

Original Research Article

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

ABSTRACT

Aims: Hokkaido islands are one of northern limit regions of rice cultivation. There is about 170 years history about cultivation and breeding of Hokkaido rice. However, English references with hard data about the history ~~were lack~~ at all 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 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 ~~was~~ 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 (~~DSF~~) 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 how degree 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. As a result, modern breeding decreased HTD by genetically modifying the first flowering time from earliness to lateness using the negative correlation relationship between HTD and DFH.

Conclusion: As shown in Results, it was clarified ~~what~~ 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 ~~has not been 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 years. Then rice had steadily expanded one of the northern limits of rice cultivation, Hokkaido islands (41° 18' – 45° 30') at last [2]. There is about 170 years history about cultivation and breeding of Hokkaido rice [2]. However, English references with hard data about the history were extremely ~~lack~~ and the cultivars studied in the reports written in English have been limited to modern cultivars [3,4]. This situation results

from which 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 cool tolerance have been genetically improved in Hokkaido islands [2, 4, 5, 6]. However, there is room for studies about how degree cool tolerance contributed to expansion of rice cultivation in Hokkaido. It is because near-isogenic lines of Taichung 65 with earliness genes showed a high fitness 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 Hokkaido rice cultivars 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 times in an individual were recorded using thirteen representative Hokkaido cultivars from initial to modern cultivars. First, I demonstrated that factors of heading time were different each other. Second, I analyzed which heading time is associated with variation of heading time of an individual. Third, to know what rice breeders have genetically modified Hokkaido cultivars, 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 was used. The seven strains of the thirteen involved one introduced cultivar, Tuguru-wase (A110), and three initial land races, Akage (A1), Akaine (A2), Akamuro (A5), and the three land race, Bozu (A9), Kokushoku-to 2 (A58) and Kurikaramochi (A60). 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 the cross breeding cultivars. They are so called modern cultivars [2].

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. 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.

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. 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: $HTD = DTH - DFH$. 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. 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.

Analysis of variance 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. It also suggest that quantitative locus analysis of heading time might missed total nature of heading time since they have treated only the first heading date [8, 9, 10, 11].

To examine how degree 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.

Compared with land races, modern cultivars more decreased HTD and its difference was significant at 1% level (Fig. 4). In contrast to the relationship, DFH of land races were averagely 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 using 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 simultaneously flower and have genetically modify rice cultivar to flower in the small high-temperature period [7]. Since photoperiod sensitivity, which is a device that makes rice cultivars simultaneously flower, 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 simultaneously flower in high latitude. But excess of fine-tuning of flowering time would sit side by side with cool weather damage in this region.



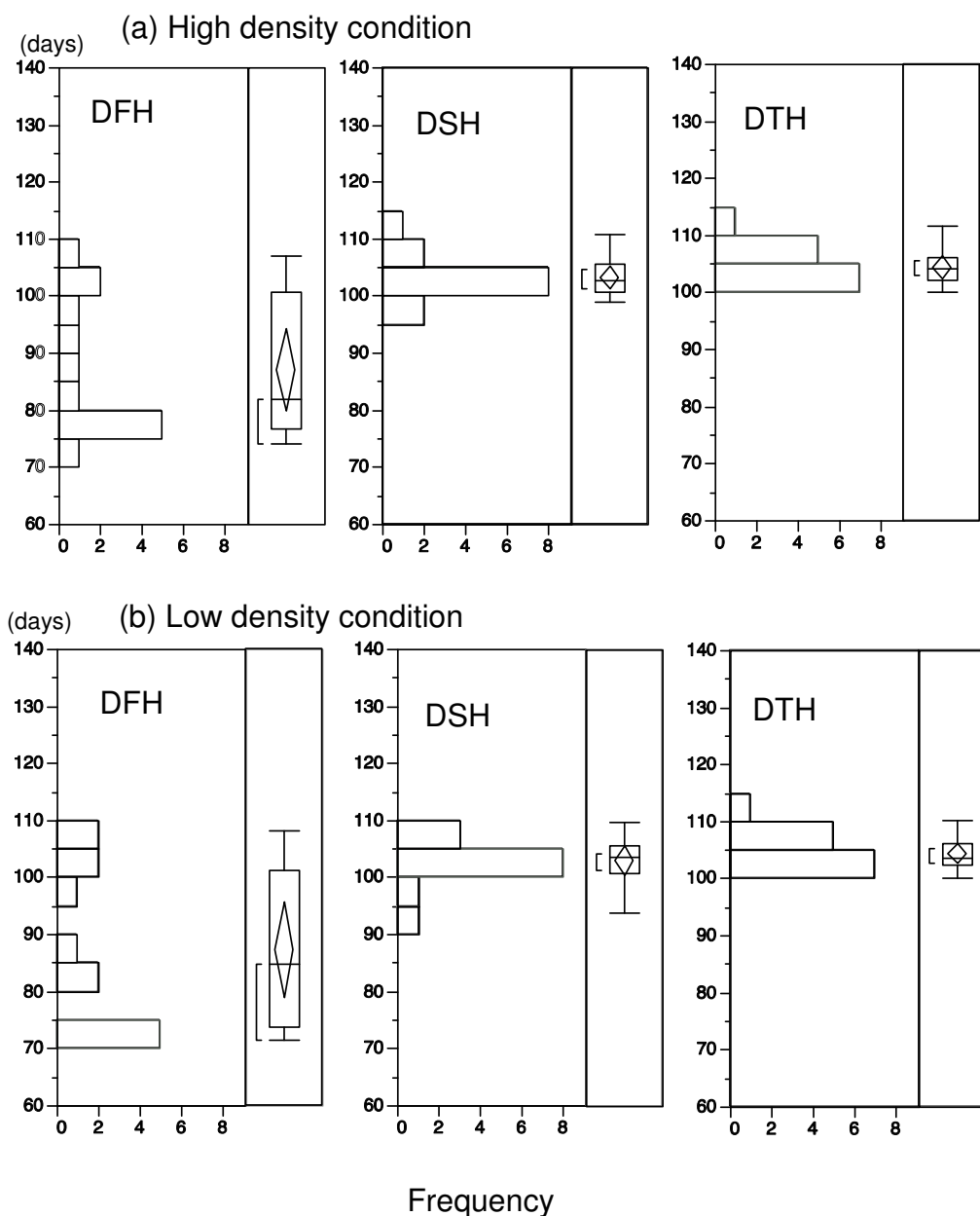


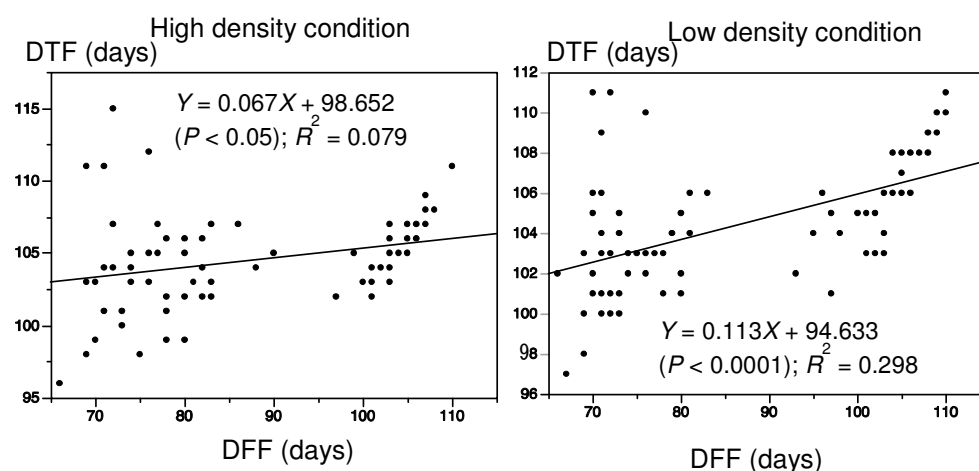
Fig. 1

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 - $1.5 \times$ interquartile range, while the vertical line extended on the box is calculated from the formulation: the third quartile - $1.5 \times$ interquartile range

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

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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*

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1) ****, ** and * indicates significance at 0.01%, 1% and 5% level, respectively. NS indicates non-significance.

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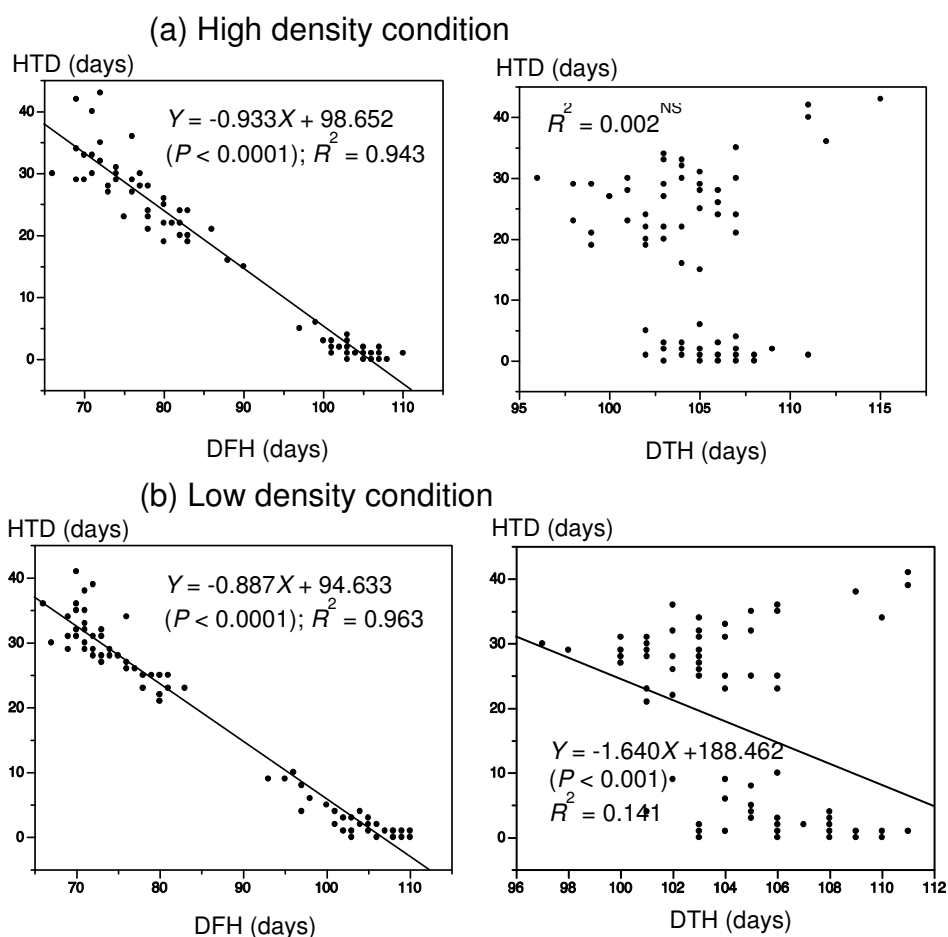
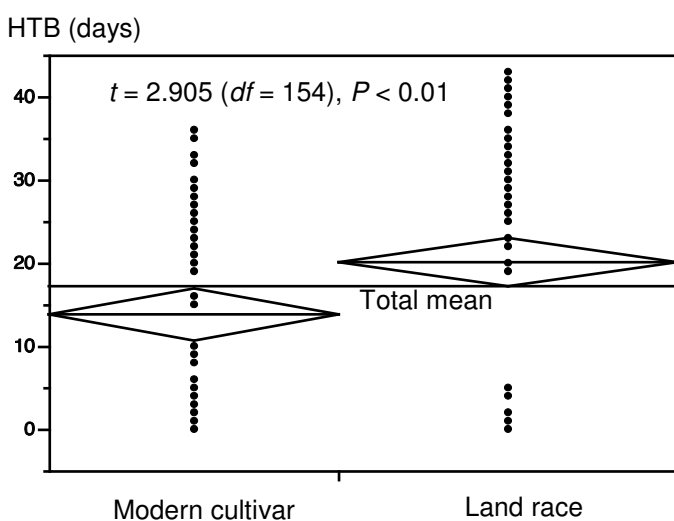


Fig. 3. Regression analysis between DFH and HTD and between DTH and HTD under high-density (a) and low-density (b) condition



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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.

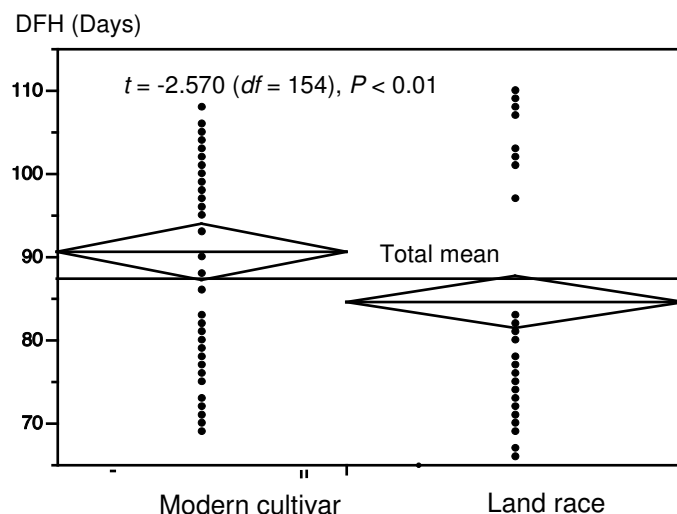


Fig. 5. Comparison of DFH between modern cultivar and land race

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 what 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.

REFERENCES

1. Oka HI. Origin of cultivated rice. Tokyo: Elsevier; 1988
2. Takahashi M. The history and future of rice cultivation in Hokkaido. Japanese Experience of the UNU Human and Social Development Program series. 1980; 22. Available: http://d-arch.ide.go.jp/je_archive/english/society/wp_je_unu22.html9.
3. Okumoto Y, Ichitani K, Inoue H, Tanisaka T. Photoperiod insensitivity gene essential to the varieties grown in the northern limit region of paddy rice (*Oryza sativa* L.) cultivation. Euphytica 1996 ;92 (1): 63-66. DOI: 10.1007/BF00022829
4. Fujino K. Photoperiod sensitivity gene controlling heading date in rice cultivars in the northernmost region of Japan. Euphytica 2003; 131 (1): 97-103. DOI: 10.1023/A:1023088810701
5. Baruah AR, Ishigo-oka N, Adachi M, Oguma Y, Takizono Y, Onishi K, Sano Y. Cold tolerance at the early growth stage in wild and cultivated rice. Euphytica 2009; 165 (3): 459-470. DOI: 10.1007/s10681-008-9753-y

- 192 6. Baruah AR, Onishi K, Oguma Y, Ishigo-oka N, Uwatoko N, Sano Y. Effects of a acclimation on chilling
 193 tolerance in Asian cultivated and wild rice. *Euphytica* 2011; 181: 293-303. DOI: 10.1007/s10681-011-
 194 0427-9
- 195 7. Itoh Y. Effect of the two-early-flowering-time genes, *Ef1* and *m-Ef1*, founded in low latitude were
 196 reexamined in one of the origin of donor parent under fluctuating field environments as to earliness effect
 197 and fitness. *Journal of Agriculture and Ecology Research International* 2015; 4 (2): 43-52. DOI:
 198 10.9734/JAERI/2015/18360
- 199 8. Sato S, Ogata K, Shinjoh C. Thermo-sensitive action of earliness gene *Ef-x* in rice, *Oryza sativa* L. *Jpn*
 200 *J Genet.* 1992;67(6): 473-482.
- 201 9. Tsai KH. Studies on earliness genes in rice, with special reference to analysis of isoalleles at the *E*
 202 locus. *Jpn J Genet.* 1976; 51(2):115-128.
- 203 10. Fujino K, Yamanouchi U, Yano M. Roles of the *Hd5* gene controlling heading date for adaptation to
 204 the northern limits of rice cultivation. *Theor Appl Genet.* 2013;126(3):611-618. DOI: 10.1007/s00122-012-
 205 2005-5.
- 206 11. Hori K, Nonoue Y, Ono N, Shibaya T, Ebana K, Matsubara K et al. Genetic architecture of variation in
 207 heading date among Asian rice accessions. *BMC Plant Biology* 2015;15: 115. DOI: 10.1186/s12870-015-
 208 0501-x
- 209 12. SAS Institute Inc. JMP® 11. Cary, NC; 2012.