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Citation	FIELD CROPS RESEARCH, 116(1-2), 38-45 https://doi.org/10.1016/j.fcr.2009.11.006
Issue Date	2010-03-03
Doc URL	http://hdl.handle.net/2115/44085
Type	article
File Information	Kossonou_etal_FCR-REVISE2.pdf



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1 **Genetic improvements for high-yield and low soil nitrogen tolerance in rice (*Oryza Sativa***
2 **L.) under a cold environment**

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31

1 **Abstract**

2

3 Eight rice cultivars released in 1905, 1919, 1941, 1954, 1971, 1984, 1987 and 1988 were
4 investigated to identify the traits that contributed to high yield and low soil nitrogen tolerance
5 breeding under cold environment. They were grown in fields at three different nitrogen (N)
6 fertilizer treatments, 0, 6 and 12 g N m⁻² (0 N, 6 N and 12 N) in Sapporo, Northern Japan, in 2001
7 and 2002. All cultivars increased their grain yield (GY) with the increase in soil N availability,
8 and better response to N was observed in modern cultivars released during 1984 to 1988
9 compared to old ones (1905 to 1954). Irrespective of N treatments, the modern cultivars showed
10 better GY than the older ones. Absolute genetic gain was 2.15 or 2.94 g m⁻² year⁻¹ at 6 N and 12
11 N. Under 0 N treatment, although the magnitude of yield increase was small, the genetic gain in
12 GY was still observed at 0.78 g m⁻² year⁻¹. The GY increments were achieved mainly through
13 increasing the number of spikelets (SPK) which depends on the number of panicle (PAN) at any
14 level, and the PAN could be increased by enhancing the number of tillers. The extinction
15 coefficient (*k*) showed that the older cultivars had a spreading plant type, on the other hand, the
16 modern cultivars had an erect plant type which seemed to be a better plant structure in terms of
17 light distribution. This change on plant structure would allow the modern cultivars to have a
18 larger LAI with improved light capturing resulting in better GY in modern cultivars than the old
19 cultivars having similar LAI with modern cultivars. These breeding strategies could work for the
20 high-yielding rice breeding program under cold environments irrespective of soil nitrogen
21 conditions.

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23 *Key words:* Rice, Genetic improvement, Nitrogen, LAI, Coefficient extinction, Grain yield.

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1 **1. Introduction**

2
3 World-wide rice is one of the most important food crops that is feeding more than a half of
4 the world population. Global rice production needs to grow at a rate of ca.1.6% year⁻¹ for the
5 period 1990-2030 to meet the projected demand by the continuously increasing world population
6 (Oga and koyama, 1995; FAOSAT, 2003). With the land suitable for agriculture being limited,
7 increasing the rice productivity under optimal as well as moderate stress environments would be
8 one of the promising strategies rather than unreasonably expanding the cultivation area to
9 marginal land where the environments are fairly stressful (Evans, 1999).

10 A holistic and intensive analysis of world rice improvement efforts reveal that the grain
11 yield improvement of rice had been principally achieved by nitrogen (N) fertilizer application and
12 by development of high N responsive rice cultivars (Hossain et al., 1995; Klush et al., 1995; Peng
13 et al., 1999; Peng and Klush, 2003). However, high N response had lead to more tillering and
14 consequently more mutual shading, resulting in an unhealthy plant growth and eventual failure
15 due to lodging. Introducing traits of semi-dwarfism with firm stems, compact and erect leaves to
16 avoid lodging had brought in a dramatic success with quantum leaps in the grain yield during
17 1960-1970 (Hossain et al., 1995; Klush, 1995; Klush et al., 2001; Peng et al., 2003). Four decades
18 after, although several rice cultivars had been developed, only marginal improvements in the
19 grain yield had been achieved. The stagnation or slow down of yield improvement in rice had
20 been interpreted as genetical yield limit (Hossain et al., 1995; Klush et al., 1995; Klush et al.,
21 2001; Peng et al., 2003). Therefore, it becomes important to identify relevant trait(s) that could
22 contribute to break this yield barrier under optimal cultivation environments.

23 There is a good scope for improving rice productivity in cold environment as large rice
24 areas are potentially available and the ecosystems are not that fragile for agriculture. However,
25 cultivating rice in cold environments had been a big challenge. In Hokkaido, Northern Japan (43°
26 04' N), efforts have been made for long (since 1898) to grow rice (Nagai, 1959; Tanaka et al.,
27 1968). At the beginning of the 20th century, the average grain yield of rice in Hokkaido was 1.75 t
28 ha⁻¹ which was far lower than the national average (2.47 t ha⁻¹). This yield gap was filled by new
29 rice cultivars which were suitable for cold environments, and it reached the national average of
30 5.40 t ha⁻¹ in year 2000 (MAFF, 2002). Those modern high-yielding rice cultivars in Hokkaido
31 were developed based on better performances to high N fertilizer application (8-10 g m⁻²), and
32 therefore, little is known about their performances in low N under cold environments.

33 The objective of this study was to identify the traits that would contribute to yield
34 improvement under optimal as well as suboptimal environments for further yield gain in rice.

2. Materials and methods

2.1. Environment and cultivars

Field trials were conducted at the experimental farm of Field Science Center for Northern Biosphere of Hokkaido University, Sapporo, Japan (43°04' N), on alluvial soil in 2001 and 2002. Eight rice cultivars (year of release in parenthesis), Akage (1905), Bozu 5 (1919), Norin 20 (1941), Shinsetsu (1954), Ishikari (1971), Yukihihikari (1984), Kirara 397 (1987) and the breeding line Joiku 404 (1988), developed by the Hokkaido Rice Research Program (HRRP) were used. Akage was introduced in 1905 for Hokkaido, and it was the most popular cultivar in the primary era of rice production in Hokkaido. Bozu 5 was bred from Akage in HRRP as a pure line selection in 1919. It was suitable for direct-seeding with its awn-less characteristic, and contributed to the spread of rice cultivation throughout Hokkaido and became the leading cultivar from 1925 to 1935. Norin 20 was bred in 1941 by HRRP. It had been far more superior in yield potential and eating quality than the cultivars previously grown in Hokkaido, and was widely cultivated from 1943 to 1950 throughout Hokkaido. Shinsetsu was bred in 1954 from a cross between local cultivars of Hokkaido. It has conferred strong tolerance to cold weather damage and blast disease, and was the leading cultivar during 1957 to 1961 in Hokkaido. Ishikari was bred in 1971, and this was a special cultivar as the target trait for which it was bred happened to be better eating quality than the usual high grain yield. Ishikari, an improved eating quality cultivar was a leading one in Hokkaido during 1973 to 1981. Yukihihikari was the first successful HRRP bred cultivar released in 1984 which also had a good eating quality. Kirara 397, one more good eating quality cultivar, was released in 1987 and became a leading cultivar in Hokkaido since late 1980's. Joiku 404 was a breeding line developed in 1987, and had high grain yield with good eating quality.

2.2. Planting, nitrogen treatments and experimental design

Pre-germinated seeds were sown on seedbeds (60 × 30 cm, three seeds hill⁻¹) in a greenhouse in late April 2001 and 2002. The seedlings were grown up to the four-leaf stage and then were transplanted to paddy fields in late May of both years in a planting density of 13 × 33 cm (23.3 hills m⁻²). The water level was maintained at 3–8 cm above from the soil surface until three weeks before harvest. Weeds and diseases were prevented by applying herbicides and pesticides as

1 needed. About two weeks after panicle initiation, all experimental plots were covered with a net
2 (20 cm × 20 cm meshes) to protect from bird damage.

3 Total N content of the soil at this experimental farm before starting the trials was 0.25%
4 with an inorganic-N of 14.7 mg/100g-soil. Three nitrogen (N) fertilization treatments were tested;
5 1) non-N application (0 N), 2) 6 g N m⁻² application (6 N), and 3) 12 g N m⁻² application (12 N).
6 Phosphorous (P₂O₅; 9.6 g m⁻²) and potassium (K₂O; 7.2 g m⁻²) were applied on all plots. All these
7 fertilizers were applied as basal dressing. The experiments were conducted in a split-plot design
8 with three replications. N treatments were assigned to main-plots and cultivars to sub-plots. The
9 dimension of each sub-plot was 3.0 m × 3.0 m in 2001 and 4.5 m × 3.0 m in 2002.

10 2.3. Measurements

11
12
13 The number of emerged panicles was recorded in 10 consecutive hills in each sub-plot
14 once in every two days, and the full heading stage was defined as a date when more than 90% of
15 the tillers had their panicles emerged. The maturity was defined as the date when the whole
16 panicle turned yellow. At the full heading stage, the plant height and the number of tillers were
17 recorded in consecutive five hills in each sub-plot. The leaf area was measured on the three hills
18 by using an automatic leaf area meter (AAM-7, Hayashi Denko Co. Ltd., Tokyo, Japan), and then
19 the leaf area index (LAI) was calculated as the ratio of leaf area measured / ground area sampled.

20 At the final harvest time, 21 hills in about 0.90 m² of each sub-plot were cut at ground
21 level. The harvested entire shoots were kept in dry-air oven at 80 °C for three days, and then the
22 total aboveground biomass (TB) was measured. After that, the number of panicles was counted.
23 The panicles were threshed carefully to collect all spikelets (filled and non-filled) and the total
24 spikelets weights recorded. A sub-sample of 100 g of spikelets were soaked in an ammonium
25 sulfate solution with 1.06 specific gravity, and the number of shrunk and floating spikelets was
26 counted with a seed counter (Count-A-Pak model 77, Co. Ltd., Tokyo) to determine filled
27 spikelets percentage (FS). The filled spikelets were hulled and 1000 grains weight (1000-GW)
28 and GY (brown rice) was determined after the moisture content of grains was adjusted to 14%.
29 Number of panicles m⁻²(PAN), spikelet number panicle⁻¹ (SPK PAN⁻¹), and the number of
30 spikelets m⁻² (SPK) were calculated. Harvest index (HI) was determined as the ratio of GY / TB.

31 The coefficient of light extinction was estimated at full heading stage in 2002 to understand
32 the leaf canopy structure. The light intensity above the canopy (I_o) and below the canopy (I_b) were
33 measured at consecutive point intervals of 1 m apart in the inter-row space and in the inter-hill
34 space simultaneously by using two light sensors (LI-250, LI-COR, Lincoln, NE), each to measure

1 I_o and I_b when the sun was at the zenith and sunlight incidence was near vertical (1100 to 1300 h).

2 The coefficient of light extinction (k) was calculated as follows (San-oh et al., 2004);

$$3 \quad k = \ln (I_b / I_o)$$

4 The plants were sampled from three hills for the N content measurements. The N content
5 of grains (N_{grain}) and shoots (N_{shoot}) in each cultivar were estimated by Kjeldahl method, and the
6 plant N content (N_{plant}) was calculated as $N_{\text{grain}} + N_{\text{shoot}}$. The N uptake ability (NUA) was, then,
7 calculated as the N_{plant} derived by the area sampled. The internal N use efficiency (NUE) was
8 calculated as $(N_{\text{grain}} / N_{\text{plant}}) \times 100$.

9 The weather data such as mean daily air temperature, net solar radiation, and sun shine
10 duration during growth period (May-September) in 2001 and 2002 were recorded by a data logger
11 set up at 1.5 m above-ground level in the experimental field. The climatic data during 1981 to
12 2002 (21-years) were recorded at the Agro-meteorological station of Hokkaido University.

14 2.4. Statistical analysis

15
16 Analysis of variance (ANOVA) was performed on statistical software (CoHort software
17 6.0, Monterey, USA) following the procedure of McIntosh (1983) for combining the data across
18 two years for GY, yield components, and other agronomic traits measured in this study. The years
19 and replications were treated as random factor, and N treatments and cultivars were treated as
20 fixed factors. Simple ANOVA was also performed for coefficient of extinction as it was
21 measured in only one year (2002).

23 2.5. Genetic gain in grain yield and the contribution of each trait

24
25 The genetic gain of GY was estimated by using the following equation (Ortiz-Monasterio et
26 al., 1997).

$$27 \quad Y_i = a + bX_i + u \text{ absolute}$$

28 where Y_i is the mean GY for the two years at each rate of applied N (g m^{-2}) of cultivar i , and X_i is
29 the year in which cultivar i was released. The linear equation provides an estimate of grain yield
30 increase based on the cultivar release date in absolute term (i.e., b measures GY in $\text{g m}^{-2} \text{ year}^{-1}$).
31 The intercept and residual error are estimated by a and u , respectively.

32 From the morphology point of view, the GY could be expressed by an empirical formula
33 ($\text{GY} = \text{SPK} \times 1000 - \text{GW} \times \text{FS}$), and as $[\text{SPK} \times 1000 - \text{GW}]$ constitute the yield capacity (Y_{cap}) the
34 GY could be simplified as $\text{GY} = Y_{\text{cap}} \times \text{FS}$. By taking the log of all components, the relationship

1 among them is changed from multiplicative to additive, viz., $\text{Log (GY)} = \text{Log (Y}_{\text{cap}}) + \text{Log (FS)}$.
2 The contribution to GY could be determined by the standardized coefficient using the multiple
3 regression analysis. The same method was applied to analyze the contribution of other traits to
4 GY.

6 **3. Results**

8 *3.1. Seasonal climate*

10 The climatic conditions at some key growth stages of rice in Hokkaido were different
11 between 2001 and 2002 (Fig. 1). The mean solar radiation varied less when comparisons were
12 made between 2001 and 2002 or with long term (21-years) averages. It was $17.0 \text{ MJ m}^{-2} \text{ d}^{-1}$ in
13 2001 and $16.5 \text{ MJ m}^{-2} \text{ d}^{-1}$ in 2002 compared to a long term average of $16.2 \text{ MJ m}^{-2} \text{ d}^{-1}$. The solar
14 radiation at the panicle initiation and heading stage of 2002 was lower than the long term average
15 but higher than in 2001 even during the ripening stage. The mean air temperatures of $16.4 \text{ }^{\circ}\text{C}$ in
16 2001 and $16.1 \text{ }^{\circ}\text{C}$ in 2002 was similar to the long term average ($16.3 \text{ }^{\circ}\text{C}$). However, it was still
17 lower than the other major rice cultivating regions; for example, the mean temperature in Hunan
18 province of China, one of the intensely rice producing areas in the world, is $22.4 \text{ }^{\circ}\text{C}$ (World
19 Metrological Organization, 2009). The mean temperature at the panicle initiation stage in 2002
20 was lower than the long term average, and was higher in 2001. And it was relatively cooler in
21 both 2001 and 2002 than the long term average at the heading stage. It declined during the
22 ripening stage in 2002, but went up in 2001.

24 *3.2. Grain yield*

26 Analysis of variance for the yield of brown rice (GY) showed no significant effects of
27 year cultivated (Y), and the interaction effects of $Y \times \text{cultivar (C)}$, $Y \times \text{nitrogen treatments (N)}$
28 and $Y \times N \times C$ were also not significant (Table 1). Despite the observed variation in climatic
29 conditions during some key growth stages, the cultivar ranking for GY did not vary across the
30 years. Therefore, the GY and its other related parameters were analyzed with the mean across 2
31 years.

32 The GY increased significantly with the advancing years of cultivar release ($p < 0.001$),
33 and also with the increase in N applications ($p < 0.001$) (Table 1). The modern rice cultivars
34 (released in 1984-1988) showed better response to N than their predecessors. The modern

1 cultivars showed 38% increase in GY at 6 N and 39% at 12 N over the old cultivars (released in
2 1905-1941), and 13% increase over the intermediate cultivars (released in 1954-1971). At 0 N,
3 the difference in GY among cultivars became smaller compared to other two N treatments as 0 N
4 application seemed not to allow the rice to exhibit their potential growth. Interestingly, at 0 N, the
5 GY increased significantly with advancing cultivars ($p < 0.05$), viz., the GY of the modern
6 cultivars was 23% better than the old cultivars, and 19% better than the intermediate one. A
7 significant $C \times N$ interaction was the result of higher N use efficiency in the modern day cultivars.
8 The N uptake ability (NUA) was different between the cultivar groups of year released. The NUA
9 of intermediate and modern cultivars were 6.3 g N m^{-2} , and it was better than that of old cultivars
10 (5.6 g N m^{-2}). In addition, internal N use efficiency (NUE) of the modern cultivars was 60.0%, on
11 the other hand, it was 54% in the intermediate cultivars and 57.6% in the old cultivars. From N-
12 use point of view, the GY could be expressed as $GY = NUA \times NUE$, and the multiple regression
13 analysis showed the determination coefficient of each trait as 1.20 for NUA, and 0.60 for NUE.
14 This means that the successful GY improvement under cold environments have been achieved
15 through greater enhancements on NUA.

16 17 3.3. Phenology

18
19 The mean days to heading was 69 across all cultivars at all N levels, and N application
20 significantly delayed it (Table 2). It was 69 days at 6 N, which was delayed by a day at 12 N but
21 was early by 2 days at 0 N. The days to heading were significantly different among cultivars at
22 any N levels. It varied by 1.5% at 0 N, 1.8% at 6 N and 2.0% at 12 N. Since strong $C \times Y$ and $C \times$
23 N interactions were observed, the days to heading response to climate and soil N is likely to be
24 cultivar specific. There was no clear trend between the days to heading and the year of cultivars
25 released. However, it correlated well with the yield under 6 N treatment ($r = 0.55$, $p < 0.001$), but
26 this correlation could not be seen in other N treatments.

27 28 3.4. Morphology for yield capacity improvements

29
30 Multiple regression analysis revealed the relative importance of Y_{cap} over FS for the GY
31 improvements under cold environments as $\text{Log}(GY) = 1.14 * \text{Log}(Y_{\text{cap}}) + 0.31 * \text{Log}(FS)$. As a
32 further dissection of Y_{cap} , the correlation analysis showed that the Y_{cap} had a closer correlation
33 with the SPK, and with 1000-GW at 12 N (Fig. 2a and b). With a multiple regression analysis, the
34 relationship between Y_{cap} and these two traits at 6 N could be expressed as $\text{Log}(Y_{\text{cap}}) = 1.09 * \text{Log}$

1 (SPK) + 0.21*Log (1000-GW), and at 12 N as $\text{Log}(Y_{\text{cap}}) = 0.92*\text{Log}(\text{SPK}) + 0.23*\text{Log}(1000\text{-GW})$.
2 GW). Even at 0 N, it was $\text{Log}(Y_{\text{cap}}) = 0.93*\text{Log}(\text{SPK}) + 0.17*\text{Log}(1000\text{-GW})$. These larger
3 determination coefficients in SPK indicated that the improvements of Y_{cap} had been made through
4 SPK rather than 1000-GW. The SPK of modern cultivars was 18% more at 0 N, 31% more at 6 N
5 and 27% more at 12 N than that of old cultivars, and 10% more at 0 N, 4% more at 6 N and 13%
6 more at 12 N than that of intermediate cultivars. These indicated that the SPK increase was
7 through the combined effects of genetic improvements and N applications. The 1000-GW was
8 associated with Y_{cap} at 12 N, but not at 0 N and 6 N. Thus, incomplete grain filling could have
9 been the cause of 1000-GW reduction at 0 N and 6 N, which would cancel the significant
10 contribution of 1000-GW to Y_{cap} .

11 The SPK had a linear correlation with the number of panicles (PAN) (at 0 N, $r=0.58$,
12 $p<0.001$, at 6 N, $r=0.60$ $p<0.001$, and at 12 N, $r=0.65$ $p<0.001$), and with the spikelets number
13 panicle⁻¹ (SPK PAN⁻¹) (at 0 N, $r=0.41$, $p<0.1$, at 6 N, $r=0.33$ $p>0.05$, and at 12 N, $r=0.03$ $p>0.05$).
14 The multiple regression analysis showed an equation for SPK at 6 N as $\text{Log}(\text{SPK}) = 1.14*\text{Log}(\text{PAN}) + 0.96*\text{Log}(\text{SPK PAN}^{-1})$,
15 at 12 N as $\text{Log}(\text{SPK}) = 1.15*\text{Log}(\text{PAN}) + 1.12*\text{Log}(\text{SPK PAN}^{-1})$ and also at 0 N, as $\text{Log}(\text{SPK}) = 1.01*\text{Log}(\text{PAN}) + 0.94*\text{Log}(\text{SPK PAN}^{-1})$. This
16 PAN⁻¹) and also at 0 N, as $\text{Log}(\text{SPK}) = 1.01*\text{Log}(\text{PAN}) + 0.94*\text{Log}(\text{SPK PAN}^{-1})$. This
17 indicates that the SPK had closer relationship with PAN than SPK PAN⁻¹ at any N level. The
18 PAN was associated with the number of tillers (at 0 N, $r=0.66$, $p<0.01$, at 6 N, $r=0.92$, $p<0.001$,
19 and at 12 N, $r=0.70$ $p<0.001$). Across the three N treatments, the PAN varied from 212 to 376,
20 while the SPK PAN⁻¹ ranged from 77 to 48. These indicate that the SPK improvement was
21 achieved through an improved tillering capacity which in turn increased the PAN in new rice
22 cultivars at any N fertilization condition, and the N application could also increase the SPK
23 through increased PAN.

24 The plant height and tillering capacity were also modified with advances in cultivars at
25 any N level (Fig. 3a and b). The plant height of cultivars released during 1905 to 1971 declined
26 significantly (ca. 25% at 0 N, 26% at 6 N and 25% at 12 N) ($p<0.001$). Thereafter, it increased
27 slightly with ca. 5% at 0 N, 7% at 6 N and 3% at 12 N from 1984 to 1988 which was not
28 significant (Fig. 3a). These changes were well correlated with Y_{cap} at 12 N ($r=-0.57$, $p<0.05$). On
29 the contrary, the number of tillers of cultivars from 1905 to 1971 significantly increased at any N
30 level, ca. 23% at 0 N, 22% at 6 N and 38% at 12 N ($p<0.001$) (Fig.3b), and then slightly
31 increased with ca. 18% at 6 N and 8% at 12 N. This dynamics of the tillering was also well
32 correlated with the Y_{cap} at all the N levels (at 0 N, $r=0.84$, $p<0.001$, at 6 N, $r=0.58$, $p<0.001$, at 12
33 N, $r=0.66$, $p<0.01$).

34

3.5. Assimilate allocation for grain yield improvements

The Y_{cap} had a very close relationship with the aboveground total biomass (TB) (at 0 N, $r = 0.95$, $p < 0.001$, at 6 N, $r = 0.95$, $p < 0.001$, at 12 N, $r = 0.86$, $p < 0.001$), and with the harvest index (HI) (at 0 N, $r = 0.29$, $p < 0.01$, at 6 N, $r = 0.47$, $p < 0.001$, at 12 N, $r = 0.36$, $p < 0.01$). Through multiple regression, the GY could be expressed as $\text{Log (GY)} = 0.89 * \text{Log (TB)} + 0.20 * \text{Log (HI)}$ at 6 N, and at 12 N, $\text{Log (GY)} = 0.83 * \text{Log (TB)} + 0.22 * \text{Log (HI)}$. Even at 0 N, the GY could be described as $\text{Log (GY)} = 0.82 * \text{Log (TB)} + 0.28 * \text{Log (HI)}$. These equations indicate that the GY improvements were more due to improvements in TB rather than HI. At all these N levels, the TB of the modern cultivars were greater than the old cultivars (Fig.4a). The TB was also positively associated with the tillering capacity, and the genetic gain in TB was $2.96 \text{ g m}^{-2} \text{ year}^{-1}$ at 6 N ($r=0.62$, $p < 0.01$), and $5.14 \text{ g m}^{-2} \text{ year}^{-1}$ at 12 N ($r=0.96$, $p < 0.001$). However, the genetic gain of $1.18 \text{ g m}^{-2} \text{ year}^{-1}$ at 0 N was not significant ($r=0.65$, $p > 0.05$) (Fig. 4a).

Increasing N application decreased HI, and the magnitude of HI decline at 6 N to that of 0 N was far greater in old cultivars than in modern cultivars (Fig. 4b). The genetic gain for this ratio was 0.0007 year^{-1} at 6 N ($p < 0.001$), and 0.0006 year^{-1} at 12 N ($p < 0.001$), and even at 0 N, it was 0.003 year^{-1} ($p < 0.05$).

3.6. Plant structure for grain yield improvements

Leaf area index (LAI) at the full heading stage has been significantly modified at any N level ($p < 0.001$), and larger LAI was obtained by increasing N level (Fig. 5a). Interestingly, the LAI dynamics with advance in cultivars exhibited a U-shape at any N level with the lowest during 1941 to 1954. The genetic progress in LAI from 1905 to 1954 showed negative values with a -0.01 year^{-1} at 0 N ($r=-0.82$, $p < 0.01$), -0.02 year^{-1} at 6 N ($r=-0.83$, $p < 0.01$) and -0.02 year^{-1} at 12 N ($r=-0.98$, $p < 0.01$), but from 1971 to 1988 it turned to be positive with 0.03 year^{-1} at 0 N ($r=0.78$, $p < 0.01$), 0.05 year^{-1} at 6 N ($r=0.84$, $p < 0.01$) and 0.08 year^{-1} at 12 N ($r=0.80$, $p < 0.01$).

Despite that the LAI changes at all N levels showed a typical U-shape, the light extinction coefficient (k), which could indicate the leaf architecture (leaf angle relative to its stem), decreased exponentially from the old to modern cultivars (Fig. 5b). This could have been an unconscious effort for improving the ratio of k to LAI and achieving an optimum or effective k / LAI ratio in the modern cultivars allowing them to have lower k despite possessing equal LAI compared to old cultivars. As smaller k indicates better plant structure in terms of the light diffusion and capture across leaf strata, it could significantly contribute to the Y_{cap} and number of

1 tillers at any N levels (Table 3). In addition, it showed significant correlation with the TB, HI and
2 plant height at 6 N and 12 N.

4 **4. Discussion**

6 This study showed that the grain yield (GY) had been improved successfully over years
7 through successive release of high-yielding rice cultivars. Interestingly, the modern rice cultivars
8 showed better yield than the older, even at low soil N availability, although the differences in GY
9 among them as well as the genetic gain was small. This information was unknown until now as
10 the modern cultivars have been selected under optimal growth conditions, and therefore they
11 would be guaranteed better field performances under optimal growth conditions but didn't have
12 strong tolerance against the stressful environmental conditions, e.g. low soil N availability. Our
13 results suggest that the same strategy adopted for developing the high-yielding cultivars could
14 also work for breeding new rice cultivars with low soil N.

15 Growth duration, especially, days to heading is quite an important trait in this
16 environment where rice cultivation season is short (June-August). In this study, however, all
17 cultivars that were tested had relatively early maturing characteristics, and so they could escape
18 from cold injury. Our results suggested that the heading could be delayed up to 75 days and still
19 get reasonably good yields under the cold environments.

20 Analyzing the trait-basis of yield improvement would be one of the best ways to
21 understand the breeding effects. In this study, the analysis was made from three different points
22 of view. Firstly, from the morphological point of view, the number of spikelet (SPK) was
23 identified to be a relevant component that was closely associated with the yield capacity (Y_{cap}).
24 This relationship between the SPK and Y_{cap} was observed at any soil N environment. Over years,
25 the high-yielding rice breeding programs in Hokkaido, therefore, have been focusing on
26 increasing SPK through increasing the number of panicles (PAN) which in turn had been through
27 increase in number of tillers. Actually, since 1950's it had been relatively easy to increase the
28 tiller through N, as N fertilizer was cheaper. Our results only helps to appreciate their breeding
29 strategy, and quite interestingly the SPK could be used as a target trait not only when breeding for
30 high yield but also for low N stress tolerance in rice as it was closely associated with Y_{cap} even at
31 low N level. Since the PAN has exhibited a strong negative correlation with the SPK PAN^{-1}
32 (Abeledo et al., 2003), their breeding efforts have resulted in less SPK PAN^{-1} . It is worth that
33 further breeding programs concentrate on improving SPK PAN^{-1} and try to break this negative
34 relationship. Our results had also shown that not much attention had been paid on improving the

1 filled spikelet percentage (FS) despite the fact that the FS would be more sensitive to cold
2 temperature. This would be mainly because of the likely breeding for early maturity rice cultivars
3 for escaping from the cold injury and so those cultivars had reasonably high FS. However, shorter
4 growth period is expected to penalize the biomass accumulation. Therefore, paying more
5 attention towards improving FS through cold tolerance breeding would be more relevant as a long
6 term strategy as this would allow us to extend the growing period for further increase in rice yield.

7 Secondly, from the assimilate allocation point of view, our results showed that increase
8 in the above-ground total biomass (TB) would contribute significantly more to GY than the
9 harvest index (HI). The modern cultivars had larger TB than older ones, which was mainly due to
10 increased number of tillers. As the TB obtained in our cold environments was not as high as the
11 high-yielding rice in Yunnan, China (Amano et al., 1996), further improvement in the N uptake
12 ability (NUA) and extending the cultivation period could potentially improve the TB. Although
13 less in its dimension of contribution than the TB, the HI could also contribute to the GY at any
14 soil N condition. The improvement on HI has been achieved mainly by reducing the plant height
15 (Flinthman et al., 1997; Austin et al., 1980), and it consequently added the lodging tolerance on
16 the improved cultivars. Increasing the SPK has been the principle breeding strategy for high yield
17 in rice rather than the choice of grain weight increase. Our results also supported this strategy as
18 the 1000-grain weight (1000-GW) didn't always correlate with Y_{cap} under our cold environment.
19 It is likely to be difficult to improve the GY through HI although many researchers believe that
20 the maximum HI can go up to 0.55 (Amano et al., 1996, Horie et al., 1997; Peng et al., 2000;
21 Yang et al., 2002 and 2007).

22 Thirdly, from the plant architecture point of view, it should be emphasized that the
23 modern rice cultivars could have a large leaf area index (LAI) and a better light extinction
24 coefficient (k) simultaneously. This could have been achieved by the plant architecture
25 modification via breeding. Thus, the principal breeding strategy might have been encouragement
26 of erect leaves on rice plants, otherwise larger LAI would result in mutual shading and less grain
27 yield (Peng and klush, 2003; Yamauchi, 1994; Kabaki, 1993; Patnaik et al., 1991; Song et al.,
28 1990). According to this breeding strategy, the modern cultivars have been improved for better
29 plant architecture leading to better light interception, viz. smaller light extinction coefficient (k)
30 with greater LAI. This is expected to provide a big advantage to the modern cultivars in terms of
31 the greater photosynthesis. On the other hand, the old cultivars in spite of having similar LAI, had
32 a spreading canopy structure, larger k , inefficient light utilization and as a consequence less yield
33 at every level of soil N.

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1 **5. Conclusion**

2
3 Breeding high-yielding rice for the cold environments of Hokkaido was quite successful
4 with progressive and substantial improvements over years. This seems to have been achieved
5 through i) increase in number of spikelets with more tillers that could make more panicles and ii)
6 increase in total above ground biomass and also marginally the harvest index. This total shoot
7 biomass improvement had been a likely outcome of effective maintenance of large leaf area index
8 with an improved light extinction coefficient. It is proposed that further yield improvement efforts
9 need to concentrate on improving the biomass productivity as the current levels are far below the
10 reported maximum. Our results point out that the strategies applied for developing high yielding
11 rice would also work for the rice breeding for low soil nitrogen tolerance in the cold
12 environments.

13
14 **Acknowledgments**

15 We are grateful to Mr. S. Ichikawa, the experimental farm of Hokkaido University, and
16 Mr. K. Otsubo, the Crop Science Laboratory of Hokkaido University for conducting the
17 experiments. We are also grateful to the staff of Iwamizawa Agriculture Research Station for
18 providing the rice seeds. Our heartfelt thanks should also be given to Dr. L. Krishnamurthy,
19 International Crops Research Institute for the Semi-Arid Tropics, India, for improving this
20 manuscript.

21
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24

1 **Figure captions**

2

3 Fig.1. Dynamics of the daily solar radiation and daily air temperature during the rice cropping
4 season (May-September) in 2001, 2002, and the long term averages from year 1981 to 2002 (21-
5 yrs). TP= transplanting stage, PI= panicle initiation stage, HDG= heading stage.

6

7 Fig.2. Relationship between the yield capacity and (a) the spikelets (m^{-2}) and (b) 1000-grain
8 weight in eight rice cultivars released in different times at three levels of nitrogen (0 N= \circ , 6 N=
9 \blacksquare , and 12 N= \blacktriangle) across two years (2001 and 2002). Bars stand for the standard error (se) of
10 means. Number from 1 to 8 refer to, 1 = Akage, 2 = Bozu 5, 3 = Norin 20, 4 = Shinsetsu, 5 =
11 Ishikari, 6 = Yukihihikari, 7 = Kirara 397, 8 = Joiku 404.

12 *, ***, indicate significant at 0.05, 0.001 probability levels, and NS, not significant.

13

14 Fig.3. Changes of (a) the plant height, and (b) number of tillers in eight rice cultivars released in
15 different eras at three levels of nitrogen (0 N= \circ , 6 N= \blacksquare , and 12 N= \blacktriangle) across two years (2001
16 and 2002) at the full heading stage. Bars stand for the standard error (se) of means.

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18 Fig.4. Changes of (a) the aboveground total biomass at maturity, and (b) harvest index in eight
19 rice cultivars released in different eras at three nitrogen levels (0 N= \circ , 6 N= \blacksquare , and 12 N= \blacktriangle) for
20 two years (2001 and 2002).

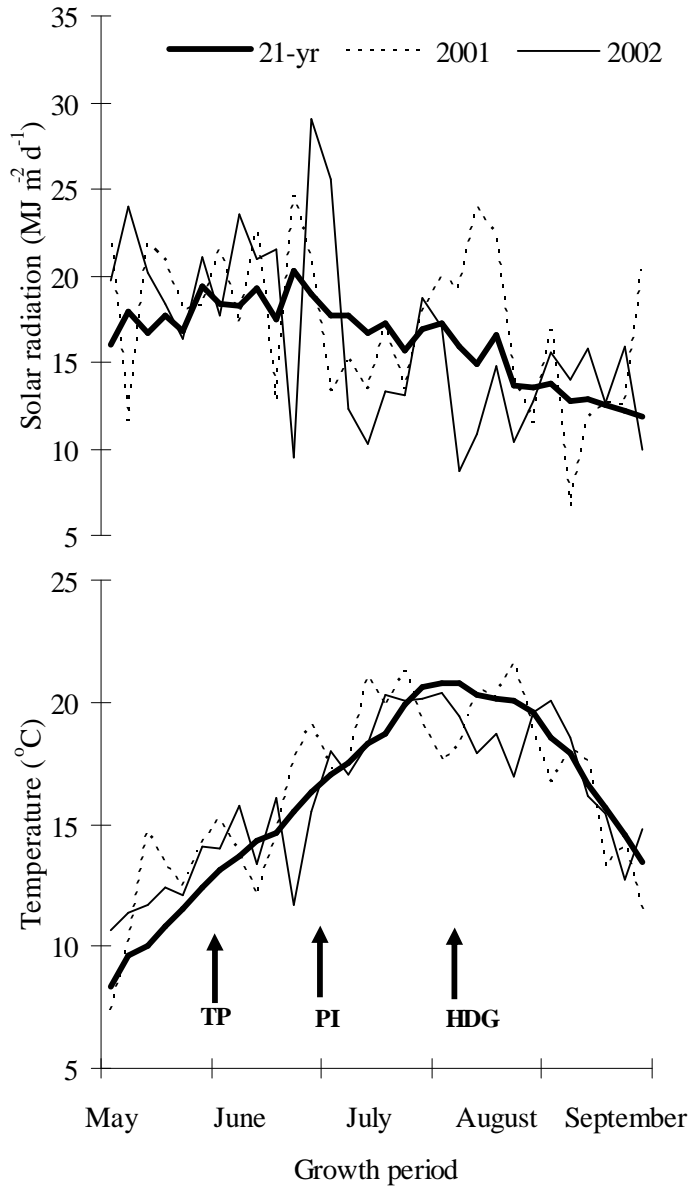
21 *, **, *** indicate significant at the 0.05, 0.01, 0.001 probability levels, respectively, and NS, not
22 significant.

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24 Fig.5. Changes of (a) the leaf area index (LAI), (b) light extinction coefficient (k) in eight rice
25 cultivars released in different eras at three levels of nitrogen (0 N= \circ , 6 N= \blacksquare , and 12 N= \blacktriangle)
26 across two years (2001 and 2002) at the full heading stage.
27 Bars stand for the standard error (se) of means.

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Fig 1



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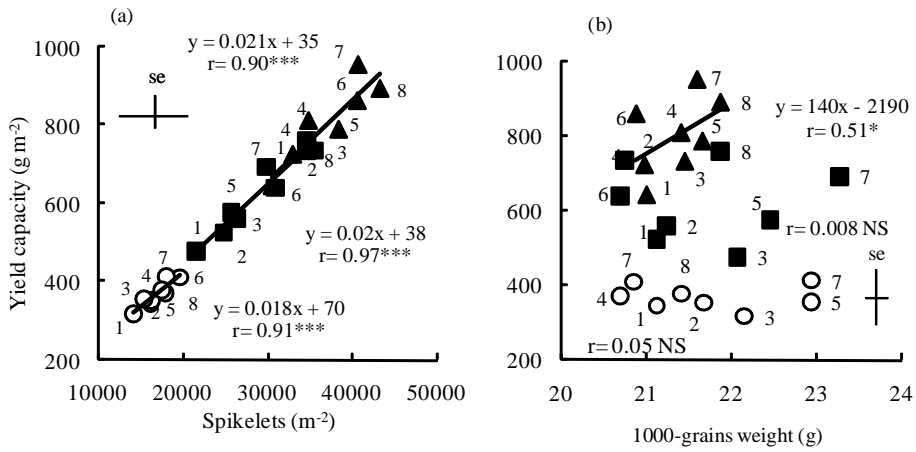
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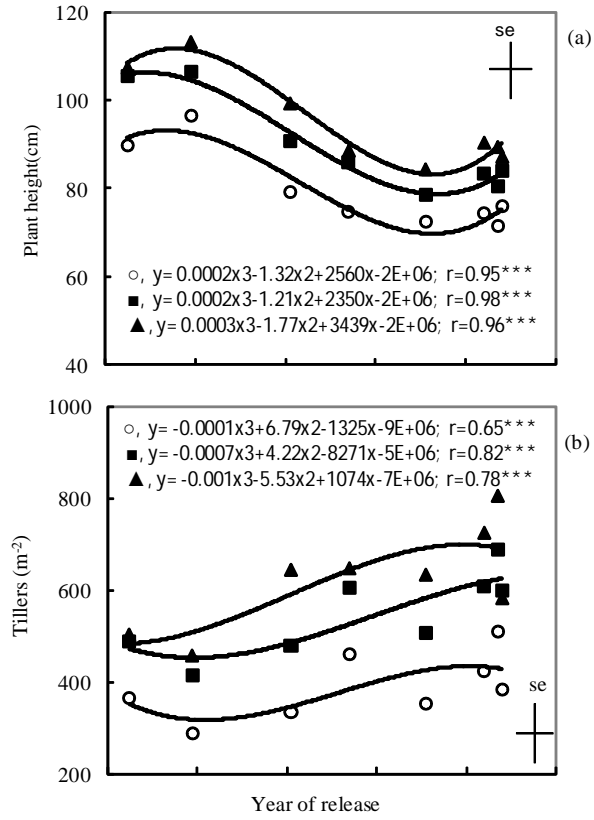
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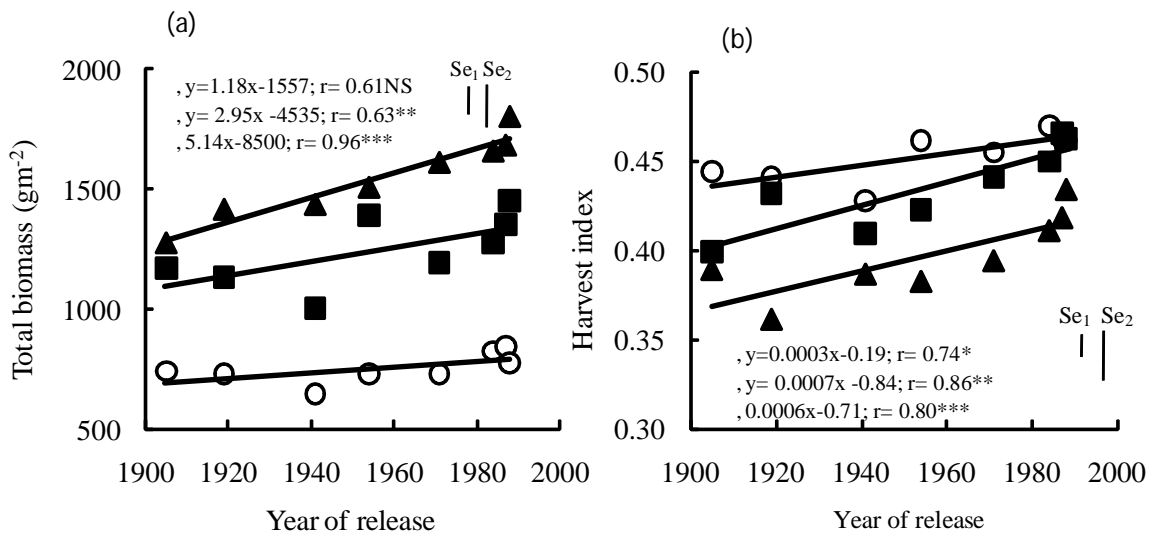
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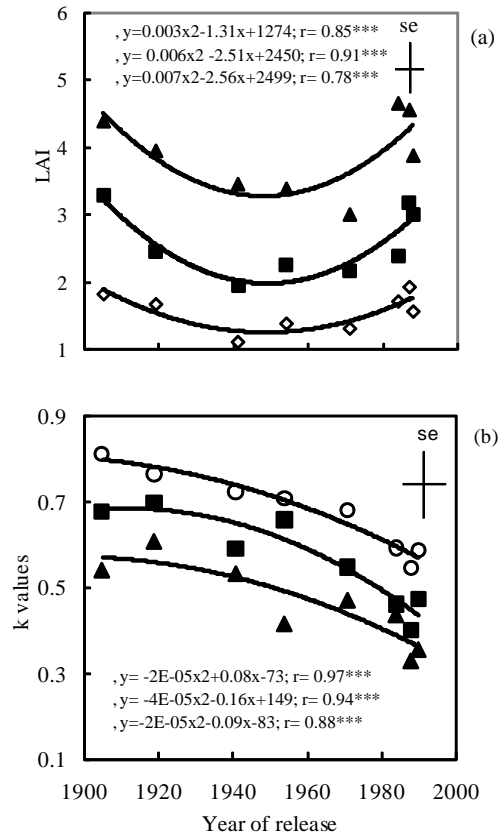
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Table 1.
Means and analysis of variance of 8 rice cultivars released at different eras at three N levels (0 N, 6 N, 12 N) across two years (2001-2002) for grain yield.

Cultivars	Year of release	Grain yield (g m ⁻²)			Mean
		0 N	6N	12N	
Akage	1905	329	464	482	425
Bozu 5	1919	319	486	515	440
Norin 20	1941	279	410	581	423
Shinsetsu	1954	339	586	584	503
Ishikari	1971	332	526	677	512
Yukihikari	1984	386	574	690	550
Kirara 397	1987	391	630	752	591
Joiku 404	1988	358	674	746	593
Mean		342	544	628	505

Lsd₁ = 23.3; Lsd₂ = 36.6; Lsd₃ = 30.0

Sources of variation	df	Mean of square
Year (Y)	1	189805 **
Nitrogen (N)	2	1042421 ***
Cultivars (C)	7	88434 ***
Y x N	2	5347 NS
Y x C	7	2509 NS
N x C	14	14772 ***
Y x N X C	14	2731 NS

*, **, *** indicate significance at 0.05, 0.01, 0.001 probability levels, respectively. NS= non significant.
Lsd₁ (P<0.05) for comparison between N, Lsd₂ (P<0.05) between cultivars and Lsd₃ (P<0.05) for cultivar × N interaction

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Table 2.
Means and analysis of variance of the days from transplanting to heading of eight rice cultivars released at different eras and grown with three N levels (0 N, 6 N, 12 N) in Hokkaido, Japan.

Cultivars	Year of release	Days to heading			Mean
		0 N	6 N	12 N	
Akage	1905	65	68	69	67
Bozu 5	1919	69	71	72	70
Norin 20	1941	63	66	69	66
Shinsetsu	1954	71	73	75	73
Ishikari	1971	65	66	67	66
Yukihikari	1984	67	70	72	69
Kirara 397	1987	68	68	69	69
Joiku 404	1988	70	72	71	71
Mean		67	69	70	69

Lsd₁= 0.80; Lsd₂= 0.76; Lsd₃= 1.12

Sources of variation	df	Mean of square
Year (Y)	1	38 ns
Nitrogen (N)	2	57 ***
Cultivars (C)	7	74 ***
Y x N	2	1.55 ns
Y x C	7	4.96 ***
N x C	14	4.45 ***
Y x N x C	14	1.71 ns

*, **, *** indicate significant at the 0.05, 0.01, 0.001 probability levels, respectively. NS= non significant. Lsd₁ (P<0.05) for comparison between N, Lsd₂ (P<0.05) between cultivars and Lsd₃ (P<0.05) for cultivar × N interaction.

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Table 3. Correlation coefficients between leaf area index (LAI) or light extinction coefficient (*k*) and the yield capacity, total biomass, harvest index, number of tillers, and plant height in eight rice cultivars released at different times at three nitrogen levels (0 N, 6 N and 12 N).

	Yield capacity (g m ⁻²)	Total biomass (g m ⁻²)	Harvest index	Tillers (m ⁻²)	Plant height (cm)	LAI
0 N						
LAI	0.43 *	0.45 **	0.32 *	0.36 *	0.28 *	
k	-0.33 *	-0.13 ns	-0.09 ns	-0.38 *	0.21 ns	-0.61 ***
6 N						
LAI	0.39 *	0.50 **	0.49 **	0.49 *	-0.10 ns	
k	-0.57 ***	-0.39 *	-0.69 ***	-0.69 ***	0.76 ***	-0.54 ***
12 N						
LAI	-0.16 ns	-0.05 ns	-0.18 ns	0.30 ns	0.16 ns	
k	-0.51 **	-0.63 ***	-0.42 *	-0.68 ***	0.58 ***	-0.14 ns

*, **, *** indicate significance at 0.05, 0.01, 0.001 probability levels, respectively. NS= non significant.