

Characterization of Heading Times and Duration of Heading time of an Individual Using a Wide Range of Variety of Rice (*Oryza sativa* L.) in One of the Northern-limit Regions of Rice Cultivation, Hokkaido Islands

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ABSTRACT

Aims: Hokkaido islands are one of northern limit regions of rice cultivation. There is about 170-year history about cultivation and breeding of Hokkaido rice and the history was well described in Japanese. Hence, cultivation and breeding of rice in Hokkaido islands can be a model case for the history in high latitudes. However, there is no English references about the history with hard data and the cultivars studied in the reports written in English have been limited to modern cultivars. Plant breeders in Hokkaido have mainly genetically improved earliness due to small range of suitable flowering times. Then, heading times and heading time duration need to be characterized using wide range of cultivar from introduced to modern cultivars. I provided basic knowledge of total nature of heading time of Hokkaido cultivars with hard data in this report.

Study design: The seven land races and six modern cultivars were used. In each genotype, plants were divided into high-density (H) and low-density (L) condition. Two replicates were prepared. In each of replicates, genotypes were placed according to plant height to avoid intergenotypic competition.

Place and Duration of Study: The place of this study is Hokkaido Agricultural Research Center (HARC) in Sapporo (43 °N). Duration of the study was summer season in year 2006.

Methodology: In each of replicates of a genotype, middle three plants were measured in both H and L conditions. In each plant, the first, second and third heading times were recorded based on daily observation. After the records, days to the first heading time from germination (DFH), days to the second heading time from germination (DSH) and days to the third flowering time from germination (DTH) were calculated. Heading-time duration (HTD) of each plant was conveniently defined as the equation: $HTD = DTH - DFH$.

Results: From frequency distribution of DFH, DSH and DTH, DFH showed larger variation of days to heading time than DSH and DTH, suggesting that genetic control of DFH might differ from that of DSH and DTH. Analysis of variance revealed that DFH was deterministically dependent on genotype, while the other flowering time can respond to environment, showing genotype × environment interaction, suggesting DFH specific genetic controlling. To examine that each of components of HTD, DFH and DTH, contributes to HTD, regression analyses were performed. As a result, HTD was primarily determined by

DFH. As for HTD and DFH, comparison between land race and modern cultivar was performed. Modern breeding decreased HTD by genetically modifying the first flowering time from earliness to lateness, and as a result, HTD decreased due to the negative correlation relationship between HTD and DFH.

Conclusion: As shown in Results, it was clarified that rice breeders genetically modified rice population fitted to Hokkaido island as to heading time. This report will be essential reference of heading time of Hokkaido cultivars. Total nature of heading time of Hokkaido cultivars was unknown because existence of land race has been neglected until the present study.

Keywords: Heading times, Heading time duration, Historical Viewpoint of rice breeding, Northern Limit of rice cultivation

1. INTRODUCTION

Rice is originated from tropical zone [1]. The northward march of rice cultivation with human migration had reached southern most tip of Japan before some 2000 year. Then rice cultivation had steadily expanded one of the northern limits of rice cultivation, Hokkaido islands ($41^{\circ} 18' - 45^{\circ} 30'$) at last [2]. There is about 170-year history about cultivation and breeding of Hokkaido rice [2] and the history was well described in Japanese. Hence, cultivation and breeding of rice in Hokkaido islands can be a model case for the history in high latitudes. However, there are a few English references with hard data about the history and the cultivars studied in the reports written in English have been limited to modern cultivars [3,4]. This situation results from that rice breeding has preceded breeding genetics and it has been recently that genetically unique feature of early heading time of Hokkaido cultivars has recognized [3]. Then, reference with hard data giving a historically bird-eye view of Hokkaido rice cultivars is needed.

In Hokkaido islands, environment is mainly characterized by long period of low temperature, and hence, suitable period of rice-flowering time is extremely short [2]. Therefore, it was mentioned that early-flowering time and tolerance for cool weather have been genetically improved in Hokkaido islands [2, 4, 5, 6]. However, there is room for studies about tolerance for cool weather that contributed to expansion of rice cultivation in Hokkaido since near-isogenic lines of Taichung 65 with earliness genes showed a high fitness in Hokkaido although Taichung 65 is fitted to sub-tropical regions [7]. As for earliness genes of Hokkaido cultivar, some genes have been identified [8,9,10,11]. However, heading times of an individual varied, and as a result, the variation enlarged flowering time duration of an individual. Therefore, identification of the first flowering time cannot reveal whole nature of heading time of Hokkaido cultivar. This variation is well-known common experience among rice breeders in Hokkaido islands but English reference, which can be cited, has not existed. Another feature of environment in the high latitudes is large changes in temperature in a crop season [2, 7], and it is likely that the variation of heading time of an individual might be fitted to this environmental feature.

The main objective of the present study is giving a historically bird-eye view of rice cultivars of Hokkaido islands for the common experience with hard data. Here, in the present report, two density conditions prepared in a paddy field of Hokkaido and the first, the second and the third heading time in an individual were recorded using thirteen representative cultivars of Hokkaido islands from initial land race to modern cultivars. First, I demonstrated that factors of heading time might be different among the three heading times of an individual. Second, I analyzed which heading time is associated with variation of heading time of an individual. Third, to know that rice breeders have genetically modified heading time of

cultivars of Hokkaido islands, modern cultivars and land races were compared as to the first flowering time and variation of heading time of an individual.

2. MATERIAL AND METHODS

2.1 Genetic Materials

A total of thirteen cultivar strains from initial to modern cultivars were used. The seven strains of the thirteen involved one introduced cultivar, Tugaru-wase (A110), and three initial land races, Akage (A1), Akaine (A2), Akamuro (A5), and the three land races, Bozu (A9), Kokushoku-to 2 (A58) and Kurikaramochi (A60). Akage is the origin of Hokkaido cultivars and genetically various population. A1 is selected from Akage population. Therefore, A1 does not strictly correspond to variation of Akage. Bozu is selected from Akage population by a farmer. Kokushokuto-2 and Kurikaramochi might be mutants derived from Akage since the two varieties show glutinous endosperm and glume and leaves were discolored while Akage does not show such features. Kokushokuto-2 and Kurikaramochi were also selected by farmers. They are preserved in Hokkaido University. The other six strains preserved in Hokkaido Agricultural Research Center (HARC), Fukoku, Fukuyuki, Yukara, Hoshinoyume, Nanatsuboshi and Kitaaoba, were established from cross breeding. They are so called modern cultivars [2]. Fukoku, Fukuyuki and Yukara were breeding strains for high-yielding ability in Hokkaido islands. Especially, plant type was drastically changed in the breeding of Yukara. For improving light-receiving efficiency, the plant type is elected and dwarf. This feature determined the later rice breeding in Hokkaido islands. Hoshinoyume and Nanatuboshi is elite varieties for eating quality but their yields are smaller than Fukuyuki and Yukara. Kitaaoba is only rice variety for feeding for cattle and hence the yield is the highest among rice cultivars in Hokkaido but eating quality is not so bad.

2.1 Cultivations

Germination treatments were carried out in the dark (30 °C) for three days in the late April. Ninety germinated seeds per strain were strictly implanted in a culture soil at uniform intervals according to the way the coleoptiles were above the soil surface and the horizontal directions of coleoptiles in all the rows were same in a seedbed. The seedlings after sowing were grown in a greenhouse at Hokkaido Agricultural Research Center (HARC). In the late May, sixty-six plants with showing a uniform leaf age and plant height (PH) were selected for transplanting in each genotype. At the transplanting time (i.e. the late May), the selected seedlings of a genotype were randomly divided into the high-density (H) and low-density (L) conditions. The reason why H and L conditions were prepared is that density-dependent-flowering time was often observed in the breeding process. In each genotype, the eighteen plants per replicate for H condition and fifteen plants per replicate for L one were transplanted into three rows with a spacing of 12.5 x 30.5 cm and 25.0 x 30.5 cm, respectively in a paddy field at HARC. The modern Hokkaido cultivars fitted to transplanting cultivation have been genetically improved and cultivated under the H condition regardless of the cultivated region. Fertilizers were applied at a rate of 0.40-0.49-0.35 NPK (Kg / a). To minimize the intergenotypic competitions among the rice strains, genotypes were arranged according to the genotypic mean of PH. The order in replicate I was from the genotype showing the largest PH to that showing the smallest one, while that in replicate II was the reversed order of replicate I. It is noted that land race strains were cultivated with modern cultivars, and hence, data of land race strains was obtained in the identical experiment of modern cultivars.

2.1 Trait evaluations

In each of replicates of a genotype, middle three plants were measured in both H and L conditions. Heading time is defined as the time when a panicle emerged from the uppermost leaf sheath. In each plant, the first, second and third heading times were recorded based on daily observation. The reason why the three heading times were recorded is that the first heading date is largely faster than the second and the third heading times especially in land race cultivars. After the records, days to the first heading time from germination (DFH), days to the second heading time from germination (DSH) and days to the third flowering time from germination (DTH) were calculated. This calculation was transform to easily statistical analyze. Heading-time duration (HTD) of each plant was conveniently defined as the equation: $HTD = DTH - DFH$. When the third flowering time occurs, many tillers simultaneously headed, and hence, DFH is an index that can be regarded as final time of heading time of an individual. All the statistical analyses were performed by JMP 11 [12].

3. RESULTS AND DISCUSSION

To grasp frequency distribution of DFH, DSH and DTH in the thirteen cultivars, Fig. 1 was made. DFH showed larger variation of days to heading time than DSH and DTH. The pattern of variation of DSH and DTH tended to be similar each other. This suggests that genetic control of DFH might differ from that of DSH and DTH.

To investigate the relationship between DFH and DTH, which is component trait of HTD, regression analysis between DFH and DTH was performed in each of density conditions (Fig.2). In both density conditions, regressions were significantly established but R^2 values were lower than 0.30 that is commonly minimum confidence degree of regression conformity. In addition to the result of Fig. 1, it is suggested that genetic regulation of DFH might partly differ from that of DTH. Then, analysis of variance (ANOVA) is needed for examining the suggestion.

When genotype, repeat and density were regarded as the factors, ANOVA was performed using the data of all the plants examined as to DFH, DSH and DTH. It is noted that there was no deficit in ANOVA. ANOVA revealed that DFH was deterministically dependent on genotype, while the other flowering time can respond to environment, showing genotype $\times$ environment interaction (Table 1), suggesting a specific genetic devise for controlling DFH. With the progress of the shoot development of rice, developmental fate of phytomer tended to be affected by environments [13] under an internal constraint [13, 14]. Shoots of later heading time in a plant were usually produced at higher position of phytomer from the base than early heading time in a rice plant. Hence, later heading time might be affected by environments including genotype-by-environment interactions. Therefore, the lower values of R^2 in Fig.2 might be results from that DFH was only affected by genotype while DTH was affected by repeat, genotype-by-density and genotype-by-repeat-by-density interactions in addition to genotype. The result of ANOVA also suggests that quantitative locus analysis of heading time might missed total nature of heading time since they have treated only the first heading date in an identical density condition [8, 9, 10, 11].

To examine that each of components of HTD, DFH and DTH, contributes to HTD, regression analyses were performed (Fig. 3). In Both density conditions, DFH highly explained HTD. The R^2 values showed more than 0.900. On the other hand, DTH was not associated with HTD under H condition (Fig. 3a). Under L condition, DTH barely explained HTD (Fig. 3b), but the R^2 value was far from 0.30 that is commonly minimum confidence degree of regression conformity. Hence, HTD was primarily determined by DFH. This results may be derived from that variation of DFH is larger than that of DTH (Fig. 1).

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HTD of Modern cultivars was smaller than that of land races and its difference was significant at 1% level (Fig. 4). In contrast to the relationship, DFH of land races was smaller than that of modern cultivars and its difference is significant at 1% level (Fig. 5). This indicates that modern breeding decreased HTD by genetically modifying the first flowering time from earliness to lateness based on recognition of the negative correlation relationship between HTD and DFH (Fig. 3). This also means that rice breeders in Hokkaido have intended that an identical rice cultivar (or plant) flowers in a small range using the negative correlation relationship between DFH and HTD (Fig. 3) and have genetically modify rice cultivar to flower in the short period of high temperature [7]. Since photoperiod sensitivity that makes tillers simultaneously flower in a rice plant cannot be used in high latitudes due to extremely long days, it is likely that the breeding of late-flowering-time strain might be only way to make tillers simultaneously flower of a rice plant in the sort period of high temperature in the high latitude using the negative correlation relationship between DFH and HTD (Fig. 3). But excess of fine-tuning of flowering-time duration would sit side by side with cool weather damage in this region since pattern of change of temperature different among years [7].

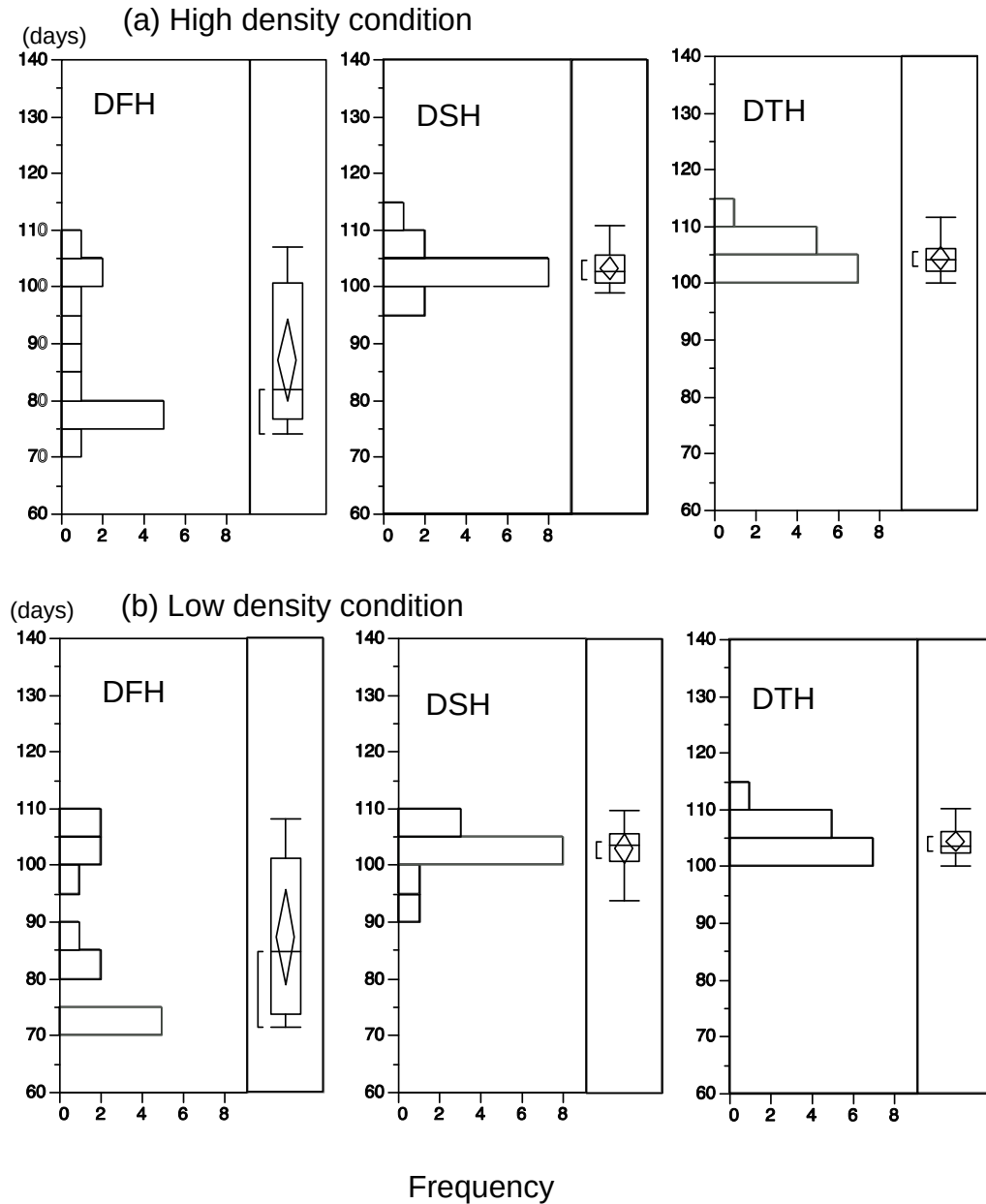
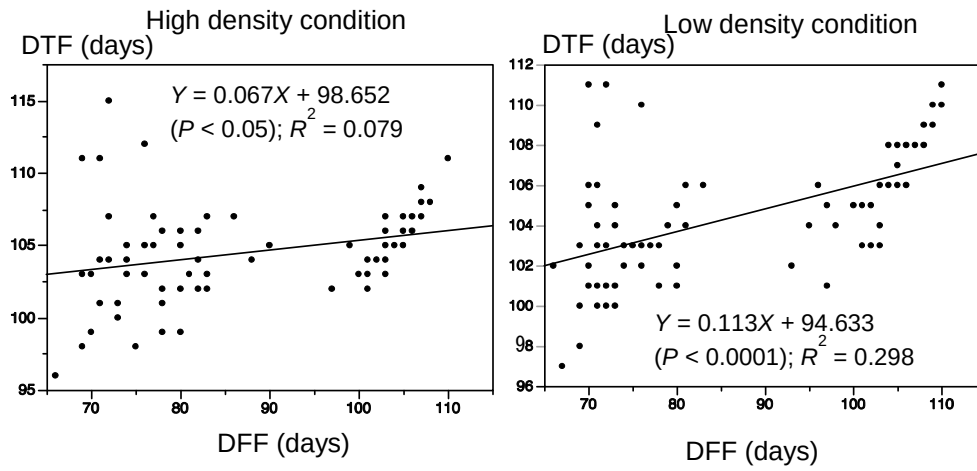


Fig. 1 Frequency distribution of DFH, DSH and DTH in the thirteen Hokkaido cultivars under high-density (a) and low-density (b) condition
A horizontal and vertical axis of rhombus indicates mean and 95% confidence interval of mean, respectively. The upper and base side of box indicates the first and third quartile, respectively. The horizontal line in the box represents median. The vertical line extended below the box is calculated from the formulation: the first quartile - 1.5 × interquartile range, while the vertical line extended on the box is calculated from the formulation: the third quartile - 1.5 × interquartile range

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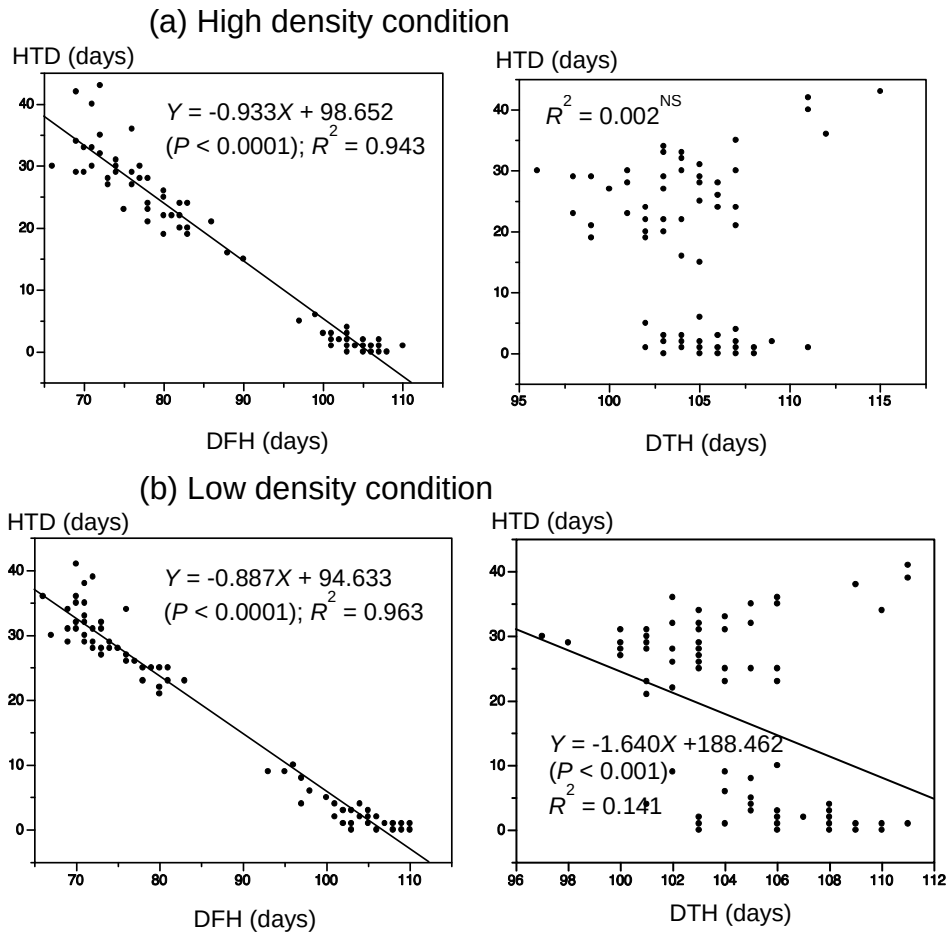
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Fig. 2. Regression analysis between DFH and DTH in high- and low-density condition

242 **Table 1. F-values for DFH, DSH and DTH**

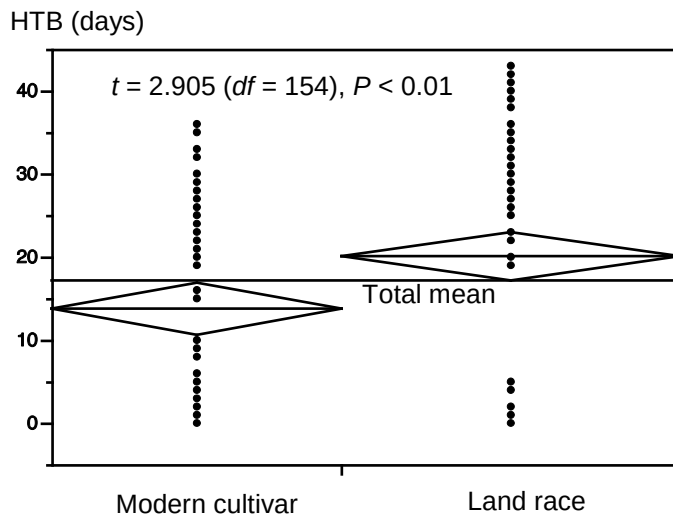
Factor	df	DFH	DSH	DTH
Genotype	12	24.9377****	38.2615****	54.5935****
Repeat	1	1.3630 ^{NS}	0.6182 ^{NS}	7.801**
Density	1	0.0361 ^{NS}	1.3910 ^{NS}	0.0288 ^{NS}
Genotype × Repeat	12	1.5554 ^{NS}	3.8699****	1.6287 ^{NS}
Genotype × Density	12	0.6505 ^{NS}	2.9549**	2.2025*
Repeat × Density	1	0.6783 ^{NS}	0.989 ^{NS}	2.0032 ^{NS}
Genotype × Repeat × Density	12	0.3851 ^{NS}	2.4263**	1.9407*

243 1) ****, ** and * indicates significance at 0.01%, 1% and 5% level, respectively. NS indicates non-
244 significance.
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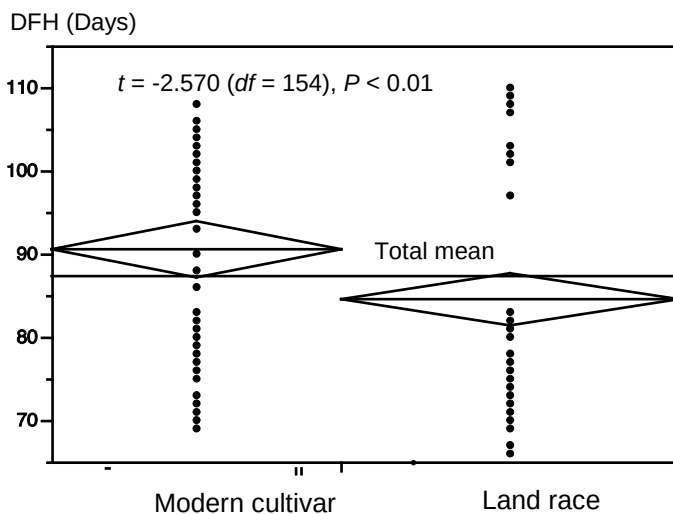
247 **Fig. 3. Regression analysis between DFH and HTD and between DTH and HTD**
248 **under high-density (a) and low-density (b) condition**
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Fig. 4. Comparison of HTB between modern cultivar and land race
Apices of rhombus indicate 95% confidence interval. Horizontal line in rhombus indicates mean of a group. The width of rhombus is in proportion to sample size.



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Fig. 5. Comparison of DFH between modern cultivar and land race
Apices of rhombus indicate 95% confidence interval. Horizontal line in rhombus indicates mean of a group. The width of rhombus is in proportion to sample size.

4. CONCLUSION

By the addition of land race in the present study, it was clarified that rice breeders of Hokkaido islands genetically modify heading times. DFH was not affected by environments including genotype × environment interaction and its nature was genetically deterministic. Hence, DFH was easy index for rice breeder to distinguish heading time variation among cultivars. DFH and HTD showed negative correlation relationship. Rice breeders of Hokkaido islands diminish HTD by genetic improvements of the first heading time toward lateness using the relationship. As a result, they succeeded in making rice genotype, which simultaneously flowered in probabilistically high-temperature period.

ACKNOWLEDGEMENTS

A paddy field was provided for the present study by Mr. Hiroyuki Shimizu (HARC). The use of genetic materials was permitted by Professor Yoshio Sano (Research Group of Breeding Science, Division of Applied Bioscience, Research Faculty of Agriculture, Hokkaido University). Y. Itoh thanks Miss Yoshie Sato (GASUTO, Fukuzumi), Assistant Professor, Rie Kameyama (Hokkaido University Hospital), Drs. Hiroshi Yorozya, Kazutoshi Mori and Yoshikazu Sato (Brown Bear Research Group of Hokkaido University) for their encouragement and discussions about the present study.

COMPETING INTERESTS

Author has declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

The sole author designed, analyzed and interpreted and prepared the manuscript.

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