Greenhouse gas emissions and agro-physiological responses of rice cultivars (*Oryza sativa* L.*)* under drip irrigation with plastic-film-mulch

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［千葉大学学位申請論文］

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January, 2020

A thesis submitted to Graduate School of Horticulture, Chiba University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph.D.)

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APPROVAL

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ACKNOWLEDGMENTS

I am grateful to Almighty God for the successful completion of this program. My profound gratitude goes to my major supervisor (Professor Akihiro Isoda) for granting me the acceptance to carry out this project in his laboratory. I am also thankful for the love, care, guidance and full support that he extended to me throughout my study. I am also indebted to my advisory committee, Professor Toru Maruo, Professor Kazuyuki Inubushi and Professor Nobuhiro Matsuoka for their contributions to this project. My cordial thanks go to Professor Kazuyuki Inubushi for his guidance and also for providing the laboratory assistance for an important aspect of this research.

My deepest appreciation goes to the Japanese Ministry of Education, Culture, Sports, Science, and Technology (MEXT) for the educational-based investment they made in me through the Monbukagakusho scholarship program to fund my study. My gratitude also goes to other sponsors of this project: Japan Society for the Promotion Science (JSPS KAKENHI) (JP 16K07570); Xinjiang Tianyuan Institute of Rice Drip Irrigation System, and Chiba University SEEDS Fund (Chiba University Open Recruitment for International Exchange Program) for the grants provided. These aided the successful completion of the research and presentation of results at international conferences.

I offer my sincere thanks to my research team (Kanako Honda, Yuki Taguchi, Motofumi, Hiramatsu, Junfa Wang) and other members of the laboratory of crop science that contributed to the success of this work. Your labor of love will forever linger in my heart.

My sincere appreciation goes to my affiliated (home) institution (Federal University of Agriculture Abeokuta, Nigeria) for the approval of my study leave without withholding other benefits. Also, I am grateful to all the staff of the Department of Pure and Applied Botany (FUNAAB) under the leadership of Professor (Mrs) I.A Kehinde for their support and assistance while staying out of the key departmental responsibilities. God bless you all.

Finally, I am indebted to my family, my in-laws, friends, members of Matsudo city church, all love beings for their prayers and good wishes. I love you all.

DEDICATION

This thesis is dedicated to my wife (Kehinde Oluwaseyi Fawibe) and my lovely twins (Taiwo and Kehinde Fawibe) whose love, sacrifice, support, and encouragement have motivated me to pursue and complete this research. You are the true champions!

ABSTRACT

The increased competition for water and the substantial environmental impacts of conventional rice production has intensified the search for the most efficient water-saving rice production system capable of mitigating greenhouse gas emissions without yield loss. A 3-year field experiment was conducted to quantify greenhouse gas emissions (GHGs) and to investigate agronomical and physiological responses of rice cultivars under drip irrigation with plastic-film-mulch. CH_4 and N_2O were quantified and contrasted in drip irrigation with direct fertilization under plastic-film-mulch (DPD) and continuous flooding (CF) systems in 2016 and 2017. Moreover, the grain yield, grain quality, and water-use efficiency (WUE) of rice as influenced by different fertilizer application methods under drip irrigation with plastic-film-mulch were investigated in 2017 and 2018. As a result, drip fertigation with plastic-mulch (DPF) was included in 2017 and 2018. Koshihikari (a lowland *Japonica* cultivar), Norin 24 (an upland *Japonica* cultivar) and Princessari (a lowland cultivar; a derivative from *Indica and Japonica* crosses) were used in 2017, whereas only two cultivars (Koshihikari and Norin 24) were used in 2018. In 2017, the cumulative CH_4 emission under both irrigation systems was higher than in 2016. Compared with CF, DPD reduced CH_4 emission by 194% and 96% in 2016 and 2017, respectively. However, low and insignificant N_2O emissions were observed under both irrigation regimes. This was attributable to low nitrogen fertilization and a probable discontinuous aerobic-soil condition. The 2-year average CO_2 equivalent (CO_2eq) emission of the CF was 9 times greater than that of the DPD. The grain yields of Koshihikari and Norin 24 were not significantly different under DPF and DPD compared with CF. These were ascribed to the comparable photosynthesis associated parameters at the grain-filling stage. The high yields obtained under DPF and DPD were attributed to the increase in the number of spikelets per panicle. However, the deficiency of available soil Fe and mild water stress decreased the leaf SPAD value and maximum quantum yield (Fv/Fm) of photosystem

II (PSII) of Princessari. These resulted in a grain yield reduction of 65% and 54% under DPF and DPD, respectively, compared with CF. The DPF and DPD increased WUEs of Koshihikari and Norin 24 by 40-74% and improved rice nutritional quality but increased the chalky grain percentage. The DPF and DPD similarly influenced the grain yield and grain quality; nonetheless, the DPD had a greater water-saving capacity. The study demonstrated that, with cultivars adaptable to Fe deficiency in the soil and mild water stress, drip irrigation with plastic-film-mulch could significantly increase WUE while maintaining grain yield that is comparable to that under CF with reduced environmental impact. Therefore, DPF or DPD could be an efficient water-saving rice production system in areas with limited water resources.

摘要

水をめぐる競争の激化と従来のイネ生産が環境に及ぼす影響により、収量を損なうことなく温室効果ガスの排出を軽減できる効率的な節水イネ生産システムの探索が強く求められてきている．本研究は、プラスチックフィルムマルチによる点滴かんがい栽培条件下での温室効果ガス排出量（GHG）を定量化し、イネ品種の農業生理学的反応を調査するために、3年間の圃場実験を実施した．実験1では、2016年と2017年に、元肥として化成肥料を用いるプラスチックフィルムマルチ点滴かんがい栽培（DPD）と湛水栽培（CF）におけるメタンと亜酸化窒素ガスの発生量を測定した．実験2では、プラスチックフィルムマルチ点滴かんがい下での異なる施肥法が収量、品質、および水利用効率（WUE）に及ぼす影響について調査した．この実験は2017年と2018年に、コシヒカリ（水稲、ジャポニカ品種）、農林24号（陸稲、ジャポニカ品種）およびプリンセスサリー（インディカとジャポニカの交雑後代、2017年のみ供試）を用いて行った．実験2は3つの栽培システム、湛水栽培（CF）、液肥を用いるプラスチックマルチ点滴かんがい栽培（DPF）、および元肥として化成肥料を用いるプラスチックマルチ点滴かんがい栽培（DPD）を用いて行った．2017年、両かんがいシステムでの累積CH_4排出量は2016年よりも高かった．CFと比較して、DPDはCH_4排出量が2016年と2017年にそれぞれ194%、96%小さかった．ただし、DPFとDPDで、ごく少量のN_2O排出が観察されたが、有意ではなかった．これは、低窒素施肥と断続的な土壌窒素飽和レベルに起因しているものと考えられた．2年平均の地球温暖化係数は、CFと比較してDPDで89%減少した．コシヒカリと農林24号の子実収量は、CFと比較してDPFとDPDで有意差はなかった．これは、登熟期での光合成関連パラメーターに大きな差異がなかったことに起因していた．しか

し、プリンセスサリーの子実収量は、DPFおよびDPDでそれぞれ65%および54%と大幅に減少した. WUEはCFと比較してDPFおよびDPDで40～74%大幅に増加した. プラスチックフィルムマルチ点滴かんがい栽培は、コシヒカリと農林24号の子実の栄養価品質を改善したが、乳白米率は増加した. 土壌肥沃度が高い場合、プラスチックフィルムマルチ点滴かんがい栽培は、子実品質は若干低下したものの、大きな節水効果共に子実収量向上の可能性を示した. また、土壌の鉄欠乏と穏やかな水ストレスとの相互作用により、品種の適応特性に応じて収量が減少する傾向が認められた. 以上の結果から、プラスチックフィルムマルチ点滴かんがい栽培は環境への影響を軽減し、大幅に水を節約し、子実品質はわずかに低下するものの、湛水栽培と同等の高い子実収量を維持することを示した. この栽培方法は、水資源が不足している地域での効率的な節水イネ栽培システムとなる可能性が大きいものと考えられた.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background of study

Agricultural practices such as irrigation and fertilization are essential for crop improvement and plant growth but these also increase greenhouse gas (GHG) emissions from the soil to the atmosphere. Greenhouse gas emissions from agricultural land have been estimated to account for 13.5% of anthropogenic emissions worldwide (IPCC, 2007). Improving agricultural practices is a recommended strategy for mitigating GHG emissions from agricultural soils without incurring yield loss (Wu *et al.,* 2014). However, this strategy greatly depends on crop types.

Rice crops require a substantial amount of fresh water because it is mostly grown under a continuous flooding system also referred to as paddy (Tuong and Bouman, 2003; Bouman *et al.,* 2007). This cultivation method is most common in Asia where more than 75% of the world's rice is being produced (Cantrell and Reeves, 2002); making irrigated rice production important to the present and future food security. It is quite infelicitous that despite the contribution of paddy field towards feeding the growing population and ensuring sustainable food production, its adverse role in the environment cannot be underestimated. Rice production has environmental impacts, largely by releasing gases to the atmosphere, therefore, becoming a significant source of greenhouse gases that contribute largely to climate change. The magnitude and pattern of methane (CH_4) emissions from rice fields are determined mainly by the water regime and organic inputs and to a lesser extent by soil type, weather, tillage practices, residue management, and rice cultivar (Dickie *et al.,* 2014).

In irrigated rice systems with good water control, nitrous oxide (N_2O) emissions are negligible except when nitrogen fertilizer rates are excessively high. In irrigated rice fields, the bulk of nitrous oxide emissions occur during fallow periods and immediately after the flooding of the soil at the end of the fallow period (Kreye *et al.,* 2007). In rainfed systems, however, nitrate accumulation in aerobic phases might contribute to considerable emission of nitrous oxide.

Water is essential for the growth and development of rice plants. Continuous flooding (CF) provides favorable water and nutrient supply under anaerobic conditions. The significance of this system relates to the high yield and quality of rice production. This could be attributed to high soil microbial biomass and soil organic matter under flooded conditions. In paddy, decomposition of soil organic matter is slow leading to a gradual release of nutrients to the rice plant. Also, the presence of high soil microbial biomass enables the soil to be rich in nitrogen. However, the conventional system consumes a large amount of water. It has been estimated that the continuous flooded rice production system takes 3000 to 5000 liters of water to produce 1 kilogram of rice. This is 2-3 times more than to produce 1 kilogram of other cereals such as wheat and maize (Bouwman *et al.,* 2002).

Water has been reported to be the most precious resource. Nonetheless, the increasing world population, industrialization, urbanization and the development of other economic indices have intensified competition for available water resources (Tuong and Bouman, 2003; Bouman, 2007). This has led to a significant decrease in freshwater availability for agricultural production. With limited water, farmers are facing a challenge to produce more rice per unit of land to meet the food demand of a growing population. Therefore, to ensure food security while reducing the agricultural impact of GHGs concentration in the atmosphere, rice cultivation systems that incorporate water-saving methods with great potential to mitigate greenhouse gases emission need to be established. Thus, enabling to cope with potential water deficit and ensuring that the demand for rice continues to be met in a sustainable environment.

1.2 Emission of Greenhouse gases

Human activities are estimated to have caused approximately 1.0˚C of global warming above pre-industrial levels, with a likely range of 0.8˚C to 1.2˚C. Global warming is likely to reach 1.5˚C between 2030 and 2052 if it continues to increase at the current rate (IPCC, 2018).

Methane (CH_4) and nitrous oxide (N_2O) are important greenhouse gases (GHGs) in the atmosphere that have been receiving more attention in recent years (Li *et al.,* 2014). This does not only result from their rapid increase in concentration in the last century caused by anthropogenic and natural emissions but also as a result of their strong infrared absorption capacity and large warming effects in the atmosphere (Fender *et al.,* 2012). The global warming potential (GWP) of a single molecule of CH_4 and N_2O is 28 times and 265 times that of carbon dioxide (CO_2) respectively, for a period of 100 years (IPCC, 2014).

Methane sources are divided into biological and non-biological sources. The biological source consists of wetlands, termites, oceans, landfills, ruminants and paddy fields. Non biological sources include biomass burning and fossil fuel origins such as hydrates, mining, and transport. Both types comprise the anthropogenic and natural emissions. The anthropogenic CH_4 emission accounts for 60-70% of the total CH_4 emission (Hein *et al.,* 1997; Fung *et al.,* 1991).

1.2.1 The contribution of rice field to anthropogenic methane emission

The increase in demand for food as a result of the steady increase in population has necessitated the increase in rice production. Therefore, a further increase in methane emission from rice fields has become a matter of concern. The lowland rice field is one of the most important sources of CH_4 emission. Although methane is not toxic when inhaled but it is an important greenhouse gas accounting for 15% of the total enhanced greenhouse effect (Jain *et al.,* 2004). The staying time of this gas in the atmosphere is relatively short (10 years) as compared to other greenhouse gases (GHGs) such as CO_2 (100 years) and N_2O (170 years). Therefore, the reduction of global methane sources offers possibilities for curtailing the increasing trend of global warming on a short time scale.

Carbon substrates for methanogenesis are supplied from applied organic matter, exudates and sloughed tissues of rice plants, and soil organic matter. CH_4 is produced by the reduction of carbon substrates by methanogens under strictly anaerobic conditions with an approximate redox potential from -150 to -160mV (Wang *et al.*, 1993). The anaerobic condition occurs in the paddy as a result of soil submergence that limits the transport of oxygen into the soil. Under this condition, microorganisms start to use alternative electron acceptors in their respiration causing further soil reduction. The redox potential reduces sharply and thus leads to methanogenesis.

Under anaerobic and reduced conditions, methanogens produce methane from either the reduction of CO_2 and H_2 (Hydrogenotrophic) or from the fermentation of acetate to CH_4 and CO_2 (acetotrophic) (Deppenmier *et al.,* 1996). Under steady-state conditions in anoxic rice fields, the acetotrophic pathway is dominant and accounts for about 78-80% of the total CH_4 emitted. These bacteria being strictly anaerobic, convert fermentation products formed by other microorganisms, notably CO_2, H_2, esters, salt of methanoic acid (HCOOH), and other substrates into CH_4 (Cicerone and Oremland, 1998). The reactions with reference to the type of methanogens involved in forming CH_4 as an end product were illustrated by Papen and Rennenberg (1990).

(a) H_2 reduction of CO_2 by chemoautotrophic methanogens

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$$

(b) Several strains of methanogens can also use HCOOH or CO as a substrate for methane production

$$4HCOOH \longrightarrow CH_4 + 3CO_2 + 2H_2O$$

$$4CO + 2H_2O \longrightarrow CH_4 + 3CO_2$$

(c) Methane can also be produced by methylotrophic methanogens, which use methyl-group containing substrates such as methanol and acetate.

$$4CH_3OH \longrightarrow 3CH_4 + CO_2 + 2H_2O$$

$$CH_3COOH \longrightarrow CH_4 + CO_2$$

In contrast, there are some aerobic microsites in rice soils which function as a sink for methane. These enable the transformation of methane to CO_2 by the oxidation process carried out by methanotrophic bacteria. Methanotrophs are a subset of the physiological group of methylotrophs, which utilize a variety of one-carbon compounds. Some of the microorganisms responsible for the oxidation of CH_4 are strictly aerobic, obligate methyl- or methanotrophic eubacteria. These microorganisms can use CH_4 and C1 compounds such as methanol and methylated compounds containing sulfur as substrates (Richard and Thomas, 1996).

1.2.2 Transfer of Methane from soil to the Atmosphere

The emission of methane from the soil to the atmosphere involves different regulating processes. However, CH_4 emission is mostly transported through rice plants. Similar to the transport of O_2 and some other gases in several aquatic plants, aerenchyma helps in the transport of gaseous CH_4 in rice. The path includes diffusion into the root, conversion into gaseous CH_4 in the root cortex, diffusion through cortex and aerenchyma and the release to the atmosphere through micropores in the leaf sheath (Schutz *et al.*, 1989). A shift in the CH_4 transport pathway was observed by Wang *et al.* (1997) that about 50% of CH_4 were released from the leaf blades before shoot elongation, whereas only a small amount was emitted through leaves as plants grew older. In addition to the presence of the micropores of the leaf sheath, Wang *et al*. (1997) identified cracks in junction points of internodes. Although CH_4 can also be released through panicles, this pathway is negligible as long as leaves and nodes were not submerged. Nouchi *et al.* (1990) have also reported that CH_4 was mostly released from the culms of rice plants. Moreover, the ebullition of gases entrapped in sediments and peats has also been identified as a possible path for methane emission to the atmosphere. The ebullition process could be influenced by many factors like wind speed, flood water-temperature, solar radiation, flood water level, and atmospheric pressure. The contribution of ebullition to the total CH_4 emission is not more than 20%. The emissions from the unplanted fields were almost exclusively due to ebullition. In Japan, Takai and Wada (1990) had observed that the ebullition of CH_4 is important during the early stage of flooding when rice plants are small, whereas vascular transport becomes more important as the rice plants get older.

In addition, diffusion is another pathway. Nonetheless, this has an infinitesimal contribution to CH_4 emission in comparison to the other pathways. The diffusion of gases in water is 104 times slower than in air, therefore the exchange of gases almost stops when soil is waterlogged. The diffusion of CH_4 from the rice fields depend on the CH_4 concentration in the flood water and the prevailing wind speed (Sehacher *et al.,* 1983). Diffusion through the floodwater is usually less than 1% of the total flux (Conrad, 1993). However, a considerable amount of CH_4 trapped in the soil is emitted by direct diffusion during drainage (Minamikawa *et al.,* 2005).

1.2.3 Nitrous oxide emission from rice field

Soils are the principal source of nitrous oxide with agricultural soils representing the single largest source of anthropogenic N_2O production (Del Grosso *et al.,* 2006). Nitrous oxide accounts for approximately 6% of the total anthropogenic greenhouse gas (GHG) emissions

(Weller *et al.,* 2015). The direct and indirect emissions of N_2O from agricultural ecosystems to the atmosphere contribute approximately 6 Tg N yr^{-1} (IPCC, 2007). Direct emissions of N_2O are considered emissions from the soil that occurs as a direct result of additional fertilizer N application to the soil (e.g. a producer's field within a defined boundary). Indirect emissions of N_2O are considered to be those produced off-site (beyond the boundary) (IPCC, 2006). However, N_2O emissions can be reduced through improved fertilizer management, alternative irrigation, and crop management techniques (Parkin and Kasper, 2006).

Nitrous oxide is primarily produced from the microbial processes of denitrification and nitrification and is affected by many factors: environmental, managerial, and their interaction (Millar *et al.,* 2010). Nitrification is the aerobic process in which ammonium (NH_4^+) is oxidized to nitrite (NO_2^-) and further oxidized to nitrate (NO_3^-). Denitrification is an anaerobic process in which NO_3^- is reduced to N_2O and N_2O is reduced to dinitrogen gas (N_2). This depends on factors such as soil pH, soil degree of anaerobicity, soil carbon content, NO_3^- content, and water content (Dalal *et al.,* 2003). Larger emission from the soil tends to be associated with the denitrification pathway whereas nitrification-derived N_2O flux rate is smaller (Skiba and Smith, 2000). However, conditions favorable for the nitrification process tend to be more common. These include the greater soil aeration, good soil drainage, and more aerobic conditions.

1.2.4 Effects of irrigation and fertilization on N_2O fluxes

Water and Nitrogen are the two of the main limiting factors affecting crop production within arid and semi-arid regions. Both of these factors are intrinsically linked to the production of N_2O emissions from soils in agricultural ecosystems. The addition of synthetic fertilizers is a major source of N_2O production. The production of both natural and fertilizer-derived N_2O from agricultural soil can be greatly influenced by N fertilizer (rate, type, timing, and application method), irrigation timing, and microbial processes (Parkin and Kaspar, 2006). Others include local climate conditions and soil properties including soil water and N dynamics, thus making the quantification of N_2O emissions from specific cropping systems a challenging task.

Irrigation does not only stimulates plant growth but also accelerate microbial turnover of C and N (Davidson, 1991). This increases the potential of N_2O production. Many studies have assessed net N_2O fluxes under different cropping systems (Aguilera *et al.*, 2013; Han *et al.*, 2017), and it has been shown that there is a strong relationship between irrigation and the stimulation of N_2O production. In semi-arid and arid regions, the soils are often low in available nutrients and have low microbial activity when dry (Steenwerth *et al.*, 2010). However, following soil moistening, especially after irrigation events, the microbial population within the soil greatly increases and N mineralization takes place at a rapid rate (Appel, 1998). In addition, the wet-dry cycles that often accompany irrigation events are known to affect soil microbial processes and subsequently C and N cycling. These regulate both the production and consumption of N_2O between the soil and atmosphere interfaces.

1.3 Irrigation and crop production

Irrigation is an artificial application of water to the soil, primarily to meet the water needs of crops. Water from rivers, reservoirs, lakes, or aquifers is pumped or flows by gravity through pipes, canals, ditches or even natural streams for irrigation purposes. Applying water to fields enhances the quality and reliability of crop production. According to the World Bank (2017) irrigated agriculture represents 20% of the total cultivated land, but contributes 40% of the total food produced worldwide. However, the pressure for the most efficient use of water for agriculture is intensifying with the increasing competition for water resources among various sectors coupled with an increasing population (Fanish *et al.*, 2011). This emphasized the need to maximize crop production per unit drop of water. Various irrigation methods have been developed over time to meet the irrigation needs of certain crops in specific areas. The irrigation methods have been categorized into three main methods: (i) surface (furrow, border), (ii) sprinkler and (iii) drip/micro-irrigation.

Surface irrigation enables water to flow over the soil by gravity. However, sprinkler irrigation applies water to the soil by spraying water droplets from fixed or moving systems. Drip irrigation (trickle or micro irrigation) on the other hand supplies water in drops directly to the rhizosphere with the aid of drip tubes set on the soil surface (surface drip) or few centimeters beneath the ground (subsurface). The drip irrigation system has proved its superiority over other methods of irrigation. This is especially so for irrigating fruit and vegetable crops as a result of its precise and direct application of water to the root zone. A study conducted at Agricultural College, Madurai, revealed that the increase in seed cotton yield under drip irrigation was 24%, 35%, 45% and 53% more than that under furrow, skip furrow, alternate furrow and check basin method, respectively (Sampathkumar *et al.*, 2006). A field experiment in the USA by Pruitt *et al.* (1984) also demonstrated that drip irrigation increased the yield of tomato and water-use efficiency (WUE) by 19% and 20%, respectively, as compared to furrow irrigation.

The productivity of drip irrigation has prompted a significant increase in its usage (Howell, 2001). The technique is receiving better acceptance and adoption, particularly in the arid and semi-arid regions. It is one of the innovations for applying water to row planted, widely spaced crops especially in areas with water scarcity. This method is necessary to manage the available water efficiently for maximum crop production (Solaimalai *et al.,* 2005). Drip irrigation can supply water both precisely and uniformly at a high irrigation frequency compared to surface and sprinkler irrigation; thus, potentially increasing yield, reducing subsurface drainage, providing better salinity control and better disease management (Hanson and May, 2007). Other benefits of drip irrigation include: improved crop quality, enhanced efficiency of fertilizer and other chemicals, limited weed growth, decreased energy requirements and improved cultural practices (Wang *et al.*, 2012).

Drip irrigation technique saves a considerable amount of water since water can be applied almost precisely and directly in the root zone without wetting the entire area. In a drip irrigation system, only a fraction of the soil surface generally between 15 to 60% is wetted (Fanish *et al.*, 2011). This is caused by the stable moisture content maintained at the root zone of the crop by the way of frequent irrigation at short intervals; thereby, reducing water losses through surface evaporation and deep percolation (Aujla *et al.,* 2005). Maintaining available soil moisture at low water tension and almost constant during the entire growth period through micro-irrigation saved up to 50% of irrigation water (Aujla *et al.*, 2005).

1.3.1 Effect of drip fertigation on crop improvement

Drip irrigation has the potential to improve two of the most common contributing factors to N leaching i.e. over-fertilization and over-irrigation. Fertigation is an innovative cultural method that supplies water and fertilizer simultaneously in a drip irrigation system, feeding a crop by injecting soluble fertilizer into the water and then transporting them to the root zone (Hagin and Lowengart, 1996). This results in higher fertilizer-use efficiency besides increasing the crop yields (Hagin and Lowengart, 1996). Fertigation enables the harmonization and integration between the application of water and plant nutrients. This technique forms a critical measure required to meet the mounting demands on water resources and the acute need for the efficient use of nutrients (Malakouti, 2004). Fertigation ensures substantial savings in fertilizer usage and reduces leaching losses (Hebber *et al.,* 2004). A properly designed drip fertigation system delivers water and nutrient at a rate, duration, and frequency, so as to maximize crop water and nutrient uptake while minimizing the leaching of nutrients (Gardenas *et al.,* 2005). The ability of drip fertigation to reduce nutrient losses to the environment makes it an eco-friendly system. The application of NPK from liquid fertilizer significantly increased the concentration and total uptake of N, P and K in tomato crop over solid fertilizer (Dhake *et al.,* 2009). Janat and Somi (2001b) reported that the yield of fertigated-cotton increased by 50% compared with surface-irrigated cotton. The fruit yield of tomato was 20-30% higher in drip fertigation than in furrow irrigation (Hebbar *et al.,* 2004). According to Tumbare *et al.* (1999), the fruit yield of okra was significantly increased, when fertilizer was applied through fertigation than band placement. The fertigation of chili with 100% recommended N saved 40% water and produced 52% higher yield over check-basin and only 50% N applied through fertigation produced the equivalent amount of fruit to check-basin (Singh et al., 1999).

1.3.2 Effect of mulch on crop production and improvement

Mulching is one of the good management practices among others to improve water-use efficiency of crops. This plays a pertinent role in maintaining the soil water status along with other benefits. Mulching materials have been grouped into two categories: organic and inorganic. The most frequently used inorganic mulch is the plastic-film. The effective usage of plastic-mulch increased crop yield by contributing to the improvement of soil fertility, conservation of soil moisture, and the control of weed growth (Zhang *et al.*, 2005; Bu *et al.*, 2002). The use of mulch lessening the impact of raindrops on the soil surface (Farooq *et al.*, 2011a, Seguy *et al.*, 2012); thereby, improving soil physical properties such as soil texture, porosity, and infiltration rate. Also, the improvement of organic matter and soil structure has been reported for soil mulched with organic materials or plastic-film (Saroa and Lal, 2003). The use of polyethylene mulch in vegetable production was reported to control weed incidence, reduce nutrient loss and improve hydrothermal regimes of soils (Asworth and Hurrison 1983).

1.3.3 Rice cultivation and water management

Rice is one of the most important cereal crops in the world, grown annually on areas more than 140 million hectares and consumed more than any other staple food (Dickie *et al.*, 2014). Rice is a profligate user of water. Its production consumes about 30% of all freshwater used worldwide. Interestingly, the physiological demand of the rice crop for transpiration accounts for only 10-12% of the total water use in paddy rice production. Moreover, 16-18% and 50-72% are being lost in evaporation and seepage respectively (Wang and Zhou, 2000). This accounts for the low water-use efficiency of paddy rice compared with other crops. However, to safeguard food security and preserve precious water resources, ways must be explored to grow rice using less water.

The evolution of water-saving practices has called into question the common belief that rice is an aquatic plant. Rice has been grown under flooded conditions mostly because these make weed control easier or even unnecessary, thereby saving labor. It is true that rice grows more abundantly in standing water than it does under upland (unirrigated) conditions. But this does not make continuous flooding the best practice. Recently, attention has been paid more on the optimization of the middle range of water management in which the soil is kept moist but mostly aerobic through controlled water application. These practices focus on the reduction of water usage, an increase in water-use efficiency, and maintenance or increase of production for rice-based systems (Tuong and Bhuiyan, 1999).

The most commonly practiced techniques include alternate wetting and drying (AWD) irrigation (Oo *et al.,* 2018; Bouman and Tuong, 2001); the most widely adopted water-saving practice in Asia (Li, 2001). In this system, the rice field is allowed to dry for a few days in between irrigation events, including midseason drainage in which the field is allowed to dry for 7-15 days at the end of the tillering stage. The potential of AWD to reduce water input and its effect on yield and water productivity depends on soil type, groundwater table depth and climate (Bouman and Tuong, 2001).

Tabbal *et al.* (2002) reported reduced water inputs and increased water productivity of rice grown under saturated soil conditions (saturated soil culture) compared with traditional flooded rice. This system involves growing rice on raised beds with shallow water depth in the furrows. In a semi-arid environment, Thompson (1999) reported no gain in water productivity since both irrigation input and yield declined by 10%.

It has also been suggested that rice could be grown aerobically under irrigated conditions just like upland crops such as wheat or maize (Bouman, 2001). The potential for water saving is large, but aerobic cultivation using conventional lowland rice varieties almost leads to yield reduction (McCauley, 1990). Therefore, a special type of rice is required to produce high yields under non-flooded conditions in non-puddled and unsaturated (aerobic) soil. Bouman (2001) named this “aerobic rice”. It has been reported to be responsive to high inputs, can be rainfed or irrigated and tolerates occasional flooding.

The System of Rice Intensification (SRI) developed in Madagascar is another water-saving rice production system. This offers the opportunity to improve food security through increased rice productivity by changing the management of plants, soil water, and nutrients while reducing external inputs like fertilizers and herbicides (Uphoff, 2003). The SRI field is not continuously flooded but treated with intermittent irrigation, usually a form of alternate wetting and drying. The system proposes the use of a very young seedling, wider planting space, intermittent wetting and drying, use of a mechanical weeder for soil aeration and enhanced soil organic matter to improve soil fertility and water holding capacity of the soil (Pascaul and Wang, 2017).

Despite its obvious benefits and intensive extension efforts by an indigenous non-governmental organization, SRI has not been taken off as expected. This was because the adoption rates have generally been low. The average rate of dis-adoption (practice abandonment) has been high at 40% and those who adopt and retain the technology rarely put more than half of their rice land under SRI (Moser and Barrett, 2002). The main constraint to SRI use has been the requirements for more labor, skill and water control. Chapagain *et al.* (2011) conducted a crop budget analysis for SRI versus conventional rice farming using organic and inorganic management. They concluded that labor input required in SRI-organic plots was double (90 man day/hm^2) than in conventional inorganic plots (45 man day/hm^2), and was primarily affected by the weed requirement of (50 man day/hm^2). The combination of wide spacing and fewer waters use in SRI provides ideal conditions for weed growth, therefore making frequent weeding a necessity. The studies of its labor requirements show that SRI requires an estimated 38% to 54% more labor than traditional methods (Moser and Barrett, 2002).

The Ground Cover Rice Production System (GCRPS) has gained popularity as a successful new approach to save water and increase nutrient-use efficiency. This cultivation technique involved the irrigation of lowland rice to 80-90% of water holding capacity. Subsequently, the soil surface is covered by plastic film or plant mulch and the soil is kept at 70-90% of water-holding capacity depending on the crop development stage (Wu *et al.*, 1999). The GCRPS has been reported to use only 40% of the amount of water usually needed to grow rice in submerged conditions while grain yields remain at 90% of those of the high-yielding submerged systems (Liang *et al.*, 1999). Nonetheless, the GCRPS was reported to have more effects on global warming. This was exclusively due to the higher nitrous oxide emission after fertilizer application (Kreye *et al.*, 2007).

1.3.4 Rice cultivation with drip irrigation and plastic-film-mulch.

Rice cultivation under field conditions with drip irrigation and plastic-film-mulch was first introduced in 2009 by Professor Wang and his team at Shihezi Agricultural and Environmental Institute for Arid Areas in Central Asia (presently known as Urumqi Agricultural and Environmental Institute for Arid Areas in Central Asia). This is a semi-arid region located in Northern Xinjiang, China on the edge of the Dzungar desert (Miyauchi *et al.*, 2012). The amount of precipitation and evaporation in Shihezi is less than 200 mm and more than 1500 mm per year, respectively. The drip irrigation with plastic-film-mulch was developed with the goal to save water and increase rice production in the semi-arid region. The results of their large-scale field experiments (22 ha and 135 ha in 2009 and 2010, respectively), showed that high water-use efficiency and relatively high yields of 6.0 t/ha and 7.5 t/ha in 2009 and 2010, respectively, were obtained (Wang, 2012). However, it was reported that this technique reduced cooking and taste qualities of rice.

The outcome of these experiments initiated further studies on unlocking the potentials of drip irrigation with plastic-film-mulch on rice production in China (He *et al.*, 2013 and Adekoya *et al.,* 2014) and the selection of suitable cultivars for this techniques in other regions of the world (Rao *et al.,* 2017; and Beser *et al.,* 2015) to ensure food security.

1.3.5 Physiological response of rice cultivars to stress

Conditions such as extreme air temperature, changes in photoperiod, light intensity and quality, nutrient abundance and starvation, drought, flooding or excessive salts hinder the full expression of the genetic potential of plants and are limiting to crop yield and quality (Ciais *et al.,* 2005). Drought may delay the phenological development of the rice plant (Inthapan and Fukai, 1988) and affect physiological processes like transpiration, photosynthesis, respiration, and translocation of assimilates to the grain (Tuner, 1986). Photosynthetic pigments allow plants to absorb energy from light making foliar chlorophyll content a key factor affecting the performance of plant photosynthesis (Croft and Chen, 2018). Previous reports have explained that drought stress significantly decreases the chlorophyll a, chlorophyll b and total chlorophyll content of different crops (Mafakheri *et al.,* 2010). However, the presence of low levels of chlorophyll in leaves (Chlorosis) is a general symptom of stress and might be poorly related to water status in the field when other environmental factors interact. It is well known that the net assimilation by leaves decreases as water potential decreases (Lawlor, 2002). Stomatal closure is one of the first responses of plants to the lack of water in the soil (Flexas *et al.,* 2002a). After stomatal closure, water deficit induces a decline in photosynthetic activity by decreasing internal CO_2 concentration which may limit carboxylation (Meyer and Genty, 1998; Flexas *et al.,* 2002a).

The analysis of chlorophyll fluorescence parameters is considered as an important approach to evaluating the health or integrity of the photosynthetic apparatus within a leaf, and this provides a rapid and accurate technique for detecting and quantifying the interaction of plants to stress (Lichtenthaler *et al.,* 2005). Photosystem II (PSII) is accepted to be the most vulnerable part of the photosynthetic apparatus to light-induced damage which results in the manifestation of stress in a leaf. Although fluorescence measurements may sometimes provide a useful measure of the photosynthetic performance of plants, its strength lies in its ability to give insights into the capacity of a plant to tolerate environmental stresses and into the extent to which those stresses have damaged the photosynthetic apparatus (Maxwell and Johnson, 2000). The principle underlying chlorophyll fluorescence analysis is relatively easy. Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates: it can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat or it can be re-emitted as light-chlorophyll fluorescence. These three processes occur in competition, such that any increase in the efficiency of one will result in a decrease in the yield of the other two. Therefore by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained (Maxwell and Johnson, 2000). Chlorophyll fluorescence has been commonly classified into two parameters: photochemical quenching parameters and non-photochemical quenching. The photochemical quenching includes the actual quantum yield (ΔF/Fm'), the proportion of open PSII (qP), and maximum quantum yield (Fv/Fm). The actual quantum yield measures the proportion of the absorbed energy by chlorophyll associated with PSII that is been used in photochemistry. The qP shows that the proportion of PSII reaction centers that are open. An alternative expression of this is (1-qP), the proportion of centers that are closed and is sometimes termed “excitation pressure” on PSII (Maxwell *et al.,* 1994). The maximum quantum yield is a measure of the intrinsic (maximum) efficiency of PSII. The dark-adapted values of Fv/Fm reflect the potential quantum efficiency of PSII and are used as a sensitive indicator of plant photosynthetic performance. Under field conditions, it is generally measured before dawn when photosynthetically active radiation (PAR) is almost zero. Schreiber (1997) reported that the Fv/Fm value of 0.75~0.85 indicates that a leaf or plant is photosynthetically active and non-stress. The NPQ measures a change in the efficiency of heat dissipation, relative to the dark-adapted state. This occurs as a measure to protect the leaf from light-induced damage. Heat dissipation is closely related to the stress resistance of rice in that NPQ level in rice leaf increases under stress conditions of heat, chilling, nitrogen deficiency and drought (Zhao *et al.*, 2016).

The fluorescence variables measured in dark-adapted leaves may be strongly affected by other factors such as Fe deficiency chlorosis (Bavaresco *et al.,* 2006). A low chlorophyll concentration (chlorosis) in young leaves is a visible symptom of Fe deficiency which has a significant negative effect on photosynthesis activity (Maxwell and Johnson, 2000). A shortage of physiologically Fe leads to a decrease in the electron transport rate, as well as to a lowering of the efficiency of PSII (Bavaresco *et al.*, 2006).

1.4 Justification of the study

Water-saving techniques that improve water productivity at the expense of grain yield can be justified in some cases. Nevertheless, there are needs to identify the conditions where improved water productivity can be achieved without yield penalties.

Although previous studies have shown that GCRPS effectively reduced CH_4 emissions and produced grain yield similar to those produced with the continuous flooding system; however, the system was also found to emit high N_2O (Kreye *et al.,* 2007). It has been observed by Kreye *et al.* (2007) that the inverse relationship between emissions of CH_4 and N_2O can be altered based on soil properties, fertilization regimes, and irrigation management. However, there are only a few studies on the synergistic effect of drip irrigation and plastic-film-mulch on grain yield, grain qualities, and water-use efficiency of different rice cultivars.

Also, its effect on the emission of greenhouse gases (GHGs) from the rice field has been scarcely reported.

1.5 Aim of the study

The objectives of this research are:

1. To quantify greenhouse gases (CH_4, and N_2O) emissions under drip irrigation with plastic-film-mulch compared with continuous flooding rice cultivation system.
2. To evaluate grain yield, grain quality and water-use efficiency of rice cultivars under drip irrigation with plastic-film-mulch in comparison with continuous flooding system.
3. To evaluate physiological characteristics of the different rice genotypes under drip irrigation with plastic-film-mulch in comparison with continuous flooding system.

CHAPTER TWO

Greenhouse gas emissions from rice field cultivation with drip irrigation and plastic-film-mulch

2.1 Introduction

Global warming caused by increasing potent greenhouse gas (GHG) concentrations in the atmosphere has become a great environmental concern. Methane and nitrous oxide are the key greenhouse gases that contribute towards global warming at 15, and 5% respectively (Watson *et al.,* 1996). The increasing rate of the concentration of these gases in the atmosphere is at 3.0 and 0.22% per annum respectively (Battle *et al.,* 1996). The irrigated lowland-rice whose production plays a critical role in feeding the world's growing population has contributed substantially to the emission of CH_4, and N_2O. This was reported to be due to the anoxic condition created by flooded water and the concomitant increase in the use of inorganic fertilizer (Cai *et al.*, 1997; Kanno *et al.*, 1997). However, the agricultural sector is expected to face increased competition for water from other sectors in the future ascribable to environmental considerations and economic growth (Mancosu *et al.,* 2015). This will put a great deal of strain on the availability of irrigation water, especially for high water-intensive crops such as lowland rice (Kima *et al.,* 2014). Therefore, to feed a growing population with limited water resources in a sustainable environment has become an urgent task for crop production.

Various reports have ascertained that crop management practices such as fertilizer management, irrigation timing, and soil properties play a major role in the emission of GHGs (Wu *et al.,* 2014). Nevertheless, the trade-off that co-exists among GHGs emissions in rice production systems remains viable without overlap in the production of the two gases. The waterlogged condition in continuous flooding rice production system creates an anoxic environment which is conducive for methane production by the anaerobic methanogenic archaea (Cicerone and Oremland, 1998), while N_2O emission has been linked to the microbial processes of denitrification and nitrification which majorly depend on degree of anaerobicity of soil and nitrate content (Suddick *et al.,* 2011). Previous studies reported that alternative irrigation practices during rice production effectively reduced CH_4 emission at the expense of N_2O emission (Yao *et al.,* 2014; Ahn *et al.,* 2014). This inverse relationship was reported to be governed by soil moisture and the addition of synthetic nitrogen fertilizers. It was estimated that approximately 1.25% of all nitrogen fertilizer added to the soil was emitted as N_2O (Barker *et al.,* 2007). Previous studies suggested that this relationship can be altered by the optimization of nitrogen fertilizer and irrigation regimes (Berger *et al.,* 2013; Kreye *et al.,* 2007; Cai *et al.,* 1999).

The potential of drip irrigation in improving water and nutrient use efficiency makes it an attractive system to consider. Drip irrigation makes it possible to supply a relatively large amount of nitrogen and water to the rhizosphere effectively (Tilman *et al.,* 2002). This could have a large influence on nitrogen and carbon turn over in the soil and possibly reduce carbon-related greenhouse gases (CH_4) and nitrogen-induced N_2O (Wu *et al.,* 2014). Several studies have reported the improvement of yield and reduction of greenhouse gas emission by drip irrigation on crops such as cotton (Li *et al.,* 2014), olive (Maris *et al.,* 2015) but there are few reports on the role of drip irrigation on the emission of GHGs in rice cultivation. Drip irrigation with mulching is an example of Ground Cover Rice Production System (a modern water-saving technique) (He *et al.,* 2013). This technology is employed due to the threat of the shortage of irrigation water on crop productivity. It allows growing traditional lowland rice cultivars at nearly saturated soil conditions with no standing water. Plastic film mulch has been reported to contribute to the improvement of hydrothermal regimes of the soil (Wang *et al.,* 2011; Yao *et al.,* 2014). But little is known about the combinational effect of drip irrigation and plastic-film-mulch on greenhouse gas emissions from the rice field. Also, various reports have demonstrated the trend of fluxes of GHGs (CH_4, and N_2O) in long-existing rice paddies, but little attention has been given to newly established paddies. Therefore, the objective of this study is to quantify the fluxes of CH_4, and N_2O in a newly established rice paddy and to determine if the drip irrigation with plastic-film-mulch could reduce water usage, GHG emissions and at the same time promote rice grain yield.

2.2 Materials and Methods

2.2.1 Site description

The field study was conducted at the experimental farm of Faculty of Environmental Horticulture, Chiba University, Japan. This region has a warm and temperate climate with mean annual temperature and precipitation of 15 °C and 1399 mm, respectively. Meteorological data (rainfall and temperature) of the site of about 16 km to the experiment site (35˚42.7' N and 140˚2.6' E) was obtained from the Japan Meteorological Agency (Figure 2-1).

The soil in this region is the low-humic Andosol as classified by FAO (2006). The physicochemical properties of the soil sampled at 0-20 cm depth were as follow: Soil pH (5.8), electrical conductivity (EC) 0.15 mS cm^{-1}; cation exchange capacity (CEC) 24.6 meq $100g^{-1}$; soil total carbon and total nitrogen content was 37.7 g kg^{-1} and 3.8 g kg^{-1}, respectively.

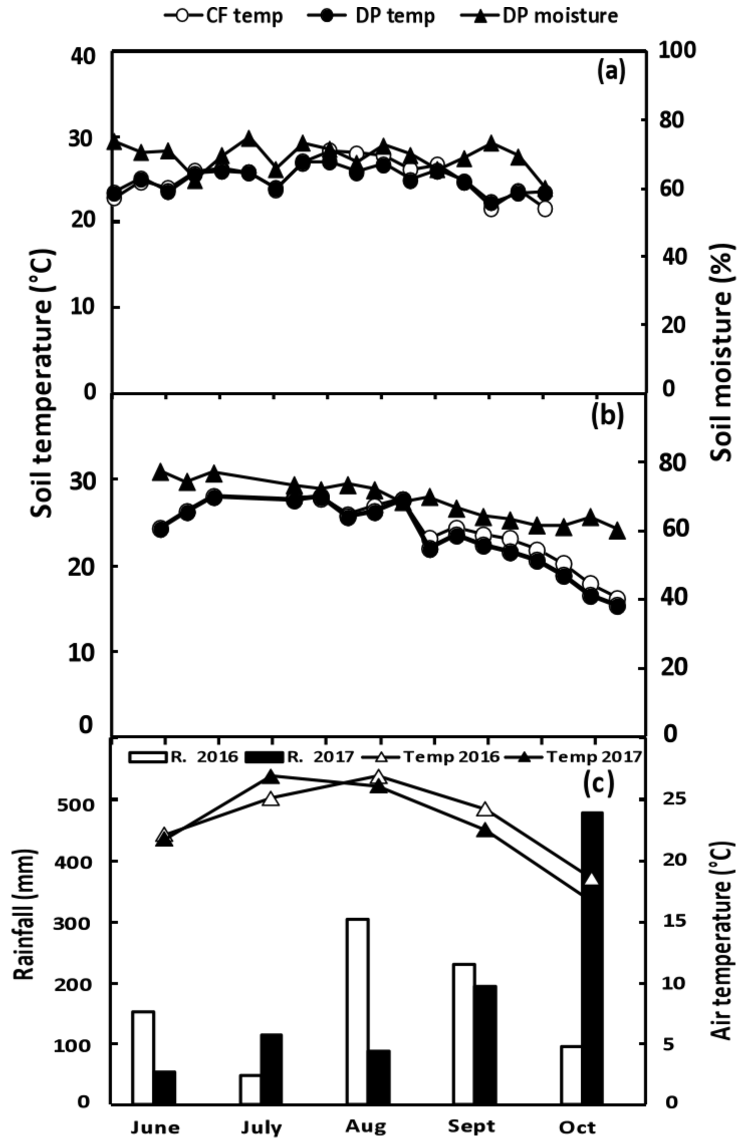

Figure 2-1: Weekly averages of soil temperature and soil moisture during the rice-growing season in 2016 (a) and 2017 (b). The monthly amount of rainfall and average air temperature during the rice-growing season in 2016 and 2017 (c)

2.2.2 Treatments and field management

The field experiment comprised of two treatments during the rice-growing season of 2016 and 2017. These included (1) continuous flooding system (CF) (2) Plastic-mulched drip irrigation with a direct fertilizer application system (DPD). The experiment was set up in a randomized complete block design (RCBD) with three replicates. The size of each experiment plot in CF and DPD systems was 7.5 m^2 and 31.2 m^2 respectively in both years. The CF treatment consisted of three plots of a concrete frame. In 2016, the CF plots were filled with 0 to 20 cm soil depth of the upland field situated about 10 m away from the DPD experimental plots. This was analyzed to have similar physicochemical properties to that of the DPD plots. Seeds of *Oryza sativa* L. cv. Koshihikari (lowland japonica cultivar) was planted with a planting density of 66.7 hills m^{-2} on June 6 and June 9 in 2016 and 2017 respectively. Direct seeding was employed in all treatments in both years. In 2016, 5 seeds per hill were sown manually at 5 cm interval in a narrow row spacing of 10 cm, wide row spacing of 20 cm and 30 cm distance between adjacent films (Figure 2-2). Approximately 7 seeds were sown per hill in 2017 using a grain planter (HS 300, Japan) in the order of the planting distance of 2016. A black plastic mulch of 135 cm was used to cover four rows in each plot before planting and after planting in 2016 and 2017 respectively. Plastic films were cut along the planting line in 2017 and thus ensured sprouting of the seedlings. In the DPD plots, drip pipes were laid beneath the plastic mulch. The DPD plots were sited 20 m away from the CF plots to avoid the horizontal exchange of water and mineral nutrients. During the land preparation, hairy vetch (*Vicia villosa*) grown as green manure were incorporated into the soil in all plots. In both years, inorganic fertilizer consisting of N (40 kg ha^{-1}), P_2O_5 (40 kg ha^{-1}) and K_2O (40 kg ha^{-1}) was applied directly as basal to CF and DPD plots. An additional 20 kg ha^{-1} of nitrogen as urea was applied to both treatments via foliar spraying method at the panicle initiation stage of rice growth in both years. Other field management practices were carried out when necessary. Table 2-1 illustrates a summary of the field management practices in CF and DPD treatments.

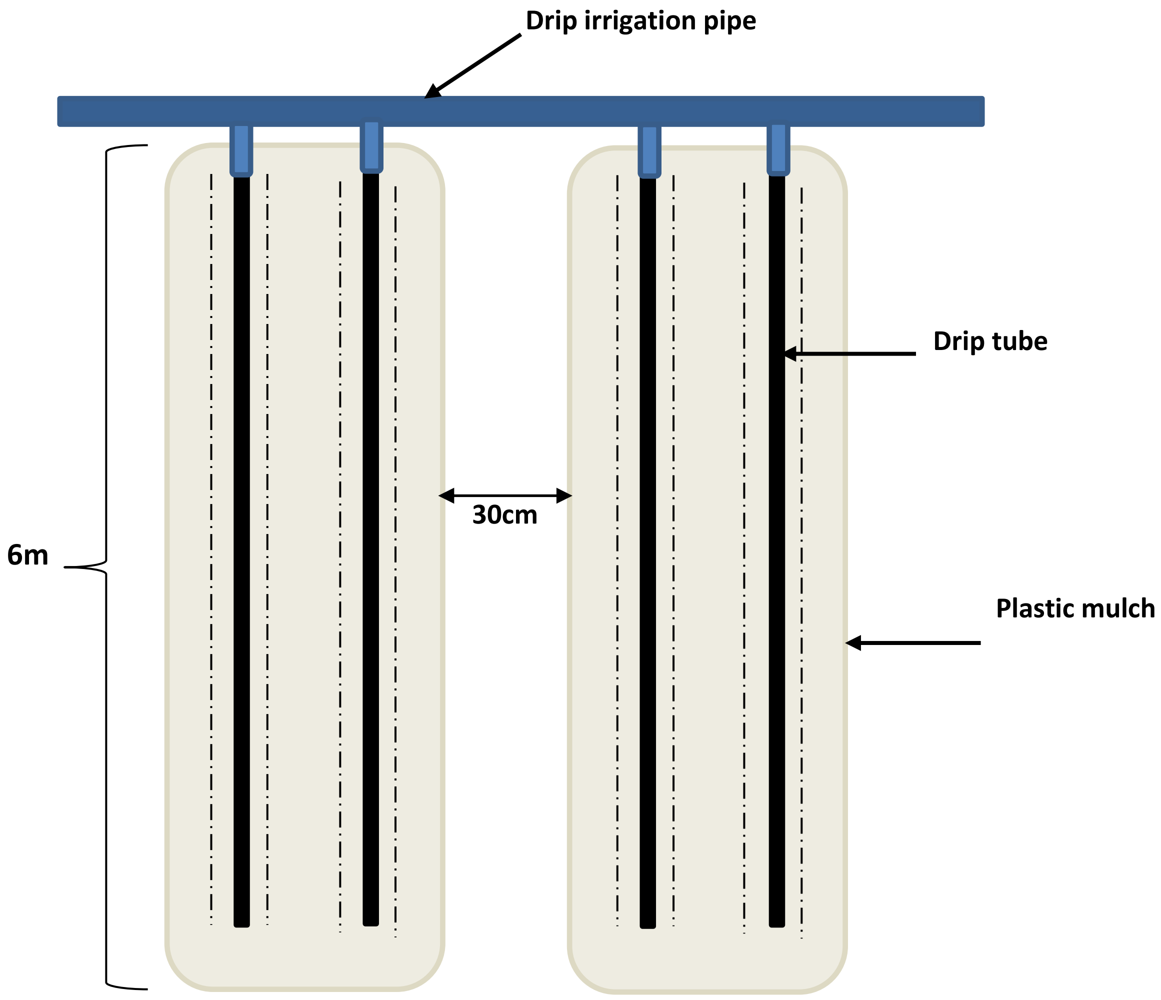

Figure 2-2: Drip irrigation with plastic-film-mulch layout with planting row-spacing configuration in an experiment plot.

Table 2-1: Field management practices during rice growing seasons of 2016 and 2017

		CF	DPD
2016	**Pre-planting operations and crop management**	Incorporation of green manure (*Vicia villosa*), ploughing, and harrowing before seeding. 5 Seeds were sown per hill on June 6 with a planting density of 66.7 hills m^{-2}	Land preparation and seeding method were the same as that of CF. Planting date and density were June 6 and 66.7 hills m^{-2}, respectively.
	Fertilization rate and method of application	Basal application of N (40 kg ha^{-1}), P_2O_5 (40 kg ha^{-1}) and K_2O (40 kg ha^{-1}). An additional 20 kg ha^{-1} of nitrogen was applied as urea through the foliar spray at the panicle initiation stage.	Basal application of N (40 kg ha^{-1}), P_2O_5 (40 kg ha^{-1}) and K_2O (40 kg ha^{-1}). Foliar application of 20 kg ha^{-1} of nitrogen as urea at the panicle initiation stage.
	Mulching	The black plastic mulch was laid before seeding through perforations but removed after seedling establishment (3-leaf stage) before flooding.	The black plastic mulch was laid before seeding through perforations and maintained throughout the rice-growing season.
	Irrigation	Flooded (maintaining 4-5 cm water level above the soil) throughout the experiment period except for 2 weeks before harvest (drainage period).	Irrigation was supplied through drip tubes throughout the rice-growing period.

	Weeding	Hand weeding (2 twice).	Hand weeding (2 twice).
2017	**Pre-planting operations and crop management**	Incorporation of green manure (*Vicia villosa*), ploughing, and harrowing before seeding. 7 Seeds coated with fungicide (Benomyl) were sown per hill on June 8 with a planting density of 66.7 hills m^{-2}	Land preparation and seeding method are the same as that of CF. Planting date and density are June 9 and 66.7 hills m^{-2} respectively.
	Fertilization rate and method of application	Basal application of N (40 kg ha^{-1}), P_2O_5 (40 kg ha^{-1}) and K_2O (40 kg ha^{-1}). Additional 20 kg ha^{-1} of nitrogen was applied as urea through foliar spray on August 17 (panicle initiation stage)	Basal application of N (40 kg ha^{-1}), P_2O_5 (40 kg ha^{-1}) and K_2O (40 kg ha^{-1}). Foliar application of 20 kg ha^{-1} of nitrogen as urea on August 17 (panicle initiation stage)
	Mulching	The black plastic mulch was laid after seeding and cut along the seeding line but removed after seedling establishment (3-leaf stage) before flooding.	The black plastic film was laid after seeding and cut along the seeding line. This was maintained throughout the rice-growing period.
	Irrigation	Flooded (maintaining 4-5 cm water level above the soil) throughout the experiment period except for 2 weeks before harvest (drainage period).	Irrigation was supplied through drip tubes throughout the rice-growing period.
	Weeding	Hand weeding (2 twice)	Hand weeding (2 twice)

2.2.3 Water management

CF plots were flooded on June 26 and July 5, respectively, when the rice plants were at the 3-leaf stage. The water level was monitored and maintained at 4-5 cm above the soil surface using an embedded vertical ruler until two weeks before harvest (September 22 and September 25 in 2016 and 2017, respectively) when the plots were drained. In 2017, a high percolation rate was observed attributable to bricks leakages in one of the CF plots. This resulted in a higher supply of irrigation water compared to 2016 (Table 2-2). In the DPD plots, irrigation water was supplied to the rice plants through the drip tubes of 20 cm distanced emitters connected to the irrigation system. The total amount of irrigation water supplied to each treatment was monitored by a flow meter connected to the irrigation pipeline.

Soil moisture contents of the DPD plots were maintained close to saturation by an FDR soil moisture meter (DIK, 321A). The FDR probes were embedded in the soil layer (0-6 cm). Moisture threshold was taken as 75% and supplementary irrigation was applied when soil moisture falls below 70% in both years (Figure 2-1a and b). In both treatments, soil temperature at 20 cm soil depth was measured and recorded at 10 minutes interval by sensors connected to a data logger (CADAC3, 9300 series, Eto Denki, Japan).

Table 2-2: Irrigation, rainfall, and total water input in continuous flooding and drip irrigation with plastic-mulch systems during rice-growing seasons of 2016 and 2017

	2016			2017		
	Irrigation (mm)	Rainfall (mm)	Total (mm)	Irrigation (mm)	Rainfall (mm)	Total§ (mm)
CF†	807	827	1634	1144	924	2068
DPD‡	599	827	1426	556	924	1480

† CF = continuous flooding

‡ DPD = drip irrigation with plastic-film-mulch

§ Total = irrigation + rainfall

2.2.4 Preparation of evacuated glass vials

Glass vials equipped with butyl rubber stoppers and aluminum caps were prepared for gas sample collection and storage (Plate 2-1). Glass vials were evacuated using vacuum-pumped equipment (Freeze Dryer FDU-506) specifically designed to prepare evacuated vials (Plate 2-2). The vacuum-connected equipment is made up of an inbuilt vacuum gauge meter and a detachable glass-closure consisting of the bottom plate and an inner plate. Forty 30 mL glass vials were arranged in the bottom plate of the apparatus with the rubber stoppers lightly placed on them (Plate 2-2). The vacuum pump was put on for about 1 hour to create a vacuum in the glass enclosure of the apparatus. The inner plate was moved down manually to close the vials with the rubber stoppers after the completion of the evacuation process. The aluminum caps were fixed using a capper.

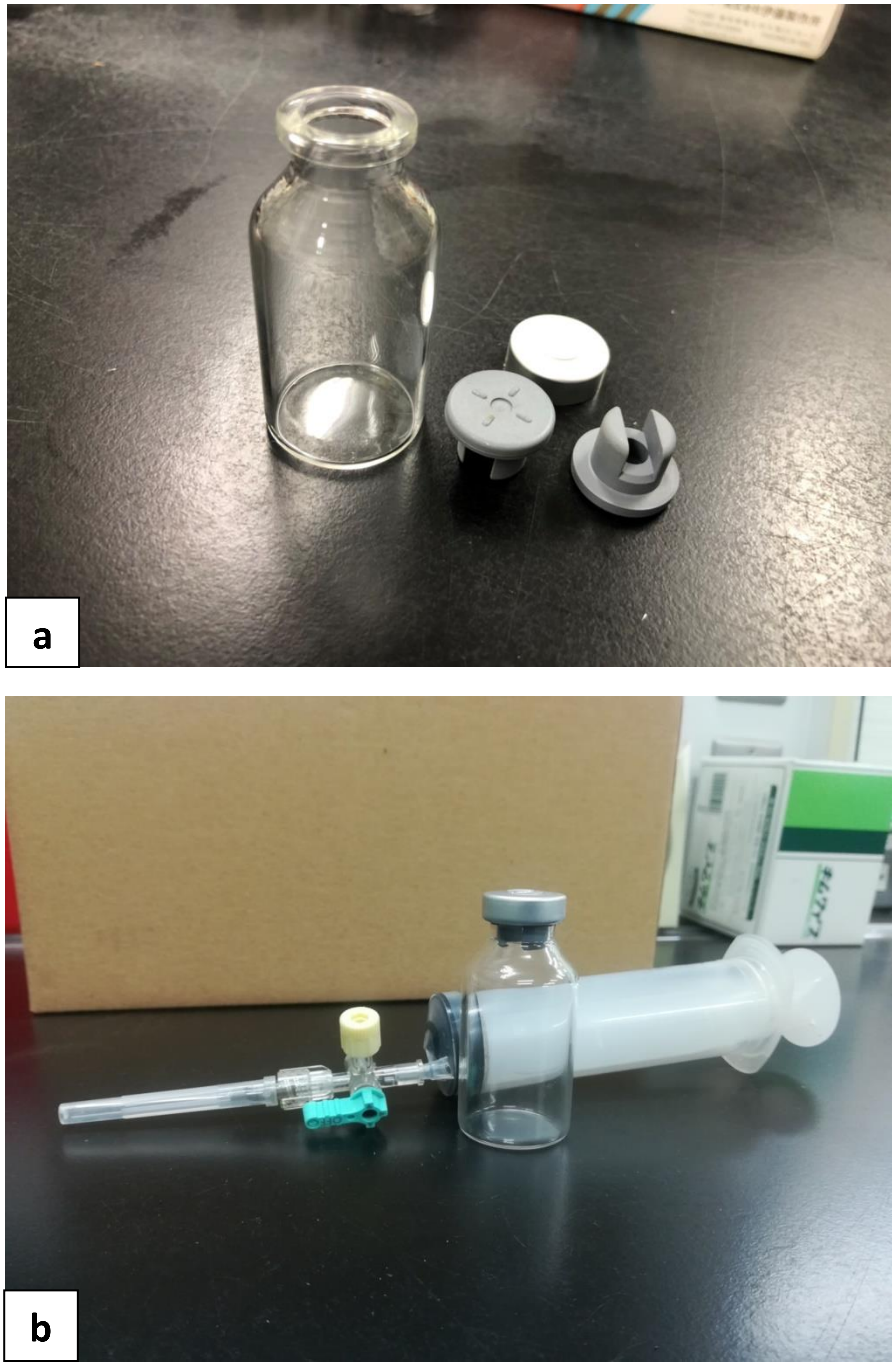

Plate 2-1: Preparation of evacuated glass vials. (a) = a glass vial, butyl rubber stopper, and aluminum cap and (b) = a prepared glass vial for gas sampling.

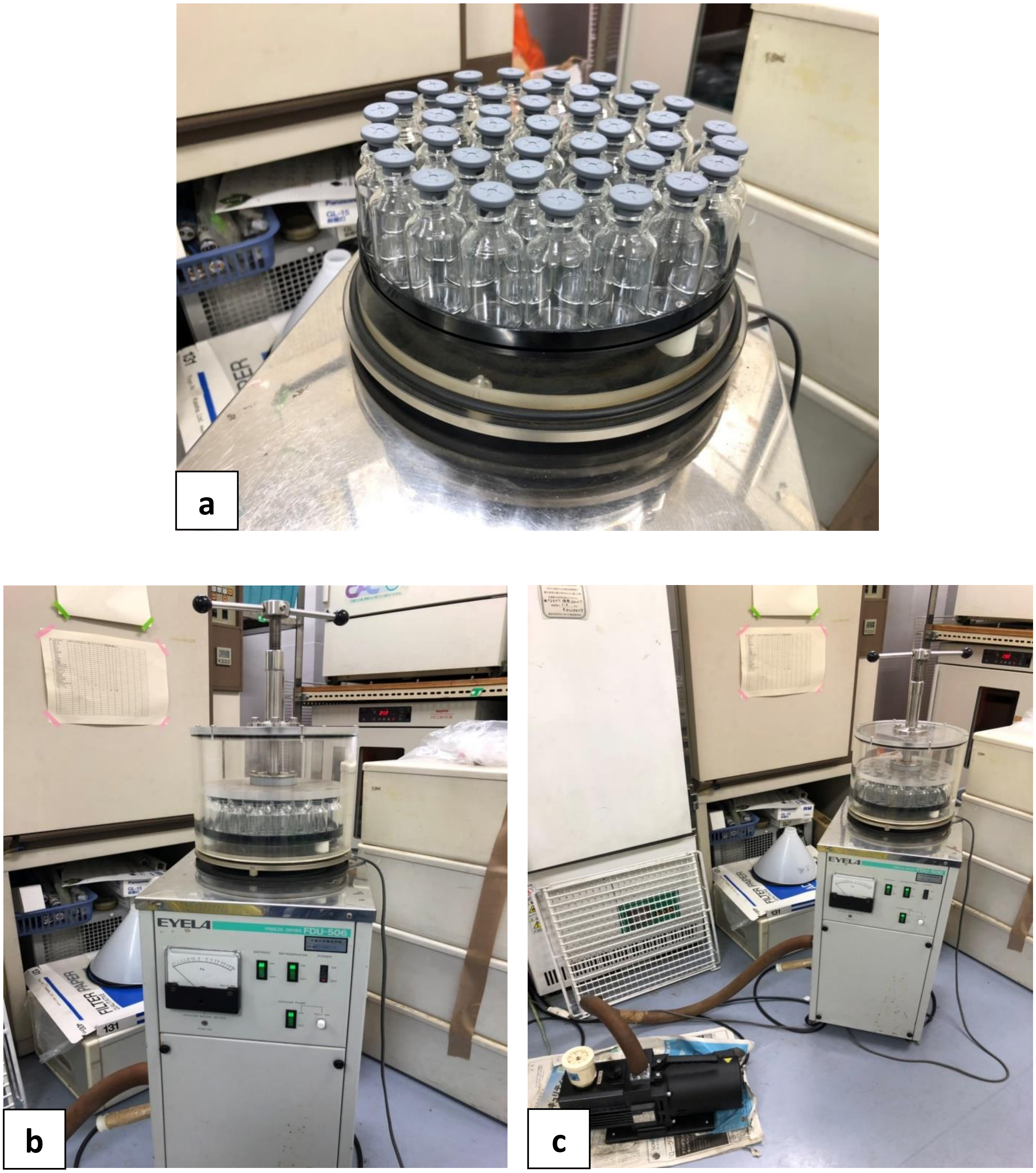

Plate 2-2: Glass-vials evacuation process using vacuum-pumped apparatus. (a) = arrangement of glass vials on the vacuum apparatus's plate; (b) = closure of apparatus chamber and (c) = chamber air evacuation process with vacuum pump.

2.2.5 Gas sampling

Air was sampled using a closed chamber method and its GHG concentrations were analyzed using gas chromatography (Inubushi *et al.,* 2003). Gas samples were collected over rice plants (Plate 2-4). A cylindrical acrylic chamber (15 cm diameter, 1 m high and 0.3 cm thick) equipped with a digital thermometer was used. Gas was collected over 15 - 20 rice tillers at three different points within each replicate of the treatment. Biweekly gas sampling was carried out between 9:00 and 11:00h as soil temperatures and gas fluxes are expected to be representative of their average daily values during this time period (Adviento-Borbe *et al.*, 2013). The chamber deployment was from (June - October) and (July - September) in 2016 and 2017 respectively. A suitable chamber measurement sequence with an appropriate time window as described by Minamikawa *et al.* (2015) was adopted (Table 2-3). At each sampling date, 30 mL of gas samples were drawn from the chambers through a three-way stopcock fixed to an airtight syringe of 60 mL capacity at 0, 10, and 20 minutes after chamber closure and transferred into a 30 mL vacuum glass container. The sample at 0 minutes was taken immediately after chamber placement.

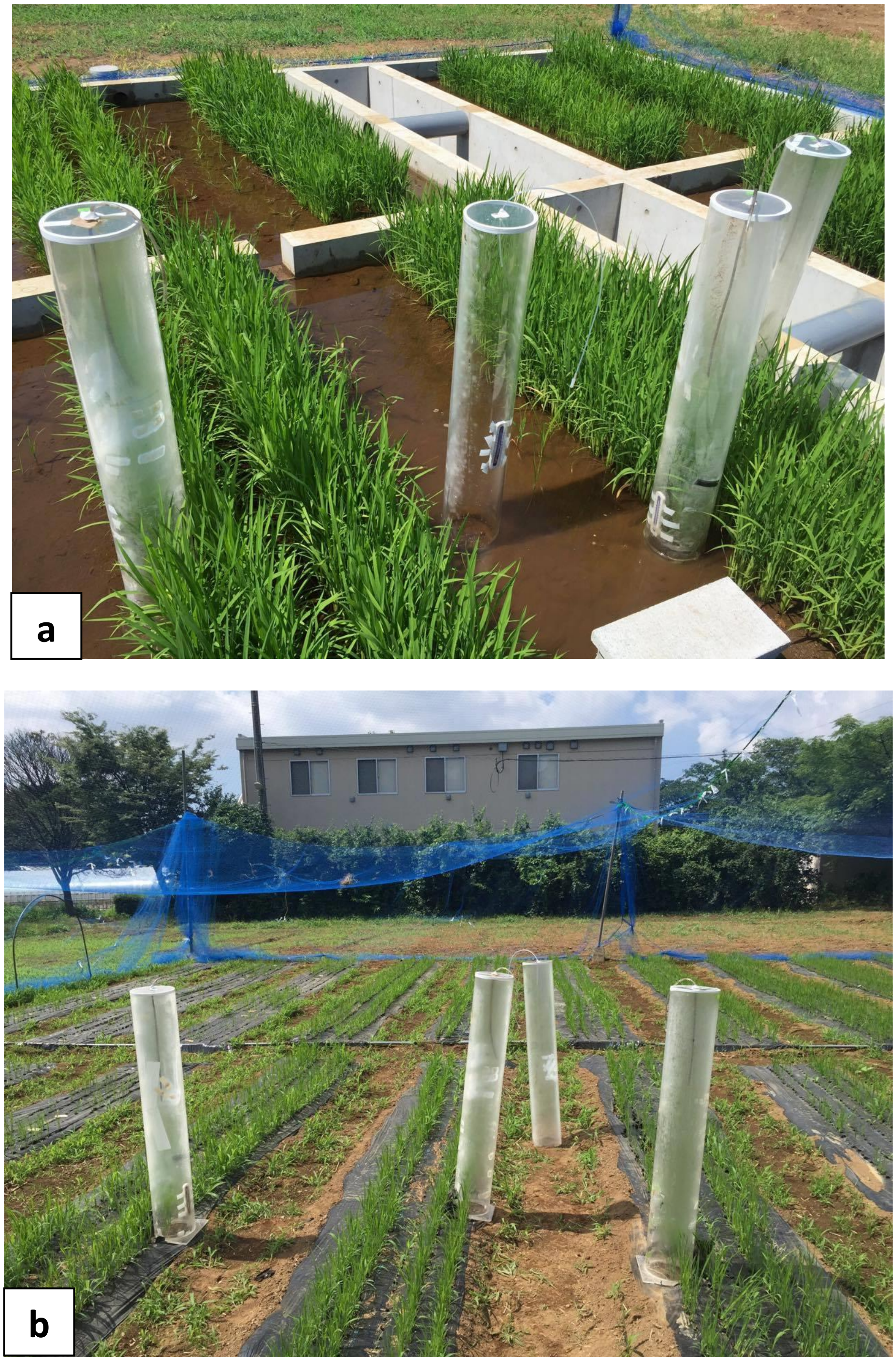

Plate 2-3: Gas sampling using close chamber method. (a) = continuous flooded system and (b) = drip irrigation with plastic-film-mulch system

Table 2-3: Time schedule for sample measurements during a 20-min closure with three chambers in an experimental plot

Chamber	**Placement**	**1st sampling**	**2nd sampling**	**3rd sampling**
1	9:00	9:01	9:11	9:21
2	9:04	9:05	9:15	9:25
3	9:08	9:09	9:19	9:29

2.2.6 Preparation of standard gas and sample analysis

Plastic bag with a 2-way stopcock was evacuated using a gas evacuating mini pump (MP-2N, Sibata Co., Ltd.) (Plate 2-5a). This was filled up with standard gas with the aid of a pressure gauge connected to the gas jar. The plastic bag was capped with a rubber stopper to avoid gas leakage. To minimize errors due to contamination, a new standard gas was prepared for every sample analysis period.

The gas samples were analyzed for the concentration of CH_4 and N_2O in 2016 and 2017. Gas chromatography (GC-14B, Shimadzu Co., Ltd) equipped with a Flame Ionization Detector (FID) and Electron Capture Detector (ECD) was used for CH_4, and N_2O analysis respectively (Plate 2-5b). Standards were analyzed periodically at 20 samples intervals to account for the errors associated with GC drift. Fluxes were estimated using linear regression. Hourly fluxes of CH_4 ($mg\ m^{-2}\ h^{-1}$) and N_2O ($\mu g\ m^{-2}\ h^{-1}$) were calculated with the equation: $Flux = \rho \times (V/A) \times (\Delta C/\Delta t) \times [273 / (273 + T)] \times 60$ (where $\Delta C/\Delta t$ is the concentration change over time (ppm-CH_4, ppb-N_2O h^{-1}); V is the chamber volume (m^3), A is the chamber area (m^2), ρ is gas density ($\rho CH_4 = 0.717\ kg\ m^{-3}$, and $\rho N_2O = 1.977\ kg\ m^{-3}$), T is the air temperature inside the chamber (°C), 273 is the correction factor between C and K (Minamikawa *et al.,* 2015).

The total GHGs emissions were sequentially cumulated from two adjacent measurements (Wang *et al.*, 2011b). The equivalent CO_2 (CO_2-eq) emission for total methane (TCH_4) and total nitrous oxide (TN_2O) emissions over a 100-year time horizon was calculated using the equation: $CO_2\text{-eq} = (TCH_4 \times 28) + (TN_2O \times 265)$ (IPCC, 2014).

Plate 2-4: Preparation of a standard gas and sample analyses. (a) = prepared standard gas and (b) = Gas chromatograph with a flame ionization detector (FID) for CH_4 and Electron Capture Detector (ECD) for N_2O.

2.2.7 Soil analyses

Soil samples (0 - 20 cm depth) were collected at each experiment plot with a 2 cm diameter stainless steel auger in 2017. Each sample was collected close to the area covered by the gas chamber at three different stages of rice growth: tillering (July 6), panicle-initiation (August 14), and grain-filling (September 13). Soil samples were air-dried, separated from stones and pebbles, pulverized and sieved through 2 mm sieve. The mineralizable nitrogen (ammonium and nitrate) concentrations were determined from 10 g air-dried soil samples using a 1M KCl extraction procedure and analyzed by the nitroprusside method and hydrazine-reduction-naphthyl ethylenediamine method respectively. Total nitrogen (TN) and total carbon (TC) were analyzed from 3 g dried soil samples oven-dried at 105 $^{\circ}$C for 48 hours. The percentages of TC and TN were determined using a CN corder (MT-700, Yanaco Co., Ltd).

2.2.8 Grain yield analysis

At maturity, grain yield of unhusked rice was determined from a 0.3 m^2 sampling area. The harvested aboveground parts of rice were further separated into the straw, filled and unfilled spikelets. Panicles of the sampled plants were counted, and grains were separated by hand-threshing to determine the number of spikelets per panicle. The number of spikelets per panicle was taken as the summation of both filled and unfilled spikelets. Filled spikelets were defined as the filled grains at gravity $\geq$ 1.06 g cm^{-3}. Percentage filled spikelet was calculated as (filled spikelets/ total spikelets) × 100. One thousand grain weight was expressed as 1000 grain weight of filled spikelets at 14% moisture content.

2.2.9 Statistical analysis

Analysis of variance (ANOVA) was carried out using the Statcel3 statistical package. The statistical model used included sources of variation due to year, irrigation regimes and the interaction of year and irrigation regime. Data for each parameter were analyzed separately. Means were separated by Tukey test at 1% and 5% probability level.

2.3 Results

2.3.1 Irrigation volume and soil characteristics

The total water supply (irrigation and rainfall) throughout the rice-growing seasons were (1634 mm and 1426 mm in 2016) and (2068 and 1480 in 2017) for CF and DPD respectively (Table 2-2). In both years, there was no significant difference between soil temperature of CF and DPD at $P \leq 0.05$ (Figure 2-1a and 1b). Soil total carbon was not significantly different between CF and DPD at the different rice growth stages observed in this study except the flowering stage where soil carbon was significantly higher in DPD than CF. Soil total nitrogen was significantly different at the different stages of rice growth between the CF and DPD (Table 2-4). The soil nitrate showed a strong dominance between the studied mineralizable nitrogens (NH_4^+ and NO_3^-) in both treatments. It was observed that NH_4^+ in DPD was significantly higher ($P \leq 0.05$) than in CF at the different rice growth stages. Moreover, the difference between the NO_3^- content of CF and DPD was not significant at the different stages of rice growth (Table 2-4).

Table 2-4: Effect of drip irrigation with plastic-film-mulch on soil total carbon, total nitrogen, and mineralizable nitrogen at different stages of rice growth in 2017

	Tillering stage	Flowering stage	Grain filling stage
C content (g kg^{-1})			
CF†	34.84 ± 2.67a	31.8 ± 0.89b	34.77 ± 3.23a
DPD‡	39.81 ± 0.59a	40.14 ± 1.61a	40.72 ± 0.39a
N content (g kg^{-1})			
CF	3.15 ± 0.09b	2.86 ± 0.06b	3.09 ± 0.12b
DPD	4.13 ± 0.13a	4.14 ± 0.02a	4.07 ± 0.11a
NH_4^+ (mg kg^{-1})			
CF	6.14 ± 0.52b	7.70 ± 0.20b	6.77 ± 0.18b
DPD	8.43 ± 0.30a	9.43 ± 0.25a	7.38 ± 0.29a
NO_3^- (mg kg^{-1})			
CF	227.11 ± 30.26a	16.20 ± 1.03b	24.22 ± 0.33b
DPD	184.41 ± 57.57a	72.54 ± 2.39b	54.07 ± 15.22b

Means (n=3) followed by the same letter within each column under each soil nutrient component was not significantly different at 5% probability level in Tukey’s test.

† CF = continuous flooding

‡ DPD = drip irrigation with plastic-film-mulch

2.3.2 Methane fluxes

In this experiment, the range of methane fluxes observed between treatments was -0.36 to 0.43 mg m^{-2} h^{-1} and -0.77 to 4.66 mg m^{-2} h^{-1} in 2016 and 2017, respectively (Figure 2-3). In 2016, a rise in CH_4 emission was observed under CF after flooding with the highest peak on August 8. In contrary to 2016, the highest emission was observed at the first sampling date after flooding (July 19) in 2017. In both years, the lowest fluxes in CF were recorded during the drainage periods. The cumulative CH_4 emission was significantly different between 2016 and 2017 with the same irrigation regime. The cumulative CH_4 emissions were higher under both treatments in 2017 than in 2016. These were 16 times and 5 times higher under CF and DPD, respectively. Significant differences were also observed in the accumulated methane emissions between CF and DPD in both years at $P \leq 0.01$ (Table 2-5). DPD reduced cumulative CH_4 flux by 194% and 69% in 2016 and 2017, respectively, compared with CF.

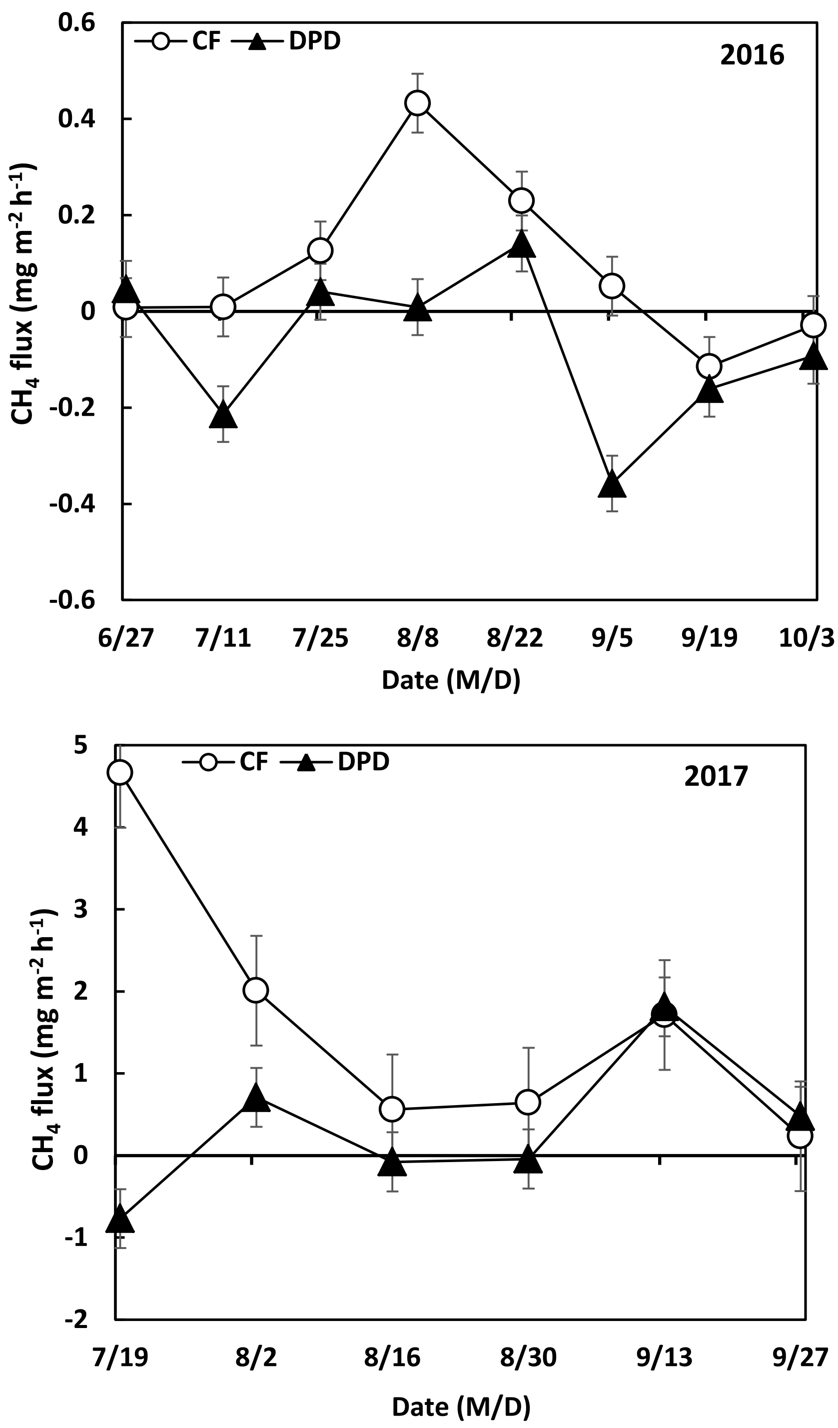

Figure 2-3: Biweekly methane emissions from the drip irrigation with plastic-film-mulch (DPD) and continuous flooding (CF) systems during rice-growing seasons of 2016 and 2017. Different scaling for 2016 and 2017

2.3.3 Nitrous oxide fluxes

This study shows a wide variance of N_2O fluxes in the rice-growing season of 2016. N_2O fluxes ranged between -41 to 387 µg m^{-2} h^{-1} and -134 to 87 µg m^{-2} h^{-1} in 2016 and 2017, respectively. Low N_2O fluxes were recorded in the two irrigation regimes. The N_2O fluxes in the CF and DPD of both years were close to zero except a sudden high flux recorded at the first sampling date in the CF during the rice-growing season of 2016 (Figure 2-4). Table 2-5 shows that year and irrigation regime had no significant effect on the accumulated N_2O emissions.

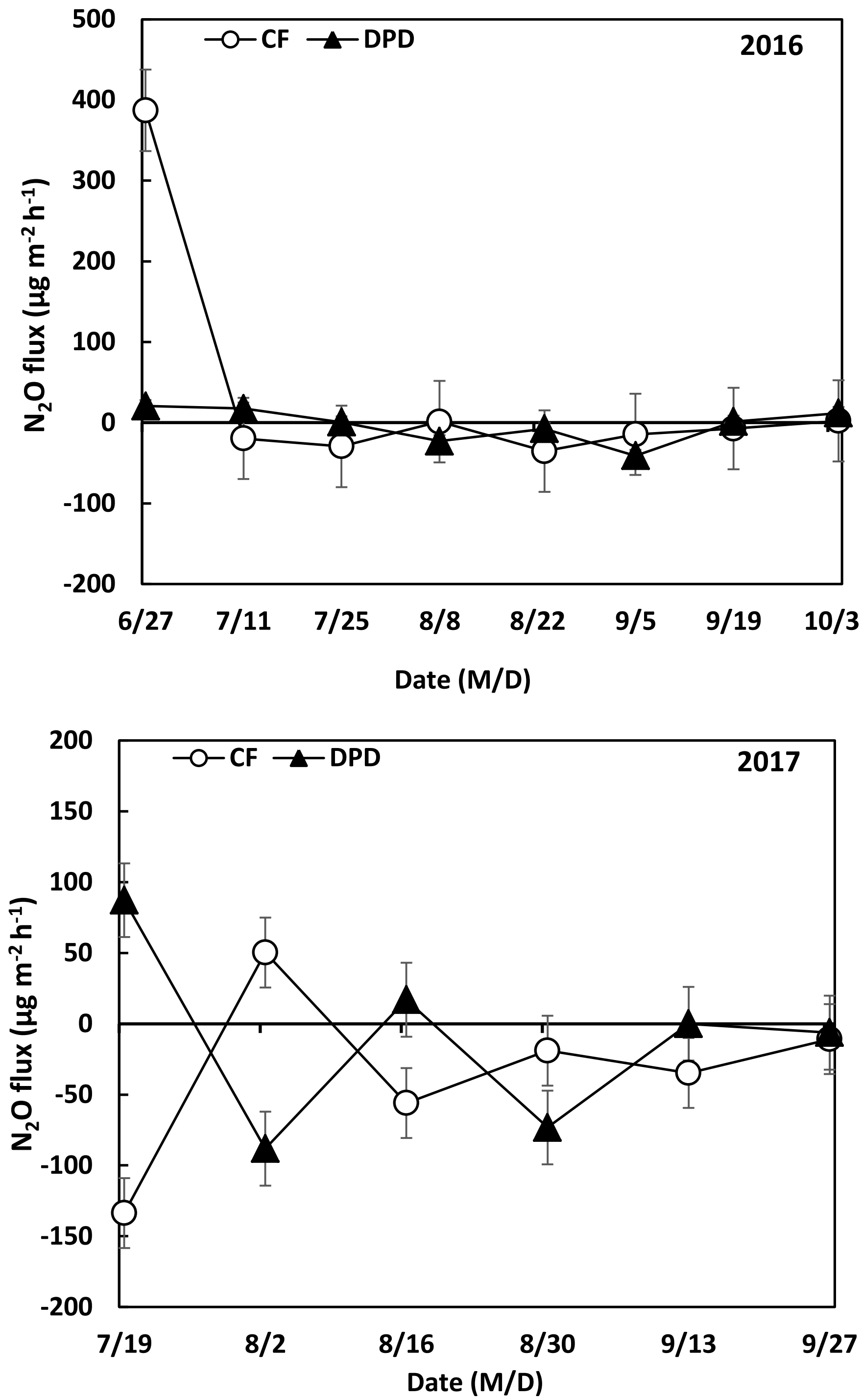

Figure 2-4: Biweekly nitrous oxide emissions from drip irrigation with plastic-film-mulch (DPD) and the continuous flooding (CF) systems during rice-growing seasons of 2016 and 2017. Different scaling for 2016 and 2017

2.3.4 CO_2 equivalent emission

The equivalent CO_2 (CO_2-eq) emission was significantly influenced by the year and the irrigation regime (Table 2-5). The CO_2-eq emission was reduced by 127% and 79% in DPD compared with CF in 2016 and 2017, respectively. The result of the 2-year average shows that the irrigation regime had a significant effect on the CO_2-eq emission (Table 2-5). The average CO_2-eq emission of CF was 9 times higher than that of DPD. It was observed that the significant increase in methane emissions from the CF plots had a great effect on the climate system. The average cumulative N_2O emissions from the two years' study resulted in a non-significant low emission from CF and DPD (Table 2-5).

Table 2-5: Effect of drip irrigation with plastic-film-mulch on accumulated CH_4 and N_2O emissions and CO_2 equivalent emission during the 2-year rice growing seasons

Year/irrigation regime	TCH_4 (g $m^{-2}season^{-1}$)	TN_2O (g $m^{-2}season^{-1}$)	CO_2-eq emission § (CH_4 + N_2O) (g m^{-2} $season^{-1}$)
2016			
CF†	0.16 ± 0.12	0.037 ± 0.03	14.11 ± 10.47
DPD‡	-0.15 ± 0.03	0.001 ± 0.01	-3.74 ± 2.84
2017			
CF	2.48 ± 0.13	-0.044 ± 0.004	57.64 ± 4.01
DPD	0.76 ± 0.63	-0.035 ± 0.04	11.98 ± 6.24
Year	**	ns	*
Irrigation regime	**	ns	*
Year × irrigation regime	*	ns	ns
2-year average			
CF	1.32 ± 0.01	-0.004 ± 0.01	35.87 ± 3.81
DPD	0.31 ± 0.32	-0.017 ± 0.01	4.12 ± 2.52
	*	ns	*

*, ** and ns indicate 5%, 1% level of probability, and no significance respectively. Values represent mean ± standard error (n=3).

† CF = continuous flooding.

‡ DPD = drip irrigation with plastic-film-mulch.

§ CO_2-eq = (TCH_4 × 28) + (TN_2O ×265); where CO_2-eq is the total amount of CO_2 emission (g CO_2-eq m^{-2}); TCH_4 and TN_2O is the total amount of methane emission and nitrous oxide emission, respectively; 28 and 265 are the global warming potentials of CH_4 and N_2O, respectively, to CO_2 over a 100-year time horizon (IPCC, 2014)

2.3.5 Grain yield and yield components

The grain yield in 2017 was significantly higher than in 2016, but the difference observed between the grain yields of the irrigation regimes of each year was not significant. The significance recorded between the years was as a result of the low number of spikelets per panicle, filled grain percentage, and 1000 grain weight in 2016 compared with 2017 (Table 2-6). The yield in CF and DPD ranged from 3.5 t ha^{-1} in DPD (2016) to 7.4 t ha^{-1} in paddy (2017). Grain yield of Koshihikari cultivar under DPD decreased by 19% and 5% in 2016 and 2017, respectively, compared with CF but differed insignificantly at $P \leq 0.05$ (Table 2-6).

Table 2-6: Effect of drip irrigation with plastic-film-mulch on grain yield and yield components of rice during the 2-year rice growing seasons

Year/irrigation regime	Panicles (m^{-2})	Spikelets ($panicle^{-1}$)	Filled grains (%)	1000 grain weight (g)	Grain yield ($t\ ha^{-1}$)
2016					
CF†	414	81	65.6	23.4	4.3
DPD‡	378	82	60.6	22.3	3.5
2017					
CF	498	86	81.3	20.8	7.4
DPD	456	104	75.2	19.4	7.0
Year	ns	*	*	*	*
Irrigation regime	ns	ns	ns	*	ns
Year × irrigation	ns	ns	ns	ns	ns

* and ns indicate a significant difference and no significance at 5% level of probability, respectively.

† CF = continuous flooding.

‡ DPD = drip irrigation with plastic-film-mulch.

2.4 Discussion

The water regime of soil is an important factor for gas exchange between soil and atmosphere and this has a direct impact on the processes involved in methane emission (Jain *et al.,* 2004; Hadi *et al.,* 2010). In this study, CF had higher CH_4 fluxes in 2016 and 2017 compared with the DPD. This agrees with previous studies that reported high CH_4 emission from flooded rice fields due to the decomposition of organic matter by methanogens under an anaerobic condition (Rath *et al.,* 2000; Oo *et al.,* 2018). However, the cumulative emission of CH_4 from CF plots in 2016 and 2017 was lower than those reported in the other previous studies in Japan (Yagi and Minami, 1990) (0.60 to 44.88 g m^{-2} $season^{-1}$) and (Naser *et al.,* 2007) (4.04 to 40.80 g m^{-2} $season^{-1}$). This could be ascribed to the period of usage of the soil for flooded rice cultivation. The CF plots used in this study have not been previously used for paddy rice cultivation. This could have had a great effect on the population of methanogenic archaea capable of CH_4 production.

The sudden upsurge of methane flux at the first sampling date after flooding in 2017 (Figure 2-3b) probably resulted from a combined effect of the utilization of available carbon from remaining plant-residue in 2016 and the presence of enough viable methanogenic archaea in the air-dried soils. Although CH_4 production in soils is a strongly anaerobic process, methanogenic microorganisms can survive in well-aerated soils over a long time and can be activated by short-term induced anaerobiosis (Ruser *et al.,* 2008). Methanogens and methanotrophs are microorganisms that play an important role in the absorption and production of CH_4 in the soil depending on the presence or absence of oxygen (Angel *et al.,* 2011) and several biotic and abiotic factors. It was observed that DPD treatment absorbed and emitted CH_4 at different sampling periods in both years. This finding indicates changes in the community structure and metabolic activity of methanogenic archaea associated with alternate aerobic and anaerobic cycling as reported by Watanabe (2010). This could be due to the wet condition in deeper soil depth (close to saturation) (Figure 2-1a and 1b) capable of creating a thriving environment for methanogens. Also, the relatively similar soil temperature between the DPD and CF at the shallow depth in both years further explains the intermittent CH_4 emissions in the DPD. The significant increase in the cumulative CH_4 emission in 2017 compared to 2016 under both irrigation regimes could probably be due to the increase in the degree of anaerobicity in 2017. The significant increase in the total water (irrigation and rainfall) supplied in 2017 further support this argument (Table 2-2).

The emission of N_2O to the atmosphere has a significant effect on climate change. According to IPCC (2007), direct and indirect emissions of N_2O from agricultural ecosystems to the atmosphere contribute approximately 6 Tg per year. For the CF and DP systems, the total N_2O emissions recorded during the rice-growing seasons were -0.044 to 0.037 g m^{-2} and -0.035 to 0.001 g m^{-2} respectively. These were at the lowest range compared to other previously reported emissions in the flooded rice field (Yao *et al.*, 2014) and ground cover rice production system (Kreye *et al.*, 2007; Yang *et al.*, 2012). The low emission rates were attributed to the relatively low quantity of nitrogen fertilizer, rates of application and techniques (foliar spray at panicle initiation stage) adopted in this study. This was further explained by the non-significant nitrate contents observed between the CF and DPD at the different stages of rice growth (Table 2-4). The wide variance in the emission of N_2O observed in CF treatment between the first sampling date and other sampling periods in 2016 (Figure 2-3c) could partly depend on the denitrification of soil NO_3^- as reported by (Skiba and Smith, 2000). Previous studies showed that denitrification becomes the highly significant source of N_2O once the soil has exceeded field capacity or has a water-filled pore space in the range of 60-90% (Davidson and Swank 1986; Schjonning *et al.*, 2003). The absence of oxygen suppressed the nitrification of ammonium (NH_4^+-N). Therefore, the nitrate reaction rate (NO_3^- to N_2O to N_2) presumably determined the sudden emission and absorption observed under CF in 2016.

The ground cover rice production system was observed in previous studies (Kreye *et al.*, 2007; Yao *et al.*, 2014) to support high N_2O emission. The result of the overall assessment of our two years' experiment is at variance with this argument. The inconsistency might be related to the differences in soil type, mulch type, agricultural management, and climatic condition. Moreover, the DPD system under this study was considered to have a discontinuous aerobic soil condition because of the soil saturation level and probably the temperature-increasing-effect of plastic film on the soil denitrifiers activities as reported in previous studies (Millar and Baggs 2005; Qin *et al.*, 2010). Perhaps, this had an acceleration-effect on soil organic matter decomposition and root respiration rates resulting in the depletion of oxygen thereby responsible for the methane emissions and low accumulated N_2O emissions (Li *et al.*, 2014).

The cumulative fluxes of the trace gases were converted to the CO_2 equivalent for the assessment of the combined effect of these gases on global warming (IPCC, 2014). The significant reduction of CO_2-eq emission under DPD during the rice-growing seasons of 2016 and 2017 was in agreement with the report of Weller *et al.* (2015) who also reported low GWP in aerobic soil compared with flooded rice cultivation system. This study was also in concert with their assumption of the potential losses of soil organic carbon (SOC) caused by higher soil aeration which is capable of increasing CO_2-eq emission of the non-flooded system. Nevertheless, the use of plastic-film-mulch in this study could have possibly mitigated the loss of SOC. Therefore, we recommend further studies on the quantification of SOC in relation to GWP under drip irrigation with plastic-film-mulch system of rice cultivation. The CO_2-eq emission of the 2-year average was 36 and 4 g m^{-2} $season^{-1}$ for CF and DPD respectively. This implies that the CO_2-eq emission was 89% reduced under DP compared with CF.

The significant decrease in grain yield of Koshihikari in 2016 compared to 2017 could be linked to decreased panicle per square meter resulting from the number of seeds sown per hill in both years. It was observed in this study that there was no significant difference in grain yield between the DPD and CF in each year. These comparable yields were attributed to the insignificant values of the grain yield components except 1000 grain weight between the two irrigation regimes resulting from the irrigation management, water conservative ability of the plastic-film and sufficient rainfall during the rice-growing seasons of 2016 and 2017. These could have lightened the prospective negative effect of water stress during the critical growth stages of the rice plants most especially from August to October (Figure 2-1). Tao et al. (2015) and Rickman *et al.* (2001) documented that with proper management practices, GCRPS has the potential to close the yield gap between aerobic rice and flooded rice.

Minamikawa *et al.* (2012) reported that biweekly measurement of GHGs especially methane, yielded an acceptable estimation (i.e ± 10%) under the flooded condition in paddy rice cultivation. The use of this measurement approach was considered as a limitation to this study as it remains insufficient to capture the rapid changes in the GHGs flux potentials under both irrigation regimes. Therefore, further studies that incorporate intensive gas sampling (daily to every other day) were recommended to elucidate the impact of this promising technique on global warming.

2.5 Conclusion

This field study revealed the GHGs emission pattern on a biweekly basis under newly established continuous flooding rice cultivation system and drip irrigation with the plastic-film-mulch system over the duration of 2 years. The result of this study shows that CH_4 emission was more predominant between the GHGs quantified in both irrigation regimes. Cumulative methane emissions in 2017 were also greater than in 2016 on the same irrigation regime. Our findings demonstrated that the DPD mitigated GHGs as a result of low nitrogen fertilization and intermittent soil saturation level; this lower methane emissions, act as a sink for N_2O emissions and in-turn reduced the resulting CO_2-eq emission. Also, the comparable grain yield with the reduced water-usage of the DPD system to CF further suggests it as a promising water-saving rice technology with the potentials to mitigate greenhouse gases without yield loss.

CHAPTER THREE

The agro-physiological responses of rice cultivars (*Oryza sativa* L.) as influenced by different fertilization methods under drip irrigation with plastic-film-mulch

3.1 Introduction

Rice, as a staple food, plays a significant role in global food security. More than 60% of the world's population largely depends on rice, making it the most widely consumed crop (Patel *et al.* 2010). Global food security is challenged by increasing food demand and declining water availability (Mancosu *et al.* 2015). Rice requires a substantial amount of fresh water because the most predominant system of rice cultivation is continuous flooding (Tuong and Bouman 2003; Bouman *et al.* 2007). This system is the most common in Asia, where more than 75% of the world's rice is being produced (Cantrell and Reeves 2002). However, in recent years, labor shortage and the increasing competition for water from non-agricultural sectors pose a threat to the sustainability of irrigated flooded rice systems. Hence, there is an urgent need to increase rice production with less water to ensure continuity in meeting the demand for rice.

To alleviate increasing water scarcity, water-saving rice production systems, which focus on the reduction of water usage and on increasing water-use efficiency and grain yield, have been reported (Tuong and Bhuiyan 1999). The most commonly practiced techniques include alternate wetting and drying (AWD), aerobic rice, the system of rice intensification (SRI) and ground cover rice production system (GCRPS) (Oo *et al.* 2018; Y. Zhang *et al.* 2017; Belder *et al.* 2004). Nonetheless, it remains unclear if water-saving techniques in rice production could accomplish the dual goal of increasing grain yield and resource-use efficiency (Fan *et al.* 2005; Liu *et al.* 2005; Lu *et al.* 2007 and Zhang *et al.* 2008).

The results from previous research have been controversial relative to the response of grain yield under different water-saving rice cultivation techniques. Some studies pointed out that shifting from continuous flooding to water-saving cultivation systems decreased grain yield (George *et al.* 2002; Peng *et al.* 2006, Tao *et al.* 2006). The yield gaps were commonly attributed to the differences in the number of productive tillers, sink formation, and spikelet sterility. Other reports suggested that water-saving rice production techniques produced yield comparable to that produced by the conventional method (Liu *et al.* 2013; Tao *et al.* 2015). However, it remains debatable if the yield obtained is related to the cultivation systems or to the adaptive potential of the studied cultivars.

The GCRPS has gained attention in recent years. This system uses materials such as a plastic-film or rice-straw as a barrier between the soil and the atmosphere; thus, preventing evaporative losses and enhancing soil water retention with no standing water during the rice cultivation period (Seguy *et al.* 2012). To promote resource-use efficiency and improvement of grain yield under the GCRPS, direct fertilizer application under drip irrigation with plastic-film-mulch has been introduced as a modification to the GCRPS (He *et al.* 2013). With the production of higher grain yield, water-productivity, and greater economic benefits, drip irrigation with plastic-film-mulch was considered to be a better water-saving rice-cultivation system compared with the furrow and sprinkler irrigation techniques in arid and semi-arid regions (He *et al.* 2013). The direct fertilizer application method under drip irrigation with plastic-film-mulch has also been reported to mitigate greenhouse gas emissions compared with continuous flooding system (Fawibe *et al.* 2019). However, achieving comparable yield with the prevailing conventional method remains debatable.

In addition, the quality of rice grain is a major factor in rice production evaluation and economic benefits to farmers. Different cultivation techniques have shown varied effects on the grain quality of rice (Zhang *et al.* 2008; Bouman *et al.* 2005; Kozak *et al.* 2007). The level of soil moisture, especially during the grain-filling period has a great influence on grain quality (Dingkuhn and Gal 1996). Nonetheless, there is a knowledge gap regarding the grain quality assessment of different rice cultivars under drip irrigation with plastic-film-mulch. Moreover, among major plant nutrients, nitrogen is usually limited in crop growth because of leaching loss, ammonia volatilization and denitrification, thus, decreasing its utilization efficiency considerably. The drip fertigation incorporates soluble fertilizers into irrigation lines which enabled for the first-time the harmonization and integration between the application of water and plant nutrients under protective plastic-film cover. Fertigation splits at weekly intervals produced higher fruit yield and improved the quality of tomatoes as compared to the fertilizer applied just before planting (Singandhupe *et al.*, 2003). It has been rarely reported in literature if this approach of fertilizer application could bring about improvement to the grain yield and quality of rice. Therefore, the objectives of this research were:

(a) to determine the grain yield, grain quality and water-use efficiency of rice cultivars under drip irrigation with plastic-film-mulch in comparison with the continuous flooding system.

(b) to evaluate the agro-physiological responses of different genotypes under drip irrigation with plastic-film-mulch.

(c) to determine if different fertilizer application methods could have a significant influence on rice productivity under drip irrigation with plastic-film-mulch system.

3.2 Materials and Methods

3.2.1 Plant materials and site description

A field experiment was conducted at the farm of the Faculty of Environmental Horticulture, Chiba University, Japan, during the summer (June to October) of 2017 and 2018. The soil was a low-humic andosol, as classified by FAO (2006). The soil properties at the soil depth of 0-20 cm were: pH = 5.8, electrical conductivity (EC) = 0.15 mS cm^{-1}, cation exchange capacity (CEC) = 24.6 meq $100g^{-1}$, soil total carbon = 37.7 g kg^{-1}, and soil total nitrogen = 3.8 g kg^{-1}. The weekly averages of daily air-temperature and daily precipitation during the rice-growing seasons recorded at a weather station close to the experimental site (35˚42.7'N 140˚2.6'E) are illustrated in Figure 3-2.

The seeds of Koshihikari, Norin 24 and Princessari were obtained from the Agricultural seed company (K-nouken, Japan). In 2017, these three cultivars of *Oryza sativa* L. were used: (i) Koshihikari (most popular lowland *Japonica* cultivar in Japan), (ii) Norin 24 (upland *Japonica* cultivar) and (iii) Princessari (lowland cultivar, a derivative of the *Indica* and *Japonica* crosses). The selected cultivars had crop growth stages of similar duration. Days to maturity for these cultivars were: Koshihikari, 120-125 days; Norin 24, 115-120 days; and Princessari, 115-120 days. In 2018, only Koshihikari and Norin 24 were considered for further experimentation as a result of the poor yield outcome for Princessari under drip irrigation with plastic-film-mulch in 2017.

3.2.2 Experimental design and treatments

The treatments were: three cultivation systems that were assigned to main plots and three rice cultivars (in 2017) and two rice cultivars (in 2018) were assigned to subplots. Thus, the experimental design was a split-plot with three replications. The rice cultivation systems were continuous flooding (CF), drip fertigation with plastic-film-mulch (DPF), and drip irrigation with direct fertilizer application under plastic-film-mulch (DPD). The site of the continuous flooded treatment was about 20 m away from the non-flooded plots in both years. The CF plots had been under conventional paddy a year before. Each CF plot was made of a concrete frame with an area of 7.5 m^2 (3 m by 2.5 m). This was designed to have water inlet and outlet channels, which allowed flooding and drainage, respectively (Plate 3-1). In 2017 and 2018, the CF was flooded to maintain 4-5 cm of water above the soil surface after seedling establishment until two weeks before harvest (drainage period). This was in line with the common farming practices in the region. The DPF and DPD had an equal area of 31.2 m^2 (6 m by 5.2 m) in both years (Plate 3-2). In the DPF, the soil was covered with black plastic-film and soluble liquid fertilizer was supplied to the rhizosphere through the drip tubes. The DPD system was similar to the DPF but solid inorganic fertilizer was broadcasted and incorporated into the soil as a substitute to the soluble liquid fertilizer. The plots in the non-flooded treatments were separated by an installed-plastic film at the soil depth of 40 cm to prevent the exchange of water and nutrients among plots. In each non-flooded plot, two drip tubes, each with an emitter discharge rate of 0.13 L min^{-1} m^{-1} (Uni-Ram CN17, Sumika Agricultural Materials Ltd) and emitter spacing of 20 cm were laid beneath the plastic film. The amount of irrigation water supplied was monitored with a flow meter installed in the irrigation pipelines. Soil-moisture level in the non-flooded plots was recorded with an FDR soil moisture meter (DIK-321A, Daiki Rika Kogyo Co., Ltd., Kounosu, Japan) connected to a multi-system data logger (CADAC, Eto Denki Co., Ltd., Mitaka, Japan).

The FDR sensor was buried 6 cm beneath the soil surface in each non-flooded plot (Plate 3-3). The volumetric soil-moisture threshold was maintained at 75% and 70% in 2017 and 2018, respectively. Additional irrigation was supplied whenever soil moisture content decreased below 70% and 65% in 2017 and 2018, respectively.

The direct seeding method was used in all treatments. Prior to seeding, viable seeds were selected using a salt solution at density ≥ 1.13 g cm^{-3}. Seeds were further treated with fungicides (benomyl) of 0.5% concentration. Seeds were sown in 2017, as described by Fawibe *et al.* (2019). In 2018, holes were perforated on the mulch at 5 cm intervals using a hand-made tool, and 5 seeds were sown per hill manually after mulching. Eight rows of rice were planted in each plot, with a planting density of 66.7 hills m^{-2}. Every four rows were covered with a black-plastic –polyethylene-film of 135 cm width (Plate 3-2). The row-spacing configuration was "10-20-10-30" in the sequence of "narrow row spacing - wide row spacing - narrow row spacing - distance between adjacent films." In the CF plots, the plastic film was removed at the 3-leaf stage of rice plants before flooding. However, the plastic film was maintained throughout the rice-growing seasons under the DPF and DPD (Plate 3-4). The sowing dates were 9 June 2017, and 4-5 June 2018; and grains were harvested on 20 October 2017 and 13 October 2018.

The continuous flooding plots and the drip irrigation with plastic-film-mulch plots were shielded with nets. These were done to eliminate the effect of birds and other bigger herbivores on the growth and development of the rice cultivars.

Plate 3-1: Experimental plots of the continuous flooding rice production system with direct seeding.

Plate 3-2: Experimental plots of the drip irrigation with plastic-film-mulch systems (a) drip irrigation set up (b) DPF and DPD after mulching.

Plate 3-3: Volumetric soil moisture measurement with FDR probe sensor under drip irrigation with plastic-film-mulch systems.

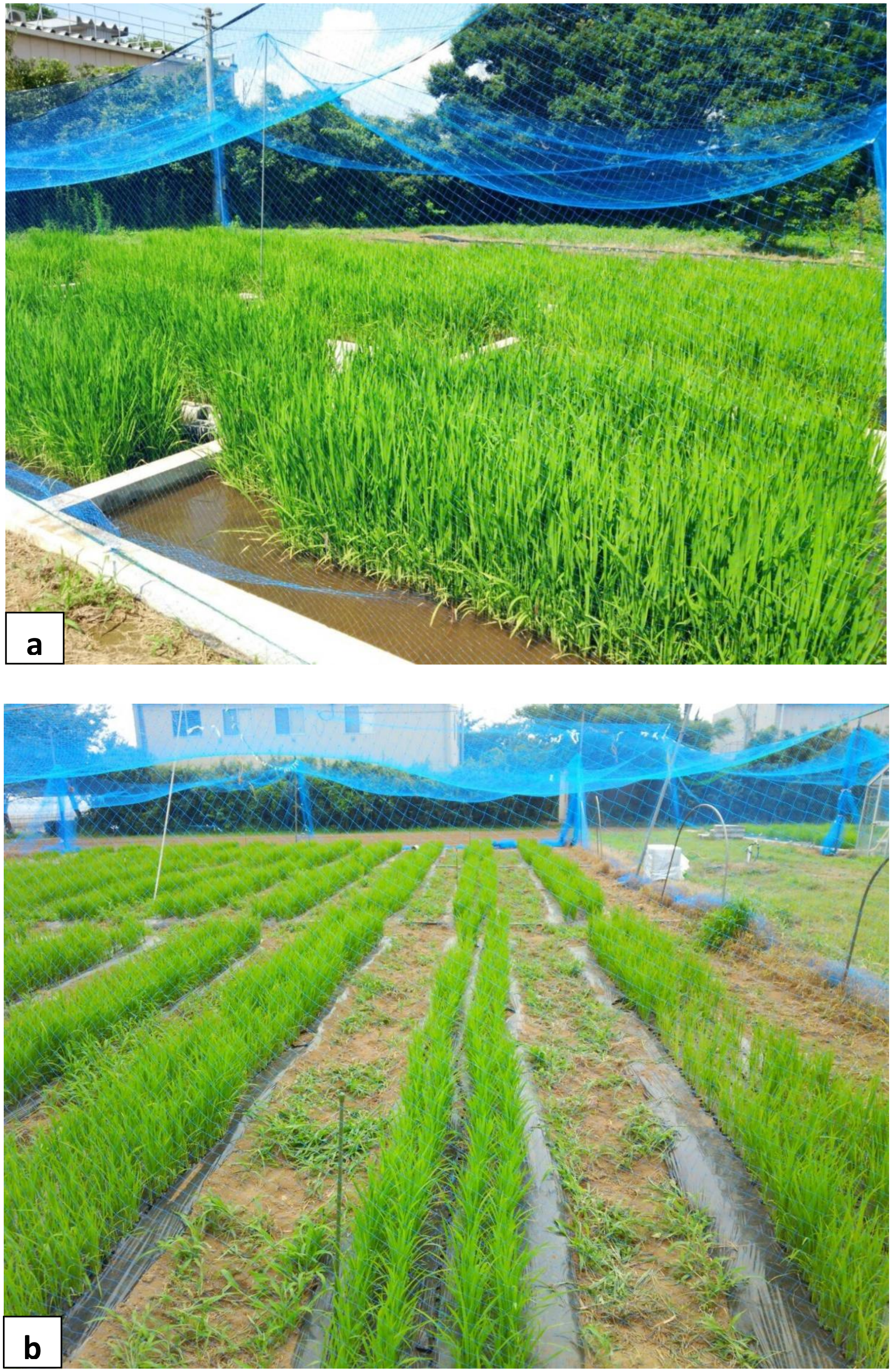

Plate 3-4: The tillering stage of rice growth under varying irrigation treatments (a) continuous flooded system (b)) drip irrigation with plastic-film-mulch systems.

3.2.3 Fertilization procedure under drip irrigation with plastic-film-mulch

The fertilizer application rates for CF and DPD plots were similar in both years. Inorganic fertilizer consisting of N (40 kg ha^{-1}), P_2O_5 (40 kg ha^{-1}) and K_2O (40 kg ha^{-1}) was applied as basal dressing before mulching. In the DPF plots, an equal amount of soluble liquid fertilizer was fertigated at 1% concentration using an injector (DR06GL, Dosatron) installed in the irrigation pipeline (Plate 3-5). This was supplied at a split fertigation rate of 25% each, at the one-week interval for one month after seedling establishment in both years. At the panicle-initiation stage, an additional 20 kg ha^{-1} of nitrogen was applied in the form of urea to all treatments through foliar spray using a garden sprayer (MS-252C, Koshin, Kyoto, Japan) in both years.

Interveinal-chlorosis was observed on emerging leaves at the mid-tillering stage under the DPF and DPD (Plate 3-6). Consequently, comparative soil analysis of available soil Fe was carried out among treatments (CF, DPF, and DPD) at the mid-tillering stage in 2017. Five replicates of soil were randomly sampled in each treatment. The available soil Fe was determined according to the method of Lindsay and Norvell (1978), using diethylenetriamine pentaacetic acid (DTPA) solution. 10 g of soil was extracted using 20 mL of 0.005 mol L^{-1} DTPA solution (pH = 7.3) in Erlenmeyer flasks covered with stretchable parafilm and secured upright on a shaker (TAITEC NR-3, Japan) at a speed of 180 rpm for 2 hours. These were filtered by gravity through Whatman #42 filter paper. The concentration of available Fe in the soil extracts was measured using an atomic absorption spectrophotometer (AA-6600F, Shimadzu, Kyoto, Japan) (Figure 3-1).

Nano-size iron oxide (Fe_2O_3) (450 ml of 0.1% concentration) was applied through foliar spray at the late-tillering stage across treatments in 2017. In 2018, 1000 ml of 0.1% nano-size Fe_2O_3 was foliar-sprayed at the mid-tillering stage and late-tillering stage of rice growth across treatments. Other important field management practices were carefully observed.

Plate 3-5: Drip fertigation process using liquid fertilizer injector (DOSATRON)

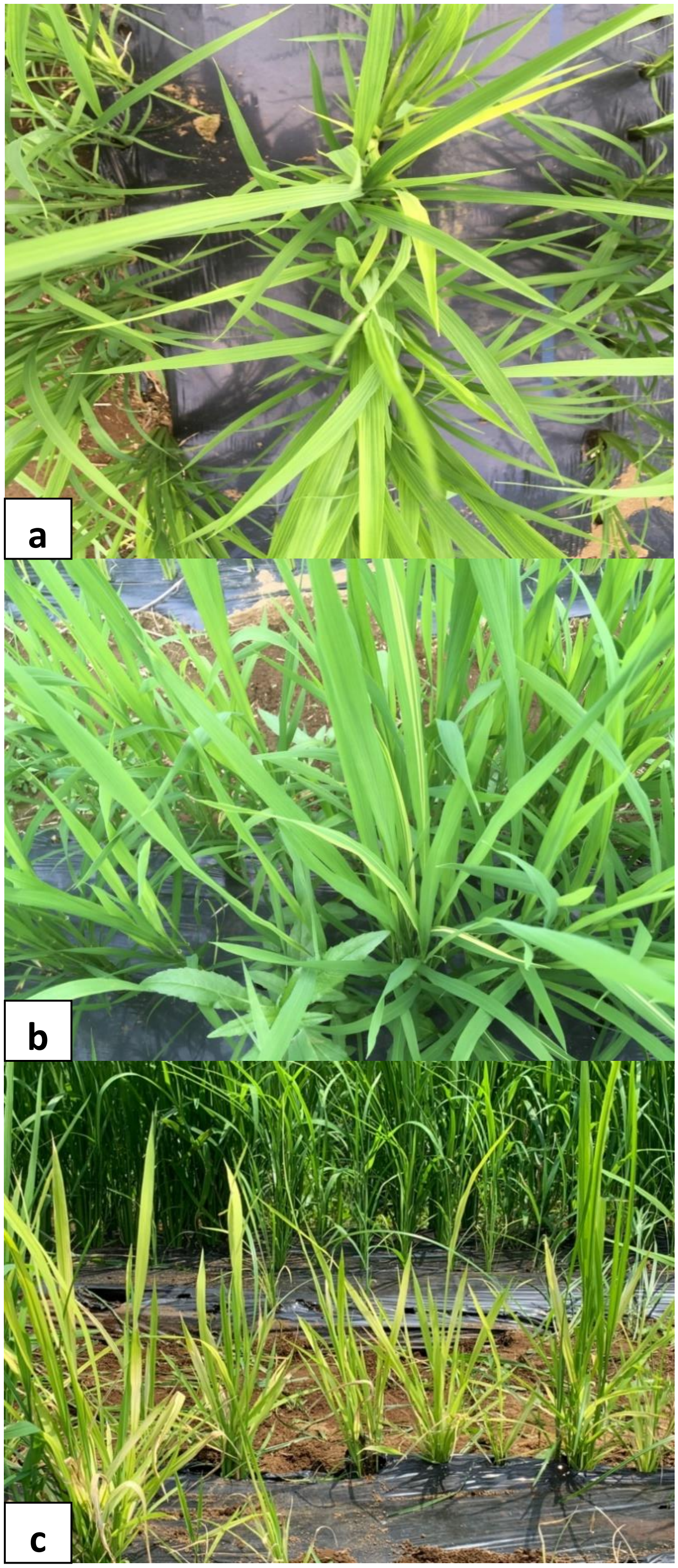

Plate 3-6: Fe chlorosis at the mid-tillering stage of rice cultivars under drip irrigation with plastic-film-mulch system in 2017. Koshihikari, lowland *Japonica* (a); Norin 24, upland *Japonica* (b); and Princessari, lowland cultivar, a derivative of *Indica* and *Japonica* crosses (c).

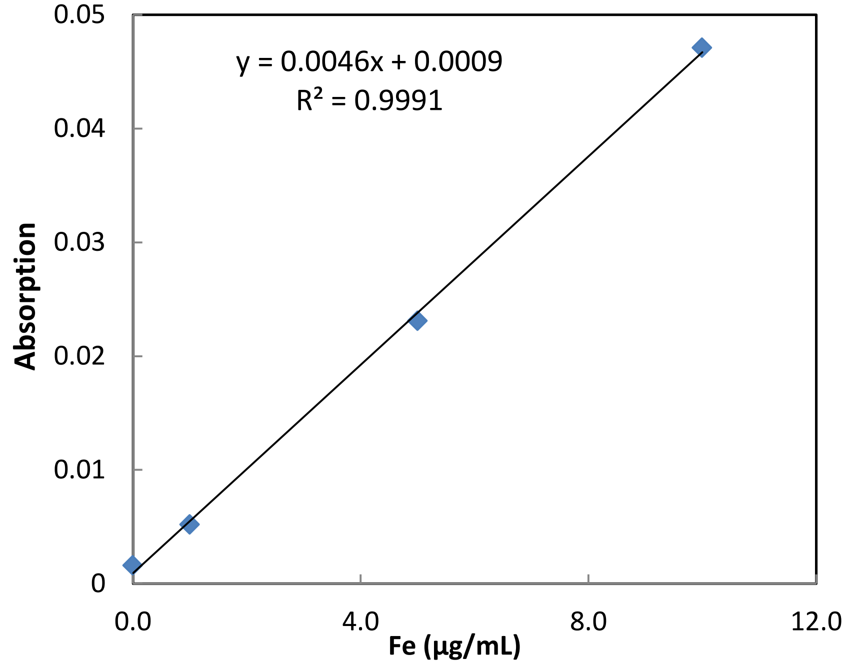

Figure 3-1: The standard absorption curve for the determination of available soil Fe concentration under varying irrigation systems in 2017.

3.2.4 Plant sampling and analyses

Agronomic parameters which included the number of tillers per unit area and the relative chlorophyll contents were examined in both years. Considering the border effect, the tiller number per square meter was determined from 10 hills sampled at the middle row at 7 days interval. The relative chlorophyll content (SPAD values) of five randomly selected plants per treatment was measured at different stages of rice growth. The SPAD values of the uppermost fully expanded leaves were obtained at the early-tillering (ET) and late-tillering (LT) stage. However, the SPAD values were obtained from the flag leaves at the grain-filling (GF) stage using a SPAD meter (SPAD-502, Konica Minolta Co., Ltd., Japan). To determine the leaf area index (LAI), and the dry matter accumulation; 10 hills were sampled from the middle row in each treatment at two-week intervals. This was done from 14 July to 29 September in 2017 and from 29 June to 21 September in 2018. Samples were separated into leaves, stems, roots, and panicles (when present). Leaf area of the sampled plants was determined using leaf area meter (AAM-8, Hayashi Denkoh Co., Ltd., Tokyo, Japan). Total dry matter was determined by oven-drying samples at 70ºC to a constant weight.

At maturity, uniform rice plants from a $1m^2$ area were sampled in each plot to determine the grain yield. Grain yield components were determined from randomly selected 10 hills per treatment (excluding border plants). Panicles were counted and separated by hand-threshing. Filled spikelets were defined as the filled grains at density ≥ 1.06 g cm^{-3}. The number of spikelets per panicle was taken as the sum of both filled and unfilled spikelets. Aboveground total biomass was the total dry weight of straw, rachis, and filled and unfilled spikelets. Grain-filling percentage was computed as follows:

$$\text{Grain-filling (\%)} = (\text{number of filled spikelets} / \text{total number of spikelets}) \times 100$$

Harvest index (HI)was computed as follows:

Harvest index = (filled spikelet weight / aboveground total biomass) × 100

Unhusked grain yield and one thousand grain weight were expressed at 14% grain moisture content. Water use efficiency (WUE in kg m^{-3}) was expressed as the grain yield per unit of total water input including irrigation and precipitation.

3.2.5 Physiological measurements

The CO_2 assimilation rate, transpiration rate, and stomatal conductance were measured on the flag leaf at the grain-filling stage using a gas exchange analyzer (LI-6400, LI-COR Co., Ltd., Lincoln, USA). These parameters were measured between 9:00 and 12:00 local time when active photosynthetic radiation above the canopy was 1100~1200 µmol m^{-2} s^{-1}. The measurement of chlorophyll fluorescence parameters which included the actual and maximum quantum yields of photosystem II (PSII) was carried out on the flag leaves at the grain-filling stage using a chlorophyll fluorometer with a leaf holder (PAM-2000, Walz Co., Ltd., Effeltrich, Germany). The actual quantum yield of PSII was measured at midday (11:00 to 13:00 hours, local time), whereas the maximum quantum yield of PSII (Fv/Fm) was measured on dark-adapted leaves after sunset (21:00 to 22:00 hours, local time). The leaves were dark-adapted by covering the randomly selected leaves with aluminum foil for four hours before the measurements were taken. The chlorophyll fluorescence parameters were expressed as follows:

Actual quantum yield = ΔF/Fm'; ΔF = Fm' - F.

Maximum quantum yield = Fv/Fm; Fv = Fm – Fo.

where Fo and Fm represent the minimum and maximum fluorescence in dark-adapted leaves, respectively; Fm' is the maximum fluorescence of leaf under light conditions; F represents steady-state fluorescence yield measured at any given time. The measurements of CO_2 assimilation rate, transpiration rate, stomatal conductance, and chlorophyll fluorescence parameters were replicated three times.

3.2.6 Grain quality measurements

One-thousand grains of brown rice were randomly selected to determine the chalky grain percentage, grain length-width ratio, and grain thickness using a grain-quality analyzer (RGQ120A, Satake Co., Ltd., Tokyo, Japan). Apparent amylose content of brown rice at 14% moisture content was determined using the procedure given by Tamura *et al.* (2014) following modifications. Grain samples were ground to powder and sieved (using a 500 µm sieve) in preparation for colorimetric analysis. A 50-milligram powdered sample was placed in 0.5 mL of 95% ethanol and gelatinized using 4.5 mL of 1N NaOH for 20 min in boiling water; distilled water was added to the solution to achieve 50-mL volume. Subsequently, a 2.5 mL aliquot of the solution was added to 0.5 mL of 1M acetic acid and 1 mL of iodine solution; distilled water was added to the solution to make the volume 50 mL. The solution was incubated for 20 min at 30ºC, and absorbance was measured at 620 nm using a spectrophotometer (V-630 BIO, Jasco Co., Ltd., Tokyo, Japan). Potato amylose (A0512, Sigma-Aldrich) was used as a standard.

Brown rice samples were oven-dried at 135ºC for 3 h. A 0.15 g aliquot from each dried sample was taken and its nitrogen content was determined using a CN corder (MT-700, Yanaco Co., Ltd., Kyoto, Japan). The crude protein content was calculated using a 5.95 nitrogen-protein conversion factor. Hippuric acid was used as the standard nitrogen material.

3.2.7 Statistical analysis

Data were analyzed using the mixed ANOVA procedure (SPSS 16.0 analytical software). The statistical model used included sources of variation due to year, cultivar, system, and interactions of year × cultivar, year × system, cultivar × system, and year × cultivar × system. Differences between means were detected by use of the least significant difference (LSD) at $\alpha = 0.05$.The grain quality data were processed statistically by two-way analysis of variance (ANOVA).

The available soil Fe concentration data were analyzed statistically by one-way analysis of variance (ANOVA). The differences between means were compared by the least significant difference (LSD) at $\alpha = 0.05$.

3.3 Results

3.3.1 Weather and hydrological conditions

The climatic data at the experimental site during the 2017 and 2018 growing seasons showed different temperature dynamics, precipitation frequency, and distribution (Figure 3-2). The weekly average temperature ranged between 14˚C and 28˚C in 2017 and between 20˚C and 30˚C in 2018. The total rainfall during the rice-growth period was 924 mm in 2017 and 570 mm in 2018 (Table 3-1). The total water input (irrigation and precipitation) in drip irrigation with plastic-film-mulch systems was between 1213-1489 mm across the years. This saved 28-36% of the total water input compared with continuous flooding system (Table 3-1). On average, total water input in DPF was 3% higher than that supplied to the DPD across the years due to the additional water used during fertigation. The weekly average temperature between the panicle initiation and flowering stage of rice growth (7/27-9/7) in 2018 was 2˚C to 5˚C higher than in 2017. During this period, less precipitation was recorded in 2018 than in 2017 (Figure 3-2). The soil moisture contents at the 0-6 cm soil depth in the DPF and DPD show a similar trend in both years. Moisture threshold set at 70% in 2018 saved 16% of total water supplied compared with 2017 (Figure 3-3).

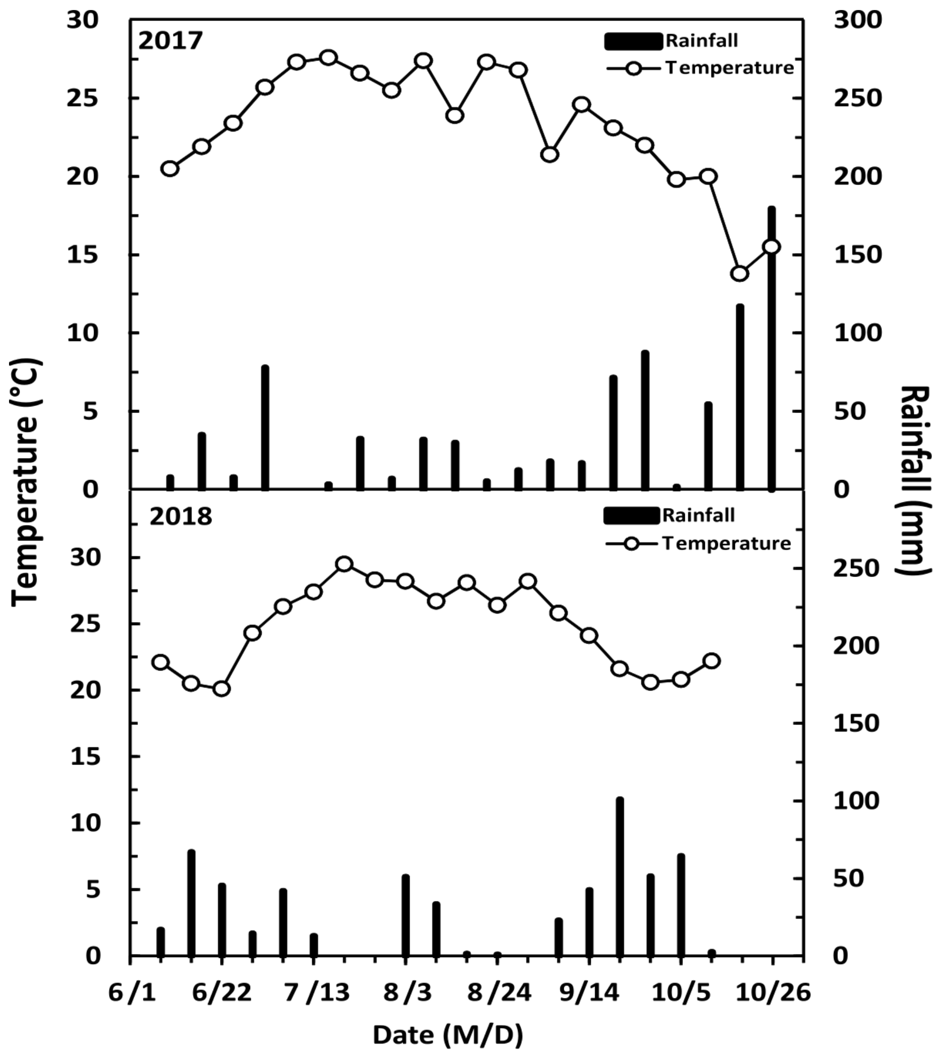

Figure 3-2: Weekly averages of daily air-temperature and daily rainfall during the rice-growing seasons of 2017 and 2018

Table 3-1: The amount of irrigation water, rainfall and total water input under varying irrigation systems in 2017 and 2018

	Irrigation (mm)	Rainfall (mm)	Total water input¶ (mm)
2017			
CF†	1144	924	2068
DPF‡	565	924	1489
DPD§	556	924	1480
2018			
CF	1312	570	1882
DPF	703	570	1273
DPD	643	570	1213

† CF = continuous flooding

‡ DPF = drip fertigation with plastic-film-mulch

§ DPD = drip irrigation with direct fertilizer application under plastic-film-mulch

¶ Total water input = irrigation + rainfall

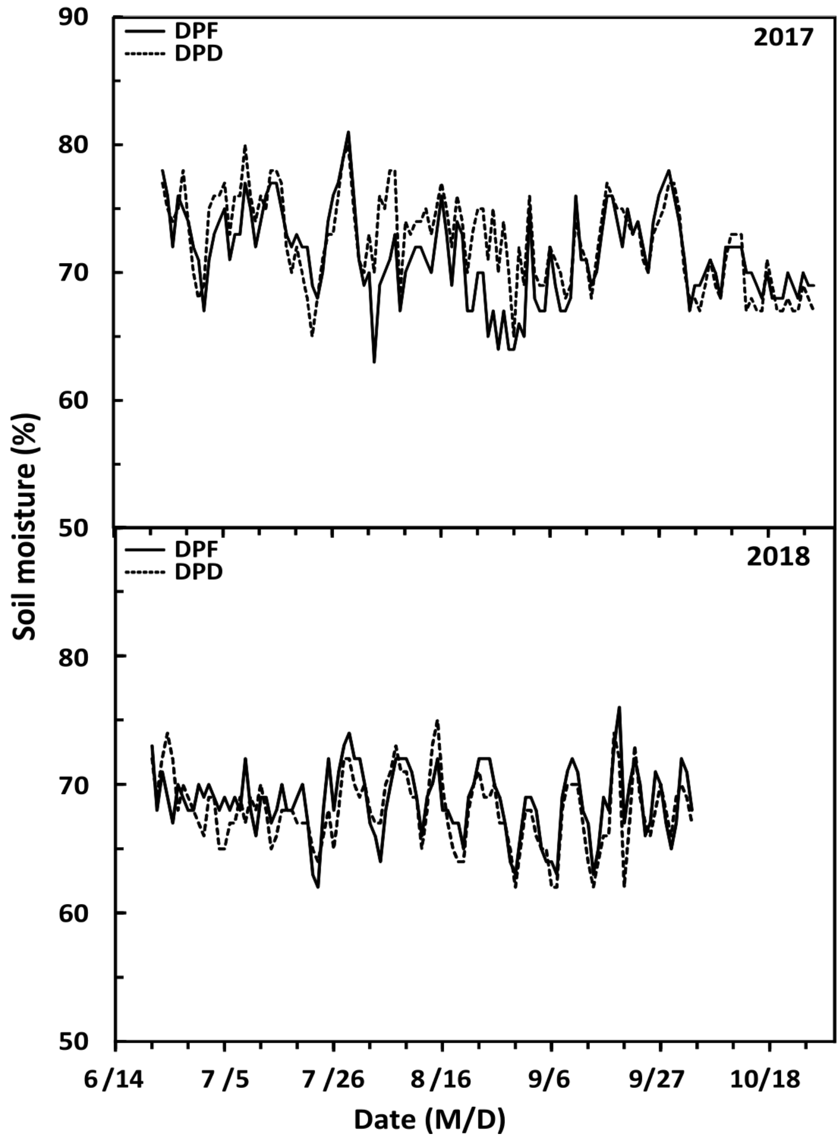

Figure 3-3: Volumetric soil moisture content under drip irrigation with plastic-film-mulch systems. Straight and broken lines indicate DPF and DPD respectively during the rice-growing seasons of 2017 and 2018. Data were the means across four replicates of each treatment.

DPF = drip fertigation with plastic-film-mulch

DPD = drip irrigation with direct fertilizer application under plastic-film-mulch

3.3.2 The number of the tiller of rice cultivars under varying irrigation systems

The average tiller number per unit area across cultivars and irrigation systems was not significantly different between years. In both years, a similar trend was observed in the tiller formation of each cultivar. However, significant differences were observed among irrigation systems and cultivars. At the maximum-tillering stage in 2017 (7/27) and 2018 (7/25), Koshihikari and Princessari produced significantly higher number of tillers under CF compared with DPF and DPD in both years and in 2017 respectively (Figure 3-4 a,c,d). Conversely, Norin 24 showed higher numbers of tillers under DPF and DPD in both years compared with CF. This was significantly higher at the maximum-tillering stage in 2018 (Figure 3-4 e). Among cultivars under DPF and DPD, Norin 24 produced the highest number of tillers per square meter. However, in 2017 Princessari decreased significantly under both DPF and DPD in comparison with CF; depicting its poor tillering ability under these systems. The different fertilizer application methods under drip irrigation with plastic-film-mulch had no significant effect on each of the examined cultivar.

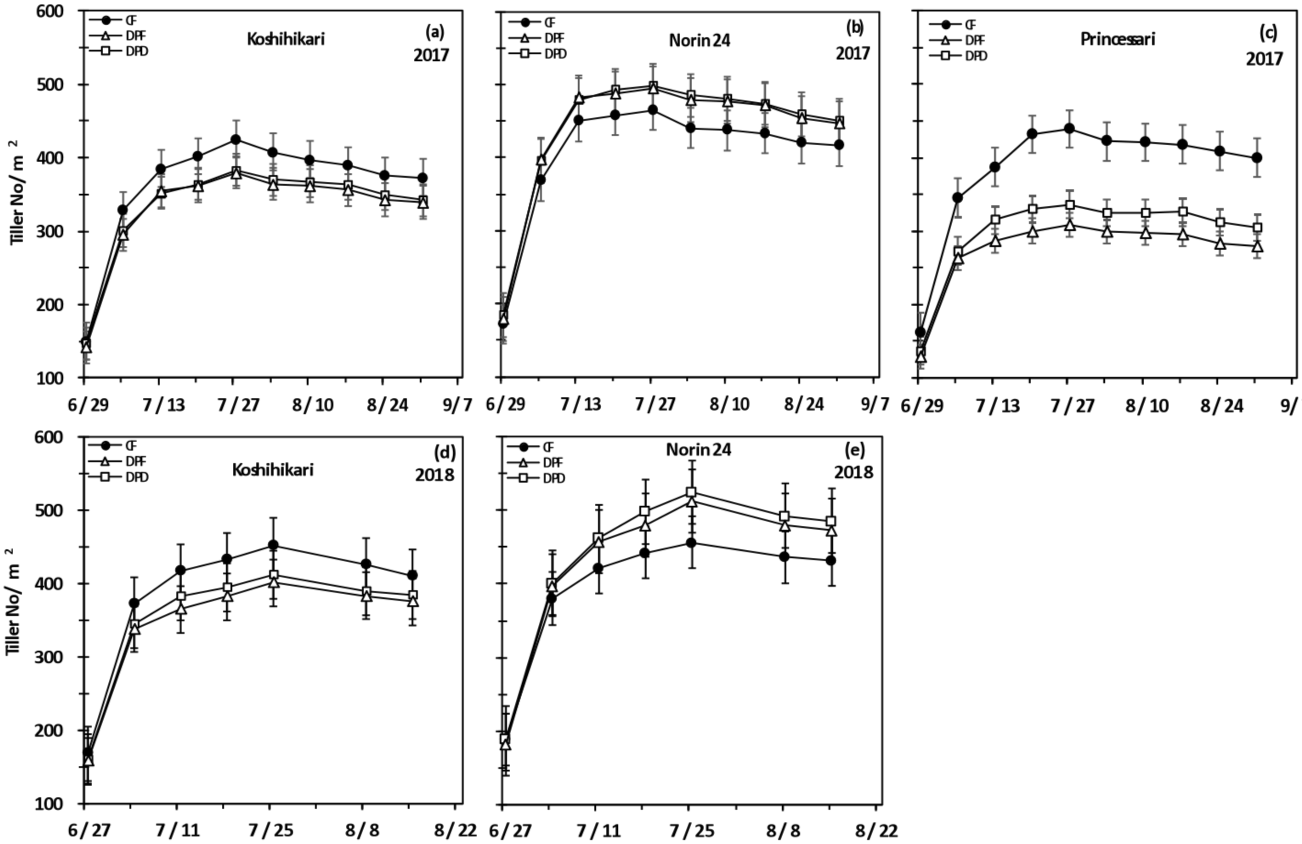

Figure 3-4: The number of tillers per square meter of rice cultivars during rice-growing seasons of 2017 and 2018. CF, continuous flooding; DPF, drip fertigation with plastic-film-mulch; and DPD, drip irrigation with direct fertilizer application under plastic-film-mulch. Koshihikari, lowland *Japonica* cultivar (a, d); Norin 24, upland *Japonica* cultivar (b, e); and Princessari, lowland cultivar, a derivative of *Indica* and *Japonica* crosses (c) under varying irrigation systems. Vertical bars represent ± S.E of the mean. The S.E was calculated across three replicates for each year.

3.3.3 Soil-available iron concentration under varying irrigation systems

The average Fe concentration in soil samples was 69.58 µg g^{-1}, 28.67 µg g^{-1}, 28.96 µg g^{-1} in CF, DPF, DPD, respectively (Figure 3-5). The average Fe concentration in the CF soil was higher by 143% and 140% than that of DPF and DPD, respectively. However, the different fertilizer application methods under drip irrigation with plastic-film-mulch system had no significant influence on Fe concentration in the soil. Therefore the significantly low Fe concentration of the upland soil was considered responsible for the interveinal-chlorosis observed on young emerging leaves of the cultivars at the mid-tillering stage under DPF and DPD.

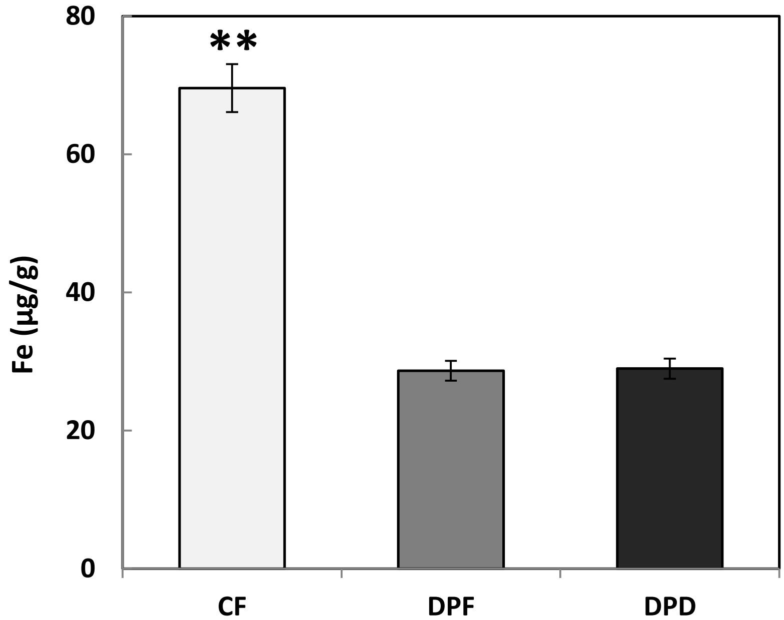

Figure 3-5: Soil-available Fe concentration under varying irrigation systems at the mid-tillering stage of rice in 2017. CF, continuous flooding; DPF, drip fertigation with plastic-film-mulch; and DPD, drip irrigation with direct fertilizer application under plastic-film-mulch. ** indicates a significant difference at $P < 1\%$.

3.3.4 Chlorophyll contents of rice cultivars under varying irrigation systems

At the early-tillering stage of rice growth (ET), there was no significant difference in the SPAD values of all the cultivars under different irrigation systems across years. The exception was Norin 24 which had higher chlorophyll content under DPD than CF in both years. At the late-tillering stage (LT), a significant decrease in chlorophyll content was observed in all cultivars' leaves under DPF and DPD compared with CF in 2017 (Figure 3-6). This was attributable to the available soil Fe deficiency determined at the mid-tillering stage of rice under DPF and DPD (Plate 3-6). There was no significant difference in the chlorophyll content of each cultivar under different irrigation systems in 2018. At the grain-filling stage, the chlorophyll contents of Koshihikari and Norin 24 under different irrigation systems were statistically the same in both years. However, in 2017, Princessari exhibited a decrease in chlorophyll content at the grain-filling stage under DPF and DPD compared with CF but was only significant for DPF (Figure 3-6).

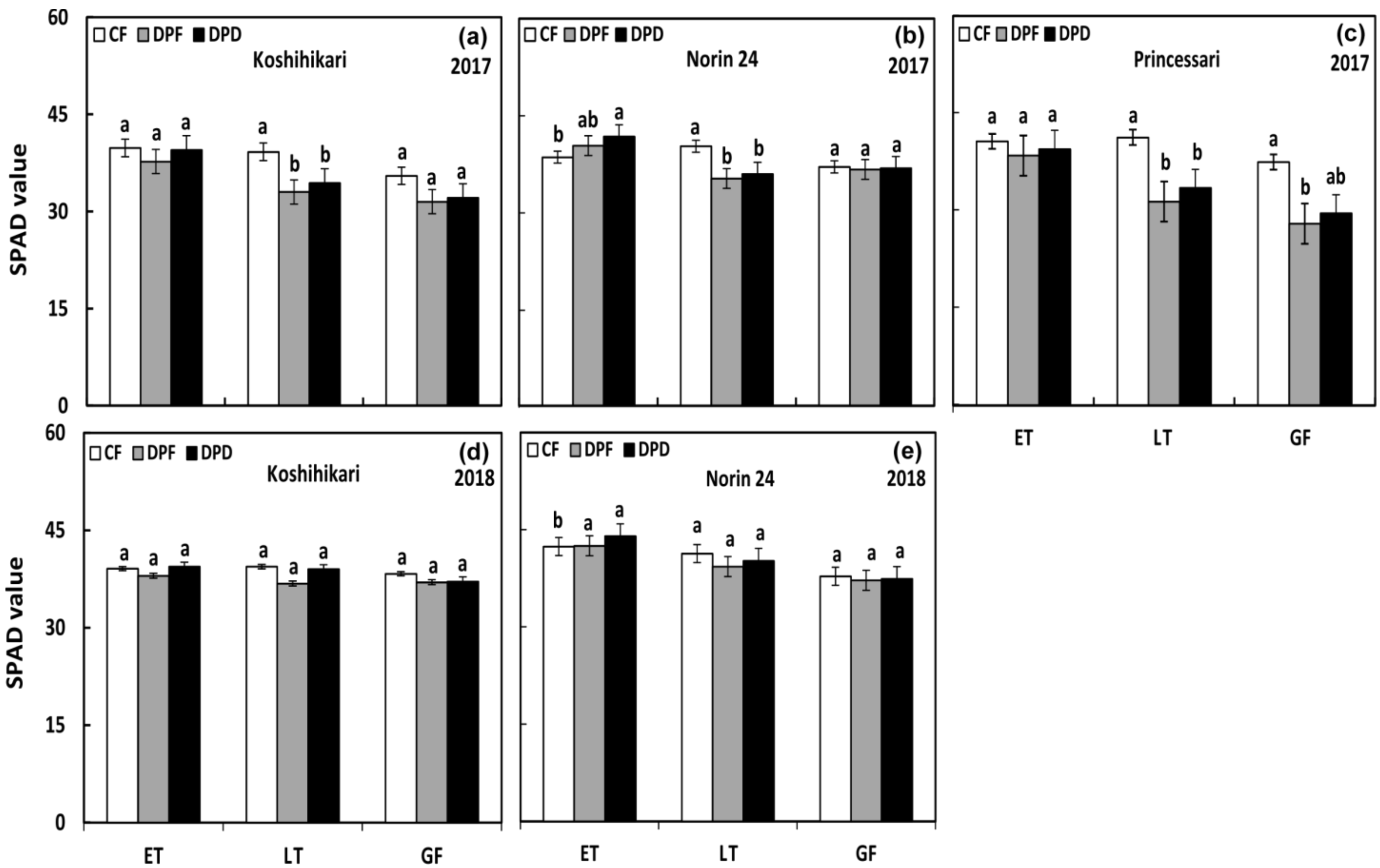

Figure 3-6: SPAD values of rice cultivars under varying irrigation systems during rice growing seasons of 2017 and 2018. CF, continuous flooding; DPF, drip fertigation with plastic-film-mulch; and DPD, drip irrigation with direct fertilizer application under plastic-film-mulch. Koshihikari, a lowland *Japonica* cultivar (a, d); Norin 24, an upland *Japonica* cultivar (b, e); and Princessari, a lowland cultivar, derivative of *Indica* and *Japonica* crosses (c). ET, LT, GF denote the stages of early-tillering, late-tillering, and grain-filling, respectively. The different lowercase letters indicate a significant differences among the irrigation systems at $P < 5\%$. Vertical bars represent ± S.E of the mean. The S.E was calculated across three replicates for each year.

3.3.5 Leaf area index and dry matter accumulation of rice cultivars under varying irrigation systems

There were no significant differences in leaf area index of Koshihikari and Norin 24 under CF compared with DPF and DPD in both years (Figure 3-7). Nevertheless, the leaf area index of Princessari in 2017 decreased significantly under DPF and DPD. Also, a significant difference was not observed in dry matter accumulation among cultivars in both years (Figure 3-7). Nonetheless, the dry matter was significantly affected by irrigation system. The dry matter of Koshihikari and Princessari in 2017 increased significantly under the CF than DPF and DPD. Conversely, the dry matter of Norin 24 was higher under drip irrigation with plastic-film-mulch compared with CF in both years regardless of the fertilization method (Figure 3-7). Across cultivars in both years, total dry matter accumulation was not significantly different under DPF and DPD but slightly higher in DPD than DPF.

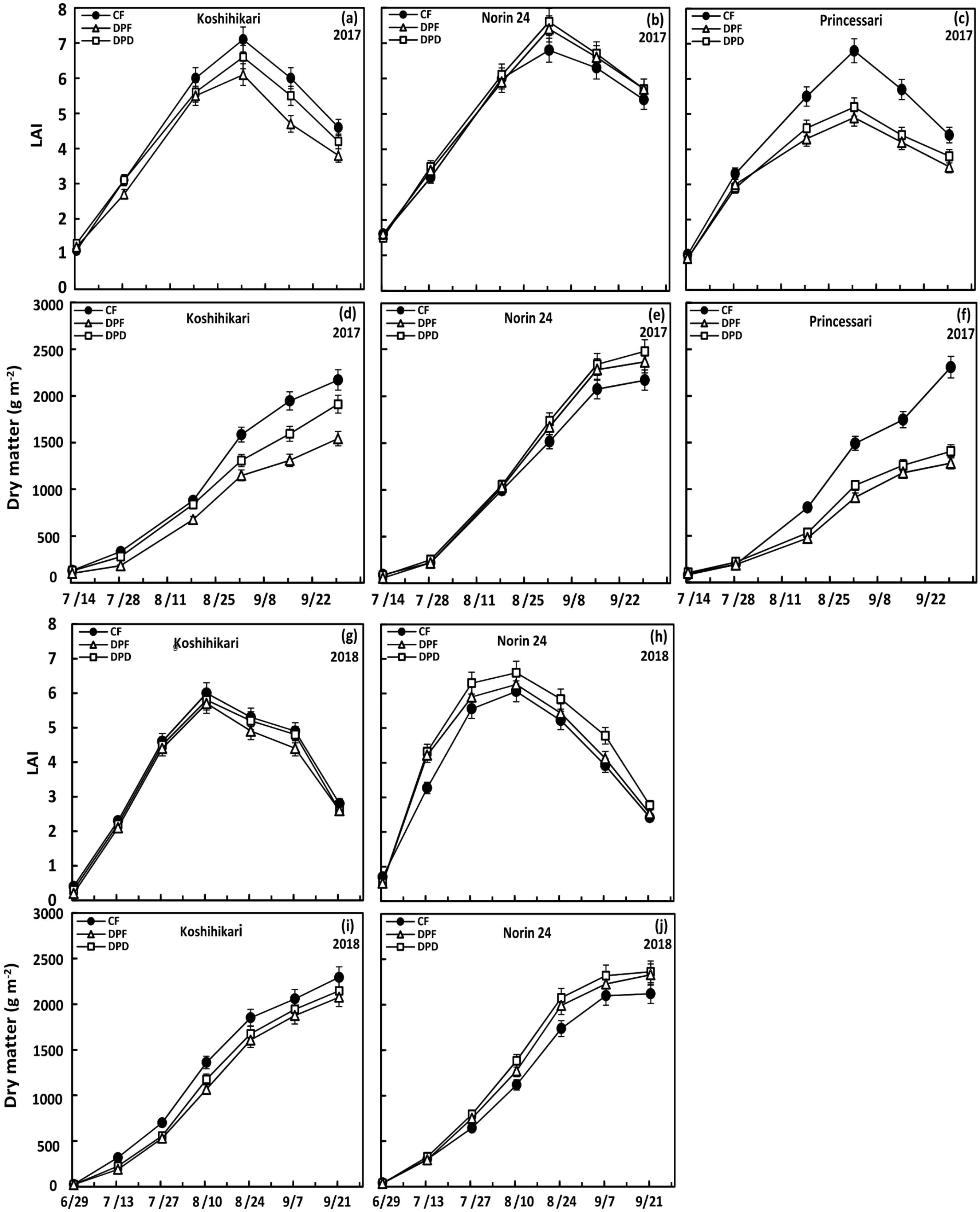
Koshihikari
(a)
2017
Norin 24
(b)
2017
Princessari
(c)
2017
Koshihikari
(d)
2017
Norin 24
(e)
2017
Princessari
(f)
2017
Koshihikari
(g)
2018
Norin 24
(h)
2018
Koshihikari
(i)
2018
Norin 24
(j)
2018
CF
DPF
DPD
LAI
Dry matter (g m-2)
7 /14
7 /28
8 /11
8 /25
9/8
9/22
6/29
7 /13
7 /27
8 /10
8 /24
9/7
9/21

Figure 3-7: Leaf area index (LAI) and dry matter of rice cultivars under varying irrigation systems during rice growing seasons of 2017 and 2018. CF, continuous flooding; DPF, drip fertigation with plastic-film-mulch; and DPD, drip irrigation with direct fertilizer application under plastic-film-mulch. LAI (a, b, c, g, h), and dry matter (d, e, f, i, j). Koshihikari, a lowland *Japonica* cultivar (a, d, g, i); Norin 24, an upland *Japonica* cultivar (b, e, h, j); and Princessari, a lowland cultivar, a derivative of *Indica* and *Japonica* crosses (c, f). 2017 (a, b, c, d, e, f) and 2018 (g, h, i, j). Vertical bars represent ± S.E of the mean. The S.E was calculated across three replicates for each year.

3.3.6 Photosynthesis associated parameters of rice cultivars under varying irrigation systems

At the grain-filling stage, the CO_2 assimilation rates of the cultivars under varying irrigation systems were not significantly different in both years. The exception was Princessari with 88% and 82% higher CO_2 assimilation rate under CF than under DPF and DPD, respectively (Table 3-2). Like the CO_2 assimilation rate, the transpiration rate and stomatal conductance of the leaves of rice cultivars under varying regimes exhibit similar patterns other than those of Princessari's leaves which were significantly lower under DPF as compared with CF. However, the transpiration rate and stomatal conductance of the different cultivars showed no significant difference between DPF and DPD.

It was important to ascertain the integrity of the photosynthetic apparatus of the leaves under varying irrigation systems. Table 3-2 contains information showing that Koshihikari and Norin 24 had comparable $\Delta F/Fm'$ and Fv/Fm with no significant differences among irrigation systems. Nonetheless, the $\Delta F/Fm'$ and Fv/Fm of Princessari were significantly lowered by 50% and 22%, respectively, under DPF compared with CF. In general, the different fertilizer application methods had no significant effect on both photosynthesis and associated parameters under drip irrigation with plastic-film-mulch system.

Table 3-2: CO_2 assimilation rate, transpiration rate, stomatal conductance, and chlorophyll fluorescence parameters of rice cultivars at the grain-filling stage under varying irrigation systems in 2017 and 2018

Cultivar†	System‡	CO_2 assimilation rate (μmol m^{-2} s^{-1})	Transpiration rate (mmol m^{-2} s^{-1})	Stomatal conductance (mmol m^{-2} s^{-1})	ΔF/Fm'§	Fv/Fm¶
2017						
Koshihikari	CF	15.7a#	5.8a	256a	0.53a	0.79a
	DPF	15.2a	5.3a	240a	0.55a	0.79a
	DPD	15.3a	5.5a	246a	0.53a	0.80a
Norin 24	CF	16.1a	6.1a	269a	0.41a	0.78a
	DPF	15.6a	5.8a	247a	0.43a	0.78a
	DPD	15.8a	5.7a	251a	0.42a	0.78a
Princessari	CF	15.8a	5.9a	258a	0.50a	0.78a
	DPF	8.4b	3.1b	151b	0.25b	0.61b
	DPD	8.7b	3.5b	169ab	0.27b	0.63b
2018						
Koshihikari	CF	16.1a	6.0a	272a	0.52a	0.80a
	DPF	15.8a	5.7a	258a	0.54a	0.81a
	DPD	16.0a	5.7a	261a	0.55a	0.79a
Norin 24	CF	16.5a	6.5a	293a	0.48a	0.81a
	DPF	16.0a	5.9a	276a	0.49a	0.79a
	DPD	16.3a	6.1a	281a	0.51a	0.81a
Year (Y)		ns	ns	ns	*	ns
Cultivar (C)		***	***	*	***	***
Irrigation system (I)		**	***	ns	**	**
Y×C		ns	ns	ns	*	ns
Y×I		ns	ns	ns	ns	ns
C×I		**	**	ns	***	***
Y×C×I		ns	ns	ns	ns	ns

*, **, *** significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. ns = non-significant.

†Koshihikari, a lowland *Japonica* cultivar; Norin 24, an upland *Japonica* cultivar; Princessari, a lowland cultivar, a derivative of *Indica* and *Japonica* crosses.

‡CF, continuous flooding; DPF, drip fertigation with plastic-film-mulch; and DPD, drip irrigation with direct fertilizer application under plastic-film-mulch.

§ΔF/Fm' = actual quantum yield of photosystem II

¶Fv/Fm = maximum quantum yield of photosystem II

#Within a column for each cultivar within a year, different lowercase letters indicate a significant difference ($P < 0.05$) among irrigation systems.

3.3.7 Grain yield and water-use efficiency of rice cultivars under varying irrigation systems

Table 3-3 shows that year had no significant effect on the grain yield, WUE, and harvest index (HI). However, the interaction between cultivar and irrigation system significantly affected the grain yield and water productivity. The grain yields of Koshihikari and Norin 24 were not significantly different between CF and the non-flooded irrigation systems across years (Plate 3-7). The average yield of Norin 24 across years was significantly higher by 16% and 60% than Koshihikari and Princessari, respectively. These results indicate that Norin 24 was the highest yielding cultivar among the three cultivars.

The year had a significant influence on the grain yield components except on the grain weight. In 2018, there was no significant difference in the number of panicles of Koshihikari and Norin 24 under the varying irrigation systems. However, in 2017, the number of panicles of Norin 24 was significantly higher under drip irrigation systems (DPF and DPD) compared with CF. Conversely, number of panicles of Koshihikari significantly increased under CF compared with DPF. Table 3-3 shows that the number of spikelets per panicle of Koshihikari and Norin 24 was consistently higher in both years under the DPF and DPD compared with CF. Nonetheless, the number of spikelets per panicle and filled-grain percentage of Koshihikari were not significantly different among the irrigation systems in both years. Norin 24 also showed a similar result in 2017, with no significant differences in the number of spikelets per panicle and filled-grain percentage under varying irrigation systems. However, in 2018, filled-grain percentage and grain weight of Norin 24 decreased significantly under DPF and DPD compared with CF (Table 3-3). This was attributable to the trade-off effect between these components and the number of spikelets per panicle. This indicates that the high number of spikelets per panicle of Norin 24 produced under DPF and DPD resulted to the reduction in the grain-filling percentage and grain weight of Norin 24.

The grain yield of Princessari decreased by 65% and 54% under DPF and DPD, respectively, in comparison with CF (Table 3-3). The reduction of the yield components (number of panicles per unit area, spikelets per panicle and the grain-filling percentage) of Princessari under both DPF and DPD compared with CF in 2017 resulted in the significant decrease in grain yield and HI observed.

The average water-use efficiency under DPF and DPD was 0.52 and 0.65 kg m^{-3}, respectively. These were 1.2 and 1.5 times higher than that of CF across years. The WUE was significantly higher for both Koshihikari and Norin 24 under DPD by 61% and 68% (2017), 40% and 76% (2018), respectively in relation to CF (Table 3-3). However, there was no significant difference in the WUE of Koshihikari and Norin 24 under DPD and DPF in both years except in 2018 where WUE of Norin 24 decreased significantly by 35% under DPF. The WUE of Princessari was not significantly different among irrigation systems (Table 3-3). Moreover, Norin 24 had the highest WUE among cultivars. This increased significantly under the drip irrigation with plastic-film-mulch systems compared with CF. The different fertilizer application methods had no significant effect on WUE of the cultivars except in 2018 where WUE of Norin 24 decreased significantly by 35% under DPF compared with DPD.

Among cultivars, Norin 24 showed the highest HI in both years. The HI of each cultivar was not significantly different among irrigation systems except Princessari whose HI was higher under CF compared with DPF and DPD. Also, the result suggests the existence of considerable variation among cultivars to yield and WUE under the drip irrigation with plastic-film-mulch. The upland cultivar (Norin 24) with the highest LAI, biomass accumulation, and HI had the highest grain yield and WUE under the DPF and DPD while Princessari (lowland cultivar; a derivative of *Indica* and *Japonica* crosses) with the lowest LAI, biomass accumulation and HI recorded the lowest yield and WUE under DPF and DPD.

Table 3-3 Grain yield and its components, water-use efficiency and harvest index of rice cultivars under varying irrigation systems in 2017 and 2018

Cultivar†	System ‡	Panicles per m^2	Spikelets per panicle	Filled grains (%)	1000 grain weight (g)	Grain yield (t ha^{-1})	WUE§ (kg m^{-3})	HI¶
2017								
Koshihikari	CF	440a#	85a	81.3a	24.3a	7.40a	0.36b	0.36a
	DPF	349b	99a	67.8a	22.5b	5.27a	0.44ab	0.24b
	DPD	391ab	104a	75.2a	22.9b	7.02a	0.58a	0.32ab
Norin 24	CF	418b	114a	81.0a	23.7a	9.09a	0.44b	0.41a
	DPF	509a	107a	68.3a	22.9ab	8.58a	0.71a	0.34a
	DPD	527a	112a	68.8a	21.7b	8.98a	0.74a	0.32a
Princessari	CF	531a	105a	75.0a	21.6a	8.99a	0.43a	0.39a
	DPF	380b	69b	55.6b	21.3a	3.14b	0.26a	0.21b
	DPD	353b	79b	65.9ab	22.3a	4.13b	0.35a	0.25b
2018								
Koshihikari	CF	548a	93a	74.4a	24.1a	9.04a	0.48b	0.34a
	DPF	488a	99a	72.8a	22.4b	7.78a	0.61ab	0.28a
	DPD	492a	100a	72.0a	23.2ab	8.18a	0.67a	0.30a
Norin 24	CF	546a	89b	81.3a	24.1a	9.26ab	0.49b	0.37a
	DPF	585a	154a	47.3b	21.5b	7.53b	0.59b	0.34a
	DPD	575a	151a	54.4b	22.1ab	10.48a	0.86a	0.37a
Year (Y)		***	**	*	ns	ns	ns	ns
Cultivar(C)		***	***	**	***	***	***	*
System (S)		*	*	***	***	**	**	*
Y × C		ns	*	ns	ns	ns	ns	ns
Y×S		ns	**	ns	ns	ns	ns	ns
C×S		***	**	ns	**	*	*	ns
Y×C×S		ns	***	ns	ns	ns	ns	ns

*, **, *** significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. ns = non-significant.

†Koshihikari, a lowland *Japonica* cultivar; Norin 24, an upland *Japonica* cultivar; Princessari, a lowland cultivar, a derivative of *Indica* and *Japonica* crosses.

‡CF, continuous flooding; DPF, drip fertigation with plastic-film-mulch; and DPD, drip irrigation with direct fertilizer application under plastic-film-mulch.

§WUE = water-use efficiency

¶HI = harvest index

#Within a column for each cultivar within a year, different lowercase letters indicate a significant difference ($P < 0.05$) among irrigation systems.

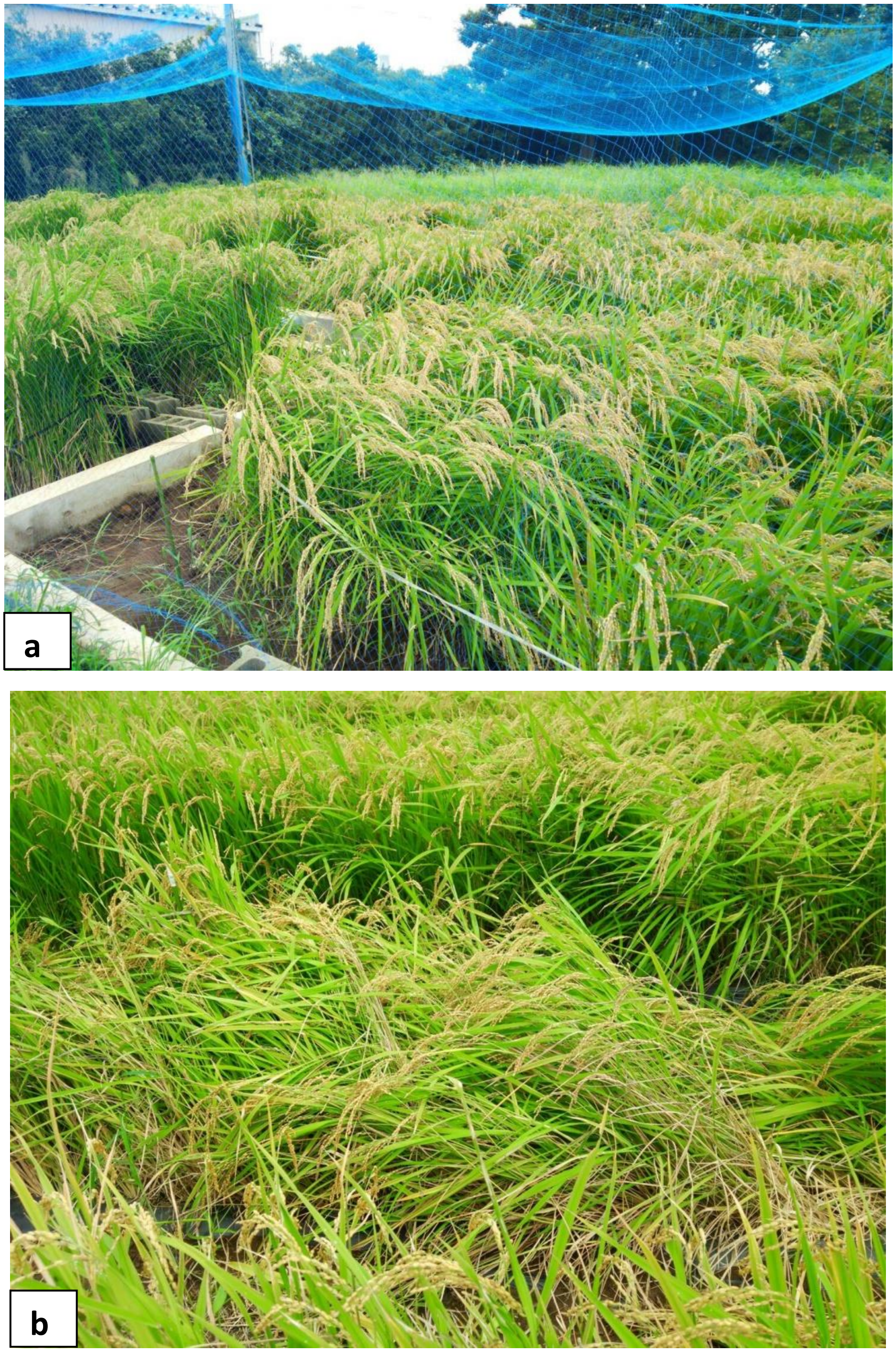

Plate 3-7: Rice grain yield at maturity stage under varying irrigation systems (a) continuous flooding (b) drip irrigation with plastic-film-mulch with different fertilization methods (DPF and DPD).

3.3.8 Grain qualities of rice cultivars under varying irrigation systems

The chalky grain percentage, grain length and width ratio, grain thickness and protein content were significantly affected by the varying irrigation systems and cultivar types. However, only the irrigation system had a significant effect on amylose content. On average, the chalky grain percentage of Koshihikari was lowered by 64% compared with Norin 24 (Table 3-4). This depicts that Koshihikari under the varying irrigation systems had a better appearance than Norin 24 under the same irrigation systems. There was no significant difference in the chalky grain percentage of Koshihikari under varying irrigation systems. However, the chalky grain percentage of Norin 24 was lowered by 34% and 32% under CF compared with DPF and DPD respectively. The difference in the chalky grain percentage of Koshihikari and Norin 24 between DPF and DPD was not significant.

The average grain length and width ratio was higher in Norin 24 by 8% compared with Koshihikari; however, the grain thickness of Koshihikari had an average thickness that is significantly higher by 6% than that of Norin 24. The grain length-width ratio and thickness of both cultivars under DPF and DPD were similar. Moreover, the grain length and width ratio and grain thickness of Norin 24 were insignificant under DPF and DPD compared with CF. There was no consistent difference in the grain length and width ratio and grain thickness of Koshihikari among irrigation systems.

The amylose contents of Koshihikari and Norin 24 were not significantly different (Table 3-4). The amylose content of Koshihikari under DPF was not significantly different from that under CF but was 17% reduced than that of DPD (Table 3-4). However, the amylose content of Norin 24 under DPF and DPD were comparable to that under CF. Protein content was significantly higher in Norin 24 compared with Koshihikari. Koshihikari and Norin 24 produced 32% and 22% higher protein content under DPF than under CF respectively. The different fertilizer application methods under drip irrigation with plastic-film-mulch had no significant effect on the protein contents of both cultivars. The average protein content of Koshihikari and Norin 24 was 27% and 17% higher under DPF and DPD, respectively, than under CF. This indicates that drip irrigation system irrespective of the fertilizer application methods significantly increased the protein contents of the rice cultivars.

Table 3-4: Appearance, cooking and nutritional qualities of rice cultivars under varying irrigation systems during the rice-growing season of 2018

Cultivar†	System‡	Chalky grain (%)	Grain length/width	Grain thickness (mm)	Amylose content (%)	Protein content (%)
Koshihikari	CF	11.4a§	1.84a	1.83b	21.18ab	9.41b
	DPF	17.7a	1.77b	1.87a	20.15a	12.40a
	DPD	16.6a	1.77b	1.90a	24.40b	10.88ab
Norin 24	CF	31.7a	1.94a	1.77a	21.15a	11.56b
	DPF	48.2b	1.95a	1.77a	18.33a	14.12a
	DPD	46.5b	1.94a	1.77a	21.78a	13.71a
Cultivar (C)		***	***	***	ns	**
System (S)		**	**	*	*	**
C × S		ns	***	*	ns	**

*, **, *** significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. ns = non-significant.

†Koshihikari, a lowland *Japonica* cultivar; Norin 24, an upland *Japonica* cultivar; Princessari, a lowland cultivar, a derivative of *Indica* and *Japonica* crosses.

‡CF, continuous flooding; DPF, drip fertigation with plastic-film-mulch; and DPD, drip irrigation with direct fertilizer application under plastic-film-mulch.

§Within a column for each cultivar, different lowercase letters indicate a significant difference ($P < 0.05$) among irrigation systems.

3.4 Discussion

Water is a crucial resource for the growth and productivity of rice plants. However, continuous flooding has been reported to results in a large number of unproductive water outflows through evaporation, seepage, and percolation (Wu *et al.*, 2017). The introduction of the water-saving techniques has shown that flooding is dispensable for rice production. Nonetheless, apart from saving water, the ability of non-flooded irrigation systems to produce yield that will be comparable to that under continuous flooded system remains debatable.

Drip-irrigated rice suffers Fe-chlorosis mainly at the early vegetative stage in alkaline soil (J. Zhang *et al.*, 2017). This was primarily because Fe^{3+}, the most common of Fe in aerobic soil has low solubility, especially in calcareous or alkaline soil. The available-soil Fe deficiency determined under slightly acidic soil (pH = 5.8) in this study (Figure 3-5) was within the range of 4.6 to 6.9 reported by Fageria et al. (2009) in acidic soils that decreased Fe uptake of upland rice. However, the application of nano-size iron oxide (Fe_2O_3) alleviated the Fe-chlorosis effect under DPF and DPD in both years as shown by the non-significant SPAD values of Koshihikari and Norin 24 under the varying irrigation systems at the grain-filling stage. The reduction in Fe chlorosis resulted from the high penetration of Fe_2O_3 into the leaves due to the fast dissolution kinetics of the Fe_2O_3 nano-size particles (Alidoust and Isoda, 2014). However, the recovery of the chlorophyll content of the cultivars (Koshihikari and Norin 24) under DPD was more effective in 2018 than in 2017, probably as a result of the higher frequency and quantity of Fe_2O_3 supplied in 2018.

In this study, the response of Koshihikari and Norin 24 with respect to grain yield was similar in both years. Consistent with this similarity, water-use efficiency, and harvest index were not affected by the differences in soil moisture conditions of the years. This shows that the adopted range of soil moisture conditions, 70-75% and 65-70% volumetric soil moisture content in 2017 and 2018 respectively, had a similar effect on yield. The absence of yield loss for Koshihikari and Norin 24 under DPD and DPF compared with CF indicated that both lowland and upland high-yielding varieties could achieve the grain yield potential of the continuous flooding system under drip irrigation with plastic-film-mulch. This study showed that the increase in the number of spikelets per panicle was a major contributor to the high yield obtained under drip irrigation with plastic-film-mulch irrespective of the fertilizer application methods compared with CF. This result was in contrast with the report of Zhang *et al.* (2008) who reported fewer spikelets per panicle for *Japonica* and *Indica* F_1 hybrid cultivars under non-flooded, plastic-mulch cultivation system. The discrepancies in these observations might be caused by the difference in stress sensitivity at the spikelet-differentiation stage as a result of the differences between F_1 hybrid and inbred cultivars.

The significant decrease in the Fv/Fm of Princessari in 2017 under DPF and DPD compared with CF indicated partial damage to PSII by photoinhibition as a result of mild water stress. The damage to PSII suggests that the decrease in the grain yield of Princessari under DPF and DPD was attributable to the effect of Fe deficiency in the soil and mild water stress interaction. This synergy resulted in a significant reduction of the number of tiller per m^2, leaf area index (LAI), dry matter accumulation and the resulting grain yield (Saitoh, 1993).

The different methods of fertilizer application adopted in this study (DPF and DPD) similarly influenced grain yield and grain qualities of the understudied cultivars. This could be attributed to the high natural fertility of the soil in the experiment location. The soil in this region has been classified as the low-humic andosol (volcanic ash soils), reported having a high soil organic carbon level and high water and nutrients retention capacity (Takahashi and Shoji, 2002). This probably aided the sufficiency of the direct fertilizer application method (DPD) to sustain crop growth during the vegetative period before additional fertilizer was applied at the panicle initiation stage. The soil total nitrogen is an important index for soil fertility evaluation (Sui *et al.,* 2005). The high nitrogen content (3.8 g kg^{-1}) of the soil before the experiment also indicates the neutralization of the effect of fertilizer application methods on cultivars' agro-physiological characteristics under drip irrigation with plastic-film-mulch systems. This implies that DPF could be a better rice production system than DPD under soil with low fertility, provided that higher nutrient-use efficiency would be attained.

The advent of GCRPS has shown great potential (Liu *et al.,* 2013; Zhang *et al.,* 2017). However, a high amount of water is still required to fill furrows between the raised beds. In this study, the DPF and DPD saved 87-103% and 104-106% of irrigation water, respectively compared with CF in both years. On average, DPF and DPD saved 31% and 51% of irrigation water more than the GCRPS (transplanting) and GCRPS (direct seeding), respectively (Tao *et al.,* 2006; Tao *et al.*, 2015), and 69% more than the AWD compared with continuous flooding system (Yao *et al.* 2012). The water-saving capacity of the DPD was influenced by the direct supply of water to the rhizosphere by the drip irrigation system in addition to the water-conserving-effect of the plastic-film-mulch. This was in line with Dong *et al.* (2009) and Jabran *et al.* (2015) in that plastic-film-mulch has a great ability to conserve moisture, resists the growth of weeds and raises soil temperature. These lead to the improvement of rice productivity under water-saving rice production system. The range of WUE of 0.4 to 0.9 kg m^{-3} achieved in this study under DPF and DPD was higher than the common values of 0.2 to 0.5 kg m^{-3} for flooded rice reported elsewhere (Kato *et al.,* 2009). The average WUE was 32% higher than that reported by He et al. (2013) under DPD in a semi-arid region. The difference in WUEs could be attributable to the decrease in grain yield (Liu *et al.,* 2019) which could be responsible for reduction in water productivity. He *et al.* (2013) reported a drastic yield decline under DPD which was ascribed to the dry ecotype effect. However, long-term studies involving more cultivars under different environmental conditions are needed to better understand the response of cultivars under drip irrigation with the plastic-film-mulch system.

The quality of rice grains under non-flooded cultivation system remains a major concern (Zhang *et al.*, 2008). This study revealed that both the DPF and DPD improved the nutritional quality of rice. The amylose contents of Koshihikari and Norin 24 under different irrigation systems were within the range of intermediate amylose content (18-25%). Rice cultivars with amylose content in this range were reported to have good cooking and eating characteristics (cooked rice was soft and less sticky) (Zhang et al. 2012). Chalky grain formation increased significantly as a result of high temperature and environmental stresses during the critical period of 12 to 16 days after heading (Counce et al. 2000; Wu et al. 2016). Therefore we assumed that the significantly higher chalky grains of Koshihikari and Norin 24 under DPD and DPF compared with CF, resulted from the low tolerance-ability of the cultivars to high air-temperature after the heading stage in 2018.

The mechanism that illustrates the effect of the non-flooded rice production system on rice quality is yet unclear. In this study, it was observed that the chlorophyll content, CO_2 assimilation rate, actual quantum yield and maximum quantum yield of Koshihikari and Norin 24 were high and statistically similar at the grain-filling stage under DPF, DPD, and CF in both years. This resulted in the supply of more assimilates to the sink organ (spikelet) and consequently produced grains with good qualities.

Previous studies have reported that the response of rice to irrigation regimes varied with rice genotypes (Chu *et al.,* 2014; Wang *et al.,* 2016). This study showed that the existing variation among cultivars under drip irrigation with plastic-film-mulch and the continuous flooding did not depend on whether the cultivars were lowland or upland but on their ability to cope with soil Fe-deficiency and mild water stress.

Also, the higher number of spikelets per panicle produced under DPF and DPD compared with CF was offset by the differences in the grain-filling percentage and grain weight caused by the irrigation systems. This indicated that cultivars under DPF or DPD have the potential to produce grain yield that could be more than that under CF. Therefore, to maximize the potential of the drip irrigation with plastic-film-mulch system irrespective of fertilizer application methods, source strength rather than sink size is required to supply resources necessary for the development of grains (Sheehy *et al.,* 2001). To achieve this, a cultivar's adaptation to soil-Fe deficiency and mild water stress would be essential to prevent severe photoinhibition and reduction in CO_2 assimilation which could lower assimilate production and grain yield under DPF or DPD.

3.5 Conclusions

These results indicated that high chlorophyll contents and high photosystem II efficiency at the grain-filling stage of rice were the key characteristics for obtaining rice grain yield under DPF and DPD that is comparable to that under CF. Among the yield components, the high number of spikelets per panicle was responsible for the comparable yield obtained under DPF and DPD in relation to CF. The DPF and DPD increased WUE and improved the nutritional quality of rice grains; however, it increased the chalky grain percentage compared with CF.

The fertility of the experimental soil influenced the effect of the fertilizer application methods under drip irrigation with plastic-film-mulch, with both DPF and DPD producing similar grain yield and grain quality. However, the DPD produced higher water-use efficiency than DPF. Therefore the DPD would be an efficient water-saving technique for rice production in areas with high soil fertility but with limited water resources.

Moreover, there was considerable variation in the response of cultivars to the different irrigation systems. Cultivar response under DPF and DPD depended on the cultivar's adaptability to Fe deficiency in the soil and mild water stress. Therefore, knowledge of the variation in cultivar adaptability and the adaptive mechanisms of rice cultivars to Fe deficiency, mild water stress, and their interaction would help in the selection and breeding of suitable cultivars for drip irrigation with plastic-film-mulch system.

CHAPTER FOUR

GENERAL DISCUSSION AND CONCLUSION

4.1 General discussion

Global agriculture in the 21st century is expected to double food production to meet the demand from a growing population while simultaneously reducing its environmental footprint in the face of challenges like water scarcity and climate change (Foley *et al.*, 2011). Proffering solution to this major challenge, water-saving rice production systems have been introduced in irrigated rice to reduce the environmental burden associated with rice cultivation without jeopardizing rice production and global security. However, reconciling agricultural productivity and environmental integrity is challenging due to multiple interacting proximate and distal factors that drive the process of greenhouse gas emissions especially CH_4 and N_2O (Chapin *et al.*, 2011). The contrasting emission pattern of CH_4 and N_2O with almost no overlap in production (Hou *et al.*, 2000) has made rice cultivation management that will address both rice yield improvement and greenhouse gas mitigation a difficult task. Therefore, understanding and balancing the trade-off between these two prominent gases are important steps toward advocating a rice production system on the grounds of climate mitigation.

Previous studies have reported mitigation of CH_4 with the use of water-saving rice production systems such as alternate wetting and drying (AWD) (Ahn *et al.*, 2014; Yang *et al.*, 2012). However, an increase in N_2O emissions during the drying period could possibly negate the reduction in CO_2-eq emission from reduced CH_4 emission during the wetting period under the alternate wetting and drying rice production system (AWD) (Lagomarsino *et al.*, 2016).

Agronomic and the economic performance of the water-saving techniques are not only attached to optimal yield but also to the nitrogen rates required to achieve these yields. An increase in yield at high nitrogen application rates of 180-240 kg N ha^{-1} (LaHue *et al.,* 2016) and 200-300 Kg N ha^{-1} (Wang *et al.,* 2016) have been reported in the study carried out in America and China respectively. This high nitrogen application rate has been linked to the cause of nitrogen fertilizer-use inefficiency with an apparent recovery efficiency of only 33% on average (Ju *et al.,* 2009; Garnett *et al.,* 2009). This increases ammonia volatilization, nitrification and N loss as N_2O emissions through denitrification. Thereby, making N management to achieve yield goals while minimizing environmental losses, a considerable challenge (Han *et al.,* 2017).

High grain yield, optimum water-use efficiency and nutrient-use efficiency are determined not only by irrigation regimes but also by their interaction with N rates (Wang *et al.* 2016). In this study, the use of drip irrigation with plastic-film-mulch was advocated on the premise of its water-saving ability and nutrient-utilization improvement to attain high yield. As expected, CH_4 was the major greenhouse gas responsible for the seasonal CO_2-eq emission of the continuous flooding system in both years. The dominance of CH_4 emission was attributed to the anaerobic condition, as flooding remains a critical driver to methanogenesis (Ma *et al.*, 2010). However, drip irrigation with plastic-film-mulch system reduced seasonal CH_4 emission by 194% and 69% in 2016 and 2017 respectively. The significant reduction in methane emission under DPD was attributed to the aerobic-soil conditions leading to negligible CH_4 emission. Also, the variation in methane emissions between years in each treatment was in line with a long term study in China by Dong *et al.* (2011) in that under the same treatment (except for depth in continuously flooded fields) CH_4 emissions varied by 68% over three years. This indicates that the quantity of methane produced is highly variable over space and time. It is noteworthy, that the CF plot in this experiment was previously cultivated as upland with no previous record of conventional flooding system practice. The introduction of anaerobic condition to a previously cultivated upland-soil resulted in the reduction of seasonal methane emissions in 2016 compared with 2017 under CF due to the probable fewer population of methanogenic archaea capable of methane production in 2016. Xu *et al.* (2003) reported that methane production increased when the soil had been flooded in the previous season.

Nitrogen fertilizer rate had the most significant impact on N_2O emissions. This is in line with the study of Bouman *et al.* (2002) who reported that N_2O emissions were mainly controlled by fertilizer rates. The low N_2O emissions recorded under drip irrigation with plastic-film-mulch system could be ascribed to the low nitrogen fertilizer input (60 kg N ha^{-1}). On average, the fertilizer rate in this study is 71% and 76% lower than those reported by LaHue *et al.* (2016) and Wang *et al.* (2016) respectively. Fertilizer management strategies that aim to increase crop uptake by optimizing application rate, chemical composition, timing and placement of fertilizer have been proposed by previous studies (Chen *et al.*, 2011; Venterea *et al.*, 2016) as a mitigation strategy to nitrogen losses. The fertilizer application rate, frequency, methods adopted in this study has shown that the drip irrigation with plastic-film-mulch is a system that incorporates and accommodates this strategy. Hence, qualifying it to be a potential water-saving rice production system capable of minimizing environmental consequences. This relationship between N rate and N_2O emissions is congruent with studies linking N rates to the size of soil inorganic N pool, nitrate leaching and total N losses (Boy-Roura *et al.*, 2016; Shaddox *et al.*, 2016). Other probable explanations for the low N_2O emissions under this study include: (a) an enhancement of nutrient uptake through the temperature increasing effect of plastic-film-mulch thereby reducing losses of N through N_2O emissions. (b) a discontinuous aerobic condition. The drip irrigation with plastic-film-mulch system was considered to have a discontinuous aerobic condition under this system. This could be ascribed to the optimum soil saturation level (70%) in addition to the effect of the plastic-film cover which probably depleted the oxygen concentration in the deeper soil depth. This possibly created the intermittent aerobic and anaerobic condition, thereby resulting in the negligible N_2O emissions under this system. This indicates that creating a near-equilibrium environment for CH_4 and N_2O production by optimizing both water and nitrogen content of the soil under plastic-film-mulch has the potential to reduce the CO_2-eq emission.

Temperature plays an important role in the rate of activity of soil microorganisms. The weekly averages of soil temperature of both CF and DPD were high (close to 30°C) and similar during the periods of sample collection in both years. At high temperatures, the rates of nitrification and denitrification increase, thereby reducing the production and emission of N_2O as an intermediate product. Moreover, soil-temperature at around 30°C enhances methane formation (Inubushi *et al.,* 1990). The range of soil moisture (60-80%) under favorable soil temperature contributed to the methane fluxes observed under DPD.

This study showed that the drip irrigation with plastic-film-mulch system decreased irrigation water and increased WUE when compared with CF treatment. Evaporative-loss accounts for about 16-18% of total water loss under the traditional flooded rice (Wang and Zhou, 2000). This remains a critical pathway for water loss especially in regions with high temperatures during the rice-growing seasons (Ahmad *et al.,* 2014). On average across three years, drip irrigation with plastic-film-mulch system saved approximately 36% of total water input compared with CF. This was majorly attributed to the irrigation application method and the water-conserving effect of the plastic-film-mulch. The drip pipelines supply water directly to the rhizosphere, while the plastic film act as a barrier between the soil and the atmosphere thereby reducing excessive water loss through evaporation. This agrees with previous studies that mulches are helpful in reducing water inputs for successful crop production, water conservation and increased grain yield (Jabran *et al.,* 2015; Zhang *et al.,* 2008). Moreover, the effectiveness of plastic-film-mulch in the improvement of water productivity of rice compared with the no-mulch water-saving system has been reported (Jabran *et al.*, 2015).

The low water-use efficiency of paddy rice has been ascribed to deep percolation and seepage, constituting the largest percentage of (50-72%) of water loss (Wang and Zhou, 2000). The experimental plots of the continuous flooding system in this study were made of concrete frame demarcations which contradicts the condition of the normal paddy rice production system. Therefore we assumed that the concrete-demarcated plots probably reduced unproductive water outflows in the CF system. This creates a possibility of higher water-saving potential for drip irrigation with plastic-film-mulch if compared with conventional rice production under normal field conditions. The drip irrigation with plastic-film-mulch significantly increased WUE of Koshihikari and Norin 24 as a result of high grain yield production under reduced water usage. However, Princessari showed low but non-significant WUE under DPF and DPD compared with CF. The decreased WUE was attributable to the significant decrease in grain yield under both DPD and DPF compared with CF.

The similarity in the grain yields of Koshihikari and Norin 24 under DPF and DPD compared to that produced under CF was attributed to the insignificant differences in the agronomical and physiological parameters of the cultivars under the different irrigation systems. Productive tillers, high leaf area index, and aboveground dry matter are agronomical characteristics that translate to high yield in rice. These were found to be comparable for all cultivars under drip irrigation with plastic-film-mulch systems and continuous flooding with the exception of Princessari. Koshihikari and Norin 24 showed high adaptability to stress under the DPF and DPD has shown by the non-significant transpiration rate and stomatal conductance of the cultivar under the varying systems. In addition, the high SPAD values and similar chlorophyll fluorescence results of Koshihikari and Norin 24 at the grain- filling stage under varying irrigation systems indicated the active state and degree of functionality of the photosynthetic apparatus of the cultivars' leaves. Hailemichael *et al.* (2016) reported a positive relationship between Fv/Fm and chlorophyll contents. This contributed to the high spikelets formation, high grain yield and grain quality of Koshihikari and Norin 24 irrespective of the irrigation system.

Drought avoidance and tolerance strategies are part of plant responses to control water stress and resist drought (Shahensah and Isoda, 2010). Field crops, generally avoid water stress by maximizing water uptake by increasing root depth and or minimizing water loss by stomatal control (Isoda and Wang, 2002). Under mild water stress condition, stomatal conductance, internal CO_2 concentration and/or net assimilation decline (Van Leeuwen *et al.*, 2009). The significant reduction in transpiration rate and stomatal conductance of Princessari under drip irrigation with plastic-film-mulch depicted adjustment mechanisms that allow the photochemical and biochemical systems to cope with the stress. Catalina *et al.* (2011) and Zarco-Tejeda *et al.* (2013) reported that other factors such as Fe deficiency chlorosis could strongly affect the foliar chlorophyll contents in crops. The significant reduction in SPAD value and chlorophyll fluorescence parameters of Princessari under drip irrigation with plastic-film-mulch was not only as a result of mild water stress but also strongly influenced by Fe deficiency (Zhang *et al.*, 2015; Zhang *et al.*, 2017). Drip-irrigated rice frequently exhibits chlorosis at the seedling stage of rice growth. The seedlings are stunted and may die (Zhang *et al.*, 2015). However, in this study, the application of nanosize iron oxide at the late tillering stage in 2017 alleviated chlorosis of Princessari and improved colouration. Nonetheless, the number of tillers, leaf area index, and dry matter accumulation were significantly reduced, and consequently the significant reduction in grain yield. Therefore, we conclude that the decreased yield components of Princessari were as a result of soil-Fe deficiency and mild water stress interaction and not of a solitary component.

The introduction of drip fertigation with plastic film mulch in this study showed that comparable grain yield and grain quality can be achieved under drip irrigation with plastic-film-mulch system irrespective of the method of fertilizer application (basal or fertigation). However, we assumed that there could be a further reduction in N_2O emissions under drip fertigation which could lead to lower CO_2-eq emission under this system. This assumption was based on its effective and relatively easy supply of N doses to rice; hence, avoid excess N supply which would increase the risk of N losses and luxuriant vegetative growth. Therefore we recommend extensive research on the impact of drip fertigation on GHG emissions from rice field.

4.2 General conclusion and recommendation

The drip irrigation with plastic-film-mulch system saved water while maintaining comparable grain yield with continuous flooding system. This water-saving system reduced CO_2-eq emission as a result of water and nitrogen optimization under plastic-film. This in- turn created an interchangeable environment, favorable for low methane and nitrous oxide production and emissions.

This study showed that drip irrigation with plastic-film-mulch system increased rice production with less water and simultaneously reduced environmental footprint. Therefore, this technique should be adopted for rice production, most especially in developing countries where construction of paddies and irrigation dams remain unaffordable. This system will help to boost rice production, thereby alleviating food shortage as well as mitigating the contribution of rice production to climate change. However, the adaptability of cultivars is necessary to obtain high grain yield and grain quality under this promising water-saving system.

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