Factors Explaining Differences in Yield Response to High Nitrogen Fertilization among Rice Varieties under Tropical Highland Conditions in Central Kenya

Hiroaki SAMEJIMA1,2, Keisuke KATSURA3, Mayumi KIKUTA1,4, Symon Mugo NJINJU5, John Munji KIMANI5, Akira YAMAUCHI6 and Daigo MAKIHARA1*

1 International Center for Research and Education in Agriculture, Nagoya University, Nagoya, Japan
2 Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization, Sapporo, Japan
3 Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Japan
4 Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-hiroshima, Japan
5 Kenya Agricultural and Livestock Research Organization, Mwea, Kerugoya, Kenya
6 Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

Abstract
A decrease in the filled grain ratio (FGR) under high nitrogen (N) conditions inhibits the increase in rice yield in the tropical highlands of equatorial East Africa. We hypothesized that, under high N fertilization, the decrease in FGR is due to low temperatures during the reproductive growth stages, and that high grain yield can be achieved using cold tolerant varieties. Two cold-susceptible varieties (BW196 and Komboka) and a cold-tolerant variety (NERICA 1) were grown under 57, 114, and 171 kg N ha\(^{-1}\) of N fertilization. Grain yield increased with a higher N fertilization rate only in NERICA 1. Shoot dry weight and total spikelet number increased in all varieties under high N conditions. Although FGR decreased with increases in N fertilization rate in all varieties, the adverse effects of high N fertilization on FGR were least observed in NERICA 1. However, temperature did not affect FGR in all N treatments, growth stages, and varieties, except for Komboka during the ripening stage under high N conditions. The findings did not support the hypothesis that high-N-induced decreases in FGR are due to low temperatures. High-N-induced decreases in FGR in Komboka were mainly attributable to the poor filling of spikelets during the ripening stage due to excess total spikelets. Factors other than filling high-N-promoted increases in total spikelet number may affect FGR in BW196 and NERICA 1 under high N conditions in Mwea.

Discipline: Crop Science
Additional key words: East Africa, grain yield, filled grain ratio, low temperature, lowland rice

Introduction
Irrigated rice in the tropical highlands of equatorial East Africa represents one of the broad rice-ecosystem categories found in sub-Saharan Africa, in addition to the five other major rice ecosystems in West Africa (i.e., rain-fed upland rice on plateaus and slopes, rain-fed lowland rice in valley bottoms and floodplains, irrigated rice in deltas and floodplains, deep-water floating rice along major rivers, mangrove-swamp rice in lagoons and deltas) (Rodenburg & Demont 2009). The tropical highland locations in equatorial East Africa ensure high radiation and large day-night temperature differences (Njinju et al. 2018). High radiation and considerable differences in day-night temperatures are widely considered to promote rice growth (Katsura et al. 2008). Njinju et al. (2018) reported that potential biomass productivity levels in Mwea, Kenya (near the equator and approximately 1,200 m above sea level) are among the highest globally (20 t ha\(^{-1}\) or more) with adequate nitrogen (N) fertilizer applications. However, the increase in biomass did not increase grain yield due to decreased filled grain ratio (FGR) under high N fertilization (Njinju et al. 2018). It is thus necessary to...
investigate the factors causing high-N-induced decreases in FGR, which could facilitate efforts to enhance grain yield in equatorial East Africa.

In Mwea, FGR decreased and the number of spikelets per m² (total spikelet number) increased with increases in the amount of N fertilizer applied (Njinju et al. 2018). Such a trade-off could be due to multiple factors, including source limitation, sink limitation, and translocation limitation (Seki et al. 2015). In addition, such environmental stress factors as low temperature could decrease FGR, considering that plants exposed to low temperatures exhibit lower FGR under high N conditions than under standard N conditions (Hayashi et al. 2006). Cold-induced decreases in FGR are often associated with increases in sterility due to a reduction in pollen number per anther (He et al. 2018). High N fertilization is generally considered to increase total spikelet number, which reduces the production of pollen per anther, thereby increasing the susceptibility to cold stress during reproductive growth stages (He et al. 2018). Conversely, based on proteome analysis of mature anthers, Hayashi et al. (2006) suggested that high N conditions could represent some forms of stress conditions, and that the stress responses to high N conditions could enhance the damage associated with cool temperatures. Therefore, Njinju et al. (2018) suggested that low temperatures during the reproductive growth stages in the highlands caused lower FGR under high N fertilization in Mwea. Validation of the hypothesis could increase rice production in the tropical highlands of equatorial East Africa.

If the low temperatures in Mwea cause the reported high-N-induced decreases in FGR, the rate of decline would be less in cold-tolerant varieties than in cold-susceptible varieties. Accordingly, grain yield in the tolerant varieties would increase with increases in the amount of N fertilizer applied. BW196, a popular regional variety that was investigated in a previous study, was reportedly susceptible to cold (Njinju et al. 2018). Komboka was also observed to be susceptible to cold in our preliminary experiments. Komboka, which originated from the International Rice Research Institute, was released as high-yielding rice under lowland conditions in Kenya, Tanzania, and Uganda (Global Rice Science Partnership 2014, Kitilu et al. 2019). Conversely, NERICA 1 is a cold-tolerant variety (Wainaina et al. 2015, Samejima et al. 2020a). NERICA 1 was introduced in Kenya for upland conditions (Kimani et al. 2011), although its cultivation under lowland conditions is possible, similar to the cases in many upland varieties. Therefore, the present study investigated the growth and yield of cold-susceptible varieties and a tolerant variety under varying N fertilization rates in Mwea, Kenya.

**Materials and methods**

In 2015, seven field experiments were conducted at a research farm belonging to the Kenya Agricultural and Livestock Research Organization, in Mwea, Kenya (0°37′S, 37°20′E) at an elevation of 1,162 m above sea level. A weather station (Weather Bucket; Agriweather Inc. Sapporo, Japan) located on the research farm recorded air temperature and solar radiation every 10 min.

The three rice varieties—BW196, Komboka, and NERICA 1—were grown under three N treatments, including 57, 114, and 171 kg N ha⁻¹, which are hereafter referred to as 57N, 114N, and 171N, respectively. Three N applications of 19, 38, and 57 kg N ha⁻¹ were carried out in the three N treatments to achieve the 57N, 114N, and 171N treatments, respectively, as basal fertilizer and as N topdressing, 21 and 45 days after transplanting. The basal fertilizer was applied in the form of an NPK (17:17:17) compound fertilizer, and urea was used as the N topdressing. Therefore, 19, 38, and 57 kg ha⁻¹ of phosphorus (P) and potassium (K) were applied as basal fertilizer in the 57N, 114N, and 171N treatments, respectively. It is critical to note that the amounts of P and K fertilizer differed among the N treatments; however, more K and P were applied as the amount of N fertilizer increased. The N treatment was the main plot in a split-plot design with three replicates. A main plot contained three subplots (2 m × 2 m) and the varieties were assigned randomly to the subplots. The varieties were sown on the first day of every month from June to December 2015, and 21-day-old seedlings were transplanted and spaced at 20 cm × 20 cm with one seedling per hill. All experiments were conducted under continuously flooded conditions.

The number of days to 50% heading was recorded based on daily observations. We assumed that plants were in a vegetative stage from transplantation to 30 days before 50% heading, and in the reproductive and ripening stages for 30 days before and 40 days after heading, respectively. At maturity, the shoots were harvested at the ground level from 12 hills in all subplots. The harvested shoots were placed in paper bags and sun-dried for more than a month. Subsequently, the panicles were removed from the stems and threshed by hand. Filled grains were separated from unfilled spikelets by submerging them in tap water. Sunken and floating spikelets were considered filled grain and unfilled spikelets, respectively. Using the panicles and spikelets, grain...
yield (filled grain weight converted to 14% moisture content) and yield components were determined. The moisture content of the filled grains was measured using a grain moisture tester (Riceter f; Kett Electric Laboratory, Tokyo, Japan). The straws, panicle rachis, and unfilled spikelets were oven-dried at 70°C for a day and then weighed. Shoot dry weight (DW) was determined as the sum of grain yield and dry weight of the straws, panicle rachis, and unfilled spikelets.

Three-way analysis of variance (ANOVA) was performed to test the significant effects of the N treatments, variety, sowing month, and their interactions on grain yield and yield related parameters using SAS (SAS Institute Inc., Cary, NC, USA). R v3.0.2 (R Core Team 2015) was used for performing correlation analysis among FGR, mean temperatures, and solar radiation during each growth stage, and between FGR and total spikelet number using built-in cor.test.

**Results**

Figure 1 shows the grain yield, shoot DW, total spikelet number, FGR, and 1,000-grain weight in each variety sown monthly from June to December. Grain yield and yield-related parameters varied with the sowing month, N treatment, and variety. Table 1 lists the results of three-way ANOVA performed on the parameters. The effects of N treatment on grain yield and 1,000-grain weight were not significant, but those on other parameters were significant ($P < 0.001$). The three-way sowing month × N treatment × variety interaction had no significant effect on all parameters. The two-way interaction of N treatment × variety influenced grain yield, total spikelet number, and FGR significantly ($P < 0.01$ or 0.10), whereas the effect of the interaction on shoot DW and 1,000-grain weight was not significant. We evaluated the effects of N treatments on grain yield and yield-related parameters in each variety.
Figure 2 shows the average values of grain yield, shoot DW, total spikelet number, FGR, and 1,000-grain weight across the seven experiments. In BW196, grain yield was higher under the 57N treatment (370 g m\(^{-2}\)) than under the 114N and 171N treatments (309 and 348 g m\(^{-2}\), respectively). Grain yield in Komboka, which ranged from 291 to 320 g m\(^{-2}\), was similar among the N treatments. In contrast, grain yield in NERICA 1 increased from 242 to 355 g m\(^{-2}\) as the amount of N fertilizer increased (Fig. 2 (A)). Shoot DW in BW196, Komboka, and NERICA 1 increased from 939 to 1,212, from 944 to 1,319, and from 788 to 1,168 g m\(^{-2}\), respectively, with increases in the amount of N fertilizer (Fig. 2 (B)). Similarly, total spikelet number increased under high N conditions, irrespective of variety, although the difference between the 57N and 171N treatments was greater in Komboka (17,231 spikelets) than in BW196 (7,703 spikelets) and NERICA 1 (10,513 spikelets) (Fig. 2 (C)). Conversely, FGR decreased with increases in N fertilization, irrespective of variety (Fig. 2 (D)). The values in BW196 were 58.6% and 42.4% under the 57N and 171N treatments, respectively, with increases in N fertilizer, irrespective of variety (Fig. 2 (D)). The values in BW196 were 58.6% and 42.4% under the 57N and 171N treatments, respectively. The corresponding values in Komboka were 47.1% and 34.7%, and 54.3% to 48.9% in NERICA 1. The differences in 1,000-grain weight were negligible among N treatments, irrespective of variety (Fig. 2 (E)). In short, the adverse effects of high N fertilization on FGR were less severe in NERICA 1 than in the other two varieties.

Days to heading varied among varieties and increased with increases in N fertilizer amount, irrespective of variety (Table 2). In BW196, the range of mean temperatures during vegetative growth, reproductive growth, and ripening stages was 20.5-22.4, 21.3-23.8, and 21.2-23.8°C, respectively, across the seven experiments (Fig. 3 (A), (D), and (G)). The corresponding values in Komboka were 20.5-22.6, 21.2-22.9, and 21.2-23.6°C (Fig. 3 (B), (E), and (H)), and 20.4-22.8, 20.9-23.1, and 21.3-23.7°C in NERICA 1 (Fig. 3 (C), (F), and (I)), respectively. The mean solar radiation during the three growth stages across the seven experiments

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### Table 1. Effects of sowing month, nitrogen treatment, variety, and their interaction on grain yield and yield-related parameters, based on Analysis of Variance

| Sources of variation | Grain yield (g m\(^{-2}\)) | Shoot dry weight (g) | Total spikelet number per m\(^2\) | Filled grain ratio (%) | 1,000-grain weight (g) |
|----------------------|-----------------------------|---------------------|---------------------------------|-----------------------|-----------------------|
| Sowing month (M)     | ***                         | ***                 | ***                             | ***                   | ***                   |
| N treatments (N)     | ns                          | ***                 | ***                             | ***                   | ns                    |
| Variety (V)          | ns                          | ***                 | ***                             | ***                   | ***                   |
| M × N                | ns                          | ns                  | +                               | ns                    | ns                    |
| M × V                | ***                         | ***                 | ***                             | ***                   | ***                   |
| N × V                | **                          | ns                  | +                               | ns                    | ns                    |
| M × N × V            | ns                          | ns                  | ns                              | ns                    | ns                    |

***, **, *, and + indicate significance (P < 0.001, 0.01, 0.05, and 0.10, respectively) and ns indicates no significance (P > 0.10).
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were 12.1-17.2, 14.6-19.0, and 14.8-18.7 MJ m$^{-2}$ d$^{-1}$ in BW196 (Fig. 4 (A), (D), and (G)), 11.6-17.0, 14.7-18.4, and 14.8-19.0 MJ m$^{-2}$ d$^{-1}$ in Komboka (Fig. 4 (B), (E), and (H)), and 11.3-17.0, 13.7-18.1, and 14.8-18.8 MJ m$^{-2}$ d$^{-1}$ in NERICA 1 (Fig. 4 (C), (F), and (I)), respectively.

Across the seven experiments, each variety experienced virtually the same range of temperature and solar radiation in each growth stage, irrespective of N treatment (Figs. 3 and 4). During the ripening stage, the correlations between FGR and mean temperature, and

| Sowing month | BW 196 | Komboka | NERICA 1 |
|--------------|--------|---------|----------|
| June         | 57N    | 114N    | 171N    | Difference† |
| June         | 97     | 98      | 101     | 4          |
| July         | 95     | 96      | 97      | 3          |
| August       | 92     | 92      | 94      | 2          |
| September    | 94     | 96      | 98      | 4          |
| October      | 93     | 95      | 95      | 2          |
| November     | 90     | 93      | 94      | 4          |
| December     | 94     | 96      | 97      | 3          |

† Difference between the longest and shortest days to heading

Table 2. Days to heading under three nitrogen treatments in each variety sown monthly between May and December 2015

Fig. 3. Correlation between filled grain ratio and mean temperature during vegetative growth stage [(A), (B), (C)], reproductive growth stage [(D), (E), (F)], and ripening stage [(G), (H), (I)] for BW196 [(A), (D), (G)], Komboka [(B), (E), (H)], and NERICA 1 [(C), (F), (I)] in the seven experiments

* and + indicate significance (\(P < 0.05\) and 0.10, respectively).
between FGR and mean solar radiation were significant (PN < 0.05 or 0.10) in Komboka under the 114N and 171N treatments (Fig. 3 (H) and Fig. 4 (H)). In other cases, the correlations of FGR with mean temperature and mean solar radiation were not significant, irrespective of growth stage, N treatment, and variety, with two exceptions in BW196 (Figs. 3 and 4). The correlation between FGR and total spikelet number throughout the seven experiments was significant (PN < 0.001 or 0.05) negative in all varieties, but weak in BW196 (r = −0.436) and NERICA 1 (r = −0.472), and strong in Komboka (r = −0.842) (Fig. 5).

Fig. 4. Correlation between filled grain ratio and mean solar radiation during vegetative growth stage [(A), (B), (C)], reproductive growth stage [(D), (E), (F)], and ripening stage [(G), (H), (I)] for BW196 [(A), (D), (G)], Komboka [(B), (E), (H)], and NERICA 1 [(C), (F), (I)] in the seven experiments * and + indicate significance (PN < 0.05 and 0.10, respectively).

Fig. 5. Correlation between filled grain ratio and number of spikelets per m² for each variety in the seven experiments *** and * indicate significance (PN < 0.001 and 0.05, respectively).
Discussion

As expected, only NERICA 1, a cold-tolerant variety, increased its grain yield with increases in the amount of N fertilizer in Mwea, but the other results did not support the hypothesis that low temperatures during the reproductive growth stage caused high-N-induced decreases in FGR in Mwea. Across the seven experiments, shoot DW and total spikelet number increased with increases in N fertilizer amount in a cold-susceptible variety (BW196); however, grain yield did not increase because of low FGR (Fig. 2). The present study thus confirmed the high-N-induced decreases in FGR in BW196 previously reported by Njinju et al. (2018). Similarly, another cold-susceptible variety (Komboka) had results similar to those observed in BW196. In contrast, NERICA 1 increased grain yield, shoot DW, and total spikelet number, with minimal decreases in FGR, and with increases in N fertilizer amount (Fig. 2). The results suggest that there are varietal differences in the high-N-induced decreases in FGR under the tropical highland conditions in Mwea. However, temperature conditions in the present study did not influence FGR, irrespective of growth stage, N treatment, and variety, except for Komboka during the ripening stage under high N conditions (Fig. 3). In other words, the high-N-induced decreases in FGR in BW196 and Komboka were not attributable to low temperature during the reproductive growth stage.

Trade-offs between FGR and total spikelet number are often reported in rice (Seki et al. 2015). In the present study, Komboka, which had a higher total spikelet number than BW196 and NERICA 1 (Fig. 2 (C)), increased FGR under high temperature and solar radiation during the ripening stage under high N conditions (Fig. 3 (H) and Fig. 4 (H)). In Komboka, the increases in total spikelet number explained the decreases in FGR well (Fig. 5). The findings indicate that the high-N-induced decreases in FGR in Komboka were mainly attributable to poor filling of the spikelet during the ripening stage. BW196 and NERICA 1 exhibited much weaker correlation between FGR and total spikelet number than Komboka (Fig. 5). In those varieties, higher temperature and solar radiation during the ripening stage did not increase FGR (Fig. 3 (G) and (I); Fig. 4 (G) and (I)). Factors other than filling of the spikelets during the ripening stage could affect FGR in BW196 and NERICA 1 under high N conditions in Mwea.

Further studies are needed to investigate the underlying factors causing the differences between BW196 and NERICA 1 in terms of high-N-induced decreases in FGR. There was no lodging in all the experiments, irrespective of N treatment and variety. The heading delay under high N conditions did not cause considerable differences in temperature and solar radiation conditions among the N treatments, irrespective of variety (Table 2, Figs. 3 and 4). Saito (2015) reported that grain yield per N uptake, that is, nitrogen-use efficiency (NUE), was lower in NERICA 1 than in other rice varieties (Aus 257 and IR 74371-3-1-1). Although N uptake was not determined in the present study, NUE may be lower in NERICA 1 than in BW196. If this is true, the optimum amount of N fertilizer needed to achieve the highest yield in Mwea may be greater in NERICA 1 than in BW196, regardless of their differences in cold tolerance. To study whether cold tolerance has any correlation with the avoidance of a high-N-induced decrease in FGR in Mwea, grain yield response to high N fertilization is under investigation using a near isogenic line that has a quantitative trait locus for improved cold tolerance in the genetic background of Basmati 370, another popular variety in Mwea.

The presence of NERICA 1, which avoids high-N-induced decreases in FGR, motivates us to identify more varieties that achieve high yield under high N conditions in Mwea. Furthermore, it should be noted that NERICA 1, a cold-tolerant variety, showed the highest yield under high N conditions when it was sown in June and July (Fig. 1. (C)). As indicated in Table 2, NERICA 1 is an early maturing variety. Most farmers in Mwea cultivate rice once a year starting between July and August, and the sowing of cold-susceptible varieties between March and June is not recommended as it leads to suboptimal yield owing to cold stress (Samejima et al. 2020b). Sowing early maturing cold-tolerant varieties in June or earlier months under high N conditions may improve the flexibility for future multiple rice cropping in the region without reducing yield due to cold stress. Further studies are needed on cropping systems that maximize productivity and fertilizer utilization efficiency throughout the year.

In conclusion, the introduction of varieties without considering how high N fertilization influences their yield could hamper efforts to increase rice production in the tropical highlands of equatorial East Africa, such as in Mwea, Kenya. High N fertilization did not increase grain yield in five cold-susceptible varieties, including BW196, Komboka, Basmati 370, Takanari, and IR72, either in the present study or in a previous study (Njinju et al. 2018). In contrast, the grain yield in NERICA 1 increased with increases in N fertilizer amount because this variety limits high-N-induced decreases in FGR.
However, the reasons for the varietal differences in high-N-induced decreases in FGR remained unclear in the present study.

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