, 850-857.

Gu, D., Zhen, F., Hannaway, D. B., Zhu, Y., Liu, L., Cao, W., and Tang, L. (2017). Quantitative classification of rice (Oryza sativa L.) root length and diameter using image analysis. *PloS one* **12**, e0169968.

Gunawardena, A. H., Pearce, D. M., Jackson, M. B., Hawes, C. R., and Evans, D. E. (2001). Characterisation of programmed cell death during aerenchyma formation induced by ethylene or hypoxia in roots of maize (Zea mays L.). *Planta* **212**, 205-214.

Hadebe, S., Modi, A., and Mabhaudhi, T. (2017). Drought tolerance and water use of cereal crops: A focus on sorghum as a food security crop in sub-Saharan Africa. *Journal of Agronomy and Crop Science* **203**, 177-191.

Haling, R. E., Brown, L. K., Bengough, A. G., Young, I. M., Hallett, P. D., White, P. J., and George, T. S. (2013). Root hairs improve root penetration, root–soil contact, and phosphorus acquisition in soils of different strength. *Journal of Experimental Botany* **64**, 3711-3721.

Hayashi, T., Yoshida, T., Fujii, K., Mitsuya, S., Tsuji, T., Okada, Y., Hayashi, E., and Yamauchi, A. (2013). Maintained root length density contributes to the waterlogging tolerance in common wheat (Triticum aestivum L.). *Field Crops Research* **152**, 27-35.

Herzog, M., Striker, G. G., Colmer, T. D., and Pedersen, O. (2016). Mechanisms of waterlogging tolerance in wheat–a review of root and shoot physiology. *Plant, cell & environment* **39**, 1068-1086.

Hetherington, A. M., and Woodward, F. I. (2003). The role of stomata in sensing and driving environmental change. *Nature* **424**, 901-908.

Heyne, E., and Brunson, A. M. (1940). Genetic studies of heat and drought tolerance in maize. *Journal of the American Society of Agronomy* **32**, 803-14.

Hikosaka, K., Ishikawa, K., Borjigidai, A., Muller, O., and Onoda, Y. (2006). Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. *Journal of experimental botany* **57**, 291-302.

Ho, M. D., McCannon, B. C., and Lynch, J. P. (2004). Optimization modeling of plant root architecture for water and phosphorus acquisition. *Journal of Theoretical Biology* **226**, 331-340.

Hund, A., Ruta, N., and Liedgens, M. (2009). Rooting depth and water use efficiency of tropical maize inbred lines, differing in drought tolerance. *Plant and Soil* **318**, 311-325.

Huner, N., Migus, W., and Tollenaar, M. (1986). Leaf CO2 exchange rates in winter rye grown at cold-hardening and nonhardening temperatures. *Canadian journal of plant science* **66**, 443-452.

Hussain, H. A., Hussain, S., Khaliq, A., Ashraf, U., Anjum, S. A., Men, S., and Wang, L. (2018). Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. *Frontiers in plant science* **9**, 393.

IPCC (2014a). Climate change 2014: Synthesis report. *IPCC: Geneva, Szwitzerland.*

IPCC (2014b). "Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Chan," Ipcc.

Jackson, M., Fenning, Trevor Mjenins, William (1985). Aerenchyma (gas-space) formation in adventitious roots of rice (Oryza sativa L.) is not controlled by ethylene or small partial pressures of oxygen. *Journal of Experimental Botany* **36**, 1566-1572.

Kang, M. S. (1997). Using genotype-by-environment interaction for crop cultivar development. *Advances in agronomy* **62**, 199-252.

Kano-Nakata, M., Gowda, V. R., Henry, A., Serraj, R., Inukai, Y., Fujita, D., Kobayashi, N., Suralta, R. R., and Yamauchi, A. (2013). Functional roles of the plasticity of root system development in biomass production and water uptake under rainfed lowland conditions. *Field Crops Research* **144**, 288-296.

Kano-Nakata, M., Nakamura, T., Mitsuya, S., and Yamauchi, A. (2019). Plasticity in root system architecture of rice genotypes exhibited under different soil water distributions in soil profile. *Plant Production Science* **22**, 501-509.

Kartika, K., Sakagami, J.-I., Lakitan, B., Yabuta, S., Wijaya, A., Kadir, S., Widuri, L. I., Siaga, E., and Nakao, Y. (2020). Morpho-physiological response of Oryza glaberrima to gradual soil drying. *Rice Science* **27**, 67-74.

Khanthavong, P., Yabuta, S., Asai, H., Hossain, M., Akagi, I., and Sakagami, J.-I. (2021). Root response to soil water status via interaction of crop genotype and environment. *Agronomy* **11**, 708.

Khanthavong, P., Yabuta, S., Malik, A. I., Hossain, M. A., Akagi, I., and Sakagami, J.-I. (2022). Combinational Variation Temperature and Soil Water Response of Stomata and Biomass Production in Maize, Millet, Sorghum and Rice. *Plants* **11**, 1039.

Kimura, H., Hashimoto-Sugimoto, M., Iba, K., Terashima, I., and Yamori, W. (2020). Improved stomatal opening enhances photosynthetic rate and biomass production in fluctuating light. *Journal of experimental botany* **71**, 2339-2350.

KONO, Y., YAMAUCHI, A., NONOYAMA, T., TATSUMI, J., and KAWAMURA, N. (1987). A Revised Experimental System of Root-Soil Interaction for Laboratory Workr. *Environment Control in Biology* **25**, 141-151.

Lamaoui, M., Jemo, M., Datla, R., and Bekkaoui, F. (2018). Heat and drought stresses in crops and approaches for their mitigation. *Frontiers in chemistry* **6**, 26.

Lawson, T., and Blatt, M. R. (2014). Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant physiology* **164**, 1556-1570.

Lawson, T., Simkin, A. J., Kelly, G., and Granot, D. (2014). Mesophyll photosynthesis and guard cell metabolism impacts on stomatal behaviour. *New Phytologist* **203**, 1064-1081.

Li, Y., Song, X., Li, S., Salter, W. T., and Barbour, M. M. (2020). The role of leaf water potential in the temperature response of mesophyll conductance. *New Phytologist* **225**, 1193-1205.

Liao, H., Rubio, G., Yan, X., Cao, A., Brown, K. M., and Lynch, J. P. (2001). Effect of phosphorus availability on basal root shallowness in common bean. *Plant and Soil* **232**, 69-79.

Lin, C., and Sauter, M. (2018). Control of adventitious root architecture in rice by darkness, light, and gravity. *Plant Physiology* **176**, 1352-1364.

Lipiec, J., Doussan, C., Nosalewicz, A., and Kondracka, K. (2013). Effect of drought and heat stresses on plant growth and yield: a review. *International Agrophysics* **27**, 463-477.

López-Bucio, J., Cruz-Ramırez, A., and Herrera-Estrella, L. (2003). The role of nutrient availability in regulating root architecture. *Current opinion in plant biology* **6**, 280-287.

Ludlow, M., Santamaria, J., and Fukai, S. (1990). Contribution of osmotic adjustment to grain yield in Sorghum bicolor (L.) Moench under water-limited conditions. II. Water stress after anthesis. *Australian Journal of Agricultural Research* **41**, 67-78.

Lynch, J. (1995a). Root architecture and plant productivity. *Plant physiology* **109**, 7.

Lynch, J. (1995b). Root Architecture and Plant Productivity. *Plant Physiol* **109**, 7-13.

Lynch, J. P. (2007). Roots of the second green revolution. *Australian Journal of Botany* **55**, 493-512.

Lynch, J. P., and Wojciechowski, T. (2015). Opportunities and challenges in the subsoil: pathways to deeper rooted crops. *Journal of Experimental Botany* **66**, 2199-2210.

MacMillan, K., Emrich, K., Piepho, H.-P., Mullins, C., and Price, A. (2006). Assessing the importance of genotype× environment interaction for root traits in rice using a mapping population II: conventional QTL analysis. *Theoretical and Applied Genetics* **113**, 953-964.

Magalhães, P. C., de Souza, T. C., Lavinsky, A. O., de Albuquerque, P. E. P., de Oliveira, L. L., and de Castro, E. M. (2016). Phenotypic plasticity of root system and shoots of'Sorghum bicolor'under different soil water levels during pre-flowering stage. *Australian Journal of Crop Science* **10**, 81-87.

Malamy, J. (2005). Intrinsic and environmental response pathways that regulate root system architecture. *Plant, cell & environment* **28**, 67-77.

Matsuura, A., An, P., Murata, K., and Inanaga, S. (2016). Effect of pre-and post-heading waterlogging on growth and grain yield of four millets. *Plant Production Science* **19**, 348-359.

Matsuura, A., Kato, Y., Suzuki, T., Murata, K., and An, P. (2021). Hypoxia tolerance of four millet species is attributable to constitutive aerenchyma formation and root hair development of adventitious roots. *Plant Production Science*.

Maulana, F., and Tesso, T. T. (2013). Cold temperature episode at seedling and flowering stages reduces growth and yield components in sorghum. *Crop Science* **53**, 564-574.

McAusland, L., Vialet-Chabrand, S., Davey, P., Baker, N. R., Brendel, O., and Lawson, T. (2016). Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. *New Phytologist* **211**, 1209-1220.

McMichael, B., and Quisenberry, J. (1993). The impact of the soil environment on the growth of root systems. *Environmental and experimental botany* **33**, 53-61.

Müller, M. (2021). Foes or friends: ABA and ethylene interaction under abiotic stress. *Plants* **10**, 448.

Munns, R., James, R. A., Sirault, X. R. R., Furbank, R. T., and Jones, H. G. (2010). New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. *Journal of Experimental Botany* **61**, 3499-3507.

Myers, R. (1980). The root system of a grain sorghum crop. *Field Crops Research* **3**, 53-64.

Nagai, T., and Makino, A. (2009). Differences between rice and wheat in temperature responses of photosynthesis and plant growth. *Plant and Cell Physiology* **50**, 744-755.

Naredo, M., Juliano, A. B., Lu, B., De Guzman, F., and Jackson, M. (1998). Responses to seed dormancy-breaking treatments in rice species (Oryza L.). *Seed Science and Technology* **26**, 675-690.

Nations, U. (2019). World population prospects 2019: Highlights. *UN Dep. Econ. Soc. Aff. Popul. Div*.

Negin, B., and Moshelion, M. (2016). The evolution of the role of ABA in the regulation of water-use efficiency: From biochemical mechanisms to stomatal conductance. *Plant Science* **251**, 82-89.

Neira, J., Ortiz, M., Morales, L., and Acevedo, E. (2015). Oxygen diffusion in soils: understanding the factors and processes needed for modeling. *Chilean journal of agricultural research* **75**, 35-44.

Niu, S., Yuan, Z., Zhang, Y., Liu, W., Zhang, L., Huang, J., and Wan, S. (2005). Photosynthetic responses of C3 and C4 species to seasonal water variability and competition. *Journal of experimental botany* **56**, 2867-2876.

Orman-Ligeza, B., Parizot, B., Gantet, P. P., Beeckman, T., Bennett, M. J., and Draye, X. (2013). Post-embryonic root organogenesis in cereals: branching out from model plants. *Trends in plant science* **18**, 459-467.

Orzechowska, A., Trtílek, M., Tokarz, K. M., Szymańska, R., Niewiadomska, E., Rozpądek, P., and Wątor, K. (2021). Thermal Analysis of Stomatal Response under Salinity and High Light. *International Journal of Molecular Sciences* **22**, 4663.

Osborne, C. P., Wythe, E. J., Ibrahim, D. G., Gilbert, M. E., and Ripley, B. S. (2008). Low temperature effects on leaf physiology and survivorship in the C3 and C4 subspecies of Alloteropsis semialata. *Journal of Experimental Botany* **59**, 1743-1754.

Ouyang, W., Struik, P. C., Yin, X., and Yang, J. (2017). Stomatal conductance, mesophyll conductance, and transpiration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. *Journal of Experimental Botany* **68**, 5191-5205.

Pan, J., Sharif, R., Xu, X., and Chen, X. (2021). Mechanisms of waterlogging tolerance in plants: Research progress and prospects. *Frontiers in Plant Science* **11**, 627331.

Pardales Jr, J., Kono, Y., and Yamauchi, A. (1991). Response of the different root system components of sorghum to incidence of waterlogging. *Environmental and Experimental Botany* **31**, 107-115.

Pareek, A., Sopory, S. K., and Bohnert, H. (2009). "Abiotic stress adaptation in plants," Springer.

Parent, C., Capelli, N., Berger, A., Crèvecoeur, M., and Dat, J. F. (2008). An overview of plant responses to soil waterlogging. *Plant stress* **2**, 20-27.

Passot, S., Gnacko, F., Moukouanga, D., Lucas, M., Guyomarc'h, S., Ortega, B. M., Atkinson, J. A., Belko, M. N., Bennett, M. J., and Gantet, P. (2016). Characterization of pearl millet root architecture and anatomy reveals three types of lateral roots. *Frontiers in plant science* **7**, 829.

Pedersen, O., Nakayama, Y., Yasue, H., Kurokawa, Y., Takahashi, H., Heidi Floytrup, A., Omori, F., Mano, Y., David Colmer, T., and Nakazono, M. (2021). Lateral roots, in addition to adventitious roots, form a barrier to radial oxygen loss in Zea nicaraguensis and a chromosome segment introgression line in maize. *New Phytologist* **229**, 94-105.

Pickson, R. B., He, G., Ntiamoah, E. B., and Li, C. (2020). Cereal production in the presence of climate change in China. *Environmental Science and Pollution Research* **27**, 45802-45813.

Promkhambut, A., Polthanee, A., Akkasaeng, C., and Younger, A. (2011). Growth, yield and aerenchyma formation of sweet and multipurpose sorghum (Sorghum bicolor L. Moench) as affected by flooding at different growth stages. *Australian Journal of Crop Science* **5**, 954-965.

Promkhambut, A., Younger, A., Polthanee, A., and Akkasaeng, C. (2010). Morphological and physiological responses of sorghum (Sorghum bicolor L. Moench) to waterlogging. *Asian Journal of Plant Sciences* **9**, 183.

Pucciariello, C., and Perata, P. (2021). The oxidative paradox in low oxygen stress in plants. *Antioxidants* **10**, 332.

Qaderi, M. M., Kurepin, L. V., and Reid, D. M. (2012). Effects of temperature and watering regime on growth, gas exchange and abscisic acid content of canola (Brassica napus) seedlings. *Environmental and Experimental Botany* **75**, 107-113.

Rajhi, I., Yamauchi, T., Takahashi, H., Nishiuchi, S., Shiono, K., Watanabe, R., Mliki, A., Nagamura, Y., Tsutsumi, N., and Nishizawa, N. K. (2011). Identification of genes expressed in maize root cortical cells during lysigenous aerenchyma formation using laser microdissection and microarray analyses. *New Phytologist* **190**, 351-368.

Ren, B., Zhang, J., Li, X., Fan, X., Dong, S., Liu, P., and Zhao, B. (2014). Effects of waterlogging on the yield and growth of summer maize under field conditions. *Canadian Journal of plant science* **94**, 23-31.

Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., and Mittler, R. (2004). When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. *Plant physiology* **134**, 1683-1696.

Robbins, N. E., and Dinneny, J. R. (2015). The divining root: moisture-driven responses of roots at the micro-and macro-scale. *Journal of Experimental Botany* **66**, 2145-2154.

Rockström, J., Karlberg, L., Wani, S. P., Barron, J., Hatibu, N., Oweis, T., Bruggeman, A., Farahani, J., and Qiang, Z. (2010). Managing water in rainfed agriculture—the need for a paradigm shift. *Agricultural Water Management* **97**, 543-550.

Rodríguez-Gamir, J., Xue, J., Clearwater, M. J., Meason, D. F., Clinton, P. W., and Domec, J. C. (2019). Aquaporin regulation in roots controls plant hydraulic conductance, stomatal conductance, and leaf water potential in Pinus radiata under water stress. *Plant, Cell & Environment* **42**, 717-729.

Sage, R. F., and Kubien, D. S. (2007). The temperature response of C3 and C4 photosynthesis. *Plant, cell & environment* **30**, 1086-1106.

Sakagami, J.-I., and Kawano, N. (2011). Survival of submerged rice in a flood-prone region of West Africa. *Tropics* **20**, 55-66.

Sánchez, B., Rasmussen, A., and Porter, J. R. (2014). Temperatures and the growth and development of maize and rice: a review. *Global change biology* **20**, 408-417.

Sanderson, P., and Armstrong, W. (1978). Soil waterlogging, root rot and conifer windthrow: oxygen deficiency or phytoxicity? *Plant and soil* **49**, 185-190.

Sandhu, N., Raman, K. A., Torres, R. O., Audebert, A., Dardou, A., Kumar, A., and Henry, A. (2016). Rice root architectural plasticity traits and genetic regions for adaptability to variable cultivation and stress conditions. *Plant physiology* **171**, 2562-2576.

Shiono, K., Ogawa, S., Yamazaki, S., Isoda, H., Fujimura, T., Nakazono, M., and Colmer, T. D. (2011). Contrasting dynamics of radial O2-loss barrier induction and aerenchyma formation in rice roots of two lengths. *Annals of Botany* **107**, 89-99.

Singh, V., van Oosterom, E. J., Jordan, D. R., Messina, C. D., Cooper, M., and Hammer, G. L. (2010). Morphological and architectural development of root systems in sorghum and maize. *Plant and Soil* **333**, 287-299.

Sood, S., Khulbe, R. K., Gupta, A. K., Agrawal, P. K., Upadhyaya, H. D., and Bhatt, J. C. (2015). Barnyard millet–a potential food and feed crop of future. *Plant Breeding* **134**, 135-147.

Souza, R., Machado, E., Silva, J., Lagôa, A., and Silveira, J. (2004). Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (Vigna unguiculata) during water stress and recovery. *Environmental and experimental botany* **51**, 45-56.

Steuer, B., Stuhlfauth, T., and Fock, H. P. (1988). The efficiency of water use in water stressed plants is increased due to ABA induced stomatal closure. *Photosynthesis research* **18**, 327-336.

Suralta, R., Inukai, Y., and Yamauchi, A. (2010). Shoot dry matter production in relation to root growth, oxygen transport and water uptake under transient soil moisture stresses in rice. *Plant Soil* **332**, 87-104.

Tian, L., Li, J., Bi, W., Zuo, S., Li, L., Li, W., and Sun, L. (2019). Effects of waterlogging stress at different growth stages on the photosynthetic characteristics and grain yield of spring maize (Zea mays L.) under field conditions. *Agricultural water management* **218**, 250-258.

Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., and Kanno, N. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature genetics* **45**, 1097-1102.

Vadez, V. (2014). Root hydraulics: the forgotten side of roots in drought adaptation. *Field Crops Research* **165**, 15-24.

van Veen, H., Akman, M., Jamar, D. C., Vreugdenhil, D., Kooiker, M., van Tienderen, P., Voesenek, L. A., Schranz, M. E., and Sasidharan, R. (2014). Group VII E thylene R esponse F actor diversification and regulation in four species from flood-prone environments. *Plant, Cell & Environment* **37**, 2421-2432.

Vartapetian, B. B., and Jackson, M. B. (1997). Plant adaptations to anaerobic stress. *Annals of Botany* **79**, 3-20.

Vile, D., Pervent, M., Belluau, M., Vasseur, F., Bresson, J., Muller, B., Granier, C., and Simonneau, T. (2012). Arabidopsis growth under prolonged high temperature and water deficit: independent or interactive effects? *Plant, cell & environment* **35**, 702-718.

Wang, J., Vanga, S. K., Saxena, R., Orsat, V., and Raghavan, V. (2018). Effect of climate change on the yield of cereal crops: a review. *Climate* **6**, 41.

Wasaya, A., Zhang, X., Fang, Q., and Yan, Z. (2018). Root phenotyping for drought tolerance: a review. *Agronomy* **8**, 241.

Wasson, A. P., Richards, R., Chatrath, R., Misra, S., Prasad, S. S., Rebetzke, G., Kirkegaard, J., Christopher, J., and Watt, M. (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of experimental botany* **63**, 3485-3498.

Weaver, J. E., Jean, F. C., and Crist, J. W. (1922). "Development and activities of roots of crop plants: a study in crop ecology," Carnegie institution of Washington.

Wilkinson, S., Clephan, A. L., and Davies, W. J. (2001). Rapid low temperature-induced stomatal closure occurs in cold-tolerant Commelina communis leaves but not in cold-sensitive tobacco leaves, via a mechanism that involves apoplastic calcium but not abscisic acid. *Plant Physiology* **126**, 1566-1578.

Wolfe, D. W. (1991). Low temperature effects on early vegetative growth, leaf gas exchange and water potential of chilling-sensitive and chilling-tolerant crop species. *Annals of Botany* **67**, 205-212.

Wong, S., Cowan, I., and Farquhar, G. (1979). Stomatal conductance correlates with photosynthetic capacity. *Nature* **282**, 424-426.

Xangsayasane, P., Fukai, S., Mitchell, J., Jongdee, B., Jothityangkoon, D., Pantuwan, G., and Inthapanya, P. (2014). Genotypic performance in multi-location on-farm trials for evaluation of different on-station screening methods for drought-prone rainfed lowland rice in Lao PDR. *Field Crops Research* **160**, 1-11.

Yamasaki, T., Yamakawa, T., Yamane, Y., Koike, H., Satoh, K., and Katoh, S. (2002). Temperature acclimation of photosynthesis and related changes in photosystem II electron transport in winter wheat. *Plant Physiology* **128**, 1087-1097.

Yamauchi, A., Kono, Y., and Tatsumi, J. (1987). Quantitative analysis on root system structures of upland rice and maize. *Japanese Journal of Crop Science* **56**, 608-617.

Yamauchi, T., Abe, F., Tsutsumi, N., and Nakazono, M. (2019). Root cortex provides a venue for gas-space formation and is essential for plant adaptation to waterlogging. *Frontiers in plant science* **10**, 259.

Yamauchi, T., Colmer, T. D., Pedersen, O., and Nakazono, M. (2018a). Regulation of root traits for internal aeration and tolerance to soil waterlogging-flooding stress. *Plant physiology* **176**, 1118-1130.

Yamauchi, T., Colmer, T. D., Pedersen, O., and Nakazono, M. (2018b). Regulation of Root Traits for Internal Aeration and Tolerance to Soil Waterlogging-Flooding Stress. *Plant Physiol* **176**, 1118-1130.

Yamauchi, T., Noshita, K., and Tsutsumi, N. (2021a). Climate-smart crops: key root anatomical traits that confer flooding tolerance. *Breeding Science*, 20119.

Yamauchi, T., Pedersen, O., Nakazono, M., and Tsutsumi, N. (2021b). Key root traits of Poaceae for adaptation to soil water gradients. *New Phytologist* **229**, 3133-3140.

Yamauchi, T., Shimamura, S., Nakazono, M., and Mochizuki, T. (2013). Aerenchyma formation in crop species: a review. *Field Crops Research* **152**, 8-16.

Yang, M., Wang, G., Ahmed, K. F., Adugna, B., Eggen, M., Atsbeha, E., You, L., Koo, J., and Anagnostou, E. (2020). The role of climate in the trend and variability of Ethiopia's cereal crop yields. *Science of The Total Environment* **723**, 137893.

Yu, G.-R., Zhuang, J., Nakayama, K., and Jin, Y. (2007). Root water uptake and profile soil water as affected by vertical root distribution. *Plant Ecology* **189**, 15-30.

Zegada-Lizarazu, W., and Iijima, M. (2005). Deep root water uptake ability and water use efficiency of pearl millet in comparison to other millet species. *Plant Production Science* **8**, 454-460.

Zhang, F., Zhu, K., Wang, Y., Zhang, Z., Lu, F., Yu, H., and Zou, J. (2019a). Changes in photosynthetic and chlorophyll fluorescence characteristics of sorghum under drought and waterlogging stress. *Photosynthetica* **57**, 1156-1164.

Zhang, R., Zhou, Y., Yue, Z., Chen, X., Cao, X., Ai, X., Jiang, B., and Xing, Y. (2019b). The leaf-air temperature difference reflects the variation in water status and photosynthesis of sorghum under waterlogged conditions. *Plos one* **14**, e0219209.

Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D. B., Huang, Y., Huang, M., Yao, Y., Bassu, S., and Ciais, P. (2017). Temperature increase reduces global yields of major crops in four independent estimates. *Proceedings of the National Academy of Sciences* **114**, 9326-9331.