Evaluation of root distribution and nitrate leaching in sugarcane, *Erianthus*, and their intergeneric hybrid at new planting

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**ABSTRACT**

Nitrate-nitrogen leaching from farmland has adverse effects on drinking water and environmental conservation in tropical and subtropical island regions such as the Southwest Islands of Japan. Sugarcane is widely grown in these areas, and it is necessary to increase the nitrogen use efficiency of this crop to reduce nitrogen leaching. Studies on nitrogen utilization in this species have focused on yield potential and fertilizer management; however, there have been only a few breeding attempts. The relationship between root system characteristics and nitrogen utilization is also unclear, while improvement of nitrogen utilization using *Erianthus* can be expected because of its unique root system characteristics. In the present study, nitrogen leaching and root system characteristics of sugarcane × *Erianthus* intergeneric hybrid and parental genotypes were investigated using a lysimeter to verify the possibility of improving nitrogen utilization characteristics. Nitrogen leaching was significantly lower under the parental *Erianthus* from the early growth stage, while it was significantly lower in the intergeneric hybrid in the mid-growth stage than that in the parental sugarcane. The nitrogen use efficiencies of *Erianthus* and the intergeneric hybrid were significantly greater than that of sugarcane. *Erianthus* and the intergeneric hybrid exhibited lower shoot/root ratio and deeper rooting than sugarcane and consumed significant amounts of soil moisture in the deeper layers, suggesting that root mass and deeper rooting may be factors in reducing nitrogen leaching. These results indicate the possibility of improving the nitrogen utilization characteristics of sugarcane by improving its root system characteristics using *Erianthus*.

**INTRODUCTION**

In islands and coastal regions, crop production areas and the natural environment and residential areas are so close that watershed or ocean pollution is caused by soil erosion from farmlands and eutrophication, and domestic water pollution is caused by the runoff of fertilizers and agrochemicals (Thorburn et al., 2013). Nitrogen is an essential element for plant growth; however, application in excess to the plant demand and soil nutrient holding capacity, can result in underground water pollution (Ishida et al., 2011; Thorburn et al., 2011). In tropical and subtropical island regions such as the Southwest (i.e. Nansei) Islands of Japan, the islands are topographically divided into two categories (Mezaki, 1980): islands where nutrient runoff from farmland is pronounced due to steep topography (e.g. Ishigakijima Is. and Oshima Is.; Umezawa et al., 2002), and islands where nutrients easily percolate into groundwater due to flat topography and the low water-holding capacity of the soil (e.g. Miyakojima Is. and Okino-erabujima Is.; Nakanishi, 2001; Tashiro & Taniyama, 2002), both of which have problems of nutrient leaching from

**KEYWORDS**

*Erianthus arundinaceus*; introgression; nitrate-nitrogen; nitrogen use efficiency; shoot/root ratio; sugarcane
agricultural land (Nakanishi, 2017). In many of these islands, sugarcane (Saccharum spp.) is of economic importance and occupies most of the farmland area. Therefore, improving the nitrogen use efficiency (NUE) of this crop and reducing N leaching from the soil is necessary for environmental conservation in coral reefs and for improving the safety of drinking water (Nakanishi, 2017).

So far, the studies on N utilization in sugarcane have focused on agricultural measures, such as fertilization management and improvement of soil nutrient holding capacity. Cultivation trials with gradients in N fertilizer application rate and planting density have been conducted in various soils and regions to improve yield potential (optimal N fertilization to maximize yield) based on high input (Dinh et al., 2017; Irvine et al., 1980; Wiedenfeld, 1995). In contrast, in the Australian coastal production areas, including the Great Barrier Reef, concerns about the environmental impact of N leaching and runoff from farmland have pioneered a strong focus on intensive N management by reducing the use of inorganic fertilizers (Keating et al., 1997), introducing green manure (Garside & Bell, 2001; Park et al., 2010) and compost (Brackin et al., 2015), monitoring water quality (Bainbridge et al., 2009), and incorporating other methods for improving NUE in sugarcane production (Keating et al., 1997; Robinson et al., 2016; Skocaj et al., 2013). In the Southwest Islands of Japan, there are also many reports on N management in terms of environmental conservation owing to its geographical characteristics. Interviews with growers regarding fertilizer application practices and groundwater surveys in the late 1990s suggest that nutrient concentrations in the groundwater have increased since the 1960s, especially due to N leaching from the basal fertilizer applied during new planting (Nakanishi, 2001). In fact, subsequent trials using lysimeters, that is, large soil tanks that allow accurate observation of drainage water, have shown that N leaching from the basal fertilizer accounts for most of the leaching during the entire growing season in new planting (Kaji & Nagatomo, 2014; Okamoto et al., 2021).

It has also been reported that N leaching in ratoon cropping is lower (Okamoto et al., 2021), and NUE is higher than that in new planting (Kaji & Nagatomo, 2008, 2014), as in Brazil (Franco et al., 2011). This may be due to the stagnation of nutrient and water absorption during the slow initial growth period until the development of the root system in the new planting compared to ratoon cropping, where nutrient and water supplies from the underground stubble and developed root system are expected (Hiyane et al., 2021; Kaji & Nagatomo, 2008). Therefore, especially at new planting in these areas, the reduction of basal N fertilizer and the use of alternative fertilizers (e.g. compost and slow-release fertilizers) and organic materials that enhance fertilizer retention have been suggested as agronomical measures to improve N utilization (Kaji & Nagatomo, 2014; Kameyama et al., 2012; Okamoto et al., 2021).

However, there have been few breeding attempts for improving the N utilization in this crop. Based on the data from the above-mentioned previous studies, the rapid development of the root system in the early growth stage at new planting and improved ratooning ability are the desirable characteristics (and plant breeding targets) for improving N utilization in sugarcane. Robinson et al. (2014) highlighted physiological traits, such as photosynthetic NUE and N metabolism, as breeding targets, referring to examples in other crops. Sugarcane cultivars and breeding lines have been tested under a gradient of N conditions, and genetic variations in NUE and absorption characteristics have been observed (Ishikawa et al., 2009; Robinson et al., 2011; Yang et al., 2019). Genetic variation within cultivars has also been observed in root system formation ability, which is thought to be related to nutrient absorption (Fukuzawa et al., 2009; Pierre et al., 2019). However, the genetic variations in these groups of cultivars are likely to be the result of indirect breeding selection and rely heavily on the fact that the cultivars have been selected from field selection trials, including those in low-fertility areas; therefore, NUE has not been a clear breeding target (Acreche & Igartua, 2017). This may be due to the lack of established selection indices, especially simple measures and a narrow genetic base (Robinson et al., 2014; Schmidt et al., 2015). The limitations of breeding by crossbreeding using cultivars have been pointed out recently, and it is necessary to expand the genetic base by using closely related genetic resources and introducing their promising traits in the crops for the future (Jackson, 2019; Wei & Jackson, 2016). For improving N utilization characteristics as well as other characteristics, breeding improvement using closely related genetic resources should be attempted. Among the genetic resources collected from Japan, lines inhabiting sandy soils with low nutrient retention capacity (Nagatomi et al., 1984) and those with excellent N utilization characteristics have been reported (Ishikawa et al., 2009; Nose et al., 1994). It has been shown that root system development of sugarcane may be improved by interspecific hybridization with S. spontaneum (Hattori et al., 2018; Sakaiguchi et al., 2007; Terajima et al., 2005). In particular, the related species Erianthus arundinaceus not only exhibits
a vigorous root system (Matsuo et al., 2002; Sekiya et al., 2013) but also shows nitrate preference (Robinson et al., 2011; Takamizo et al., 2017), which is expected to be a promising genetic resource for the improvement of N utilization through intergeneric hybridization. However, there have been few reports on the improvement of N utilization characteristics and related root systems by intergeneric hybridization using this species. For example, although the nitrate preference of the intergeneric hybrid BC1 line (Robinson et al., 2011) and the improved root mass and root chemical components of the F1 line (Fukuhara et al., 2013) have been reported, there have been no investigations linking N utilization and root system characteristics. Therefore, in this study, the parent lines of sugarcane and Erianthus and the intergeneric hybrid F1 line were grown under indoor lysimeter conditions, and nitrate-N leaching, N uptake, and root distribution were investigated to reveal the possibility of improving the N utilization characteristics by intergeneric hybridization.

Materials and methods

Plant materials and its growth condition

The cultivation trial was conducted in a lysimeter installed in a glasshouse at the Tropical Agriculture Research Front, Japan International Research Center for Agricultural Sciences (24°22′43″ N, 124°11′4″ E), Ishigakijima Is. (one of the Nansei Islands). The lysimeter plot was designed as 0.25 m thick with a concrete frame of 1.55 m × 1.25 m size and 1.00 m soil depth (Ozawa et al., 2005; Figure 1). Each plot was filled with the local red soil (known as Kunigami mahji), and the previous crop was sugarcane. The chemical and physical properties of the tested soils are presented in Table 1. During the week before cultivation, desalination treatment by waterlogging was repeated until the nitrate-N leaching was less than 1 mg L⁻¹. Sugarcane variety ‘NIF8,’ Erianthus clone ‘JIRCAS1,’ and the intergeneric hybrid F1 (NIF8 × JIRCAS1) line ‘J08-12’ were used. J08-12 is a genetically confirmed hybrid line (Pachakkil et al., 2019). On 3 August 2020, seed canes were planted in

Figure 1. The cultivation and measurement conditions in a lysimeter in the present study.

| Soil depth | Texture grade | Sand (%) | Silt (%) | Clay (%) | Bulk density (g cm⁻³) | Saturated hydraulic conductivity (cm s⁻¹) | pH (H₂O) | Total carbon (%) | Total nitrogen (%) |
|------------|---------------|----------|----------|----------|-----------------------|------------------------------------------|---------|-----------------|-------------------|
| Top (0–0.3 m) | Sandy clay loam | 51.6 | 17.4 | 31.0 | 1.67 | 2.04 × 10⁻³ | 6.03 | 0.57 | 0.057 |
| Bottom (0.3–1 m) | Sandy clay | 47.1 | 15.5 | 37.3 | 1.48 | 6.54 × 10⁻³ | 6.59 | 0.37 | 0.051 |

Note: Soil texture was measured using a soil particle analyzer (PARIO, Meter Inc.), and its grade was classified according to US soil taxonomy. The bulk density and saturated hydraulic conductivity of the undisturbed soil sample in a 100 mL soil core were determined using a saturated hydraulic conductivity measurement kit (ML-9010C, mol Inc.). Soil pH and total nitrogen/carbon content were measured using a pH meter (D-220PC-S, Horiba) and an NC analyzer (NC22F, Sumika Chemical Analysis Service, Ltd.), respectively.
containers filled with commercial gardening soil. On 16 September 2020 (44 days after planting), two seedlings were transplanted in each plot with 20-cm plant spacing, and a total of four plots of each genotype were designed as replicates. Slow-release solid fertilizer at the rate of N: P: K = 12.0: 3.5: 5.0 g m⁻² (≈23.3: 6.8: 9.7 g plot⁻¹) was applied on 7 October 2020 (21 days after transplanting), and on 7 January 2021 (113 days after transplanting). Irrigation was performed close to the plants using an automatic drip irrigation system, and a total of 3 mm was supplied at 10 a.m. The air temperature and humidity in the greenhouse during the growing period were recorded using temperature and humidity sensors (TR-72U; T&D Co., Nagano, Japan). Solar radiation was measured using a solar radiation meter (CM21; Kipp & Zonen Inc., Delft, Netherlands) with a data logger (CR3000; Campbell Sci. Inc., UT, USA) installed in the neighboring fields outside the glasshouse. The indoor solar radiation values were obtained by converting the outside observed values taking into consideration the 52% shading ratio of the glasshouse.

**Measurement of nitrate leaching**

Percolation (drainage) water sampling from the bottom of the lysimeters and nitrate-N analysis were performed according to Okamoto et al. (2021) as follows. A water-level gauge (GYLT-01-300-BR-M8-CN; Santest Co., Ltd., Osaka, Japan) installed inside the column was used to measure the change in the water level and determine the amount of water drained. Drainage water data were recorded at 30-minute intervals using a data logger (CR1000X; Campbell Sci. Inc., UT, USA). Samples of drainage water were manually collected at a regular time every day from 16 September 2020, to 8 April 2021, and the concentration of nitrate-N was measured using a spectrophotometer (Hitachi U-2900; Hitachi, Tokyo, Japan). The daily nitrate-N load was calculated as the product of the nitrate-N concentration and the drainage water amount.

**Measurement of soil water consumption**

Soil moisture was monitored using soil moisture sensors (CS616; Campbell Sci. Inc., UT, USA) placed at 15, 30, 60, and 90 cm depth from the ground level and recorded using a data logger (CR1000X; Campbell Sci. Inc., UT, USA; Figure 1). A simple irrigation test was conducted before the harvest survey to observe the water consumption in each soil layer. A total of 50 mm of water was supplied in two splits (at 9:00, 15:00) on 23 April 2021, to saturate the soil moisture, and then irrigation was stopped for the next six days. Soil moisture at 0 days after irrigation (DAI) (15:00 on April 23) and 6 DAI (15:00 on April 29) was analyzed to observe soil water consumption.

**Measurement of biomass and N uptake**

The number of stems, culm length, number of fresh leaves, and SPAD value of the uppermost developed leaf (SPAD-502plus; Konica Minolta Inc., Tokyo, Japan) of the main stem were examined monthly for each plant from 13 November 2020, onwards. On 18 May 2021, the above-ground parts were harvested and broken down into plant parts to determine their dry matter weight. In the following two weeks, the plot soil was excavated, sieved, and washed to collect the roots and underground stems (stubble) and obtain the dry matter weights. Roots from the surface layer (30 cm) and the lower layer (deeper than 30 cm) were collected separately to calculate the root depth index (RDI) according to the equation below (Oyanagi et al., 1993), assuming a median soil depth of 15 cm and 65 cm for each layer.

\[
RDI \ (cm) = \sum (\text{Median of soil layer depth}) \times \text{Root mass percentage}
\]

The RDI represents the center of gravity of the root system and is thus used as a parameter for analyzing deep rooting ability. The dry matter obtained was ground, and the total N content was measured using an NC analyzer (NC22F; Sumika Chemical Analysis Service, Ltd., Osaka, Japan). The N uptake was calculated as the product of the total N content and the dry matter weight. Finally, the internal N use efficiency (NUE) was calculated according to the following equation (Wood et al., 1996).

\[
NUE \ (g \ gN^{-1}) = \frac{\text{Total biomass}}{\text{Total N uptake}}
\]

**Statistical analysis**

Data analysis was performed using a statistical analysis software (Excel-Tokei 2010; Social Survey Research Information Co., Ltd.). Differences among the mean values of examined parameters for each genotype were determined using the Tukey test, with statistical significance assumed at \( p < 0.05 \) (\( n = 4 \)). The t-test was performed only to determine significant differences among the mean values of soil water content at 0 and 6 DAI at each soil depth for each genotype (\( n = 4 \)).
Figure 2. Climatic conditions during the experimental period in the glasshouse. Solid black, solid gray, and dotted lines show the air temperature, relative humidity, and radiation data obtained in the experimental glasshouse, respectively. All data are shown as daily mean values.

Figure 3. Changes in nitrate-nitrogen concentration in drainage water during the growth period. Arrows indicate 1st and 2nd fertilization date. The dotted black, solid gray, and solid black lines represent the average values of NIF8, J08-12, and JIRCAS1, respectively (n = 4).

Results

Climatic conditions

The climatic conditions during the study period are shown in Figure 2. The mean air temperature of the study site showed a trend similar to that of the typical weather on Ishigaki Island during the same period. The temperature gradually decreased from December to January, reaching a minimum of 12°C, and then continued to rise from February until the end of the test. Solar radiation showed a trend similar to that of temperature. The average humidity during the study period was 69.8 ± 10.3%, although there were some fluctuations due to cloudy and sunny weather.

Nitrate leaching characteristics

The concentration of nitrate-N in the drainage water peaked approximately two months after each fertilization date (Figure 3). The peak value under JIRCAS1 after the first fertilization was 26.4 mg L⁻¹, which was lower than those under NIF8 (37.7 mg L⁻¹) and J08-12 (38.5 mg L⁻¹). After the peak, the values remained high in the order NIF8, J08-12, and JIRCAS1 until the second fertilization. In contrast, the peak value after the second fertilization was remarkable only under NIF8 (24.1 mg L⁻¹). Hereafter, to see the cumulative value during the study period, we divided the duration into three periods: from planting to the first
fertilization (P1), from the first to the second fertilization (P2), and from the second fertilization to the end of data collection (P3). The cumulative amount of drainage water for each period showed a genotypic difference only during P3, with NiF8 showing significantly higher values than those of the other genotypes (Figure 4(a)). The cumulative amount of drainage water for the entire study period was highest under NiF8, which was significantly different from that of JIRCAS1. The cumulative N leaching (i.e. N loading) for each period showed no difference during P1, whereas significantly lower values were observed under JIRCAS1 during P2, and under J08-12 and JIRCAS1 during P3 (Figure 4(b)). The total N loading during the whole study period was highest under NiF8 (44.0 kg ha$^{-1}$), followed by J08-12 (33.4 kg ha$^{-1}$; $p = 0.056$; vs. NiF8), and JIRCAS1 (18.3 kg ha$^{-1}$; $p< 0.001$; vs. NiF8). A histogram of the daily N load is shown in Figure 5. In NiF8, N loading was less than 0.1 kg ha$^{-1}$ on a few days and more than 0.1 kg ha$^{-1}$ on most days (Figure 5(a)). On the other hand, the number of days with the N load less than 0.1 kg ha$^{-1}$ was twice as high in J08-12 and JIRCAS1 compared to NiF8, although several days with N load higher than 0.6 kg ha$^{-1}$ were observed under J08-12 (Figure 5(b,c)).

Soil water consumption at different soil depths

Changes in soil moisture at each soil depth during P3 were shown in Figure 6. It appeared less changes in soil moisture at all depth under NiF8. On the other hand, soil moisture at deeper layers under J08-12 and JIRCAS1 gradually decreased until the end. In the irrigation test, soil moisture at 15 cm and 30 cm soil depths were significantly lower in all genotypes at 6 DAI compared to that at 0 DAI (Figure 7). On the other hand, there were genotypic trends in soil consumption in the deeper layers. There was no significant decrease in NiF8 at 60 and 90 cm soil depths (Figure 7(a)), whereas J08-12 and JIRCAS1 showed significant decreases (Figure 7(b,c)).

Biomass allocation and N uptake

The results of the growth survey are shown in Figure 8. Culm length tended to increase until March, in the order: JIRCAS1 > J08-12 > NiF8 (Figure 8(a)). After that, the culm length of JIRCAS1 stagnated because of heading in December, whereas that of other genotypes continued to increase. The number of stems tended to increase until March, in the order JIRCAS1 > J08-12 > NiF8 (Figure 8(b)). The number of green leaves and SPAD values showed
The results of the harvest survey are presented in Table 2. The total dry matter weight was significantly higher in J08-12 and JIRCAS1 than in NiF8. In particular, the root dry matter weight was higher in J08-12 and JIRCAS1 than in NiF8, resulting in a significantly lower shoot/root ratio. In addition, the RDI values, which represent the center of gravity of the root system, were significantly higher in J08-12 and JIRCAS1. The total N uptake was the lowest in NiF8, comparatively higher in JIRCAS1, although the difference was not significant (p = 0.111; vs. NiF8), and significantly higher in J08-12 (p < 0.05; vs. NiF8). The NUE values in J08-12 and JIRCAS1 were significantly higher than that in NiF8 (p < 0.01).

Discussion

Erianthus is a promising genetic resource for breakthroughs in sugarcane breeding, and although many pre-breeding studies have been attempted, the characteristics of sugarcane × Erianthus intergeneric hybrids have not been sufficiently evaluated. So far, there have been very few studies using these species as crossing materials that have focused on improving N utilization characteristics for environmentally friendly crop production. Robinson et al. (2011) showed the possibility of inheritance of nitrate preference using the Erianthus intergeneric hybrid BC1 line, but this was a pot test at the very early growth stage. In the present study, N leaching characteristics under sugarcane, Erianthus, and the intergeneric hybrid F1 line at new planting were evaluated using a lysimeter for approximately nine months, when the root system was considered to have progressed sufficiently. This study was conducted to verify the possibility of improving N leaching characteristics using intergeneric crosses. Briefly, the results showed that less N leaching was shown under intergeneric hybrid than under sugarcane, especially at the mid-growth stage (i.e. the grand growth stage; Figures 3, 4). In the following section, the factors that contributed to

small differences in J08-12 and NiF8, whereas they tended to decrease in JIRCAS1 after February (Figure 8(c,d)).

Figure 6. Changes in soil volumetric water content (VWC) relative to value of 2021/1/7 during P3 at each soil depth. Note: P3 means the period from 2nd fertilization to final sampling of drainage water (2021/1/7–2021/4/8). The dotted gray, dotted black, solid gray, and solid black lines represent the values at 15, 30, 60, and 90 cm depth, respectively. a, b, and c show the values for NiF8, J08-12, and JIRCAS1, respectively. Asterisk means missing data from 2021/2/17-2/19 due to a disorder of data logging system.

Figure 7. Soil water consumption at each soil depth for six days after irrigation (DAI). Note: a, b, and c indicate NiF8, J08-12, and JIRCAS1, respectively. * ** *** and ns indicate significant differences between mean values of 0 and 6 DAI in each soil depth at 5%, 1%, 0.1% level, and no significance, respectively (t-test, n = 4). Bars indicate the standard deviation (n = 4).
Figure 8. Monthly growth parameters. a, b, c, and d show the stem length, number of stems per plant, number of green leaves, and SPAD reading, respectively. The dotted black, solid gray, and solid black lines show the average values for NiF8, J08-12, and JIRCAS1, respectively (n = 8 plants). The stem length and number of green leaves were measured in the main stem. The top visible leaf of the main stem was used to read SPAD. Bars indicate the standard deviation (n = 8 plants).
this are discussed, focusing on the relationship between the N leaching characteristics and root biomass partitioning.

N leaching at each growth stage in relation to root system development

During P2, the peak of N leaching tended to be lower (Figure 3), and N loading was significantly lower under JIRCAS1 (Figure 4(b)). However, there was no genotypic difference in the cumulative amount of drainage water (Figure 4(a)). The above-ground nutrient/water requirements were considered low because the above-ground growth status in early December 2020, when the initial peak of N leaching appeared, was low (Figure 3, B). The reason for such a difference in N leaching in the face of low nutrient and water uptake could be the nitrate preference of this species (Robinson et al., 2011; Takamizo et al., 2017). Robinson et al. (2011) showed the possibility of improving nitrate preference of the intergeneric BC1 line using the 15N tracing method under pot conditions, but any reduction in N leaching in the early growth stage were not shown under the F1 line in this study, which could be attributed to less nitrate preference. Although only a single line could be used in this study, there is genetic variation in yield characteristics among the F1 population of *Erianthus* intergeneric hybrids (Pachakkil et al., 2019), which suggests that genetic variation in nitrate preference in hybrid populations should also be investigated. In addition, the experimental limitations in the present study, such as less radiation under greenhouse condition, less planting density under high input condition, and drip irrigation, may have affected the results of N leaching especially in early growth stage when plants grow slowly in poor stand and root system.

N leaching was more remarkable under NiF8 than in JIRCAS1 or J08-12 after the second application (P3; Figure 3). In contrast to P2, the second application of fertilizer was followed by an increase in stem length and number of stems (Figure 8(a,b)), indicating that P3 was a period of vigorous growth with sufficient above-ground demand for nutrients and water. Hence, the differences among the genotypes in soil N leaching during P3 were mainly due to differences in the degree of development of the root system that supports above-ground growth. In fact, root dry matter weight in JIRCAS1 and J08-12 was higher, and the shoot/root ratio was lower even though the data were obtained at the end of P3 (Table 2), suggesting that the root system was sufficiently formed to meet the requirements of above-ground growth compared to NiF8. This may have led to significantly lower water and N leaching under JIRCAS1 and J08-12 than in NiF8 after the second fertilization (P3; Figure 4).

### $N$ leaching in relation to the deep root system

The root system develops in deeper layers to acquire water/soluble N, which can easily percolate to deeper layers (Lynch, 2007). A positive effect of deep rooting gene on N uptake at the late growth stage, when N is insufficient in the shallow soil layer, has been reported in isogenic rice lines with deep rooting genes (Arai-Sanoh et al., 2014). It has also been reported that sugarcane develops deep root systems plasticly to search for N sources in the deeper layers, particularly under rainfed field and low N conditions (Otto et al., 2009). Previously, a simple comparison had shown that NUE was higher in a deep-rooted cultivar than in a shallow-rooted cultivar when grown using liquid fertilizer both under wet and dry pot conditions (Takaragawa et al., 2020). However, there is no report on the genotypic relationship between deep rooting and improved N utilization in sugarcane under field conditions (Robinson et al., 2014). Okamoto et al. (2021) showed that N leaching from basal fertilizer was significant in newly planted sugarcane, which may be due to insufficient root system formation during the initial growth period. The cultivar used by Okamoto et al. (2021), NiF8, is generally considered shallow-rooted compared to other cultivars (Fukuzawa et al., 2009; Takaragawa et al., 2020, 2018). The RDI in this study also showed that NiF8 has a shallow root system (Table 2). In contrast, JIRCAS1 and J08-12 had a larger root mass in the deeper layer and a greater RDI than NiF8 (Table 2). Trends in soil water consumption showed that NiF8 consumed less soil water in layers deeper than 60 cm, while the other two genotypes consumed significant amounts of soil water (Figures 6&7), suggesting

### Table 2. Biomass production, allocation, and nitrogen use.

| Genotype | Dry mass (ton ha$^{-1}$) | Shoot/root ratio | RDI (cm) | Total N uptake (kg ha$^{-1}$) | NUE (g gN$^{-1}$) |
|----------|--------------------------|------------------|----------|-------------------------------|------------------|
|          | Green leaves | Stem | Dead leaves | Underground shoot | Root at 0–0.3 m | Root at 0.3–1 m | Total |                  |                  |
| NiF8     | 3.3          | a    | 11.7       | a            | 0.3           | a                | 1.2    | 0.4    | a         | 0.1 | 17.0 | 0.8 | 17.0 | 30.7 | 24.8 | 104.5 | 163.5 |
| J08-12   | 5.2          | b    | 19.1       | b            | 0.7           | b                | 1.5    | 0.7    | b         | 0.4 | 27.6 | 4.8 | 24.8 | 31.6 | 20.2 | 136.3 | 202.4 |
| JIRCAS1  | 6.0          | b    | 13.3       | a            | 1.1           | c                | 2.5    | 2.1    | c         | 1.5 | 26.4 | 6.4 | 26.4 | 57.8 | 45.7 | 128.3 | 206.7 |

Note: Stem parts include stem and leaf sheaths. The dead part consists of dead leaves and a leaf sheath. Underground shoots were included in shoot when calculating the shoot/root ratio. Here, RDI and NUE are the root depth index and nitrogen use efficiency, respectively. Different letters indicate significant differences between the genotypes (Tukey’s test, $p < 0.05$, $n=4$). The bar represents the standard deviation for each parameter ($n=4$).
that roots extending into the deeper layers contribute to nutrient and water absorption. These results imply that the improvement of deep rooting character may play a considerable role in improving N load and NUE through intergeneric hybridization. However, for further evidence regarding the relationship between deep rooting and N utilization, it is necessary to validate the results using genetic populations that show diverse root system characteristics, such as isogenic lines with the same genetic background (Arai-Sanoh et al., 2014).

The discrepancy between trends in N leaching and absorption

The difference in N uptake between JIRCAS1 and the other genotypes was smaller than the difference in N leaching (Figure 4; Table 2). Even in soils with good permeability, as in this study (Table 1), all N derived from inorganic fertilization is nitrified and leached out as nitrate-N when it reaches deep soil layers (Nomura et al., 2012). In the present study, the input of N from fertilizer application was 240 kg ha⁻¹ (24.0 g m⁻²). Assuming that the N concentration in the irrigation water was 0.5 mg L⁻¹ (Tashiro & Takahira, 2001), the N input from the irrigation water was as low as 3.1 kg ha⁻¹ (data not shown), which means that a total of approximately 243.1 kg ha⁻¹ N was applied. Generally, the input N is consumed through leaching, denitrification, and plant absorption. Of the total N input, 44.0 (18%), 33.4 (14%), and 18.3 (8%) kg ha⁻¹ leached out as nitrate-N, in NiF8, J08-12, and JIRCAS1, respectively (Figure 4). Assuming that denitrification was <1% of the total input (Maeda et al., 2021), residual N was around 199.1, 209.7, and 224.8 kg ha⁻¹, which cannot account for the plant N uptake of 104.5, 136.3, and 128.3 kg ha⁻¹ in NiF8, J08-12, and JIRCAS1, respectively (Table 2). Therefore, the percentage of plant N uptake per residual N was 53, 65, and 57% in NiF8, J08-12, and JIRCAS1, respectively, suggesting the small difference in efficiency of residual N absorption among genotypes. This trend may be due to two possibilities: the selective absorption of nitrate-N by Erianthus (Robinson et al., 2011; Takamizo et al., 2017) and the high N absorption of NiF8 and J08-12 from sources other than fertilization. In NiF8, 20–30% of N uptake was reported to be derived from external N fixed by endophytic N-fixing bacteria (Hiyama et al., 2013). In addition, the mineralization of soil organic N is an important N utilization process. However, a degree of biological N fixation in the three genotypes and the dynamics of soil organic N under well remained N derived from fertilizer were not investigated in the present study. There is room for further research on N utilization in sugarcane, including the mechanisms of endogenous N fixation and mineralization capacity and their genetic improvement.

Improving N utilization characteristics by intergeneric hybridization

Similar improvements in root mass and shoot/root ratio have been reported in F₁ intergeneric hybrid between sugarcane and Erianthus using different parental genotypes (Fukuhara et al., 2013). The results of our study strongly support the possibility of root system improvement using Erianthus. This study further demonstrated the potential of improving N utilization characteristics by improving root system characteristics using Erianthus. The N leaching may be reduced by an improved root system, especially in the deep zone, during the mid-growth stage when nutrient and water demands are high. It is also important to note that for nitrate-N, which has the potential for acute toxicity to both humans and ecosystems (Camargo et al., 2005; WHO, 2011), not only the cumulative values but also the peak values and frequency for high values could be reduced by intergeneric hybridization (Figures 3–5). Owing to the limitations of the test design, only three genotypes were used in this study. For further research, it is necessary to develop a simple evaluation method to select genotypes with superior N absorption characteristics, which will contribute to screening populations for both traditional and molecular breeding approaches.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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