# Physio-Genetic Analysis for Introgression Lines with Unique Traits of Yield Components in an Indica-type Rice (*Oryza sativa* **L.**) Variety IR64

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# Physio-Genetic Analysis for Introgression Lines with Unique Traits of Yield Components in an Indica-type Rice (*Oryza sativa* L.) Variety IR64

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# Abbreviations

| | |
|---|---|
| CL | Culm length |
| CW | Culm weight |
| DH | Days to heading |
| DHL | Double haploid lines |
| DM | Dry matter (Biomass) |
| DNA | Deoxyribonucleic acid |
| DW | Dry weight |
| FAO | Food and Agricultural Organization |
| FS | Fertile seeds per panicle |
| FS/TS | Ratio of fertile seeds per panicle (fertility rate) |
| GRiSP | Global Rice Science Partnership |
| GW | Grain weight |
| IL | Irrigated lowland |
| INLs | Introgression lines |
| IPCC | Intergovernmental panel on climate change |
| IRRI | International Rice Research Institute |
| JIRCAS | Japan International Research Center for Agricultural Sciences |
| LD | Linkage disequilibrium |
| LL | Leaf length |
| LTN | Low-tillering |
| LW | Leaf width |
| MAB | Marker assisted breeding |
| MAS | Marker assisted selection |
| N- | Nitrogen |

| | |
|---|---|
| NIL | Near isogenic lines |
| NPT | New plant type |
| NUE | Nitrogen use efficiency |
| PL | Panicle length |
| PN | Panicle number (No.) |
| P/T | Ratio of panicle weight per whole plants weight (harvest index, HI) |
| PW | Panicle weight |
| QTL | Quantitative trait locus |
| RDW | Relative dry weight in percentage |
| RILs | Recombinant inbreed lines |
| RFLP | Restriction fragments length polymorphisms |
| SS | Sterile seeds per panicle |
| SSR | Simple sequence repeats (microsatellite marker) |
| TARF | Tropical Agricultural Research Front |
| TJ | Tropical Japonica |
| TN | Tiller number (No.) |
| TS | Total number of seeds per panicle |
| TW | Total weight |
| UP | Upland |
| YC | Yield components |

# Chapter I

## General Introduction

### *1.1 Rice*

Rice (*Oryza sativa* L.) is a predominantly self-pollinated and a diploid ($n$=12) semiaquatic cereal crop. It is the staple of half of the human population and growing it is the single most important economic activity in the world (GRiSP 2013). FAO (2014) stated that rice is one of the most important food crops in the world and it is the central to the food security in Asia, and the overall economic growth and political stability in this region depend on an adequate, affordable, and stable supply of this staple crop.

### *1.2 Current global issues, challenges and opportunities*

World population has been increasing, and is expected to surpass 9 billion by 2050 (UNPD 2015). In a similar pace, the global rice consumption is also rising steadily and is projected to be 873 million tons by 2030 (Purevdorj and Kubo 2005), where current production amount is 730 million tons (FAO RMM 2014). Accordingly, rice production has to increase over 20% (143 million tons) to meet the growing demand and to avoid world food crisis, just by the next fifteen years. This scenario has been exacerbated by the prediction that rice and wheat production could fall by 8% and 32%, respectively, by 2050 due to climate change (IPCC FAR note 2007). In spite of the substantial increase in rice production in the wake of the Green Revolution, decline in rice biodiversity and loss of rice heritage, global climate change, increasing competition for land, labor and water from industrial and urban sectors, changes in dietary composition with income

growth and urbanization; and changes in the demographic composition of labor in rural areas are also challenging global rice production.

Despite these challenges, several new opportunities exist to increase the impact of the rice sector in enhancing food security and reducing hunger, malnutrition and poverty. Modern scientific approaches and new technologies are making it possible to increase rice productivity in a sustainable manner, add nutritive value to rice, reduce losses from drought and flood, reduce environmental footprint of rice production and improve the production systems (FAO 2014). It is, however, increasingly important to enhance rice yields on existing land i.e. grain yield per area, than to expand paddy fields (David 1991, Cassman 1994, Khush 2013).

### 1.3 Rice ecosystems for cultivation

Rice grows in a wide range of environments, and most classifications of rice environments are based on hydrological characteristics. Rice farming is practiced at approximately 160 million hectares of land in five distinct ecotypes or ecosystems around the world. These are irrigated lowland (IL, approximately 50% of the global total cultivated area), rain fed lowland (RL, 33%), rain fed upland (UP, 10%), flood prone rice (5%), and salinity prone rice (2%) ecosystems (GRiSP 2013). IL ecosystems provide 75% of global rice production, and rice is grown in bounded fields with ensured irrigation for one or more crops a year. And, UP rice is grown under dryland conditions without irrigation and without puddling. The areas spreading in Asia, Africa, and Latin America, and can be low lying, drought-prone, rolling, or steep sloping. UP or aerobic rice cultivation reduces water use in rice production and increases the water use efficiency, and also in environmental point of view, emission of methane is lower

substantially in this condition. However, increased weed growth, poor crop stand, crop lodging, high percentage of panicle sterility and root-knot nematode infestation are the main constraints (Anandans *et al*. 2015). As global water shortage is increasing, it is now necessary to breed new cultivars with high yield potential in UP ecosystems.

### *1.4 Genetic improvement of rice yield potential*

Yield potential is defined as the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting and with pests, diseases, weeds, lodging, and other stresses effectively controlled.

There are various strategies to increase the yield potential of rice, including conventional hybridization and selection, ideotype breeding, hybrid breeding, exploitation of wild species, enhancement of photosynthesis, genomic approaches, and physiological approaches (Khush 2013). Traditional approach is based on the variability created through hybridization between diverse parents and subsequent selection of desirable individuals. This strategy could help to increase 1% per year in the yield potential of various cereals such as wheat, barley, and rice (Peng *et al.* 2000), and this is the time tested strategy. It is the basic of all other crop improvement strategies. The details of other strategies are discussed (Khush 2013). The improvement of yield potential of tall tropical rice landraces resulted in the development of semi-dwarf, high yielding modern rice variety that initiated the 'Green Revolution' in the 1960s (Peng *et al.* 2008).

Ideotype breeding aimed at modifying the plant architecture with a specific combination of characteristics favorable for photosynthesis, growth, and grain

production based on knowledge of plant and crop physiology and morphology (Peng *et al.* 2008). Donald (1968) proposed the ideotype breeding approach to plant breeding and Tsunoda (1962) compared yield potential and yield response to nitrogen fertilizer in relation to the plant type of rice genotypes. He found close association between certain morphological traits and yielding ability in response to nitrogen that led to the 'Plant Type Concept' as a guide for breeding improved varieties (Yoshida 1972).

To break the yield potential barrier, International Rice Research Institute (IRRI) scientists proposed modifications to the high yielding Indica Group in the late 1980s and early 1990s (Khush 1995). The proposed new plant type (NPT) has low tillering capacity (3–4 tillers when direct seeded), few unproductive tillers, 200–250 grains per panicle, a plant height of 90–100 cm, thick and sturdy stems, leaves that are thick, dark green, and erect; a vigorous root system; 100–130 days growth duration; and increased harvest index (Peng *et al.* 1994). Breeding efforts to develop 1$^{st}$ generation NPT were initiated in early 1990s by crossing between Tropical Japonica Group (TJ) varieties called 'Bulus' from Indonesia and semi-dwarf Japonica Group breeding line 'Shen Nung 89-366', and evaluated these NPT lines (Khush 1995, Peng *et al.* 2008, Khush 2013). As intended NPT lines had large panicles, few unproductive tillers, and lodging resistance. However, grain yield was disappointing because of low biomass production and poor grain filling, susceptible to disease and insects, and grain quality was not acceptable by the consumers in tropical and subtropical countries. Later, to improve the acceptability of these NPT-TJ lines for tropical condition and improve their yield potential, they were crossed with elite Indica Group lines and varieties with disease and insect resistance and good grain quality, and developed 2$^{nd}$ generation NPT lines. Many of these lines out yielded the best improved Indica Group varieties such as IR 72

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by as much as 1.0 to 1.5 tons per hectare. These breeding lines are very useful to increase genetic diversity and the improvement of Indica Group rice cultivars.

## *1.5 Yield component and biomass traits in rice*

Yield components (YC) refer to the structures of the rice plant that directly translate into yield. Heading date (HD) is critical factor for determining regional and seasonal adaptation in rice cultivars. It is regulated by exogenous factors such as light, temperature, and numerous endogenous factors (Hori *et al.* 2015). Plant length is the sum of culm length (CL) and panicle length (PL), and represents the vertical growth of rice plant. It has been well known that CL and PL are involved with lodging resistance, light interception, and canopy $CO_2$ intake and directly related to plant status and yield potential (Ogi *et al.* 1993, Peng *et al.* 1994, Tanisaka 1997, Yamamoto *et al.* 2001). A plant height with 90-100cm is considered for maximum yield (Khush 1995). On the other hand, panicle number (PN), which represents the horizontal growth, is a direct determinant of grain yield together with the total spikelet number (TS) per panicle and comprised of number of fertile seeds (FS) and number of sterile seeds (SS). Generally, tiller number (TN) predetermines PN at harvest; therefore it is another important trait indicating horizontal development. The rate of fertility's seeds e.g. fertile seed percentage (FS/TS, %), describes the efficiency of fertile grain production. Dry matter (DM) or biomass is the total weight (TW) of a plant, and consists of the sum total of straw weight (SW) and panicle weight (PW). And harvest index (HI) is the ratio of PW and TW (P/T). In rice, number of spikelet per $m^2$ were highly related to dry matter accumulation from panicle initiation to flowering (Kropff *et al.* 1994), whereas grain filling largely depends on biomass accumulation from flowering to maturity (Yoshida

1981). Higher yield potential can be achieved by improving the high biomass or dry matter production. These ten traits, HD, CL, PL, CW, PW, P/T, PN, FS, TS and FS/TS (%), are major traits that compose rice plant architecture and complicated yielding processes (Peng *et al.* 1994).

## *1.6 Genetic studies on yield component traits in rice*

The YC and biomass traits are controlled genetically. Conventional genetic analysis identified a number of genes that had large effects on these traits, as summarized by Kinoshita (1995) and Kamijima *et al.* (1996). Most of these genes were identified using mutants such as dwarfness, grain size and shape, anthocyanin coloration etc. rather than actually cultivating varieties (Xiao *et al.* 1996), and were located roughly by chromosome numbers (Sasaki 2001). Statistical genetics studies however showed that numerous polygenes, which have relatively minor effects, are involved with the determination of yield component traits in cultivating varieties (Sasahara 1997, Tanisaka 1997, Yamagata 1997). Because of the minor effects, it had been difficult to clarify and locate their loci by conventional crossing techniques during the pre-molecular marker era (Ray *et al.* 1994, Yano *et al.* 1997, Xu *et al.* 1998), and it responds poorly to the direct selection due to the large environmental effects.

Usually, plant height has a genetically negative relation with PN (Gravois and McNew 1993). Other yield component traits, such as TN, FS and TS, are also genetically correlated with other (Gravois and McNew 1993, Yan *et al.* 1999). The genetic correlations are attributable to a tight linkage of genes affecting traits or pleiotropic effects of a gene (Sato *et al.* 1994, Lin *et al.* 1996). However, the tight linkage or pleiotropic effects of genes have not been identified or located in classical

gene analyses, because of the lack of appropriate experimental tools.

Rice adapts to varying growth environments by modifying plant type and yield component traits, for example rapid culm elongation in floating varieties (Ray *et al.* 1994, Hamamaru 1997). The adaptation has been indicated to occur *via* the genotype by environment (G by E) interactions (McCough and Doerge 1995, Lu *et al.* 1996, Yan *et al.* 1999, Yamamoto *et al.* 2001, Sripongpangkul *et al.* 2000). Hence G by E interaction is an important factor determining rice plant type and complicating the inheritance (Lu *et al.* 1996). Classical gene analyses, which depended on crossing combinations were not powerful for locating the gene loci responsible for the G by E interaction.

### 1.7 QTL mapping studies on rice yield component traits

Grain yield is one of the most important indices in rice breeding, which is governed by quantitative trait loci (QTL). QTL mapping of a target trait is defined as the chromosomal location and genetic characterization of QTLs for the trait through the association between genetic markers and phenotypic variations. To facilitate this mapping, development of mapping population, construction of linkage map and phenotypic evaluation are essential for QTL analysis.

Typically, mapping population includes $F_2$ plants, doubled haploid lines (DHLs) and recombinant inbred lines (RILs). $F_2$ population that carries the complete genetic information from the parents can be easily developed, but its phenotypic evaluation cannot be replicated (Ying *et al*. 2012). Due to the inherent homozygosity in the lines, both DHL and RIL populations can be planted repeatedly in different planting seasons and environment conditions as many times as necessary.

In plant breeding, various breeding designs are used by breeders to generate

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mapping populations. Recurrent backcrossing (repeated crossing to a selected parent) or introgression breeding is a traditional breeding method (Anderson 1953), commonly employed to transfer rare and useful alleles at one or more loci from a donor to an elite variety (Allard 1960, Reyes-Valdes 2000). Usually, inter or intra-specific hybrids are created to increase genetic diversity for improving the yield potential, disease and insect resistance of cultivated crops. The introgression lines (INLs) are very useful experimental materials for genetic analysis and molecular breeding and could be used to evaluate the action and interaction of genes over multiple years and environments (Tanksley and Nelson 1996). The regions associated with the agronomic traits in the INLs can be easily identified because the INLs had small introduced segments from donor parents and have been substituted for the uniform genetic background of recurrent parent.

Genetic mapping can be done by mostly in two ways (1) using the experimental population (also referred to as 'parental' mapping populations) that is called QTL-mapping as well as 'genetic mapping' or 'gene tagging' and (2) using the diverse lines from the natural populations or germplasm collections that is called LD-mapping or 'association mapping'. The details of the QTL-mapping approaches, concepts, models, and methodologies, problems and perspectives of identification, isolation and pyramiding of quantitative trait loci for rice breeding, are in Liu (1998), Doerge (2002), Collerd *et al.* (2005), Ashikari and Matsuoka (2006) andYamamoto *et al.* (2009).

Until now, there are several hundreds of QTLs and genes have been detected for yield component traits, the details of the detected QTLs for yield component traits have been reviewed by Borba *et al.* (2010), Xing and Zhang (2010), Bai *et al.* (2012) and Ying *et al.* (2012). Advanced populations such as near isogenic lines (NILs) are

efficient to further fine-map and clone target QTLs (Bai *et al.* 2012). Reasonable combination of favorable alleles has the potential to increase grain yield *via* use of functional marker assisted selection. Several major QTLs have been identified, cloned, and transferred to the modern cultivars during the last decades these increased yield potential, but they were often been recognized and better utilized by conventional breeding (Yamamoto *et al*. 2009). So, it is necessary to discover new alleles for the improvement of current rice cultivars.

## 1.8 Physiological approach to improve the yield potential

Any genetic advance of yield potential must have a physiological basis. The use of physiological traits in a breeding program, either by direct selection or through molecular markers, can then result in more accurately targeting factors limiting yield and may result in faster rate of yield improvement (Richards *et al.* 2000). Besides, improvements of current genetic yield potential in rice are too low to keep pace with future demand, and several researches suggest that physiological selection traits have the potential to improve genetic yield gains in rice (Slafer *et al.* 2005). In light of the high energy costs and increasingly scarce resources, future agricultural systems have to be more productive and more efficient in terms of inputs such as fertilizer and water. The development of rice varieties with high yield under low-nutrient conditions has therefore become a breeding priority.

In intensive agricultural systems, nitrogen is the most essential nutrient in determining the yield potential of crops, and fertilizer nitrogen is one of the major inputs to agricultural systems. Relationships among growth, yield and nitrogen utilization in rice plants are important at the physiological and molecular level (Mae

1997). The uptake and use efficiency of nitrogen affect plant growth, and genetic improvement in rice variety will be very important issue for stable and high rice production.

## *1.9 IR 64 and its yield potential improvement*

An Indica Group cultivar IR 64 is one of the most popular rice varieties bred during the second phase of the Green Revolution at the International Rice Research Institute (IRRI), and first released in the Philippines in 1985 (Dalrymple 1986, Khush 1987).

The 'Green Revolution', described as a series of research, development, and technology transfer initiatives, that increased agricultural production (by developing high-yielding varieties of rice, wheat etc.) worldwide, particularly in the developing countries, beginning most markedly in the late 1960s (Hazel 2009). It has centered on high-yielding, disease and insect-resistant rice varieties, has revolutionized rice production since the late 1960s in the tropical and sub-tropical regions (Khush 1995). However, since the initial breakthrough in yield potential when IR 8 was developed, there had been only marginal increase in yield potential, yield stability, superior cooking quality and shorter growth duration (Khush 1987). Therefore, IRRI scientists have developed the varieties (Released by IRRI as IR 5 to IR 34 and by Philippine seed board as IR 36 to IR 66) with a quantum jump in yield over that of IR 8 (Rockwood 2001). IR 64 was derived from an extensive intercrossing of 20 original farmer varieties from eight countries and improved lines whose maternal ancestors was *Cina* (formerly called Tjina) from China *via* Indonesia (Dalrymple 1986). IRRI lines IR 5657 and IR 2061, derived from the cross combinations between the known varieties IR 22 and IR 24, were used to breed IR 64. It was widely adopted in many countries in the tropics

owing to its high yield potential, shorter growth duration, good eating quality, and enhanced resistance to several diseases and pests (Khush and Virk 2005). It is still widely grown (Ballini *et al.* 2007).

Furthermore, Khush and Virk (2005) reported this variety is resistant to Bacterial blight, Tungro, grassy stunt and moderately resistant to blast disease, and explained that the long grain, slender shape, none chalkiness, non-waxy endosperm and intermediate amylose percentage and gelatinization temperature helped to attract the rice consumers. It is suitable to grow in irrigated and rain fed lowland areas, and shows tolerance to moderate levels of salinity, alkalinity, acid sulfate and acid UP soils, boron toxicity and zinc and phosphorus deficiency.

However, it has limitations. It has low agronomic nitrogen use efficiency (NUE, Diekmann *et al.* 1996) and low relative dry weight and physiological NUE (Namai *et al.* 2009). It is highly susceptible to drought (Cal *et al.* 2013, Lafitte *et al.* 2007), and drought stress during flowering dramatically decreases its yield (Wade *et al.* 1999). It is susceptible to Fe toxicity (Nozoe *et al.* 2008), blast disease (Liu *et al.* 2009), and tungro disease (Shibata *et al.* 2007). It is thus important to improve its yield potential, physiological NUE, and tolerance of biotic and resistance to abiotic stresses.

Though, IR 64 genetic background has been used to develop several genetic materials, such as DHLs (Guiderdoni *et al.* 1992), RILs (Uga *et al.* 2010), and thousands of mutant lines for genetic analysis, but quite a few materials have been developed for yield potential improvement of IR 64. Enhanced breeding materials that have similar elite traits of IR64 would be very useful for the future rice production to maintain sustainability in rice production, to reduce the existing yield gap. Recently,

Fujita *et al.* (2009) developed the 334 INLs by the introgression of chromosome segments from NPT cultivars originated from the tropical Japonica Group UP varieties and a Japanese Japonica Group cultivar 'Hoshiaoba', in the background of the Indica Group rice IR 64 to improve the yield potential and to clarify the genetic improvements.

### *1.10 Aims of this study*

Fujita *et al.* (2009) carried out genetic analysis for eight agronomic traits using these INLs at IL of IRRI, in the tropics. However, they have not been evaluated yet under different environment conditions such as temperate, and rice ecosystems such as IL and UP, and these information of yield components and dry matter production were limited. To address these gaps, this study aimed to characterize again under IL and UP in temperate environment based on the evaluation of yield and biomass related traits. Also, we tried to identify the traits and these genetic factor(s) originated from tropical Japonica Group UP cultivars. The characterization and classification of the 333 INLs using YC and biomass traits under IL and UP conditions in compared to IR 64, identification of their associated genomic regions by genotype and phenotype association analysis, confirmation of representative QTL(s) and gene mapping using segregating populations and physiological characterization of the representative INLs based on nitrogen uptake at seedling stage were carried out in this study.

This Chapter I explained the rationale and background of this study by reviewing literature in rice. In Chapter II, genetic characterization of INLs for yield component traits were studied to understand the relationships between agronomic traits and the chromosome regions introgressed. Chapter III focused on identification and analysis of gene/QTL for low tiller number (TN) which was related with adaptation to UP condition,

from an INL, YTH 34. Chapter IV referred to physiological characterization of INLs based on nitrogen uptake at seedling stage. And, Chapter V discussed about the yield potential improvement of IR 64 with its basis of genetic and physiological approaches.

This study will demonstrate the genetic variations among the INLs for agronomic (YC and dry matter production) traits under IL and UP fields condition and physiological (nitrogen uptake) traits in hydroponic cultivation, and discuss the usefulness of these materials for breeding and genetic analysis to improve the elite Indica Group cultivar. And will discuss the abilities of wide hybridizations with Japonica Group cultivars for genetic and physiological improvement of Indica Group rice cultivars.

# Chapter II

**Genetic characterization of introgression lines with the genetic background of the Indica Group rice (*Oryza sativa* L.) variety IR 64 for yield components**

## *2.1 Introduction*

IR 64, a mega variety, was cultivated by the largest number of Asian farmers due to its quality traits (Ballini *et al.* 2007). However, it has limitations including low agronomic nitrogen use efficiency (NUE, Diekmann *et al.* 1996) and low relative dry weight (RDW, %) and physiological NUE (Namai *et al.* 2009). It is susceptible to drought (Cal *et al.* 2013, Lafitte *et al.* 2007) and drought stress during flowering dramatically decreases its yield (Wade *et al.* 1999). It is susceptible to Fe toxicity (Nozoe *et al.* 2008), blast disease (Liu *et al.* 2009), and tungro disease (Shibata *et al.* 2007). Due to the higher adaptability and elite characters, it is thus important to improve yield potential, NUE, and stress tolerance of IR 64, to maintain the global higher yield to feed the ever increasing population.

Fujita *et al.* (2009) developed 334 INLs with chromosome segments introduced from several tropical Japonica Group NPT cultivars and a Japanese Japonica Group high yielding cultivar, Hoshiaoba, in the IR 64 genetic background, by recurrent backcross breeding to improve the yield potential and clarify these genetic mechanisms and, identified 54 QTLs for DH (7 QTLs), CL (8), leaf width (8), leaf length (4), PL (6), PN (3), 100-grain weight (7) and TS (11) in 10 INLs sib-groups located mostly on

chromosomes 1, 2, 4, 5, and 6, by genotype and phenotype association analysis of plants grown in wet and dry seasons under IL in tropical climate at IRRI. However, this evaluation in one condition (tropics) and rice ecosystem (IL) was not sufficient to describe the overall improvement of yield potential of these INLs and for better line selection. Also, characteristics of these INLs under different climate conditions e.g. temperate, and rice ecosystems e.g. UP are unknown. And the information regarding dry matter productions of INLs were limited. But, these information could open new avenue for yield improvement of Indica Group varieties e.g. IR 64, and higher silage production in temperate climate. To fill these gaps, chapter II aimed to, first, characterize these INLs under IL and UP in temperate climate based on the evaluation of yield component and dry weight (biomass) traits; and second, to identify the underlying genetic factor(s) that originated from tropical Japonica Group UP varieties.

## 2.2 Materials and Methods

### 2.2.1 Plant materials and cultivation

Fujita *et al*. (2009) developed 334 INLs ($BC_3F_9$) which were bred by the backcross breeding with the Indica Group rice variety, IR 64, as the recurrent parent, and these were introduced unique agronomic traits from NPT cultivars (Khush 1995, Peng and Khush 2003, Peng *et al.* 2008). Here, 10 agronomic traits related to yield components and dry matter traits of 333 INLs (data of one INL could not be recorded) were evaluated to characterize and clarify the modified traits in comparing with IR64.

IR64 and the INLs were cultivated under two different conditions, IL and UP, at the

fields of Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Ibaraki, Japan (36°2′N 140°4′E) in temperate region, in two growing seasons from May to October in 2011 and 2012. It varied from 13.5 to 14.5 hr. in bi-weekly average day length and, from 22 to 32℃ in bi-weekly average temperature during these growing periods in two years (GAISMA 2012).

In IL condition 4-week-old seedlings of each line were transplanted, one plant per hill, 18 cm apart, in two rows 36 cm apart. In UP condition seedlings were transplanted one plant per hill, 20 cm apart, in a single row, with 60cm apart between lines. In 2011, chemical fertilizer (45 kg nitrogen-based (N/ha) was applied in the IL condition, and organic fertilizer (9 kg N/ha) in the UP condition. In 2012, 48 kg N/ha and 77 kg N/ha of organic fertilizer were applied in the IL and UP fields, respectively.

### 2.2.2 Agronomic traits investigated

Ten agronomic traits, days to heading (DH), culm length (CL), panicle length (PL), culm weight per plant (CW), panicle weight per plant (PW), ratio of panicle weight per whole plant's weight (harvest index: P/T), No. of panicle per plant (PN), No. of fertility's seed per panicle (FS), total seeds of one panicle (TS) and ratio of fertility's seed per panicle (fertility rate: FS/TS) were investigated

A total of 12 individuals per line were investigated for each trait, and the average was used as the representative data of each line. From sowing to panicle exertion among half plants in each line was investigated as the days to heading (DH). After 30 days of the heading, whole plants were harvested, and dried up at a well- ventilated room until 14% moisture content, to measure the YC and dry matter production. CL and PL were

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measured as the lengths from soil surface to the neck node of panicle and from the neck to top spikelet of panicle of the longest tiller in each plant, respectively. CW and PW were measured as the weights of culm and leaf, and panicles including spikelet and rachis branch, respectively, of all tillers in each plant. Harvest index (P/T) was calculated as the ratio of average of panicle weight by total of panicle and culm weights. PN was counted as the number of productive panicles per plant. FS was calculated from the abundance of well-developed spikelet's (or fertilized pollen grains) in the representative panicle. TS were computed as the sum of fertile and sterile seeds of the representative panicle in each plant. Fertility rate was calculated by as the ratio of FS by TS.

### 2.2.3 Statistical analysis & classification of INLs based on the agronomic traits

Excel statistics were used to calculate various elementary descriptive statistics, prepare histogram, and regression analysis between two years data. The traits Pearson correlation coefficients were calculated at $P<0.05$, $P<0.01$ and $P<0.001$ using SPSS statistics version 19.

The data of ten agronomic traits at IL and UP in 2011 and 2012 were used separately for the cluster analysis, and classified the INLs based on their genetic variations in two years and seasons. Cluster analysis was carried out following Ward's hierarchical analysis (Ward 1963), and significant test by Tukey's HD for cluster group variations were performed using JMP7. 2 (SAS institute Inc., Cary, NC, USA).

### 2.2.4 Association analysis for agronomic traits to find the genetic factors harboring INLs

The genotype data of these INLs were obtained from Fujita *et al.* (2009), and the chromosomal locations of introgressed segments associated with agronomic traits were determined by single marker analysis (SMA). A total of 262 SSR markers distributed among the 10 sib groups, 30 in YP1, 11 in YP3, 15 in YP4, 46 in YP5, 22 in YP6, 28 in YP7, 24 in YP8, and 16 in YP9, 37 in YP10 and 33 in YP11-INLs were used for genotyping.

The SMA in each sib-INLs group which was originated the chromosome introgressions from common NPT donor variety, was performed using Windows QTL cartographer V2.5 (Wang *et al.* 2012). A probability level of $< 0.001$ was used as the threshold value to detect significant mean differences between the two genotype groups, IR64 and donor types. When multiple markers on the same chromosomal segments were associated with an agronomic trait, the marker that showed the highest *F-* score among the multiple markers was selected as QTLs (associated markers), and designated following the standard QTL nomenclature by McCough *et al.* (1997). The proportion of observed phenotypic variation attributable to a particular chromosomal region was estimated by the co-efficient of determination ($R^2$). The genetic distance of each SSR marker was revealed by high density genetic linkage map of $F_2$ population derived from a cross between Nipponbare and Kasalath (Harushima *et al.* 1998). The approximate genetic distance of the SSR markers represented the genetic distance of the nearest RFLP markers in the high-density genetic linkage map because the linkage map in each sib-group could not be made by materials of the $BC_3$ generation. The nearest RFLP marker to the SSR marker was searched based on a Nipponbare genome sequence.

*2.3 Results*

*2.3.1 Genetic variation of agronomic traits in the INLs*

The 10 agronomic traits including DH, CL, PL, CW, PW, P/T, PN, FS, TS and FS/TS in the 333 INLs and IR 64, were investigated at two different rice ecosystems, in IL and UP, in 2011 and 2012.

Wide variations were observed in each trait among them, ecosystem and season. All the ten traits of INLs showed continuous distribution, and seven of them showed a normal distribution where the other three, P/T, FS/TS (for both 2011 and 2012), and CL (for 2012) skewed left (Fig. 1 and 2). Traits variations were higher in UP than IL. Mean, standard deviation, range (minimum and maximum), and coefficient of determination values of all traits are shown in Table 1, Fig. 3, and 4. The average of PW, P/T, FS, TS and FS/TS in whole INLs were significantly greater than those of IR 64, and no significant differences found for these of CL and PL. In contrast, DH, CW, and PN of them were significantly smaller than those of IR 64. These results indicated that INLs were modified genetically in heading and plant types, except for plant height in compared with IR 64. And these traits, HD, CW, PW, P/T, FS, PN, FS and TS, in UP were higher than those of IL, and the late heading in UP might influence the increment of these trait's value.

In details, the average DH of INLs in IL (107.5±3.1 d) and UP (136.9±5.6 d) was significantly shorter than that of IR 64 in both ecosystems (108.0±2 d in IL and 144.0 ±3.2 d. in UP) in 2011, and similar tendency was found in 2012 (Table 1). DH was significantly longer in UP. The differences of CL of INLs in IL (79.7±5.7 cm) and UP (54.3±7.3 cm) were not significant than IR 64 (78.3±0.8 cm and 40.8±3.9 cm) in

both 2011. CL was significantly shorter in UP. Similarly, the PL of INLs in IL (25.8±1.9 cm) and UP (26.0±2.1 cm) were not significant than IR 64 (24.7±1.3 cm) in 2011. Similar tendency was found in 2012. Also, no significant difference was found for PL between IL and UP. The CW of INLs in IL (47.0±6.7 g) and UP (45.7±12.5 g) was significantly lighter than that of IR 64 in both ecosystems (61.3±8.9 and 69.3±17.0 g), in 2011. Similar tendency was found in 2012, but the CW increased markedly in UP. However, PW of INLs in IL (27.2±6.2 g) and UP (35.1±2.4 g) was significantly heavier than that of IR64 in both ecosystems (14.2±2.4 and 10.6 g) in 2011. Similar tendency was found in 2012, and significantly heavier PW found in UP. average P/T of INLs in IL (36.5±7.1 %) and UP (42.8±9.0 %) was significantly higher than that of IR 64 in both ecosystems (19.2±3.0 and 12.9 %) in 2011. Similar tendency was found in 2012 and no clear difference found between IL and UP. PN of INLs in IL (16.5±4.2) and UP (20.1±4.8) was significantly smaller than that of IR64 in both ecosystems (26.6±7.8 and 20.1±6.5) in 2011. Similar tendency was found in 2012, and PN was significantly larger in UP in this year. Average FS of INLs in IL (87.1±26.9) and UP (122±39.5) was significantly higher than that of IR64 in both ecosystems (20.6±9.6 and 24.7±31.5) in 2011. Similar tendency was found in 2012, but IR 64 has higher FS in this year. TS of INLs,155.5±26.1 was significantly higher than that of IR 64 (130.1±10.2) in IL, but no clear difference in UP (166.1±39.4) than IR64 (166.2±26.6) in 2011. Similar tendency was found in 2012, but TS in UP was significantly higher than IL in this year. Average FS/TS of INLs in IL (56.0±14.6 %) and UP (74.2±18.7%) was significantly higher than that of IR 64 in both ecosystems (20.0±7.0 and 20.0±19.0 %) in 2011. Similar tendency was found in 2012.

Additionally, traits variations of INLs were higher in UP than IL as revealed by

coefficient of determination values by regression between two years. Five traits showed strong relationship under both conditions such as, DH (30.90 and 46.4%), CL (29.4 and 52.5 %), PL (46.6 and 56.0 %), FS (15.5 and 16.3 %) and TS (50.2 and 63.9 %) in both 2011 and 2012. The $R^2$ were relatively smaller in CW, PW, and P/T, PN, and FS/TS (Fig. 3 and 4).

### *2.3.2 Trait correlation*

In all conditions, DH showed correlation with all traits, for example, significant positive correlations were observed with PL and TS ranged from 0.23 to 0.49, and significant negative correlation were found with PN and FS/TS (-0.13 to -0.64) in both IL and UP (Table 2 and 3). DH showed significantly positive correlation with CL and PW in all ecosystems (0.11 to 0.42), except in UP in 2011 where correlated negatively (-0.011 to -0.28). Strong negative correlation of DH with P/T in all ecosystems, except a weak positive correlation in IL in 2011. Similarly with CW, DH showed positive correlation in all conditions except 2012 in UP. Interestingly, positive correlation FS in IL, but negative correlations in UP, indicates that delayed DH has direct influence on FS. CL and PL showed significantly positive correlation with each other and with PW, CW, FS, TS, FS/TS (0.23 to 0.98) in all conditions, indicating that plant length has direct influence on yield. However, CL showed inverse relationship with P/T and PN (-0.05 to -0.37) in IL, but positive in UP (0.17 to 0.46). Similarly, PW showed significant positive correlations with PN and TS, and had positive correlation with CW in conditions except IL in 2011. PW is negatively correlated with P/T, FS and FS/TS (-0.18 to -0.43) except UP (0.77) in 2011. CW showed significantly positive correlations with FS, TS in all conditions and, with PN and FS/TS showed positive correlation in all conditions except negative in IL for PN and UP for FS/TS in 2011. CW had high positive correlation with

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P/T in IL 2011 and FS/TS in all condition except UP 2011. PN was negatively correlated with FS, TS and FS/TS in IL and positively in UP with the exception TS in UP 2012. FS was strongly positive correlation with TS and FS/TS, but TS was negatively correlated with FS/TS with the exception in IL 2011.

Based on the variations of ten traits and reaction patterns of them between UP and IL, it was clarified that 333 INLs were improved basically for panicle types especially heavy panicle weight and low panicle No., and these six traits, DH, PL, PW, P/T, FS and TS were contributed mainly the variations among INLs, particularly.

### 2.3.3 Classification of INLs based on agronomic traits

INLs were classified into four groups (I-IV), based on the 10 traits' data from IL in 2011 and 2012, and three (A-C) from UP, by cluster analysis using Ward's method of hierarchical analysis (Ward 1963) (Fig. 5, Table 6). In IL, cluster group II showed long DH, PL, PW, P/T, short PN, and high FS and TS. Group IV had the low traits' values such as DH, CL, PL, CW, FS and TS. In UP, group A had the long DH, CL and PL, and low PN, but high FS and TS. In opposite, group C showed low traits' values, such as DH, CL, PL, PW, P/T and FS, but higher PN. It had the highest fertility rate. The detail characters of each group are shown on Tables 4 and 5. And then these INLs were reclassified into six groups, A-I, A-II, B-II, B-III, C-III and C-IV, based on these classifications of UP and IL (Fig. 5).The number of INLs among cluster groups varied from 12 to 131 which were consisted of different donor varieties from one to five, and IR 64 was categorized into B-III (Table 6).

### 2.3.4 Characterization of cluster groups

All cluster groups showed significantly higher values in four traits, FS/TS, FS, P/T and PW, and lower in two traits, CW and PN, than those of IR 64, under both of the

conditions, IL and UP. And there were variations in the other four traits, HD, CL, PL and TS, among cluster groups and cultivated condition (Fig. 6). These results indicated that INLs were modified into the heavy panicle type basically and distributed into all INLs. Six cluster groups were characterized on the basis of the differences of ten traits of cluster group mean from the whole mean values of all INLs including IR64 (Fig. 6).

Cluster group A-I comprised 131 INLs derived from donor cultivars YP1, YP3, YP4, and YP11. Within A-I, CL and PL were longer and PN was lower than the whole mean in both IL and UP. FS/TS was higher in IL, DH was longer in UP, and PW was lower in UP than the whole mean. A-II comprised 58 INLs derived from YP11 and YP5. Within A-II, the values of PL, PW, P/T, FS, and TS were higher than the whole mean in both IL and UP. CW and PN were lower than the whole mean in IL, but there were no significant differences in DH, FS/TS, or CL in either IL or UP. These results indicate that within A-II, five yield traits (PL, PW, P/T, FS and TS) behaved consistently between conditions, and CW and PN decreased in IL.

B-II comprised 18 INLs derived from YP5 and YP6. Most differences between the B-II INLs and the whole mean were not significant. Only DH was longer in IL, CW and PN were higher in UP, and CL was shorter in UP. B-III comprised IR64 and 93 INLs derived from YP6, YP7, YP8, YP9 and YP10. Within B-III, PL was shorter and PN was higher than the overall value in both IL and UP. CW was higher than the overall value in IL. There were no significant differences in the other eight traits. B-II and B-III had the fewest significant differences from the whole mean.

C-III comprised 21 INLs and C-IV comprised 12 INLs, all derived from YP10. These groups showed unique reactions in both conditions. In both groups, the values of DH, CL, PL, CW and TS were lower than the whole mean in both IL and UP. P/T and

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FS/TS were commonly higher than the whole mean in UP. Traits in both groups showed the same responses in UP. But within C-III only, FS was lower and PN was higher in IL.

These results indicate that group A-II INLs had increased PL, PW, P/T, FS and TS, in both IL and UP conditions. The traits in the other groups changed according to the condition conditions and were not as stable. The details of the variations are shown in Table 7.

## *2.4 QTL for yield related traits detected by association analysis*

The association analysis was performed separately in all conditions and years. A total of 166 QTLs were detected associated with DH, CL, PL, CW, P/T, PN, FS, TS and FS/TS in the 333 INLs. Of these, 31 QTLs were only detected in IL and 60 only in UP. The other 75 QTLs were detected in 26 different combinations of both conditions and seasons. The number of QTLs detected varied from 1 to 60 among the 10 INL sib-groups (Fig. 7-16, Table 8).

The YP1 sib-group, with 35 INLs, had introgressions in 6 regions on chrs. 1, 2, 4, 5, 7 and 8; 23 QTLs for DH (2), CL (3), PL (3), CW (2), PW (4), P/T (5), PN (2) and FS/TS (2) were detected on chrs. 4, 5, 7 and 8 (Fig. 7). Among them, 18 QTLs for CL (3), PL (2), CW (2), PW (4), P/T (3), PN (2) and FS/TS (2) were detected in the central region of chr. 7 from 78.6 to 123.8 cM near SSR markers *RM505*, *RM5847*, and *RM1132*; the YP1 alleles decreased the trait values ($F = 16.9$–$91.4$, $R^2 = 0.35$–$0.75$). Of these, 8 detected QTLs were found in UP and 10 in both conditions on chr. 7. These results indicate that variations in the YP1 INLs were due mainly to QTLs on chr. 7.

The YP3 sib-group, with 23 INLs, had introgressions in 4 regions on chrs. 1, 5, 6,

and 12; 11 QTLs for DH, CL, PW, P/T, PN, FS (2), TS and FS/TS (3) were detected on chrs. 1, 6, and 12 (Fig. 8). Among them, 7 QTLs were detected only in UP in the region of chr. 12 from 17.4 to 109.4 cM, near *RM17* and *RM1227*. The YP3 alleles of the QTLs for DH, PN and FS (2) increased trait values, and those for CL, PW,and P/T decreased the values. Two detected QTLs were found in IL and 5 in UP conditions. Thus, variations in the YP3 INLs were due mainly to the QTLs on chr. 12.

The YP4 sib-group, with 45 INLs, had introgressions in 8 regions on chrs. 1 (2), 4 (2), 5, 6, 9 and 12; 20 QTLs for DH (2), CL (4), PL (5), CW, P/T, PN, FS, TS (4) and FS/TS were detected in 6 regions on chrs. 1, 4 (2), 6, 9 and 12 (Fig. 9). We detected 10 QTLs in IL and 10 in UP conditions. Among them, 11 QTLs for DH (2), PL (3), P/T, PN, FS and TS (3) were detected on chr. 4 in the region from 99.0 to 116.9 cM, near *RM252*, *RM303* and *RM3836*; the YP4 alleles of 8 QTLs for DH (2), PL (3) and TS (3) increased the trait values, and those for P/T, PN and FS decreased the values. Thus, variations in the YP4 INLs were due mainly to QTLs on chr. 4.

The YP5 sib-group, with 56 INLs, had introgressions in 11 regions on 9 chrs. 1, 2, 4 (2), 5, 6 (2), 7, 8, 9 and 12. One QTL for PN was detected on chr. 4 in IL conditions, and 4 QTLs for DH on chr. 8, PL (2) on chr. 4, and PN on chr. 1 were detected in UP conditions (Fig. 10); the YP5 alleles of QTLs for PL and PN on chr. 4 increased the trait values, and those for DH, PL, and PN decreased the values ($F = 11.7–17.0$, $R^2 = 0.25–0.30$).

The YP6 sib-group, with 29 INLs, had introgressions in 4 regions on chrs. 2, 4, 6 and 11; only 1 QTL for PL was detected on Chr. 11 in UP condition (Fig. 11). The YP6 allele of this QTL decreased the trait value ($F = 14.5$, $R^2 = 0.34$).

The YP7 sib-group, with 21 INLs, had introgressions in 6 regions on chrs. 2 (2), 6,

7, 11 and 12; 4 QTLs for DH (2), PW, and P/T were detected on chr. 7, and 2 QTLs for CL on chrs. 7 and 12 (Fig. 12). These QTLs were detected in the terminal region of the long arm of chr. 7, near *RM5455* and *RM248* ($F$ = 18.7–24.8, $R^2$ = 0.48–0.55), and on chr. 12, at *RM1337* ($F$ = 20.2–25.1, $R^2$ = 0.52–0.57). All QTLs except 1 for DH were detected in UP conditions, and the YP7 alleles decreased the trait values. Thus, variations in the YP7 INLs were due mainly to QTLs on chrs. 7 and 12.

The YP8 sib-group, with 29 INLs, had introgressions in 7 regions on chrs. 1 (2), 2 (2), 4, 7 and 11; 12 QTLs for P/T (2), PN (2), FS (4), TS (3) and FS/TS were detected in 5 regions on chrs. 1, 2 (2), 4 and 7 (Fig. 13). The YP8 alleles of 4 QTLs for P/T (2) and PN (2) detected in IL conditions decreased the trait values ($F$ = 12.7–16.3, $R^2$ = 0.34–0.38). The other 8 QTLs, detected in UP conditions, increased the values ($F$ = 13.8–26.7, $R^2$ = 0.35–0.50). Among them, 3 QTLs for FS and TS (2) were detected on the long arm of Chr. 2, near *RM006* and *RM240*; the YP8 alleles increased the trait values. Four QTLs for FS, TS and PN (2) were detected in the central region of chr. 4 from 109.9 to 125.6 cM near *RM5503* and *RM6909*. Thus, variations in the YP8 INLs were due mainly to QTLs on chrs. 2 and 4.

The YP9 sib-group, with 16 INLs, had introgression in 5 regions of chrs. 2 (2), 4, 5 and 7; 4 QTLs for DH, CL, and FS/TS (2) were detected on chrs. 2, 4, 5 and 7 in UP conditions (Fig. 14). The YP9 alleles of QTLs for DH on chr. 5, CL on chr. 4, FS/TS on chr. 7, and FS/TS on chr. 2 increased the trait values.

The YP10 sib-group, with 39 INLs, had introgressions in 9 regions on chrs. 1 (2), 2 (2), 4, 5, 6, 8 and 12; 60 QTLs for DH (6), CL (11), PL (7), CW (6), PW (8), PN (5), P/T, FS and TS (15) were detected in 6 regions on chrs. 1, 2 (2), 4, 5 and 6 (Fig. 15). Of these, 30 QTLs were detected in IL conditions and 30 in UP conditions. The YP10

alleles of 10 QTLs for CL (3), CW (2), and PN (3) on chrs. 1, 2 and 5, and for TS (2) on chr. 6, increased the trait values, and those of the other 50 decreased the values. The YP10 sib-group had the most QTLs detected. Most were detected on chrs. 1, 5 and 6. Sixteen QTLs for DH (2), PL (2), CW, PW, CL (4), PN and TS (5) were detected in the central region of chr. 1 from 86.0 to 95.7 cM near *RM5638*, *RM009* and *RM0005*; the YP10 alleles of all QTLs decreased the trait values ($F$ = 13.6–51.1, $R^2$ = 0.26–0.58). Eighteen QTLs for PN, CL (4), PL (2), PW (2), TS (4), DH (2) and CW (3) were detected on the short arm region of chr. 5 from 4.6 to 24.7 cM near *RM3796*, *RM3334*, *RM1024* and *RM405*; the YP10 alleles of all QTLs except that for PN decreased the trait values ($F$ = 13.0–20.1, $R^2$ = 0.26–0.40). Thirteen QTLs for PW (2), PL, TS (3), DH, CL (3), CW (2), and FS were detected on the long arm of chr. 6 from 70.9 to 117.0 cM near *RM7193*, *RM3827* and *RM3343* ($F$ = 16.4–32.5, $R^2$ = 0.24–0.47). Thus, variations in the YP10 INLs were due mainly to QTLs on chrs. 1, 5 and 6.

The YP11 sib-group, with 40 INLs, had introgressions in 6 regions on chrs. 1, 4, 5, 9 and 11 (2); 24 QTLs for DH (2), CL (6), PL (2), PW, P/T (3), PN (2), FS (2), TS (4) and FS/TS (2) were detected in 5 regions on chrs. 4, 5, 9 and 11 (2) (Fig. 16). Of these, 16 QTLs were detected in IL conditions and 8 QTLs in UP conditions; the YP11 alleles of almost all QTLs increased the trait values, except for 3 QTLs for P/T on chrs. 4 and 11 (2), and for 2 QTLs for PN on chrs. 4 and 9; the other two QTLs for PN decreased the values in IL conditions. Thirteen QTLs were detected on the long arm of chr. 4 from 106.0 to 123.8 cM near *RM6909*, *RM303* and *RM1113*; the YP11 alleles of all QTLs increased the trait values. Another 7 QTLs were detected in the central region on chr. 9 from 72.1 to 74.7 cM near *RM242*, *RM3164* and *RM5535*; the YP11 alleles of all QTLs except 1 for PN increased the trait values. The QTLs on chr. 4 ($F$: 12.7–61.3, $R^2$: 0.25–

0.63) and chr. 9 (*F*: 13.1–36.0, $R^2$: 0.26–0.50) showed high effects. Thus, variations in the YP11 INLs were due mainly to QTLs on chrs. 4 and 9.

In the YP1, YP3, YP5, YP6, YP7, YP8 and YP9 sib-groups, more QTLs were detected in UP conditions. In the YP4, YP10, and YP11 sib-groups, in contrast, more QTLs were detected in IL conditions. Among all 10 sib-groups, more QTLs were detected in UP conditions. These results indicate that several trait responses were specific to UP conditions. In the YP1, YP3, YP4, YP7, YP8, YP10 and YP11 sib-groups, several QTLs were detected in several common chromosome regions. These results indicate that some key QTLs or chromosome regions contributed substantially to the variation in these sib-groups.

## 2.5 Discussion

Fujita *et al.* (2009) developed a total of 334 INLs, and evaluated a total of eight traits, DH, CL, PL, LL (leaf length), LW (leaf width), PN, 100-GW (grain weight) and TSN of INLs in wet and dry seasons in IL at IRRI, Los Banos, the Philippines, under tropical condition. But they did not evaluate dry matter production, No. of fertile spikelet's, and seed fertility rate of panicle of INLs and different cultivate conditions e.g. UP. We evaluated again these 333 INLs (one line could not be used) to characterize for 10 agronomic traits, DH, CL, PL, CW, PW, P/T, PN, FS, TS and FS/TS, at IL and UP fields, JIRCAS, Tsukuba, Japan, in temperate condition in 2011 and 2012, to confirm the variation of yield component and dry matter traits of INLs and the contribution of genetic improvement by introgression of NPT chromosomes into IR 64 genetic background.

The PW, P/T, FS, TS and FS/TS of all the INLs were greater than those of IR 64;

the CL and PL values were similar; and the DH, CW and PN values were less than those of IR 64 in both condition. These results indicate that the INLs were genetically improved for lower tillering and heavier panicles, in comparison with IR 64. Fujita *et al.* (2009) and Kobayashi *et al.* (2010) also identified lower PN, heavier GW, and higher TS in the same INLs in their tropical studies. Here, we have newly identified lower DH and CW, and higher PW, FS, and FS/TS. In addition, DH, PW, PN and TS were much higher in UP than in IL condition. And, stronger traits correlation in UP e.g. PW with P/T, FS, TS and FS/TS, supports these INLs produce more dry matter in UP. These trait differences suggest the occurrence of unique phenotypes of these INLs in both UP and IL condition, under temperate conditions.

The NPT traits distributed among the INLs and the cluster analyses based on the respective trait values in IL and UP, classified these lines into six groups: A-I, A-II, B-II, B-III, C-III and C-IV. Heavy panicle traits such as PW, P/T, FS and FS/TS increased, and the CW and PN decreased, in all the cluster groups, relative to IR 64. The other traits, DH, CL, PL and TS showed variations among the cluster groups, but could not clearly characterize the groups. So, we characterized each cluster group by comparing the values of 10 traits between each cluster group and whole mean of all the INLs including IR 64 (Fig. 6). The A-II INLs had higher PL, PW, P/T, FS and TS than the whole mean, and these increments were consistent in both IL and UP condition. In the other groups, the same traits varied with the condition conditions and were not as stable. B-II and B-III had similar trait values to the whole mean, with a significant differences of CL in UP. C-III and C-IV had lower DH, CL, PL, CW and TS than the whole averages, and generally had low trait values. PN differed only in IL condition, and most trait values were similar between C-III and C-IV. This classification grouped the INLs

by variation in the trait values, and by differential responses to IL and UP condition. In particular, A-II was unique in having higher PL, PW, P/T, FS and TS values in both conditions.

The association analysis resulted in a total of 166 QTLs for DH, CL, PL, CW, PW, P/T, PN, FS, TS and FS/TS; 31 of these were detected only in IL condition, 60 only in UP condition, and the other 75 in both conditions, in 42 introgressed regions among 10 INL sib groups (Fig. 7-16, Table 8). All the 10 sib-INLs were employed for association analysis separately due to their differences in developmental pedigree of donor parent. Interestingly, most of the QTLs were detected in only 12 introgressed regions on chrs. 1, 2, 4 (4), 5, 6, 7 (2), 9 and 12; where, in addition, many QTLs were detected for multiple traits with high effects (Fig. 7-16). This indicates the pleiotropic effects of gene(s) or chromosome locations for many traits, and this were previously mentioned by Fujita *et al.* (2013) and Xiao *et al.* (1996).

In comparison with previous studies (Fujita *et al.* 2009, 2010a, Kobayashi *et al.* 2010), 81 of these QTLs were unique and newly detected (38 in UP, 9 in IL, and 34 in both) in 23 regions on chrs. 1 (3), 2 (3), 4 (4), 5 (3), 7 (4), 8, 11 (3) and 12 (2). The new QTLs are shown with by double strike (‡) in Table 8. Notably, the QTLs on chr. 2 in the YP8 and YP9 sib groups, on chr. 7 in YP1 and YP8, and on chr. 12 in YP3 and YP7, were detected only in UP condition, and the donor alleles increased the values of FS, TS, FS/TS and DH, and reduced those of CL, PL, CW, PW and P/T. These three regions showed specific responses in UP condition, and might play important roles in developing plant architecture and the differentiation and adaptation to this condition. Lyu *et al.* (2014) also reported the candidate genes, *Os07g0449700* on chr. 7 and *Os12g0597000* on chr. 12, both related to UP adaptation, by comparing a large panel of

irrigated and UP rice accessions, using whole genome re-sequencing data of polymorphic SNPs. Our QTLs on chrs. 7 and 12 were detected in the same chromosomal regions. The other 85 QTLs were detected in the same 19 regions, on chrs 1 (3), 2 (2), 4 (6), 5, 6 (3), 8, 9 (2) and 12, as in Fujita *et al.* (2009, 2010a) and Kobayashi *et al.* (2010). The QTLs on the long arm of chr. 4 in the YP4, YP5, YP9, and YP11 sib groups increased the values of DH, CL, PL, and TS in both condition conditions and of P/T and PN in IL. Fujita *et al.* (2009, 2010a) and Kobayashi *et al.* (2010) also detected several QTLs that increased CL, PL, GW and TS in the same region, which we have confirmed in this study.

Moreover, the DH QTLs detected in YP1, YP3, YP4, YP5, YP7, YP9, YP10 and YP11 coincided with QTLs reported previously in rice: on chrs. 1, 4, and 5 (Hori *et al.* 2015, Oda *et al.* 2003), chr. 6 (Monna *et al.* 2002, *Hd3a*), chr. 7 (*Hd2* by Yano *et al.* 1997, Shibaya *et al.* 2011, *GHd7.1* by Liu *et al.* 2013, *qDTH-7-2* by Hori *et al.* 2015) , and chr. 8 (Fujita *et al.* 2009, Lin *et al.* 2003). However, the QTL detected on chr. 9 in YP11 has not been reported.

The detected QTLs showed correspondence with the cluster group characters (traits variation). Group A-II, comprising YP5 and YP11 INLs, had high PL, PW, P/T, FS and TS, in both IL and UP conditions. QTLs for PW, P/T, FS/TS, PN, TS, FS, DH and CL on chr. 4, and FS, CL, PL, DH, and PN on chr. 9, were detected in YP11; and all of these QTLs increased the trait values. These two chromosomal regions may contribute to the characterization of A-II. In YP5, 5 QTLs, for DH, PL (2) and PN (2), were detected on chrs. 1, 4 and 8; however, these did not have high effects, suggesting that the QTLs contributing to the traits in A-II were not detectable in the YP5 INLs. Several limitations, due to missing or over-counting alleles, may factor in such association

analysis, and result in low detection of respective QTLs (Fukuta *et al.* 2012). In addition, only one QTL might be directly associated with a given trait, and the other QTLs might be under the shadows of this single QTL. Therefore, hidden QTLs might be present in undetected regions of YP5.One YP5 INL, YTH183, out-yields IR 64 (Fujita *et al*. 2012, Kato *et al*. 2011). In YTH183, Obara *et al.* (2014) found a QTL, *qRL6.4*, related to root elongation and nitrogen uptake, in the terminal region of chr 6, where we could not have detected any QTLs. Thus, A-II INLs might house other genetic factors related to high yield and trait stability.

Group A-I, comprising YP1, YP3, YP4 and YP11 INLs, showed higher CL and PL, and lower PN, in both IL and UP condition, as well as higher FS/TS in IL conditions, and higher DH in UP conditions. There were multiple QTLs for PL, CW, PW, PN, FS/TS, CL and P/T on chr. 7 in YP1; for DH, PW, PN, FS/TS, CL, P/T and FS on chr. 12 in YP3; for DH, PL, P/T, PN, FS and TS on chr. 4 in YP4; and for PW, P/T, FS/TS, PN, TN, FS, DH and CL on chr. 4, and FS, CL, PL, DH and PN on chr. 9 in YP11; and all these QTLs, except for those on chr. 7 in YP1, increased the trait values. Among these, the QTLs on chr. 7 in YP1, chr. 12 in YP3, chr. 4 in YP4 and YP11, and chr. 9 in YP11, may contribute to the characterization of A-I, in both conditions. These results indicate that the phenotypes of the A-I INLs were due to these introgressed genetic components.

B-II and B-III comprised YP5, YP6, YP7, YP8, YP9 and YP10 INLs, and IR 64. The PN values of B-III increased in both conditions, and the DH of B-II and CW of B-III in IL conditions, as well as the CW of B-II in UP conditions, also increased. Fewer QTLs were found in YP5, YP6, YP7, YP8 and YP9 than in YP1, YP2, YP4, YP10 and YP11. Only 3 QTLs whose donor alleles increased trait values were found:

for DH on chr. 9 in YP9, and for DH and CW on chr. 6 in YP10; and no QTLs were associated with high PN. B-II and B-III did not show large differences in trait averages from the whole averages of the INLs. These results indicate that the INLs of B-II and B-III (with the exception of YP10) did not include many genetic factors for improving agronomic traits.

C-III and C-IV, comprising only YP10 INLs, had many QTLs on chrs. 1, 2, 4, 5 and 6. Almost all the QTLs reduced trait values, except for some on chr. 6, suggesting that the low DH, CL, PL, CW and TS values of the C-III and C-IV INLs were due mainly to the QTLs on chrs. 1, 2, 4 and 5. In addition, more QTLs were detected in UP condition than in IL condition. The donor NPT lines were bred from tropical UP japonicaGroup cultivars, and our results showed the effects of QTLs from these UP cultivars. In summary, the cluster groups characterized by yield components and dry matter production revealed relationships with the QTLs introgressed from donor cultivars, and the genetic variations in the INLs were due to these QTLs. Thus, the cluster groups characterized by yield components and dry matter production revealed relationships with the QTLs introgressed from donor cultivars, and the genetic variations within the INLs were due to these QTLs.

The 333 INLs with chromosome segments from tropical Japonica Group cultivars and with IR 64 genetic background, were characterized based on comparing of data between IL and UP. And the association analyses using SSR marker on introgressed segments and 10 agronomic traits of INLs were carried out to detect the genetic factors which contributed the variations among INLs in compared with IR 64. And unique chromosome regions which were detected only under the UP condition on chrs. 2, 7 and 12 in sib-INLs from YP8, YP1 and YP3.

We could demonstrate that the chromosome segments originated from tropical UP varieties modified or improved the biomass and yield components of IL variety IR 64, and these were useful breeding materials. The NPT segments introgressed on chrs. 2 in YP8-INLs, and 7 in YP1-INLs, and 12 in YP3-INLs were UP specific and contributed for long CL, higher FS, TS, and FS/TS, and these increased the adaptation to UP condition. The QTLs on chr. 7 in YP1-INLs for low PN will be use full for direct seeding or stress conditions to maintain the yield product by minimizing of reproductive tillers; and will provide insight into the development of plant structure for the adaptation in UP. The QTLs on chr. 4 in YP4-, and YP11-INLS contributed the genetic improvement of IR 64 for long HD's, CL, PL and higher TS. These QTLs and the corresponding INLs are useful breeding materials for enhancing the yield potential of Indica Group rice varieties under IL and, UP ecosystems, and near isogenic lines (NIL) of each yield traits can be easily developed by marker assisted selection (MAS) which can be used as research materials to dissect the loci for with economic and agricultural important traits.

This study had some limitations including the data variations between two years which direct us to conduct statistical analyses including cluster and association analysis separately. But, average of two years data for each cluster group was used for characterization; because of they showed similar patterns in both years. Also, comparisons of the detected QTLs were made with previous studies using same INLs instead of all other reports on the other populations of rice except for DH QTLs.

Because of association mapping provides little insights into the mechanistic basis of detected QTLs, genomic localization and cloning of genes is not so successful (Semagn *et al.* 2010). Besides, its reliability is affected by many factors including population

structure, outcrossing, genome rearrangement etc., and it requires larger amount of markers. To segregate and confirm the QTL on the introgressed segments, advance genetic study using new hybrid populations derived from the cross between selective INLs and IR 64 as the genetic background are necessary.

# Chapter III

## Identification of QTLs for low tiller number in an INL, YTH 34

### *3.1 Introduction*

Low tillering plant type with high density grain was desirable for dry seeding condition to maintain a proper plant density; rainfed condition to avoid episodic drought and maintain the yield by minimizing of reproductive tillers (Vergara *et al.* 1990, Farooq *et al.* 2011). Also, the unique plant architecture of low tiller/panicle might be the first priority trait for adaptation to upland under the serious environmental conditions. In Chapter II, we could identify 18 QTLs for yield component traits including the QTL for low panicle No. (as indicator of tiller number), *qPN7*, on chr. 7 by genotype and phenotype association mapping using 35 INLs originated from the donor YP1 (IR 65600-87-2-2-3). Several INLs from the same donor parent showed low tillering trait. We selected an INL, YTH 34, which significantly lowered its tiller and it appeared more dramatic in UP condition than in IL (Table 9).

The present study conducted to confirm the detected QTLs or gene for low tiller No. or related traits in UP using $F_3$ mapping population derived from a cross between the IR 64 and introgression line YTH 34.

### *3.2 Materials and Methods*
### *3.2.1 Development of mapping populations*

We could identify a total of 18 QTLs for YC traits on chr. 7 under two ecosystems,

IL and UP, by genotype and phenotype association mapping using 35 INLs from the donor parent YP1 (Fig. 7, Table 8). We then selected an INL, YTH 34, that showed unique characteristics by lowering its tiller number than IR 64, and dramatically in UP (Table 9).

We developed and genotyped a total of 88 $F_2$ plants and selfed $F_3$ lines derived from a cross between IR 64 and YTH 34, and evaluated these generations in UP and IL fields to confirm the detected gene or QTLs by linkage analysis. In addition, we selected a $F_2$ progeny line, $F_2$JII-IV-10 which was heterozygous in target locus on chr. 7, and evaluated 72 selfed $F_3$ plants at greenhouse in UP-like condition in greenhouse and employed advanced QTL analysis as additional materials. Population development scheme is shown in Fig. 17.

### 3.2.2 Cultivation of hybrid populations

YTH 34 was selected in UP field for low PN and made cross with the normal panicle type recurrent parent IR 64, and harvested $F_1$ seeds in 2013. These $F_1$ seeds were then selfed, and harvested $F_2$ seeds in 2014. A total of 88 $F_3$ family lines together with the parents, IR 64 and YTH 34, were cultivated under two different conditions, IL and UP fields of Tropical Agricultural Research Front (TARF), JIRCAS, Ishigaki, Okinawa, Japan (24.44°N, 124.22°E ), in the growing seasons from March to June in 2015.

A total of 20 seedlings (4-week-old) were transplanted into a single row with 20 cm apart per hills, and 60cm apart between lines in UP. In the IL, 22 seedlings were transplanted into two rows, single plant per hill, 18 cm apart between plants and 36 cm between lines. Among them, 18 plants excluding the border lines were considered for visual scoring and counted PN for genetic analysis at full heading stage. The total of

30.0 kg/ha. (N- basis) of chemical fertilizer was applied in both of UP and IL.

A total of 72 $F_3$ plants selfed from a $F_2$ plant, $F_2$JII-IV-10 were sown in a container (50cm×35cm×9cm) in soil (pH 5.0, 0.5 g N, 1.4 g P, 1.3 g K kg$^{-1}$, from Sumitomo Chemical Co. Japan) following the 6×7cm density in the tropical greenhouse maintaining 26-28º C temperature at the JIRCAS, Tsukuba, Ibaraki, Japan (36°2′N 140°4′E) from January to April, 2015. Also, a total of 13 plants of each parents were also grown in a positive (similar to UP), and negative (similar to IL) condition. The container was watered up to field capacity and then allowed to surface drying by withholding watering (every 1-2 days) to simulate 'UP' growing condition in greenhouse following Pariasca-Tanaka *et al.* (2009), starting after 20 days from sowing and continued until harvesting. The irrigation interval varied depending on the weather condition.

### 3.2.3 Phenotypic evaluation

The PN was counted at full heading stage, in both UP and IL in $F_3$ family lines and parents. Because we found clear segregation among the lines in UP, visual scoring was also carried out for tillering in that condition. A total of 18 individuals per line were investigated for each trait, and the average was used as the representative data of each line. In Tsukuba, Number of tiller per plant (TN), Culm length (CL) and Culm weight (CW), were evaluated. TN was counted, and CL was measured from the soil surface to the tip of the longest leaf during harvesting at 62 days after sowing. CW was determined after five days oven dry at 65°C.

### 3.2.4 DNA Extraction and SSR genotyping

Whole genomic DNA was extracted from fresh leaves of 88$F_2$ plants and 72$F_3$ plants derived from the selfed of $F_2$ plants, $F_2$JII-IV-10, using a simple method as described by Wang *et al.* (1993) with minor modification. Approximately, 1cm length of rice leaf chips was collected and ground in 100µl NaOH using mixer mill MM200 (Retsch) and then added and mixed well with 400 Tris-HCl (pH 8.0). After centrifugation, the supernatant was recovered and stored in the freezer at -20$^o$C. The extracted DNA was diluted with sterile water into 1/20 concentration and used as template DNA for PCR reaction.

Specific DNA fragments were amplified by using PCR with Quick *Taq* HS DyeMix (TOYOBO, Osaka, Japan) in accordance with the manufacturer's instructions. PCR amplifications were performed in a 10µl reaction mixture containing 3.4µl of DNA template, 1µl primer mix, 0.6µl distilled water and 5µl of *Taq* enzyme. The amplified DNA fragment was separated by electrophoresis on a 3.0 % (w/v) agarose gel containing 0.05 % (v/v) ethidium bromide. After electrophoresis, the DNA signal was scanned with a Pharos FX (Bio-Rad, Tokyo, Japan) molecular imager.

### 3.2.5 Linkage map development and gene mapping

We selected SSR markers from the four introgressed regions in introgression line YTH 34, to detect the QTL which were related with phenotypic traits. We also increased polymorphic markers to narrow down the target regions, when QTL for trait were found. Finally, a total of 19 SSR markers, ten on chr.e 7, three on chr. 2, and two of each on chrs. 4, 5 and 8, were used for genotyping, and linkage analysis in $F_3$ family lines. And, 14 SSRs were used in advanced QTL mapping in $F_3$ population derived from $F_2$ plant, $F_2$JII-IV-10. Recombination frequencies between SSR marker genotypes and genes

were analyzed following Maximum-likelihood method (Allard 1956), and converted into genetic distances as Centi Morgans by Kosambi function (Kosambi 1944) using Join Map Version 4.1 software in $F_3$ lines, and constructed linkage map in the region on chr. 7. Advanced QTL analysis was performed using version 2.5 of the QTL cartographer software (Wang *et al.* 2012) by single marker analysis (SMA). A probability level of ＜0.05 was used as the threshold value to detect significant differences between the two genotype groups, IR 64 and YTH34 for SMA, and when multiple markers on the same chromosomal segments were associated with agronomic traits, the marker that showed the highest *F-* score among the multiple markers was selected as associated markers.

### 3.3 Results

### 3.3.1 Characterization of YTH 34

The average of PN of YTH 34 was 4.0 and that of IR 64 was 14.6, which varied significantly in UP field (Fig. 19 A), but no significant difference was found in IL where PN of YTH 34 was 7.5 and IR64 was 9.5, grown at TARF, JIRCAS, Ishigaki (Fig. 19 B). Variation of PN of both parents is shown in Fig. 18. As the traits value of YTH34 reduced or increased than IR 64, in both of the cultivation systems, we calculated the relative rate (RR) of reduction or increment for each trait (Table 9). The RR of TN. of YTH34 was significantly lower (36%) in UP than IL (68%) compared to IR 64, grown at JIRCAS Hachimandai field, Tsukuba in 2013 (Table 9). Also, RR (%) of other traits such as CL (77%), PL (88%), TS (74%), PW (28%), CW (34%) and P/T (78%) reduced significantly under UP, and DH (107%) and FS/TS (105%) were slightly increased than

IR64. In IL, RR of DH (100%), CL (100%) and PL (102%) were similar, and PN (68%), TS (83%) and CW (84%) decreased slightly. RR (%) of PW (128%), P/T (151%) and FS/TS (177%) increased higher than IR 64 (Table 9).

### 3.3.2 Segregation of tillering in $F_3$ family lines

The TN of the $F_3$ family lines could be clearly differentiated into low tillering and normal tillering types by visual observation in UP, but not in IL. Among the $88F_3$ lines and 86 lines were tested for genetic analysis, and rest two were not germinated. The segregation in each $F_3$ line was investigated and confirmed under the upland field. All plants in 17 lines showed normal tiller and 48 lines segregated, whereas 21 lines showed low tillering type (Table 10, Fig. 19 A) in UP. This segregation showed good fit to a 1:2:1 ratio ($\chi^2$= 1.53, $P$= 39.0 %), and indicated that a single gene controlled the TN in the $F_3$ population. This gene was found recessive and designated as *ltn2*.

In contrast, PN in IL of the $F_3$ plants were found segregated and showed continuous distribution from 8 to 14 (Fig. 19 B). The relationships of PN between IL and UP in $F_3$ family lines is shown in Fig. 20.

### 3.3.3 Mapping of low tillering gene

By co-segregation analysis, the linkage between low tillering gene and the SSR markers were investigated in $F_3$ family lines. The close linkages were detected with SSR markers RM505, MRG5344 and RM21950 on chr.7. And the *ltn2* gene was mapped at the neighbor of RM21950 on chr.7 (Fig. 22 C). The genetic distances between *RM505* and *ltn2,* was 3.4 cM, and between RM21950 and *ltn2* was 1.1 cM (Fig. 22 C).

### 3.3.4 Candidate genes for tillering

Based on the available rice genome sequence and annotation databases, (NCBI:

http://www.ncbi.nlm.nih.gov/gene/; TIGR: http://rice.plantbiology.msu.edu/), we found accurate physical locations of *MRG5433* on chr. 7. There were three putative and expressed genes close to the target region of the Japonica Group rice genome (cultivar: Nipponbare), such as, *Os07g0607500*, *Os07g0607700*, and *Os07g0607800*, which were related to abiotic stresses tolerance (Huang *et al.* 2009, Sottosanto *et al.* 2007, Zhang *et al.* 2008, Kera *et al.* 2012), and might be the causal gene for low TN. especially in UP.

### 3.3.5 Advanced QTL mapping using $F_3$ population derived from a heterozygous $F_2$ plant

We evaluated three agronomic traits- TN, Culm length (CL) and Culm weight (CW) in 72 $F_3$ plants from a plant, $F_2$JII-IV-10, as the hybid population of those were advanced, grown in UP-like condition at JIRCAS tropical greenhouse with the parents. The parents, IR 64 and YTH 34, showed significant differences in TN, and CW, but not in CL (Fig. 21). The $F_3$ plants showed continuous variation for all the measured traits. The range of variations for TN was from 10 to 23 with the average of 15.3, for CL from 81.1 to 99.0 with the average of 91.9 cm, and for CW from 2.1 to 5.4 with the average of 3.4 g (Fig. 21).

Single-marker analysis revealed four SSR markers on chr. 7 that were significantly associated with CW and TN. Among these, marker *RM5847* for CW and *RM505* for TN had the highest *F* values (7.0 for *qCW7* and 6.5 for *qTN7*; Table 11, Fig. 22 B). No associated SSRs were found for CL. These results confirm the existence of the QTLs in the introgression region on chr. 7.

### 3.4 Discussion

Tillering or production of lateral branches (e.g. culm) is an important agronomic

trait that determines shoot architecture and grain production in grasses (Hussain *et al.* 2014). It is also a key component in the expression of phenotype plasticity of the plant in response to transient changes to growing conditions (Fujita *et al.* 2010b). In rice, PN is largely depends on the tiller number, and breeding cultivars with high or moderate tillering abilities is an important breeding objectives during the post green revolution period to achieve higher grain yield (Jennings *et al.* 1979, Nanda and Coffman 1979, Yoshida 1981). Besides, breeders also showed interest on low tillering and high productive tillers ratio, and developed NPT cultivars to improve the crop adaptation to direct-seeding conditions (Vergara *et al.* 1990, Khush 2000, Fujita *et al.* 2010b).

In this study, the location of a QTL for low TN was confirmed by advanced QTL analysis in a region on chr. 7 introgressed from the NPT cultivar, where several QTLs were previously detected by association analysis (Fig. 22 B, Table 11). The single recessive gene's segregation was confirmed (Table 10), and it was designated *ltn2* as a novel gene for low tiller (Fig. 22 C), as no reports had been published on this position on chr. 7 before in rice and a low-tiller number gene, *Ltn,* from a Japonica Group cultivar, Aikawa 1 had been previously reported on chr. 8 by Fujita *et al.* (2010). Linkage analysis of *ltn2* and SSR markers using 86 $F_3$ lines revealed the neighbor SSR marker RM21950 on chr 7.

In the greenhouse, YTH34 harboring *ltn2* showed the low tiller at 45 days after sowing. Similar results were observed in the field also, and confirmed that the low tiller occurred in early vegetative stage of rice. The *ltn2* region on chr. 7 showed particularly unique trait responses in UP conditions: low TN, low dry matter production, low TS, short PL and CL (Tables 9). This indicating that *ltn2* gene may have influences in constriction of plant architecture as a multiple effects' gene or locate several genes

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related these traits in the same chromosome region. And it was expected that *ltn2* or the region of chr.7 might be related with the adaptation or differentiation of rice cultivar in IL and UP. Previously, some stress tolerance QTLs such as for cold (Zhou *et al.* 2010), high temperature (Murata *et al.* 2014), seed shape (Qiu *et al.* 2012), higher spikelet number (Koide *et al.* 2013), and high TN (Thomson *et al.* 2003) were reported in this region, linked to the SSR markers *RM1132* and *RM505*, in IL condition. Lyu *et al.* (2014) found UP-specific, ecotype-differentiated genomic regions and identified a candidate gene (*Os07g0449700*) in a region on chr. 7 under drought stress. The region of *ltn2* on chr. 7 was same with those previous reports.

In rice, several genes isolated previously for TN through the use of mutant rice lines, including *tdr2* (Hasegawa *et al.* 2005) and *rcn9* (Jiang *et al.* 2006) on chr. 1; *OsTB1* (Takeda *et al.* 2003) on chr. 3; *HTD1* (Zou *et al.* 2005) on chr. 4; and *D3* (Ishikawa *et al.* 2005), *MOC1* (Li *et al.* 2003), and *rcn8* (Jiang *et al.* 2006) on chr. 6, and have been associated with branching mechanisms. Also, many QTLs for PN or TN have been identified on chrs. 2, 4, 5 and 6 (Lin *et al.* 1996), on chrs. 1, 3 and 5 (Wu *et al.* 1998), on chrs. 1, 2, 3, 4, 5, 6, 7. 8 and 12 (Yan *et al.* 1998), on chr. 1 (Nagata *et al.* 2002), on chrs. 1, 3, 4 and 12 (Hittalmani *et al.* 2003), on chrs. 2, 5, 6 and 8 (Miyamoto *et al.* 2004), on chrs. 1, 2, 3,4, 6, 7, 8, 9 and 12 (Liu *et al.* 2008, 2010, 2012), and on chrs. 1, 2, 4, 5, 6, 7, 8 and 10 (Bian *et al.* 2013). But no reports found for tillering QTL, in comparing between different conditions IL and UP.

The detail chromosome position of *ltn2* will need to confirm by fine mapping, and the development of NIL for it will be carried out to clarify the multiple effects to the other traits based on the physiological and genetic characterization.

# Chapter IV

## Physiological characterization of introgression lines with an Indica Group rice (*Oryza sativa* L.) variety IR 64 genetic background based on nitrogen uptake

### *4.1 Introduction*

Nitrogen is one of the essential nutrients for rice growth and development. A deficiency of nitrogen leads to dramatic yield losses. Only around 33% of applied nitrogen fertilizer can be used by cereals (Raun and Johnson 1999, Tilman *et al.* 2002), and the remained is lost in the combination of surface runoff, gaseous release from soil, leaching and de-nitrification processes which causes eutrophication of freshwater estuaries and coastal water ecosystems (Raven and Taylor 2003), and increased emission of greenhouse gases, such as nitrous oxide ($N_2O$) (Matson *et al.* 1998). If this practice of surplus fertilization continues, nutrient leaching and atmospheric contamination will soon become widespread problems around the world (Ortiz-Monasterio *et al.* 2001). High cost of nitrogen fertilizer is also another important concern to reduce the production cost. Therefore, for a balanced and sustainable use of fertilizers, it is most important to use nitrogen fertilizer and water in an efficient manner (Zhang *et al.* 2007, Stanger and Lauer 2008, Bundy *et al.* 2011).

The development of rice varieties with high NUE, through physiologically, that is, increased carbon gain per unit nitrogen and time, or agronomically, that is, greater dry matter production and protein yield per unit nitrogen (Laungani and Knops 2009), become breeding priority. The uptake and use efficiency of nitrogen affect plant growth, and genetic improvement in rice variety will be very important issue for stable and high rice production. Nitrogen efficient genotypes are those that possess higher uptake efficiency and dry matter production. In rice, Peng *et al.* (1994) showed that efficient plants are capable of accumulating N in the first 35 days of transplanting.

Crop nutrient uptake rates are different at each growth stage, and crop growth rates are vary with crop, variety, and growing conditions (Clain *et al.* 2011). Also, plant-available nitrogen released forms soil organic or applied fertilizer are highly susceptible to loss from soil-plant system. So, significant reduction of fertilizer use can be achieved by optimizing the rate and timing of fertilizer application based crop demand and uptake patterns (Peng and Bouman 2007, Chen *et al.* 2011), synchronized with plant-available forms of nutrient.

Besides, pre-cultivation period is very critical for better plant selection and before nutrient (nitrogen) treatment for genetic variations. Previously, Namai *et al.* (2009) used 22 days old seedlings, Hanh *et al.* (2013) used 14 days old seedlings, and Obara *et al.* (2010) used 0 days (started treatment immediately after sowing) for nitrogen treatment to get genetic variations and genetic analysis for nitrogen use efficiency. And, no suitable pre-cultivation period was found. And many reports have shown that plant growth and yield is superior on mixtures of $NO_3^-$ and $NH_4^+$ compared with nitrogen source alone (Kronzuker *et al.* 1999, Qian *et al.* 2004, Duan *et al.* 2007), but most studies used few selective cultivars for their experiments. Therefore, the objectives of

the Chapter IV are to determine the genetic variations of rice cultivars and INLs for responses to different nitrogen forms, $NO_3^-$, $NH_4^+$, and $NH_4NO_3$ at various seedling stages, and discuss the relationships between genetic variations of agronomic traits (YC) and responses of nitrogen.

## 4.2 Materials and Methods

### 4.2.1 Plant materials

A total of thirteen rice cultivars including Indica and Japonica Groups, and landrace and improved types from five cluster groups I to V, which showed different nitrogen responses in Namai *et al.* (2009) were selected and used in this study (Table 12). They concluded that the Indica Group landraces, such as Kasalath, are more sensitive to nitrogen with high RDW and PNUE, and improved varieties (Indica or Japonica Group) are most insensitive, UP varieties are intermediate in response to nitrogen.

Also, a total of 25 INLs representing each cluster groups based on agronomic traits from Chapter II were used in this study to evaluate the variation in responses to different nitrogen forms and seeding stage to clarify the relationships between classification under field condition and response to nitrogen in INLs. Details of the groups and number of lines are given on Table 15.

### 4.2.2 Seed preparation and sowing

A total of 180 (6g) seeds of each variety were pre-selected to ensure their potential for good growth before germination. Well-filled seeds were selected by soaking in a

NaCl solution ($d$= 1.13 g/mL) with gentle shaking. Submerged seeds were washed 20 minutes with running tap water, drained, and spread on filter paper at room temperature until they were completely dry. Seed selection with NaCl solution is known to provide a stable germination rate in rice. Seeds were sterilized in a two-step process (Obara *et al.* 2010). First, seeds were soaked 10 min in distilled water with gentle shaking at 60°C, and then washed 20 min with distilled water. Secondly, the wet seeds were soaked 20 min in a 2% (v/v) sodium hypochlorite solution (NaOCl) and then washed at least four times with large amount of distilled water. The seeds of each cultivars and lines were imbibed in distilled water at 30°C in the dark until the tip of the plumule had barely emerged.

### 4.2.3 Growth conditions and nitrogen treatment

Seedlings of rice cultivars were hydroponically grown in greenhouse conditions controlled the temperature from 28 to 35°C and humidity from 42 to 45% under 14 hours of day length. The basal nutrient solution was used following the method of Mae and Ohira (1981) with minor modifications, such as distilled water without any contaminations with additional nutrients and Good's buffer, MES (5mM), to maintain pH 5.5. Germinated seeds were sown in nylon nets (160×220mm) that floated on 40L of quarter strength nutrient solution without nitrogen, and then grown in four or three kinds of treatment 21, 14, 7 and 0 days old, before nitrogen treatment. After these duration rice seedlings were cultivated with and without nitrogen nutrient for 14 days (Fig. 23). As nitrogen sources, these forms of $NH_4Cl$ for $NH_4^+$ (1.43mM/L), $KNO_3$ for $NO_3^-$ (1.43mM/L), and $NH_4NO_3$ for $NH_4^+ + NO_3^-$ (0.714mM/L), were used and rice seedlins were cultivated for 14 days. Around 160 mL of nutrient solution was supplied

per plants and the solution was renewed in every 4 days interval according to Obara *et al.* (2010).

### 4.2.4 Evaluation of dry weight (DW) and relative dry weight (RDW)

The shoots (leaves and stems) of all plants were collected 14 days after the initiation of nitrogen treatment and dried for 5 days (120 hours) at 65°C in dry oven. Then dry matter weight of each plant was measured and calculated the relative dry weight (RDW, %), by comparing with the non-nitrogen control. Finally, the average of relative dry weight in compared with non-nitrogen control treatment was calculated as the representative data of each rice cultivars. Responses of nitrogen and seedling stages were categorized based on RDW.

$$\text{RDW (\%)} = \left| \frac{DW\ of\ N\ treatment}{DW\ of\ non\text{-}nitrogen} \right| \times 100$$

Where, DW is the dry matter weight of the leaves and stems (mg).

### 4.3 Results

Wide ranges of variations of RDW were found among the cultivars and INLs in each different nitrogen form and seedling stage (Table 12, Table 16). RDW in young seedlings (0 and 7 days) showed bigger values than those of old stages (14 and 21 days) in all nitrogen forms (Fig. 25, Fig. 28). It indicated that the uptake started after the seeding, and were reduced from the early to the later stages of seedlings.

Rice cultivars were classified into three groups, I, IIa and IIb, based on reactions to three nitrogen forms and four stages (Fig. 24, Table 13). The group I, Kasalath, showed

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always highest RDW in all form and stage, but showed highest reduction rate (Fig. 25, Table 14). RDW of Kasalath under $NO_3^-$ was highest and was 938 at 0 day's seedling stage. Compared to 0 days old, RDW of 7 days seedlings got 33% reduction, 14 days got 40.9% and 21 days got 44.1% reduction (Fig. 25) in this nitrogen form. RDW of Kasalath was lowest under $NH_4^+$ and but reduction of nitrogen uptake was lower than other two forms. Kasalath showed medium RDW and nitrogen uptake reduction under $NH_4NO_3$. These results indicated that Kasalath prefers $NO_3^-$ forms of nitrogen. Group IIa consisted of improved Indica and Japonica Groups varieties including IR 64. It showed highest RDW under $NH_4NO_3$ and 493 at 0 days seedling stage, medium under $NH_4^+$, and lowest under $NO_3^-$. It showed lower RDW compared with other groups I and IIb. These results indicated that improved varieties are insensitive to nitrogen and prefers $NH_4NO_3$. The group IIb was higher in RDW under $NH_4^+$ and was 516.3 at 0 days seedling stage, and reduction of nitrogen uptake was low in this form. Such as, from 0 days RDW, 7 days seedlings got 6.8% increment, 14 days got 15.4% reduction and only 6% reduction at 21 days (Table 13). Indica and Japonica Groups' landraces and improved varieties including Takanari, Koshihikari, Dontokoi (harboring *sd-1* and Koshihikari background) were belong to this group. Also, IIb had high RDW than those of group IIa in all forms and stage, and low reduction of nitrogen uptake than all other groups (Fig. 25). These results indicated that there were different responses to nitrogen forms during seedling stage among rice cultivars.

The INLs also showed wide variations in responses to nitrogen in all forms, especially, under $NH_4^+$ and $NH_4NO_3$ at young (0 and 7 days) seedling stage (Fig. 26). However, older seedlings stage (21 days) showed lower genetic variations. These 25 INLs and two control varieties, IR 64 and Kasalath, could be classified into four groups,

I, IIa, IIb, and III by cluster analysis (Fig. 27, Table 16). These groups were characterized based on the amount of RDWs and reduction pattern of then during seedling stages.

Group I, Kasalath, showed highest RDW in all the nitrogen forms and stages particularly 7 days seedling stage, and showed dramatic reduction of nitrogen at the 21 days seedling stage. RDW was highest under $NO_3^-$ and smaller under $NH_4^+$ (Table 2). Medium RDW and reduction of nitrogen uptake were found under $NH_4NO_3$. IR 64 and elite line YTH183 belonged to the cluster group III which comprised of 12 INLs and showed higher RDW in all forms but reduction of nitrogen uptake was higher at 21 days seedling stage. Such as RDW under $NH_4^+$ at 0 days seedlings was 403.29, and compared to this 7 days got 18.1% increment and 21 days seedlings got 27.0% reduction. It showed highest RDW under $NH_4^+$, followed by $NH_4NO_3$ and lowest under $NO_3^-$. Group IIa consists of two INLs YTH174 and YTH187, and showed low RDW at young stage and increased at the later stage (Table 16), and showed lower reduction of nitrogen uptake. This group produce higher RDW under nitrate ($NO_3^-$) forms and similar under $NH_4^+$ and $NH_4NO_3$. Group IIb also comprised of 12 INLs, and showed medium RDW at young stage and lowest at the later stage (21 days seedlings). Highest RDW was found under $NH_4^+$ of this group and lowest under $NO_3^-$. It showed medium reduction rate compared with other groups. Although, YTH183 and YTH187 harboring chromosome segments from same parents, they moved to different cluster groups based on the production of RDW and reduction of nitrogen uptake. These results indicated that these INLs varied in responses to nitrogen response.

*4.4 Discussion*

Genetic variations for responses to different nitrogen forms; nitrate ($NO_3^-$), ammonia ($NH_4^+$) and ammonium nitrate ($NH_4NO_3$), were evaluated at seedling stage among 13 rice cultivars and 25 selected INLs; and these for 0, 7, 14 and 21 days old seedlings were grown in quarter strength nutrient solution of hydroponic culture for 14 days in JIRCAS's tropical greenhouse under natural sunlight. These RDW (%) in compare with non-nitrogen control were investigated in each seedling stage and nitrogen.

Wide variations were observed among the cultivars under different nitrogen forms and stages, and RDW of young seedlings stages were bigger than those of old stages in all nitrogen forms which indicating that the uptake started after the seeding, and were reduced according to the seedling age. This might be due to higher nitrogen uptake by the young roots of young seedlings than of the older roots of late seedlings (Armstrong *et al.* 1986, Eissenstat and Volder 2005), or loss of uptake efficiency at later seedling stage. Gao *et al.* (1998) found that 10-15 days older roots maintain a steady, slowly decreasing nitrogen uptake rate as a function of age than the young roots. Lower RDW at the later stages may be due to the nitrogen starvation during pre-cultivation before nitrogen supply.

This study clarified the genetic variations of responses to nitrogen using 13 rice cultivars. Indica Group landrace, Kasalath, showed unique sensitivity to nitrogen, and always the highest RDW in all nitrogen forms and stages, also highest reduction of nitrogen uptake than other cultivars. It preferred $NO_3^-$ nitrogen to $NH_4^+$. Obara *et. al.* (2010) reported a QTL, *qRL6.1* for root length elongation from Kasalath. Other

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landraces in group IIb, also showed higher RDW, but low reduction of nitrogen uptake, and they preferred $NH_4^+$ than $NO_3^-$. Improved Indica and Japonica Groups' varieties including IR 64 in IIa, showed low RDW and high reduction of nitrogen uptake than group IIb. These patterns of responses of Indica and Japonica Groups landraces and improved type rice cultivars to nitrogen corresponded to the results reported by Namai *et al.* (2009).

INLs showed the wide variation for responses to nitrogen. These variations of RDWs under $NH_4^+$ and $NH_4NO_3$ were wider than those under $NO_3^-$. This indicated that nitrogen uptake in the INLs varied among nitrogen forms. Kasalath was unique as usual, and the INLs on the cluster group III including IR 64 and YTH183, showed higher RDW at young stage and higher reduction rate. This indicated that these INLs harbored genetic factors that contributed high RDW at early stages. INLs on cluster group IIa including YTH187, showed low RDW but low reduction of nitrogen uptake. This was quite unique due to their abilities to continue nitrogen uptake even later stages, and might possess some unique genetic factors for nitrogen uptake. Cluster group IIb was the intermediate in RDW and reduction rate between clusters IIa and III. Compared with the previous cluster groups based on agronomic traits in field condition (Table 4 in Chapter II), most of the representative INLs showed high RDW, and unique group A-II separated into three different groups (Table 15), indicating that A-II is also unique in nitrogen response. INLs on the clusters III and IIb did not correspond to any specific cluster groups of field traits; instead it had INLs from all the previous cluster groups. This indicated that most INLs were improved also for nitrogen responses, though they have variations within. Interestingly, YTH183 and YTH187 had chromosome segments from same parent YP5, but they moved into clusters IIa and III, and might be due to

their variation in RDW and reduction of nitrogen uptake, and some unique genetic factors contributed for this. This result need to be confirmed by detail comparison studies.

IR 64 INLs varied with the responses to nitrogen forms according to seedling stages, and some genetic factors from NPT cultivars might contribute them. Based on these finding and materials, the genetic factors for response to different nitrogen are able to identify and make access to genetic improvement for nitrogen uptake of Indica Group rice cultivar.

# Chapter V

## General Discussion

IR 64 is one of the most popular rice varieties developed by IRRI, and renowned for its good appearance, quality, taste and resistant to several diseases of insects and pest (Dalrymple 1986, Khush 1987, Khush and Virk 2005). However, it has limitations including low agronomic and physiological NUE (Diekmann *et al.* 1996, Namai *et al.* 2009), higher susceptibility to drought (Cal *et al.* 2013, Lafitte *et al.* 2007, Wade *et al.* 1999), Fe toxicity (Nozoe *et al.* 2008), and blast and tungro diseases (Liu *et al.* 2009, Shibata *et al*. 2007). Being the most adaptive farmer's variety, it was effective and necessary to improve the yield potential including nutrient use efficiency, tolerance to abiotic and resistance to biotic stresses of this variety to sustain higher yield.

A total of 334 INLs were bred to improve the yield potential of IR 64 by introducing of chromosome segments from NPT cultivars which were developed from the tropical Japonica Group UP cultivars and a Japanese Japonica Group cultivar Hoshiaoba (Fujita *et al.* 2009). Eight traits, DH, CL, PL, leaf length (LL), leaf width (LW), PN, grain weight (GW) and total spikelet No. (TSN) were evaluated under IL condition in wet and dry seasons at IRRI, in the Philippines, and conducted genetic analysis and gene isolation for some selected traits (Fujita *et al.* 2009, 2010a, 2012, 2013, Kobayashi *et al.* 2009). However, this evaluation with limited number of traits and only one cultivate condition was not sufficient to describe all improvements of yield potential of these INLs and better line selection. The characteristics of these INLs under

different climatic conditions such as temperate, and ecosystems for rice cultivation such as IL and UP, and their adaptability were unknown. Also, the information on the INLs dry matter production, physiological characteristics, such as nitrogen uptake, and the relationship between the yields and physiological traits were limited. But these information's are necessary for the detail characterization of these INLs, useful to finding key or new traits under various ecosystems, isolation of novel gene(s) which improves Indica Group rice cultivars e.g. IR 64, and deepen our knowledge on traits development and process of yield formation. To address these gaps, this study conducted for the characterization of INLs under two different conditions, IL and UP in temperate area, based on the evaluation of yield components and dry matter traits, identification of these unique genetic factors which were originated from NPT (Chapter II), confirmation of low tiller QTLs by advanced genetic analysis using hybrids (Chapter III) and clarification of physiological variations among INLs based on nitrogen uptake at seedling stage (Chapter IV).

*Characterization and identification of genetic factors introduced into the INLs*

The INLs were characterized again under IL and UP conditions in temperate region (in Chapter II) for 10 yield components and dry matter traits including DH, CL, PL, PN and TS which were common traits with Fujita *et al.* (2009), and CW, PW, P/T, FS and FS/TS were new traits investigated in this study. The PW, P/T, FS, TS and FS/TS of all the INLs were higher than those of IR 64; the CL and PL values were similar; and the DH, CW, and PN values were lower than those of IR 64 in both condition. This result has asserted the genetic improvement of INLs for low tiller and heavier panicles compared to IR 64. The trait differences between the UP and IL conditions in temperate

region suggested the unique phenotypes were introduced into these INLs and made success the characterization and identification of unique traits and gene(s) by using different ecosystem evaluations. Results also revealed the key traits such as shorter DH, lower PN, and heavier PW, P/T and FS/TS in these INLs. Classification and cluster group characterizations elucidated the distribution of elite NPT traits and patterns of genetic variations among the INLs and also their responses to different cultivate conditions such as IL and UP. And, through the association analysis we detected quit higher No. of QTLs with moderate to higher effects. Many QTLs were found in some key chromosomal regions in several sib groups for multiple traits, indicating the genetic pleiotropy in these INLs that contributed for yield improvement (Fujita *et al.* 2013, Xiao *et al.* 1986). This study unraveled some key chromosome regions/QTLs such as chrs. 2, 7 and 12 for UP, and chrs. 4, 5, 6 and 9 for IL, which endowed increasing adaptation or suitability in UP condition, more efficient to dry matter production or modification of yield component traits under IL condition, and contributed the genetic improvement of IR 64. The new QTL detected for DH on chr. 9 in YP11 is completely new in this study.

We performed all statistical analyses including clustering and association analysis of two years data (2011 and 2012) separately due to their variations between two years and this might be due to the environmental effects. But we used average of two years for the characterization of cluster groups. Also, QTL analysis was conducted in all the sib-groups separately because of the differences in developmental pedigree of donor parents, and comparisons of the detected QTLs (except for DH QTLs) were done with only previous studies using same INLs instead of all other reports on the other populations.

Based on the obtained information, the INLs with unique traits under group A-II

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will be useful breeding materials for improving the yield potential of popular Indica Group rice cultivars. And NIL of each yield traits can be also easily developed by MAS and used for the characterization of detected QTL and identification of gene for the target trait in the further studies. Using the developed NILs, QTLs/gene pyramiding lines with various combinations e.g. high density grain with low tiller number, will be developed.

### *Identification of low tiller gene, ltn2*

Segregation studies (Chapter III) have confirmed the location of the QTLs for low tiller No. which was introgressed from an NPT cultivar and detected on chr. 7 by association analysis using INLs (Fig. 22B, Table 11) by advanced QTL analysis. The QTL segregated as a single recessive gene. No gene/QTL was reported for low tillering in this region of chr. 7, and only some stress tolerance QTLs such as cold stress (Zhou *et al.* 2010), high temperature (Murata *et al.* 2014), seed shape (Qiu *et al.* 2012), higher spikelet number (Koide *et al.* 2013), and high TN (Thomson *et al.* 2003) were reported. And low tiller number gene 2 was designated as *ltn2*. Lyu *et al.* (2014) found an upland-specific candidate gene (*Os07g0449700*) in a region on chr. 7 under drought stress. *ltn2* or the region on chr.7 showed multiple effects for low TN, low dry matter production, low TS, short PL and CL, and these relationships between the results in our study and previous reports will need to confirm by developing isogeneic line for *ltn2* and gene identification through the fine maping.

Low tillering plant type with high density grain was desirable for UP condition and dry seeding condition to maintain a proper plant density particularly in episodic drought (Vergara *et al.* 1990, Farooq *et al.* 2011), But, *ltn2* gene constricts the plant architecture in UP with less density grain. However, this gene might have important role in

developing plant architecture for adaptation to UP or ecotype differentiation of IL and UP cultivars. Previously, researches were focused only on tillering in IL, but this study reported tillering by comparing two conditions IL and UP.

The relationships of *ltn2* with the reported candidate genes need to be confirmed. We suspect that the segment on chr. 7 originated from the UP cultivar Ketan Lumbu, but this also needs further confirmation. To understand the effect of *ltn2* and other genes, it will be necessary to develop NILs and characterize them in more detailed physiological and genetic studies.

Identification of encoding gene of *ltn2*, and unraveling its roles in the tiller bud differentiation, promotion or inhibition of bud formation and outgrowth, shoot development, hormonal changes (auxins or strigolactones and their functions), tiller efficiency, and inflorescence branching in UP will provide new insight into the tillering processes. To clarify the role of *ltn2*, gene accumulation for positive trait which may modify the effect of *ltn2* will be needed. Based on the study, model plant type for adapting of UP will be able to reconstruct. And there information of gene pyramiding based on ltn2, will help for understanding of architecture of upland rice cultivar.

*Genetic variation for nitrogen responses among INLs*

Genetic variations of 13 rice cultivars and 25 representative INLs (please see chapter IV for details) were studied under three nitrogen sources, $NO_3^-$, $NH_4^+$, and $NH_4NO_3$, and four seedling stages with one week interval such as 0, 7, 14, and 21 days old based on the nitrogen uptake determined by RDW (%) by comparing with non-nitrogen control after growing in hydroponics.

We found that 7 days old seedlings was the most responsive and for the widest genetic variations of nitrogen among the Indica and Japonica Group, Improved and

landrace types rice cultivars. Indica Group landrace e.g. Kasalath, showed unique responses to all nitrogen forms but higher RDW in nitrate ($NO_3^-$) form. IRRI bred varieties (e.g. IR64) and Nipponbare were less sensitive and produced high RDW under $NH_4NO_3$. Popular improved cultivars e.g. Koshihikari, Dontokoi, Takanari responded uniquely to nitrogen and had high RDW under $NH_4^+$. They continued nitrogen uptake also at the later stages. Based on the RDW and reduction rates of them during young seeding stage, rice accessions showed three distinct patterns, such as high RDW with high reduction rate (e.g. Kasalath type), medium RDW with medium reduction rate (IRRI bred rice, Nipponbare type), and high or medium RDW with low reduction rate (Takanari, YTH187, Koshihikari type). These patterns might be related with the variation in nitrogen metabolism among rice cultivars that contributed the differentiation of Indica and Japonica Groups, and degree of improvement from landraces and improved types by cross breeding.

Based on the investigation, we could develop the evaluation method for response to nitrogen in young rice seeding stage and clarified the genetic variation of among rice cultivars. Using the same method, the genetic variations among INLs with IR 64 genetic background were also clarified. It meant that these INLs might harbor the different genetic factors for nitrogen responses and uptake. These findings indicated that there were several INLs which harbor the different genetic factor(s) for responses to nitrogen. These genetic factors may be originated from NPT, in other words, tropical upland or Japanese Japonica Group cultivars. However, the genetic analysis for nitrogen response gene(s) in INLs was not carried out yet, and further details studies would be necessary to make clear the relationships between the final yields in the field and the nitrogen uptake. Determination of nitrogen concentration and anatomical studies of seedlings

shoot and roots are important to get more clear insight into the effect of age on uptake efficiency.

Overall, the INLs with shorter DH and higher PW, FS, TS and FS/TS will be used as breeding materials to improving the yield potential of elite Indica Group rice cultivars. The NIL of each target traits can be easily developed by MAS and used for the characterization of detected QTL and isolation of causal gene(s) in the future. The developed NIL will further be used for QTL/gene pyramiding for the desirable traits combination.

The key gene, *ltn2*, which may be related with the UP ecosystem, was found. In mountainous UP, low tiller, thick culm, big panicle and big grain such as heavy panicle type rice have been cultivated in Asia and Africa. In this ecosystem, the low tiller may be one of the most important traits for development of plant architecture or adaptation to the short duration of rainfall, and limited water condition minimizing the No. of tiller/panicle and saving the production for next generation (seeds). Because the tiller No. is easy to get changed by environmental condition and difficult to control genetically, so strong gene which can limit tiller No. in rice might be necessary for UP. The *ltn2* for cultivation in UP, will be used for confirmation the reconstruct of rice plant to develop and find the model architecture of UP rice, as one of key gene.

To improve the IR 64, and to break down the yield barriers in Indica Group cultivar, the wide hybridization or introgressions from different ecotype rice cultivar, such as UP or Japonica Group rice, was demonstrated as one of useful ways, based on the characterizations of INLs by the investigations for yield related traits under IL and UP in temperate conditions and for responses to different nitrogen forms in young seeding stage in this study. And the identifications of genetic factors introduced from donor

parents, such as NPT and Japonica Group cultivar, by the association analyses using INLs and advance QTL analysis or co-segregation analysis between DNA markers and target traits, were also conducted. These information and materials will be useful for Indica Group breeding program and the advance analyses for gene identification and cloning in basic genetic study.

# Abstract

IR 64 is an Indica Group elite rice (*Oryza sativa* L.) variety developed during the second phase of 'Green Revolution', but it has limitations including low nitrogen use efficiency (NUE), higher susceptibility to drought, iron (Fe), blast and tungro diseases. A total of 334 INLs harboring chromosome segments from tropical Japonica Group UP varieties, such as New Plant Types' (NTPs) and a Japanese Japonica Group variety were developed to improve the yield potential of IR 64. This study aimed (1) to characterize these INLs under different rice ecosystems such as IL and UP at Temperate, based on the data of YC and dry matter production, and detect the genetic factor(s) for each traits by genotype and phenotype association analysis (2) to confirm the association (QTL) for low tillering based on advanced analyses, and (3) to clarify the variation of these INLs in nitrogen uptake at seedling stage.

These 333 INLs were characterized, and results showed that panicle weight (PW), PW/whole weight per plant (P/T), fertility spikelet (FS), and fertility spikelet No./total spikelet No. per panicle (FS/TS) of INLs were increased, and culm weight (CW) and panicle No. (PN) were decreased commonly in compared with those of IR 64. These INLs were classified into three cluster groups, A, B and C, based on the data in UP, and four, I, II, III and IV in IL. And these were reclassified into six groups, A-I, A-II, B-II, B-III, C-III and C-IV. The cluster groups were characterized mainly based on the variations of four traits, days to heading (DH), culm length (CL), panicle length (PL), and total spikelet No. per panicle (TS). Three groups, B-III including IR 64, A-I and A-II, had longer DH and PL, and higher TS, than the other three groups. Two groups

C-III and C-IV had the lowest values of DH, CL, PL and TS. BII showed intermediate values of traits among the groups. To identify the genetic factors on chromosome segments introduced into INLs, association analyses were carried out using these phenotypic and genotype data of 262 SSR makers, and a total of 166 QTLs for ten traits were detected on all rice chromosomes, except for chromosomes 3 and 10. Among them, 60 QTLs were only detected in UP, and 31 QTLs were in IL. Remained 75 QTLs were found in both conditions. Off them, 81 QTLs for DH, CL, PL, PW, P/T, PN, FS and TS, were unique and detected newly.

Advanced QTL analysis for low tillering was carried out using 72 $F_3$ plants derived from an $F_2$ plant, F2JII-IV-10, which harbored the heterozygous region locating the QTL on chromosome 7. And, a total of 88 $F_3$ family lines derived from the cross between IR 64 and YTH 34, were evaluated for panicle No. in UP and IL fields, and they showed single gene segregation in UP. The low tillering was controlled by a recessive gene and designated as *ltn2*, and mapped with a neighbor SSR marker RM21950 on chromosome 7 with 1.1cM distance.

The genetic variations of responses to different nitrogen forms; nitrate ($NO_3^-$), ammonium ($NH_4^+$), and ammonium nitrate ($NH_4NO_3$), were evaluated at seedling stage of 13 rice cultivars and 25 selected INLs. Seedlings of four stages, 0, 7, 14, and 21 days old, were grown in hydroponic culture for 14 days in the greenhouse, and relative dry weight (RDW, %) in compare with non-nitrogen treatment were investigated. RDW of young seedlings of cultivars and INLs showed bigger values than those of old stages in all nitrogen forms, and showed difference in the pattern of nitrogen uptake according to the seedling stage. Rice cultivars could be classified into three groups, I, IIa and IIb,

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based on reaction patterns to nitrogen. The group I, Kasalath, showed always the highest RDW in all forms, and stage and showed dramatic reduction of nitrogen uptake. Group IIa including IR64 showed lower RDW and high reduction of nitrogen uptake in all treatments in compared with other groups. Group IIb was higher in RDW than IIa, and also low reduction of nitrogen uptake at the later stages. Improved cultivars, Takanari and Dontokoi belonged to IIb. These indicated that the cultivars in IIb were different from the other two, and might harbor some genetic factors for nitrogen uptake in rice seedling stage. In addition, INLs could be classified into four cluster groups, I, IIa, IIb and III. Kasalath showed the highest RDW in all forms and stages, and formed independent group I. Group IIa showed low RDW, but reduction of nitrogen uptake was lower than other groups, and YTH 187 belong to this group. Group III showed the higher RDW in forms, but showed higher reduction of nitrogen uptake than IIa and IIb. Elite line YTH 183 and recurrent parent IR 64 belonged to this group. Group IIb was medium in RDW and reduction of nitrogen uptake in compared with other groups. Because two INLs, YTH 183 and YTH 187, harbored chromosome segments from same donor parents (YP 5), but went to different cluster groups due to reduction of nitrogen uptake at later stages.

Based on the investigation for reaction to different nitrogen forms, genetic variations among the INLs were found. This meaning that there are different genetic factor(s) for nitrogen responses in INLs, and these INLs will be useful for identification of them and gene sources.

This study demonstrated and confirmed that genetic and physiological improvements of Indica Group rice cultivars are possible and promising by the introgression of chromosome segments from Japonica Group cultivars through wide

hybridizations. This strategies will accelerate the breeding new cultivars of major crops.

These INLs and information of QTLs will be used for genetic studies to identify genetic factor(s) that modified IR 64, and as the breeding materials for genetic improvement of Indica Group variety. Also, improvement for nitrogen uptake of IR 64, and its management in rice cultivation.

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Table 1. Phenotypic variations of yield and biomass component traits in IR64 and INLs across different cultivated conditions

| Population | Year | Conditions | Average of traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | DH | CL (cm) | PL (cm) | CW (g) | PW (g) | P/T (%) | PN | FS | TS | FS/TS (%) |
| IR64 | 2011 | IL | 108.0±2.0 | 78.3±0.8 | 24.7±1.3 | 61.3±8.9 | 14.4±2.4 | 19.2±3.0 | 26.6±7.8 | 20.4±9.6 | 130.1±10.2 | 20.0±7.0 |
| | | UP | 144.0±3.2 | 40.8±3.9 | 24.7±1.2 | 69.3±17.0 | 10.6±4.5 | 12.9±2 | 20.1±6.5 | 24.7±31.5 | 166.2±26.6 | 20.0±19.0 |
| | 2012 | IL | 108.5±1.0 | 72.2±1.5 | 26.3±0.3 | 52.1±9.2 | 27.9±5.4 | 34.9±1 | 20.2±5.51 | 77.0±8.86 | 126.3±7.7 | 61.0±4.0 |
| | | UP | 143.6±2.3 | 65.5±4.6 | 26.0±0.6 | 89.4±21.7 | 57.9±14.6 | 38.0±2 | 43.9±12.0 | 106.5±13.0 | 160.7±8.1 | 66.0±6.0 |
| INLs | 2011 | IL | 107.5±3.1<br>(99.0-121.0) | 79.7±5.7<br>(60.5-92.7) | 25.8±1.9<br>(18.2-33.4) | 47.0±6.7<br>(30.3-76.1) | 27.2±6.2<br>(11.7-43.8) | 36.5±7.1<br>(16.1-55.0) | 16.5±4.2<br>(10.4-34.7) | 87.1±26.9<br>(15.8-158.5) | 155.5±26.1<br>(86.0-229.0) | 56.0±14.6<br>(8.8-88.4) |
| | | UP | 136.9±5.6<br>(119.0-160.0) | 54.3±7.3<br>(26.9-77.9) | 26.0±2.1<br>(18.9-31.1) | 45.7±12.5<br>(9.9-101.8) | 35.1±12.4<br>(3.0-70.5) | 42.8±9.0<br>(6.8-66.0) | 20.1±4.8<br>(6.6-38.7) | 122.6±39.5<br>(0.0-201.9) | 166.1±39.4<br>(47.8-261.8) | 74.2±18.7<br>(0.0-93.9) |
| | 2012 | IL | 109.0±3.0<br>(102.0-124.0) | 73.1±5.6<br>(53.4-88.7) | 26.2±1.8<br>(20.4-33.1) | 38.1±6.6<br>(23.6-85.6) | 36.3±5.4<br>(18.7-57.6) | 48.8±5.2<br>(28.3-59.7) | 15.8±3.1<br>(10.9-37.7) | 117.3±25.0<br>(39.1-201.3) | 147.3±29.7<br>(74.1-234.5) | 79.9±8.8<br>(34.0-97.3) |
| | | UP | 141.4±6.3<br>(126.0-165.3) | 65.7±9.7<br>(32.0-86.4) | 26.5±2.2<br>(19.2-31.3) | 80.6±22.4<br>(11.7-148.3) | 62.6±20.1<br>(2.6-103.8) | 43.1±9.0<br>(7.0-58.6) | 36.4±9.2<br>(6.0-71.0) | 125.0±38.5<br>(0.0-299.2) | 189.3±44.5<br>(81.3-320.8) | 66.8±17.7<br>(0.0-93.2) |

Average data of 20 plants of IR64 and 12 plants of each INL was calculated

IL: Irrigated lowland, UP: Upland

DH; Days to heading, CL; Culm length, PL; Panicle length, PW; Panicle weight, CW; Culm weight, P/T; Harvest index, PN; Panicle number, FS; Fertile seed,TS; Total spikelets and FS/TS; Ratio of fertile seed and total spikelets.

Table 2. Pearson`s correlation coefficient`s between yield and biomass component traits in 333 INLs grown in IL and UP in 2011

| Traits (2011) | Upland | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DH | CL | PL | PW | CW | P/T | PN | FS | TS | FS/TS |
| DH | | -0.091 | 0.266** | -0.289** | 0.169** | -0.449** | -0.180** | -0.099 | 0.300** | -0.361** |
| CL | 0.228** | | 0.613** | 0.718** | 0.448** | 0.462** | 0.341** | 0.731** | 0.536** | 0.462** |
| PL | 0.459** | 0.398** | | 0.422** | 0.398** | 0.181** | -0.108* | 0.597** | 0.721** | 0.159** |
| PW | 0.117* | 0.301** | 0.173** | | 0.429** | 0.735** | 0.647** | 0.770** | 0.367** | 0.647** |
| CW | 0.159** | 0.236** | 0.405** | -0.053 | | -0.235** | 0.664** | 0.210** | 0.533** | -0.185** |
| P/T | 0.009 | 0.043 | 0.238** | -0.610** | 0.806** | | -0.243** | 0.693** | 0.021 | 0.876** |
| PN | -0.134* | -0.373** | -0.383** | 0.267** | -0.087 | -0.255** | | 0.271** | 0.170** | 0.217** |
| FS | 0.062 | 0.331** | 0.400** | -0.263** | 0.742** | 0.741** | -0.343** | | 0.613** | 0.712** |
| TS | 0.336** | 0.251** | 0.619** | 0.108* | 0.477** | 0.333** | -0.218** | 0.554** | | -0.079 |
| FS/TS | -0.144** | 0.244** | 0.069 | -0.393** | 0.563** | 0.674** | -0.276** | 0.834** | 0.025 | |

(Left side label: Irrigated lowland)

DH; Days to heading, CL; Culm length, PL; Panicle length, PW; Panicle weight, CW; Culm weight, P/T; Harvest index, PN; Panicle number, FS; Fertile seed,TS; Total spikelet and FS/TS; Ratio of fertile seed and total seed.
Above diagonal- upland and below diagonal- Irrigated lowland condition.
 *Significant at $P$ <0.05, ** Significant at $P$ <0.01 level

Table 3. Pearson`s correlation coefficient`s between yield and biomass component traits in 333 INLs grown in IL and UP in 2012

| Traits (2012) | | Upland | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DH | CL | PL | PW | CW | P/T | PN | FS | TS | FS/TS |
| Irrigated lowland | DH | | 0.133* | 0.234** | 0.267** | -0.481** | -0.703** | -0.310** | -0.335** | 0.315** | -0.643** |
| | CL | 0.415** | | 0.658** | 0.541** | 0.488** | 0.171** | 0.267** | 0.551** | 0.626** | 0.164** |
| | PL | 0.407** | 0.463** | | 0.489** | 0.276** | 0.006 | 0.042 | 0.383** | 0.725** | -0.08 |
| | PW | 0.424** | 0.36** | 0.164** | | 0.368** | -0.263** | 0.560** | 0.091 | 0.429** | -0.181** |
| | CW | 0.066 | 0.324** | 0.259** | 0.108 | | 0.748** | 0.708** | 0.680** | 0.188** | 0.655** |
| | P/T | -0.268** | -0.05 | 0.062 | -0.69** | 0.632** | | 0.361** | 0.664** | -0.054 | 0.836** |
| | PN | -0.162** | -0.267** | -0.438** | 0.386** | 0.15** | -0.184 | | 0.230** | -0.041 | 0.344** |
| | FS | 0.247** | 0.451** | 0.512** | -0.011 | 0.548** | 0.389** | -0.379** | | 0.507** | 0.715** |
| | TS | 0.499** | 0.476** | 0.586** | 0.245** | 0.391** | 0.087 | -0.311** | 0.841** | | -0.188** |
| | FS/TS | -0.422** | 0.018 | -0.072 | -0.438** | 0.336** | 0.574** | -0.148* | 0.356** | -0.195** | |

DH; Days to heading, CL; Culm length, PL; Panicle length, PW; Panicle weight, CW; Culm weight,  P/T; Harvest index, PN; Panicle number, FS; Fertile seed,TS; Total spikelet and FS/TS; Ratio of fertile seed and total seed.

Above diagonal- upland and below diagonal- Irrigated lowland condition.

 *Significant at *P* <0.05, ** Significant at *P* <0.01 level

Table 4. Variation of agronomic traits among the 4 cluster groups in IL

| Cluster group | | DH | CL(cm) | PL(cm) | CW(g) | PW(g) | P/T (%) | PN | FS | TS | FS/TS(%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IR64 | 2011 | 108.0±2.0 | 78.3±0.8 | 24.7±1.3 | 61.3±8.9 | 14.4±2.4 | 20.0±3.0 | 26.6±7.8 | 20.4±9.6 | 130.1±10.2 | 20.0±7.0 |
| | 2012 | 108.5±1.0 | 72.2±1.5 | 26.3±0.3 | 52.1±9.2 | 27.9±5.4 | 30.0±1.0 | 20.19±5.5 | 77.01±8.8 | 126.31±7.7 | 61.0±4.0 |
| I (131) | 2011 | 108±2.4 (102.0~116.0) | 83.1±3.7 (71.2~90.6 ) | 26.4±1.8 (22.5~33.4 ) | 47.6±5.1 (38.4~60.1) | 28.2±5.8 (12.8~40.6) | 40.0±6 .0 (18.0~49.0) | 15.1±2.0 (11.0~27.2) | 89.2±25.7 (20.6~142.2 ) | 153.1±24.0 (96.5~218.7) | 60.0±13 (14.0~85.0) |
| | 2012 | 109±2.4 (103.0~116.6) | 74.6±4.1 (61.2~83.2 ) | 26.9±1.6 (22.6~33.1 ) | 36.6±4.9 (23.6~54.1) | 36.1±4.9 (18.7~48.4) | 50.0±4 .0 (35.0~59.0) | 14.5±1.7 (10.9~19.9) | 121.3±25.6 (39.1~193.5) | 148.8±32.1 (74.1~234.5) | 80.0±7.0 (52.0~97.0) |
| II (76) | 2011 | 108±3.1 (102.0~116.0) | 77.0±7.0 (60.5~89.1) | 26.4±1.9 (23.0~31.5) | 44.4±7.0 (30.2~65.1) | 29.4±7.2 (11.6~43.7) | 40.0±8.0 (16.1~55.0) | 15.5±3.0 (10.4~30.7) | 93.1±30.6 (22.2~156.1) | 162.3±28.8 (99.1~229.0) | 60.0±15 (15.6~88.4) |
| | 2012 | 110±3.0 (104.0~118.6) | 72.9±6.9 (57.8~88.7) | 26.6±1.7 (23.1~32.4) | 38.3±7.0 (26.2~64.8) | 38.4±5.5 (28.6~52.7) | 50.0±6 .0 (35.0~59.7) | 16.4±2.4 (13.0~26.5) | 121.2±24.7 (72.7~201.3) | 155.5±27.0 (109.2~220.7) | 8.0±9.0 (49.3~93.1) |
| III (114) | 2011 | 107±3.6 (99.0~121.0) | 78.2±4.7 (64.3~92.6) | 25.0±1.7 (18.2~29.8) | 48.6±7.5 (33.6~76.1) | 24.8±5.1 (12.5~36.3) | 30.0±6.0 (17.9~50.3) | 18.6±5.5 (11.0~34.6) | 80.9±25.2 (15.7~158.5) | 155.8±26.3 (86.0~226.0) | 50.0±14.0 (10.0~79.9) |
| | 2012 | 109±3.6 (102.0~124.0) | 71.9±5.5 (53.4~87.2) | 25.4±1.6 (20.4~29.1) | 40.2±7.7 (27.7~85.6) | 35.5±5.5 (19.6~57.6) | 50.0±5 .0 (28.3~57.2) | 17.2±4.0 (12.2~37.7) | 111.7±24.0 (51.8~171.5) | 142.4±27.9 (86.3~219.4 ) | 80.0±9.0 (34.0~94.3) |
| IV (12) | 2011 | 106±2.3 (103.0~111.0) | 75.2±4.9 (64.5~82.6) | 24.3±1.6 (21.4~26.7) | 44.4±5.4 (37.1~54.9) | 26.3±4.9 (19.9~32.9) | 40.0±5 .0 (26.6~47.1) | 18.9±5.4 (13.3~27.9) | 85.7±19.2 (65.3~118.1) | 136.7±16.0 (117.6~168.2) | 60.0±8.0 (47.1~81.0) |
| | 2012 | 107±1.6 (104.3~109.6) | 68.9±6.8 (56.8~77.6) | 25.0±1.7 (22.8~28.4) | 34.8±3.9 (29.8~43.0) | 33.0±5.0 (25.0~40.2) | 50.0±4.0 (41.7~55.3) | 14.3±1.17 (12.4~18.2) | 103.0±14.2 (83.6~135.8) | 124.9±15.0 (104.4~161.7) | 80.0±4.0 (75.0~89.6) |
| Mean±SD | 2011 | 107.1±3.1 (99.0~121.0) | 78.3±5.7 (60.5~92.6) | 25.5±1.9 (18.2~33.4) | 46.2±6.7 (30.2~76.1) | 27.2±6.2 (11.6~43.7) | 40.0±7 .0 (16.1~55.6) | 17.0±4.2 (10.4~34.6) | 87.2±26.9 (15.7~158.5) | 152.0±26.16 (86.0~229.0) | 60.0±14.0 (10.0~88.5) |
| | 2012 | 108.5±3.0 (102.0~124.0) | 72.1±5.6 (53.4~88.7) | 26.0±1.8 (20.4~33.1) | 37.5±6.6 (23.6~85.6) | 35.8±5.4 (18.7~57.6) | 50.0±5.0 (28.3~59.7) | 15.6±3.1 (10.9~37.7) | 114.3±25.0 (39.1~201.3) | 142.9±29.7 (74.1~234.5) | 80.0±8.0 (34.0~97.3) |

¤ SD= Standard deviation;

DH; Days to heading, CL; Culm length, PL; Panicle length, PW; Panicle weight, CW; Culm weight, P/T; Harvest index, PN; Panicle number, FS; Fertile seed,TS; Total spikelet and FS/TS; Ratio of fertile seed and total seed.

Table 5. Variation of agronomic traits among the 3 cluster groups in UP

| Cluster group | | DH | CL(cm) | PL(cm) | CW(g) | PW(g) | P/T (%) | PN | FS | TS | FS/TS (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IR64 | 2011 | 144.0±3.2 | 40.8±3.9 | 24.7±1.2 | 69.3±17.0 | 10.6±4.5 | 10.0±2.0 | 20.1±6.5 | 24.7±31.5 | 166.2±26.6 | 20.0±19.0 |
| | 2012 | 143.6±2.3 | 65.5±4.6 | 26.0±0.6 | 89.4±21.7 | 57.9±14.6 | 38±2.0 | 43.9±12.0 | 106.5±13.0 | 160.7±8.1 | 66±6 |
| A(189) | 2011 | 138.0±5.2 (124.0~160.0) | 55.2±7.9 (28.8~77.8) | 26.8±2.0 (21.5~31.1) | 45.9±13.7 (9.9~101.8) | 34.9±13.7 (4.7~70.5) | 40.0±9.0 (13.0~66.0) | 19.5±5.0 (6.9~31.8) | 124.8±41.3 (0.0~201.9) | 174.0±40.2 (69.4~261.8) | 70.0±18.0 (0.0~92.6) |
| | 2012 | 143.0±5.5 (130.3~161.6) | 67.7±10.7 (32.0~86.4) | 27.1±2.4 (19.2~31.2) | 80.0±25.4 (11.6~146.5) | 59.2±21.5 (2.6~103.1) | 40.0±9 (7.0~54.0) | 34.8±10.4 (6.0~71.0) | 123.8±39.5 (0.0~299.1) | 197.5±47.0 (81.3~320.8) | 60.0±17.0 (0.0~93.2) |
| B(111) | 2011 | 137.0±6.1 (119.0~156.0) | 53.7±6.2 (26.8~69.3) | 25.2±1.7 (19.7~29.1) | 47.4±10.7 (21.8~89.5) | 35.2±11.0 (2.9~65.7) | 40.0±9.0 (6.8~57.7) | 21.2±4.3 (6.6~38.7) | 121.5±39.1 (0.0~196.2) | 162.4±34.9 (47.7~243.8) | 70.0±2.0 (0.0~93.8) |
| | 2012 | 141.0±7.0 (126.0~165.3) | 64.8±6.5 (45.5~79.4) | 25.8±1.6 (20.5~29.0) | 85.6±16.8 (44.9~148.3) | 66.7±18.0 (8.5~102.1) | 40.0±9.0 (8.5~54.7) | 39.1±7.1 (16.1~60.1) | 129.3±40.3 (0.0~210.5) | 186.5±39.1 (104.3~285.2) | 70.0±1.0 (0.0~92.3) |
| C(33) | 2011 | 132.0±4.0 (124.0~139.0) | 50.7±5.1 (41.8~64.5) | 25.1±2.3 (18.8~29.8) | 38.7±7.5 (22.2~54.2) | 36.3±7.7 (18.6~53.4) | 50.0±4.0 (36.1~55.6) | 19.7±3.9 (12.5~27.9) | 114.0±27.6 (58.2~186.3) | 132.7±28.4 (80.3~222.3) | 90.0±7.0 (53.5~93.9) |
| | 2012 | 136.0±3.6 (130.3~143.0) | 57.0±7.3 (42.1~69.0) | 25.5±2.0 (19.8~29.1) | 67.3±12.4 (46.6~97.5) | 69.0±14.4 (44.3~103.7) | 50.0±5.0 (41.3~58.6) | 36.9±5.4 (28.5~51.6) | 117.9±23.4 (68.8~165.0) | 151.8±20.2 (117.3~195.0) | 80.0±9.0 (43.1~88.0) |
| Mean±SD | 2011 | 135.6±5.6 (119.0~160.0) | 53.2±7.3 (26.8~77.8) | 25.7±2.1 (18.8~31.1) | 44.0±12.5 (9.9~101.8) | 35.5±12.3 (2.9~70.5) | 40.0±9.0 (6.0~66.0) | 20.1±4.7 (6.6~38.7) | 120.1±39.5 (0.0~201.9) | 156.4±39.4 (47.7~261.8) | 80.0±18.0 (0.0~93.9) |
| | 2012 | 139.8±6.2 (126.165.3) | 63.1±9.7 (32.0~86.4) | 26.1±2.2 (19.2~31.2) | 77.7±22.2 (11.6~148.3) | 64.9±20.1 (2.6~103.7) | 50.0±9.0 (7.0~58.6) | 36.9± 9.2 (6.0~71.0) | 123.6±38.5 (0.0~299.1) | 178.6±44.5 (81.3~320.3) | 70.0±17.0 (0.0~93.2) |

¤ SD= Standard deviation

DH; Days to heading, CL; Culm length, PL; Panicle length, PW; Panicle weight, CW; Culm weight,  P/T; Harvest index,
PN; Panicle number, FS; Fertile seed,TS; Total spikelet and FS/TS; Ratio of fertile seed and total seed.

Table 6. Classification of INLs with IR64 genetic background based on morphological and physiological traits in UP and IL fields

| Cluster group | | No. of lines in each cluster groups (%) | | | | |
|---|---|---|---|---|---|---|
| | | Irrigated lowland | | | | |
| | | I | II | III | IV | Total |
| Upland | A | 35 [YP1]<br>23 [YP3]<br>45 [YP4]<br>28 [YP11] | 12 [YP11]<br>46 [YP5] | - | - | 189 (56.8) |
| | Sum | 131 (39.3) | 58 (17.4) | 0 (0.0) | 0 (0.0) | |
| | B | - | 10 [YP5]<br>8 [YP6] | 21 [YP6]<br>21 [YP7]<br>29 [YP8]<br>16 [YP9]<br>6 [YP10]<br>(IR64) | - | 111 (33.3) |
| | Sum | 0 (0.0) | 18 (5.4) | 93 (27.9) | 0 (0.0) | |
| | C | - | - | 21 [YP10] | 12[YP10] | 33 (9.9) |
| Total | | 131 (39.4) | 76 (22.8) | 114 (34.2) | 12 (3.6) | 333 (100.0) |

[ ] : Donor parents of introgression of INLs;  hyphen (-) indicate no INLs belonged to this group
The data of 10 kinds of traits from irrigated lowland and upland conditions in 2011 and 2012 were used for cluster analysis following Ward`s hierarchical analysis (Ward 1963) using JMP7.0
INLs were classified into 4 and 3 cluster groups using two seasons' data in 2011 and 2012 in irrigated lowland and upland, respectively.
These clusters were reclassified into six groups based on the matrix relationship between the results of irrigated lowland and upland

Table 7. Phenotypic varaiations among the cluster groups and between two ecosystems, irrigated lowland and upland

| Cluster group | Ecosystems | Agronomic traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DH | CL(cm) | PL(cm) | CW(g) | PW(g) | PW/TW | PN | FS | TS | FS/TS |
| A-I | IL | $108.6\pm2.2^a$ | $78.8\pm3.6^{**a}$ | $26.63\pm1.6^a$ | $42.1\pm3.9^{cd}$ | $32.1\pm4.4^{ab}$ | $0.43\pm0.04^{**ab}$ | $14.7\pm1.5^d$ | $105.2\pm23.2^a$ | $150.9\pm26.5^{abc}$ | $0.69\pm0.08^{*a}$ |
| (n=131) | UP | $141.1\pm5.0^{**a}$ | $62.3\pm8.3^a$ | $26.9\pm2.2^{ns,a}$ | $63.2\pm18.5^{**abc}$ | $44.6\pm16.7^{**bc}$ | $0.40\pm0.08^c$ | $26.5\pm7.7^{**c}$ | $119.5\pm37.7^{**,a}$ | $184.1\pm44.8^{**a}$ | $0.66\pm0.17^b$ |
| | UP/IL | 1.29 | 0.78 | 1.01 | 1.50 | 1.38 | 0.93 | 1.79 | 1.13 | 1.21 | 0.94 |
| A-II | IL | $108.4\pm2.5^{ab}$ | $75.3\pm6.7^{**bc}$ | $26.5\pm1.6^{ab}$ | $40.3\pm5.3^{cd}$ | $34.3\pm5.6^a$ | $0.45\pm0.06^a$ | $15.5\pm1.8^{cd}$ | $109.2\pm25.8^a$ | $159.1\pm25.8^a$ | $0.68\pm0.11^{ab}$ |
| (n=58) | UP | $138.6\pm3.1^{**b}$ | $59.6\pm9.4^{abc}$ | $26.8\pm2.0^{ns,ab}$ | $62.5\pm15.7^{**abc}$ | $52.5\pm13.3^{**a}$ | $0.45\pm0.06^{ns,ab}$ | $28.3\pm4.7^{**bc}$ | $135.0\pm32.1^{**a}$ | $189.5\pm35.3^{**a}$ | $0.71\pm0.12^{*ab}$ |
| | UP/IL | 1.27 | 0.79 | 1.01 | 1.55 | 1.53 | 0.99 | 1.82 | 1.23 | 1.19 | 1.04 |
| B-II | IL | $108.7\pm2.4^a$ | $74.9\pm6.8^{**bc}$ | $26.5\pm1.5^{ns,abc}$ | $41.3\pm7.0^a$ | $33.9\pm5.2^{ab}$ | $0.44\pm0.06^{ns,abc}$ | $15.9\pm3.7^{bc}$ | $107.2\pm21.1^{ab}$ | $158.9\pm23.4^{abc}$ | $0.67\pm0.09^{ns,ab}$ |
| (n=18) | UP | $139.5\pm4.3^{**ab}$ | $56.6\pm6.5^{abc}$ | $25.9\pm1.6^{abc}$ | $70.0\pm12.2^{**ab}$ | $48.5\pm10.3^{**abc}$ | $0.40\pm0.07^{bc}$ | $30.5\pm2.4^{**bc}$ | $113.5\pm22.7^{ns,a}$ | $176.6\pm35.0^{ns,abc}$ | $0.66\pm0.14^{ab}$ |
| | UP/IL | 1.28 | 0.75 | 0.97 | 1.69 | 1.43 | 0.90 | 1.91 | 1.05 | 1.11 | 0.97 |
| B-III | IL | $108.2\pm3.2^{ab}$ | $75.8\pm4.6^{**b}$ | $25.3\pm1.4^{ce}$ | $45.7\pm6.2^b$ | $30.3\pm4.0^b$ | $0.39\pm0.05^c$ | $17.5\pm3.9^b$ | $98.6\pm20.6^{ab}$ | $154.4\pm23.1^{ac}$ | $0.64\pm0.10^b$ |
| (n=93) | UP | $138.4\pm5.4^{**b}$ | $59.7\pm5.6^{ab}$ | $25.4\pm1.5^{ns,c}$ | $65.8\pm11.8^{**ab}$ | $51.4\pm13.5^{**a}$ | $0.43\pm0.08^{**bc}$ | $30.1\pm5.2^{**b}$ | $127.6\pm38.8^{**a}$ | $174.1\pm35.9^{**ab}$ | $0.73\pm0.18^{**a}$ |
| | UP/IL | 1.27 | 0.78 | 1.01 | 1.43 | 1.69 | 1.08 | 1.71 | 1.29 | 1.12 | 1.13 |
| C-III | IL | $105.9\pm1.5^c$ | $71.7\pm4.6^{**c}$ | $24.7\pm2.0^e$ | $38.4\pm4.3^d$ | $29.6\pm4.8^b$ | $0.43\pm0.05^{abc}$ | $19.3\pm3.5^b$ | $85.7\pm18.4^b$ | $125.7\pm21.0^d$ | $0.68\pm0.10^{ab}$ |
| (n=21) | UP | $134.5\pm3.0^{**c}$ | $53.9\pm5.1^c$ | $25.4\pm2.1^{ns,bc}$ | $52.2\pm9.5^{**c}$ | $53.3\pm9.2^{**ab}$ | $0.50\pm0.03^{**a}$ | $28.6\pm4.0^{**bc}$ | $112.9\pm24.5^{**a}$ | $141.2\pm21.7^{*c}$ | $0.80\pm0.09^{**a}$ |
| | UP/IL | 1.26 | 0.75 | 1.02 | 1.35 | 1.80 | 1.15 | 1.47 | 1.31 | 1.12 | 1.16 |
| C-IV | IL | $106.1\pm1.3^{bc}$ | $72.1\pm5.8^{**bc}$ | $24.7\pm1.6^{cde}$ | $39.5\pm4.4^{cd}$ | $29.6\pm4.4^b$ | $0.42\pm0.04^{abc}$ | $16.5\pm3.1^{bcd}$ | $94.3\pm14.9^{ab}$ | $130.8\pm14.2^{bd}$ | $0.72\pm0.05^{ab}$ |
| (n=12) | UP | $134.2\pm3.6^{**c}$ | $53.8\pm6.4^{bc}$ | $25.3\pm1.8^{ns,abc}$ | $53.0\pm5.8^{**bc}$ | $52.7\pm10.4^{**abc}$ | $0.49\pm0.04^{**abc}$ | $28.3\pm3.8^{**bc}$ | $115.8\pm22.1^{**a}$ | $142.2\pm23.6^{ns,bc}$ | $0.81\pm0.03^{**a}$ |
| | UP/IL | 1.26 | 0.74 | 1.02 | 1.33 | 1.77 | 1.15 | 1.70 | 1.22 | 1.08 | 1.12 |
| Whole INLs includin g IR64 | IL | $108.3\pm2.6^{ab}$ | $76.4\pm5.3^{**b}$ | $26.0\pm1.7^{bd}$ | $42.7\pm5.6^c$ | $31.7\pm4.8^b$ | $0.42\pm0.05^b$ | $16.2\pm3.1^c$ | $102.0\pm23.2^a$ | $151.3\pm25.9^{abc}$ | $0.67\pm1.0^{ab}$ |
| | UP | $139.2\pm5.1^{**b}$ | $59.9\pm7.9^{ab}$ | $26.3\pm2.0^b$ | $63.2\pm15.6^{**b}$ | $48.8\pm14.7^{**abc}$ | $0.42\pm0.08^{bc}$ | $28.2\pm6.3^{**bc}$ | $123.8\pm35.8^{**a}$ | $177.6\pm40.4^{**ab}$ | $0.70\pm0.16^{*ab}$ |
| | UP/IL | 1.32 | 0.78 | 1.01 | 1.48 | 1.53 | 1.01 | 1.74 | 1.21 | 1.17 | 1.04 |
| IR64 | IL | $108.2\pm1.0^{abc}$ | $75.2\pm1.0^{**abc}$ | $25.5\pm0.6^{ns,abc}$ | $56.7\pm4.5^a$ | $21.2\pm1.5^c$ | $0.27\pm0.02^{**d}$ | $23.4\pm3.7^a$ | $48.7\pm8.2^c$ | $128.2\pm8.09^{bcd}$ | $0.40\pm0.05^c$ |
| (n=24) | UP | $143.8\pm2.3^{**a}$ | $53.2\pm4.3^{bc}$ | $25.3\pm0.79^{abc}$ | $79.4\pm18.8^{**a}$ | $34.3\pm10.4^{**c}$ | $0.24\pm0.02^d$ | $32.0\pm14.1^{**c}$ | $65.6\pm16.6^{**b}$ | $163.5\pm16.7^{**ab}$ | $0.43\pm0.09^{ns,c}$ |
| | UP/IL | 1.32 | 0.70 | 0.99 | 1.39 | 1.61 | 0.87 | 1.36 | 1.30 | 1.27 | 1.06 |

Asteriscs indicate the siginificant differences between upland and irrigated lowland: *$P < 0.05$, ** $P < 0.01$; ns, not significant.

Values followed by the same letter are not significantly different within cluster groups and all INLs ($P < 0.05$ by Tukey`s HD)

Values are means of 2011 and 2012 data

Table 8. QTL detected by association analysis within sib-groups of introgression lines with the same donor cultivars

| Donor | Trait | Designation of QTL | Marker associated with trait | Chr. | Position* | 2011 IL | | | 2012 IL | | | 2011 UP | | | 2012 UP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $F$ | $R^2$ | Additive[†] | $F$ | $R^2$ | Additive[†] | $F$ | $R^2$ | Additive[†] | $F$ | $R^2$ | Additive[†] |
| YP1 | DH | *qDH5-YP1* [a] | RM3796 | 5 | 4.6 | — | — | — | — | — | — | 16.6 | 0.35 | 3.2 | — | — | — |
| | | *qDH8-YP1* [d] | RM5556 | 8 | 36.0 | — | — | — | 19.4 | 0.44 | 1.4 | — | — | — | — | — | — |
| | CL | *qCL7-YP1* [a,c,d‡] | RM5847 | 7 | 81.9 | — | — | — | 25.1 | 0.45 | 7.7 | 99.0 | 0.76 | 14.5 | 55.4 | 0.64 | 22.1 |
| | PL | *qPL4-YP1* [d] | RM1113 | 4 | 123.8 | — | — | — | 13.7 | 0.29 | -0.6 | — | — | — | — | — | — |
| | | *qPL7-YP1* [b,d‡] | RM505 | 7 | 78.6 | — | — | — | — | — | — | 21.3 | 0.41 | 1.1 | 17.6 | 0.36 | 1.2 |
| | CW | *qCW7-YP1* [b,d‡] | RM505 | 7 | 78.6 | — | — | — | — | — | — | 48.8 | 0.61 | 14.1 | 22.3 | 0.42 | 27.2 |
| | PW | *qPW7-YP1* [a,b,c,d‡] | RM505 | 7 | 78.6 | 17.4 | 0.36 | 3.5 | 24.6 | 0.44 | 5.1 | 91.4 | 0.75 | 15.2 | 54.1 | 0.64 | 29.0 |
| | P/T | *qPW/TW5-YP1* [b,d] | RM3796 | 5 | 4.6 | — | — | — | — | — | — | 16.3 | 0.35 | -0.1 | 15.9 | 0.34 | -0.1 |
| | | *qP/T7-YP1* [a,b,c‡] | RM1132 | 7 | 83.3 | 19.4 | 0.39 | 0.1 | — | — | — | 35.6 | 0.53 | 0.1 | 23.8 | 0.44 | 0.2 |
| | PN | *qPN7-YP1* [b,d‡] | RM505 | 7 | 78.6 | — | — | — | — | — | — | 88.6 | 0.74 | 6.4 | 39.7 | 0.56 | 16.6 |
| | FS/TS | *qFS/TS-YP1* [b,d‡] | RM505 | 7 | 78.6 | — | — | — | — | — | — | 24.1 | 0.43 | 1.0 | 16.9 | 0.35 | 0.1 |
| YP3 | DH | *qDH12-YP3* [c‡] | RM17 | 12 | 107.4 | — | — | — | — | — | — | — | — | — | 14.8 | 0.57 | -3.1 |
| | CL | *qCL12-YP3* [c‡] | RM1226 | 12 | 109.2 | — | — | — | — | — | — | — | — | — | 37.7 | 0.77 | 4.1 |
| | PW | *qPW12-YP3* [c‡] | RM17 | 12 | 107.4 | — | — | — | — | — | — | — | — | — | 19.4 | 0.63 | 7.7 |
| | P/T | *qPW/TW12-YP3* [c‡] | RM1227 | 12 | 109.2 | — | — | — | — | — | — | — | — | — | 15.8 | 0.59 | 0.1 |
| | PN | *qPN12-YP3* [b‡] | RM17 | 12 | 107.4 | 21.3 | 0.65 | -0.7 | — | — | — | — | — | — | — | — | — |
| | FS | *qFS12-YP3* [c,d‡] | RM1227 | 12 | 109.2 | — | — | — | 15.5 | 0.58 | -5.2 | — | — | — | 19.1 | 0.63 | -49.3 |
| | TS | *qTS6-YP3* [a] | RM586 | 6 | 6.0 | — | — | — | — | — | — | 17.8 | 0.61 | 17.6 | — | — | — |
| | FS/TS | *qFS/TS1-YP3* [b] | RM3252 | 1 | 0.3 | 20 | 0.64 | -0.1 | — | — | — | — | — | — | — | — | — |
| | | *qFS/TS12-YP3* [c,d‡] | RM17 | 12 | 107.4 | — | — | — | 15.3 | 0.58 | 0.0 | — | — | — | 36.3 | 0.76 | 0.2 |
| YP4 | DH | *qDH4-YP4* [b,c] | RM252 | 4 | 99.0 | 17 | 0.28 | -0.7 | — | — | — | — | — | — | 16.0 | 0.27 | -1.5 |
| | CL | *qCL4-YP4* [b,c,d‡] | RM8213 | 4 | 10.7 | 15.5 | 0.27 | 3.4 | 14.2 | 0.24 | 3.2 | — | — | — | 59.4 | 0.58 | 9.0 |
| | | *qCL1-YP4* [d] | RM7180 | 1 | 136.9 | — | — | — | 15.3 | 0.23 | -1.4 | — | — | — | — | — | — |
| | PL | *qPL4-YP4* [b,c,d] | RM252 | 4 | 99.0 | 13.3 | 0.24 | -0.6 | 42.6 | 0.49 | -0.8 | — | — | — | 13.9 | 0.24 | -0.8 |
| | | *qPL9-YP4* [c] | RM3808 | 9 | 79.1 | — | — | — | — | — | — | — | — | — | 16.1 | 0.27 | -0.8 |
| | | *qPL12-YP4* [c‡] | RM017 | 12 | 107.4 | — | — | — | — | — | — | — | — | — | 8.3 | 0.16 | 0.7 |
| | CW | *qCW4-YP4* [c‡] | RM8213 | 4 | 10.7 | — | — | — | — | — | — | — | — | — | 13.9 | 0.24 | 19.1 |
| | P/T | *qPW/TW4-YP4* [c] | RM252 | 4 | 99.0 | — | — | — | — | — | — | — | — | — | 15.4 | 0.26 | 0.0 |
| | PN | *qPN4-YP4* [d] | RM303 | 4 | 116.9 | — | — | — | 17.5 | 0.29 | 0.8 | — | — | — | — | — | — |
| | FS | *qFS4-YP4* [c] | RM3836 | 4 | 108.2 | — | — | — | — | — | — | — | — | — | 14.6 | 0.25 | 17.2 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TS | qTS4-YP4[b,c,d] | RM3836 | 4 | 108.2 | 17.8 | 0.29 | -9.7 | 39.7 | 0.48 | -17.2 | — | — | — | 25.6 | 0.37 | -21.5 |
| | | qTS6-YP4[a] | RM314 | 6 | 19.1 | — | — | — | — | — | — | 20.7 | 0.32 | -13.6 | — | — | — |
| | FS/TS | qFS/TS1-YP4[d] | RM7180 | 1 | 136.9 | — | — | — | 16.1 | 0.26 | 0.0 | — | — | — | — | — | — |
| YP5 | DH | qDH8-YP5[c,‡] | RM331 | 8 | 54.3 | — | — | — | — | — | — | — | — | — | 13.0 | 0.26 | 2.6 |
| | PL | qPL4-YP5[a,‡] | RM1867 | 4 | 5.4 | — | — | — | — | — | — | 17.0 | 0.30 | 1.1 | — | — | — |
| | | qPL4-YP5[a] | RM5503 | 4 | 100.7 | — | — | — | — | — | — | 11.7 | 0.25 | -1.1 | — | — | — |
| | PN | qPN1-YP5[a,‡] | RM1032 | 1 | 49.3 | — | — | — | — | — | — | 15.2 | 0.29 | 2.9 | — | — | — |
| | | qPN4-YP5[b,‡] | RM1867 | 4 | 5.4 | 13.7 | 0.26 | -1.3 | — | — | — | — | — | — | — | — | — |
| YP6 | PL | qPL11-YP6[a,‡] | RM5704 | 11 | 28.6 | — | — | — | — | — | — | 15.6 | 0.36 | 1.2 | — | — | — |
| YP7 | DH | qDH7-YP7[d,‡] | RM5455 | 7 | 99.3 | — | — | — | 22.2 | 0.54 | 1.8 | — | — | — | — | — | — |
| | | qDH7-YP7[c,‡] | RM248 | 7 | 99.7 | — | — | — | — | — | — | — | — | — | 15.6 | 0.45 | 3.9 |
| | CL | qCL12-YP7[a] | RM1337 | 12 | 51.5 | — | — | — | — | — | — | 25.1 | 0.57 | 4.2 | — | — | — |
| | | qCL7-YP7[c,‡] | RM248 | 7 | 99.7 | — | — | — | — | — | — | — | — | — | 15.6 | 0.45 | 3.9 |
| | PW | qPW12-YP7[a] | RM1337 | 12 | 51.5 | — | — | — | — | — | — | 20.2 | 0.52 | 7.2 | — | — | — |
| | P/T | qPW/TW7-YP7[c,‡] | RM234 | 7 | 93.9 | — | — | — | — | — | — | — | — | — | 18.7 | 0.5 | 0.0 |
| YP8 | P/T | qPW/TW1-YP8[d,‡] | RM3252 | 1 | 0.3 | — | — | — | 16.3 | 0.38 | 0.0 | — | — | — | — | — | — |
| | | qP/T4-YP8[d,‡] | RM1155 | 4 | 58.9 | — | — | — | 12.7 | 0.36 | 0.0 | — | — | — | — | — | — |
| | PN | qPN4-YP8[a,c] | RM6909 | 4 | 109.9 | — | — | — | — | — | — | 13.8 | 0.34 | 2.0 | 15.2 | 0.37 | 4.8 |
| | FS | qFS2-YP8[a,c] | RM006 | 2 | 125.6 | — | — | — | — | — | — | 16.2 | 0.37 | -24.1 | 17.0 | 0.39 | -35.8 |
| | | qFS4-YP8[a] | RM6909 | 4 | 109.9 | — | — | — | — | — | — | 16.7 | 0.38 | -20.2 | — | — | — |
| | | qFS7-YP8[c,‡] | RM172 | 7 | 118.6 | — | — | — | — | — | — | — | — | — | 13.8 | 0.35 | -43.3 |
| | TS | qTS2-YP8[c,‡] | RM5472 | 2 | 13.1 | — | — | — | — | — | — | — | — | — | 19.6 | 0.42 | -27.5 |
| | | qTS2-YP8[c] | RM0006 | 2 | 125.6 | — | — | — | — | — | — | — | — | — | 21.0 | 0.44 | -31.8 |
| | | qTS4-YP8[c] | RM5503 | 4 | 100.7 | — | — | — | — | — | — | — | — | — | 26.7 | 0.50 | -28.6 |
| | FS/TS | qFS/TS2-YP8[a] | RM240 | 2 | 135.5 | — | — | — | — | — | — | 18.5 | 0.40 | 0.1 | — | — | — |
| YP9 | DH | qDH5-YP9[a,‡] | RM169 | 5 | 48.6 | — | — | — | — | — | — | 13.5 | 0.57 | -3.0 | — | — | — |
| | CL | qCL4-YP9[a] | RM1113 | 4 | 123.8 | — | — | — | — | — | — | 20.5 | 0.57 | -3.9 | — | — | — |
| | FS/TS | qFS/TS2-YP9[a,‡] | RM7451 | 2 | 2.5 | — | — | — | — | — | — | 31.1 | 0.69 | 0.1 | — | — | — |
| | | qFS/TS7-YP9[c,‡] | RM1306 | 7 | 166.6 | — | — | — | — | — | — | — | — | — | 18.1 | 0.56 | -0.1 |
| YP10 | DH | qDH1-YP10[c,d] | RM5638 | 1 | 86.0 | — | — | — | 17.2 | 0.32 | 1.0 | — | — | — | 23.2 | 0.39 | 2.5 |
| | | qDH4-YP10[d,‡] | RM1155 | 4 | 58.9 | — | — | — | 16.7 | 0.29 | 1.0 | — | — | — | — | — | — |
| | | qDH5-YP10[c,d,‡] | RM1024 | 5 | 12.5 | — | — | — | 25.6 | 0.42 | 1.2 | — | — | — | 47.4 | 0.57 | 3.0 |
| | | qDH6-YP10[d] | RM3343 | 6 | 117.0 | — | — | — | 16.9 | 0.32 | -1.4 | — | — | — | — | — | — |
| | CL | qCL1-YP10[a,b,c,d] | RM009 | 1 | 95.7 | 18.1 | 0.33 | 2.9 | 21.6 | 0.37 | 4.1 | 13.5 | 0.27 | 2.7 | 20.8 | 0.36 | 5.2 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CL | qCL5-YP10 [a,b,c,d‡] | RM3334 | 5 | 6.5 | 24.3 | 0.40 | 3.2 | 39.3 | 0.52 | 4.8 | 25.3 | 0.41 | 3.4 | 38.4 | 0.52 | 6.3 |
| | | qCL6-YP10 [b,c,d] | RM3343 | 6 | 117.0 | 18.2 | 0.34 | -3.9 | 4.0 | 0.28 | -4.7 | — | — | — | 19.9 | 0.36 | -6.9 |
| | PL | qPL1-YP10 [d] | RM5638 | 1 | 86.0 | — | — | — | 28.8 | 0.44 | 1.3 | — | — | — | — | — | — |
| | | qPL1-YP10 [a] | RM005 | 1 | 98.5 | — | — | — | — | — | — | 13.8 | 0.27 | 1.2 | — | — | — |
| | | qPL1-YP10 [a‡] | RM7180 | 1 | 136.9 | — | — | — | — | — | — | 17.5 | 0.32 | 1.3 | — | — | — |
| | | qPL2-YP10 [a‡] | RM7451 | 2 | 2.5 | — | — | — | — | — | — | 13.1 | 0.27 | 1.2 | — | — | — |
| | | qPL5-YP10 [d‡] | RM3334 | 5 | 6.5 | — | — | — | 33.2 | 0.47 | 1.4 | — | — | — | — | — | — |
| | | qPL5-YP10 [a‡] | RM405 | 5 | 24.7 | — | — | — | — | — | — | 37.8 | 0.51 | 1.7 | — | — | — |
| | | qPL6-YP10 [d] | RM3827 | 6 | 79.0 | — | — | — | 23.4 | 0.35 | 1.3 | — | — | — | — | — | — |
| | CW | qCW1-YP10 [c] | RM5638 | 1 | 86.0 | — | — | — | — | — | — | — | — | — | 20.1 | 0.35 | 7.7 |
| | | qCW5-YP10 [a,c,d‡] | RM405 | 5 | 24.7 | — | — | — | 31.9 | 0.46 | 3.5 | 13.2 | 0.26 | 4.6 | 20.0 | 0.35 | 7.8 |
| | | qCW6-YP10 [a,d] | RM3343 | 6 | 117.0 | — | — | — | 32.5 | 0.47 | -4.7 | 26.6 | 0.42 | -7.6 | — | — | — |
| | PW | qPW1-YP10 [d] | RM5638 | 1 | 86.0 | — | — | — | 18.1 | 0.33 | 2.8 | — | — | — | — | — | — |
| | | qPW4-YP10 [a,c,d‡] | RM8213 | 4 | 10.7 | — | — | — | 16.9 | 0.33 | 3.5 | 13.9 | 0.28 | 5.2 | 18.9 | 0.35 | 10.2 |
| | | qPW5-YP10 [a,d‡] | RM3334 | 5 | 6.5 | — | — | — | 16.7 | 0.32 | 2.8 | 23.7 | 0.40 | 5.0 | — | — | — |
| | | qPW6-YP10 [a,d] | RM7193 | 6 | 70.9 | — | — | — | 25.2 | 0.41 | 3.2 | 13.0 | 0.27 | 4.1 | — | — | — |
| | PN | qPN1-YP10 [b] | RM009 | 1 | 95.7 | 24.9 | 0.41 | -4.0 | — | — | — | — | — | — | — | — | — |
| | | qPN2-YP10 [b‡] | RM7451 | 2 | 2.5 | 13.6 | 0.27 | -3.3 | — | — | — | — | — | — | — | — | — |
| | | qPN2-YP10 [a,d‡] | RM1347 | 2 | 26.9 | — | — | — | 23.4 | 0.39 | 1.7 | 15.8 | 0.30 | 2.3 | — | — | — |
| | | qPN5-YP10 [b‡] | RM3796 | 5 | 4.6 | <u>35.5</u> | <u>0.56</u> | <u>-4.4</u> | — | — | — | — | — | — | — | — | — |
| | P/T | qPW/TW4-YP10 [c‡] | RM8213 | 4 | 10.7 | — | — | — | — | — | — | — | — | — | 14.9 | 0.29 | 0.0 |
| | FS | qFS6-YP10 [d] | RM3343 | 6 | 117.0 | — | — | — | 16.5 | 0.31 | -12.0 | — | — | — | — | — | — |
| | TS | qTS1-YP10 [a,b,c,d] | RM009 | 1 | 95.7 | 13.9 | 0.28 | 9.3 | 23.1 | 0.39 | 15.0 | 15.5 | 0.30 | 15.8 | 18.9 | 0.34 | 13.0 |
| | | qTS1-YP10 [c] | RM005 | 1 | 98.5 | — | — | — | — | — | — | — | — | — | 15.8 | 0.30 | 12.1 |
| | | qTS2-YP10 [a‡] | RM7451 | 2 | 2.5 | — | — | — | — | — | — | 19.0 | 0.35 | 17.0 | — | — | — |
| | | qTS4-YP10 [c‡] | RM8213 | 4 | 10.7 | — | — | — | — | — | — | — | — | — | 12.9 | 0.26 | 14.1 |
| | | qTS5-YP10 [a,b,c,d‡] | RM3334 | 5 | 6.5 | 14.6 | 0.29 | 9.6 | 24.0 | 0.40 | 15.3 | 38.8 | 0.52 | 20.9 | 25.6 | 0.42 | 14.4 |
| | | qTS5-YP10 [c‡] | RM163 | 5 | 73.9 | — | — | — | — | — | — | — | — | — | 14.8 | 0.27 | 12.0 |
| | | qTS6-YP10 [c,d] | RM3343 | 6 | 117.0 | — | — | — | 18.5 | 0.34 | -18.7 | — | — | — | 17.2 | 0.32 | -16.9 |
| | | qTS6-YP10 [c] | RM3827 | 6 | 79.0 | — | — | — | — | — | — | — | — | — | 16.4 | 0.28 | 12.6 |
| YP11 | DH | qDH4-YP11 [b] | RM1113 | 4 | 123.8 | 15.7 | 0.30 | -1.4 | — | — | — | — | — | — | — | — | — |
| | | qDH9-YP11 [d] | RM5535 | 9 | 74.7 | — | — | — | 34.3 | 0.49 | -1.4 | — | — | — | — | — | — |
| | CL | qCL4-YP11 [b,c,d] | RM1113 | 4 | 123.8 | 15.8 | 0.31 | -2.3 | 35.2 | 0.49 | -2.9 | — | — | — | 18.8 | 0.34 | -3.6 |
| | | qCL5-YP11 [d‡] | RM169 | 5 | 48.6 | — | — | — | <u>0.7</u> | <u>0.02</u> | <u>-0.6</u> | — | — | — | — | — | — |

| Trait | QTL | Marker | Chr | Dist* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CL | *qCL9-YP11* [c,d] | RM3164 | 9 | 72.1 | — | — | — | 19.2 | 0.35 | -2.5 | — | — | — | 18.0 | 0.33 | -3.6 |
| PL | *qPL9-YP11* [a,b] | RM3164 | 9 | 72.1 | 19.8 | 0.35 | -1.1 | — | — | — | 13.7 | 0.28 | -0.9 | — | — | — |
| PW | *qPW4-YP11* [d] | RM6909 | 4 | 106.0 | — | — | — | 25.1 | 0.41 | -3.6 | — | — | — | — | — | — |
| P/T | *qPW/TW4-YP11* [d] | RM6909 | 4 | 106.0 | — | — | — | 25.5 | 0.41 | 0.0 | — | — | — | — | — | — |
| | *qPW/TW11-YP11* [a‡] | RM5349 | 11 | 10.0 | — | — | — | — | — | — | 19.8 | 0.35 | 0.1 | — | — | — |
| | *qPW/TW11-YP11* [a‡] | RM5961 | 11 | 80.0 | — | — | — | — | — | — | 19.8 | 0.35 | 0.1 | — | — | — |
| PN | *qPN4-YP11* [d] | RM303 | 4 | 116.9 | — | — | — | 21.3 | 0.37 | 1.0 | — | — | — | — | — | — |
| | *qPN9-YP11* [b] | RM5535 | 9 | 74.7 | 14.2 | 0.28 | 0.9 | — | — | — | — | — | — | — | — | — |
| FS | *qFS4-YP11* [a] | RM348 | 4 | 111.3 | — | — | — | — | — | — | 14.6 | 0.29 | -17.7 | — | — | — |
| | *qFS9-YP11* [d] | RM242 | 9 | 72.1 | — | — | — | 22.2 | 0.35 | -11.7 | — | — | — | — | — | — |
| TS | *qTS4-YP11* [a,b,c,d] | RM303 | 4 | 116.9 | 45.2 | 0.52 | -22.3 | 59.5 | 0.58 | -26.0 | 38.7 | 0.48 | -28.7 | 33.4 | 0.46 | -30.3 |
| FS/TS | *qFS/TS4-YP11* [d] | RM6909 | 4 | 106.0 | — | — | — | 15.8 | 0.31 | -0.1 | — | — | — | — | — | — |
| | *qFS/TS5-YP11* [d‡] | RM169 | 5 | 48.6 | — | — | — | 16.3 | 0.31 | 0.1 | — | — | — | — | — | — |

Associations between SSR markers and agronomic traits in irrigated lowland (IL) and upland (UP)conditions were detected by single marker analysis (at $P \leq 0.001$)

DH, days to heading; CL, culm length; PL, panicle length; CW, culm weight; PW, panicle weight per plant, PW/TW (total weight), harvest index; PN, panicle number per plant; FS, number of fertile spikelets per panicle; TS, total number of spikelets per panicle, FS/TS, fertility rate

QTLs were designated following standard QTL nomenclature (McCough *et al.,* 1997)

*Distance of the nearest SSR marker from the end of short arm of each chromosome according to a map derived from $F_2$ population derived from a cross between Nippanbere

[†]Positive (negative) value indicates that the increased (decreased) the trait's value.

Superscript letters indicate the culture conditions: a=upland 2011; b=irrigated 2011; c=upland 2012; d=irrigated lowland 2012. ‡ New QTLs found in this study

Table 9. Comparison of agronomic traits between parents in IL and UP in 2013

| Conditions | Parents | Panicle No./plant | Culm length | Panicle length (cm) | Spikelet No./panicl | Fertility rate (%) | Panicle weight (g) | Dry weight (g) | Harvest index (%) | Days to heading |
|---|---|---|---|---|---|---|---|---|---|---|
| Irrigated lowland | IR64 | 22.0 | 70.3 | 25.3 | 130.6 | 48.2 | 25.1 | 78.5 | 31.9 | 107.6 |
| | YTH34 | 15.0 (68) | 70.8 (100) | 25.9 (102) | 109.1 (83) | 85.4 (177) | 32.2 (128) | 66.6 (84) | 48.4 (151) | 108.3 (100) |
| Upland | IR64 | 47.0 | 51.3 | 25.9 | 138.0 | 63.4 | 51.9 | 140.0 | 38.0 | 140.0 |
| | YTH34 | 17.0 (36) | 39.9 (77) | 22.8 (88) | 102.0 (74) | 66.6 (105) | 14.6 (28) | 48.0 (34) | 30.0 (78) | 151.0 (107) |

Average of 12 plants were used
()= Relative value (%) in compared with that of IR64

Table 10. Segregation of panicle No. in $F_3$ family lines derived from a cross between IR64 and YTH 34

| No. of lines | | | | | |
| Low tiller homozygote | Heterozygote | Normal homozygote | Total | $\chi^2$ | $P$ |
| --- | --- | --- | --- | --- | --- |
| 23 | 47 | 16 | 86 | 1.88 | 0.389 |

Plants were grown in an upland field at Tropical Agricultural Research Front, JIRCAS, Ishigaki in Okinawa in 2015.

Table 11. Effects of the QTLs for culm weight, tiller No. detected in the 72 $F_3$ progenies these were self pollinated from a heterozygous $F_2$ plant on chromosome 7

| Trait | Designation of QTL[a] | Marker associated with trait | Chr. | Position (cM)[b] | $F$ | $R^2$ | Additive[c] |
|---|---|---|---|---|---|---|---|
| CW | qCW7 | RM505 | 7 | 78.6 | 7 | 9.6 | 0.27 |
| | | RM5847 | 7 | 81.6 | 7 | 9.6 | 0.3 |
| | | RM5508 | 7 | 81.9 | 6.9 | 9.4 | 0.3 |
| | | RM1132 | 7 | 83.3 | 6.3 | 8.5 | 0.29 |
| TN | qTN7 | RM505 | 7 | 78.6 | 6.5 | 8.6 | 1.25 |
| | | RM5847 | 7 | 81.6 | 6.3 | 8.1 | 1.16 |
| | | RM5508 | 7 | 81.9 | 6.2 | 8.3 | 1.2 |
| | | RM1132 | 7 | 83.3 | 5.5 | 7.5 | 1.07 |

Single-marker association analysis ($P \leq 0.05$) of SSR markers with traits in 72 $F_3$ plants derived by self-pollination of an $F_2$ plant heterozygous for a region on Chr. 7.

[a] QTLs were designated following standard QTL nomenclature.

[b] Position according to Fujita *et al.* (2009).

[c] Positive values indicate that IR64 allele increases trait value.

Table 12. Classification of rice varieties based on the response to nitrogen at seedling stage

| Cluster group | Indica type | | Japonica type | |
|:---:|:---:|:---:|:---:|:---:|
| | Landrace | Improved | Landrace | Improved |
| I | Kasalath | - | - | - |
| IIa | Surjamukhi | IR64<br><br>YTH183 (Introgression lines)<br><br>Milyang 23 | - | Nipponbare<br><br>Akihikari |
| IIb | Dular | Takanari | Azusena<br><br>Owarihatamochi | Koshihikari<br><br>Dontokoi (Harboring *sd-1* and Koshihikari genetuic background) |

Cluster analysis was carried out using Ward's hierarchical clustering method with the computer program JMP 6.0 (SAS Institute. Inc., Cary, NC, USA)

Table 13. Variation of RDW (%) among 13 varieties in various nitrogen forms at different seedling ages

| Seedling age (Days after seeding) | Mean and range of relative dry weight (%) | | |
|---|---|---|---|
| | Nitrogen forms | | |
| | $NO_3^-$ | $NH_4^+$ | $NH_4NO_3$ |
| 0 | $463.4\pm155.1^a$ | $515.1\pm105.9^a$ | $522.0\pm130.9^a$ |
| | (236.7 ~938.1) | (250.0 ~691.0) | (305.9 ~891.2) |
| 7 | $456.7\pm87.0^a$ | $516.8\pm99.1^a$ | $489.3\pm88.9^a$ |
| | (323.7 ~629.1) | (386.5 ~710.4) | (361.6 ~691.6) |
| 14 | $361.2\pm71.3^a$ | $431.1\pm100.9^a$ | $426.0\pm88.5^a$ |
| | (276.5 ~554.8) | (306.2 ~683.8) | (323.6 ~674.3) |
| 21 | $326.2\pm72.7^b$ | $453.8\pm70.8^a$ | $364.4\pm61.8^b$ |
| | (228.4 ~533.6) | (331.4 ~600.0) | (273.3 ~498.5) |

The treatments were started after the seeding days.
The letters a, and b indicates the significant differences at $P$ <0.05 based on

Table 14. Variation of RDW (%) under different nitrogen sources and seedling ages in each cluster group of 13 varieties

| Treatment | | Relative dry weight (%) | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Nitrogen source | $NO_3^-$ | | | | $NH_4^+$ | | | | $NH_4NO_3$ | | | |
| | Stage (Days after seeding) | 0 | 7 | 14 | 21 | 0 | 7 | 14 | 21 | 0 | 7 | 14 | 21 |
| Cluster groups | I [1] | 938.1 (100.0)[a] | 629.1 (67.1)[a] | 554.8 (59.1)[a] | 533.7 (56.9)[a] | 691.1 (100.0)[a] | 710.4 (102.7)[a] | 683.8 (98.9)[a] | 600.1 (86.8)[a] | 891.2 (100.0)[a] | 691.6 (77.6)[a] | 674.4 (75.6)[a] | 498.5 (55.9)[a] |
| | IIa [6] | 414.8 (100.0)[b] | 393.5 (94.9)[c] | 319.6 (77.0)[c] | 268.6 (64.8)[c] | 490.1 (100.0)[b] | 453.6 (92.5)[c] | 379.9 (77.5)[c] | 411.7 (84.0)[c] | 493.8 (100.0)[b] | 415.4 (84.1)[c] | 393.3 (79.6)[b] | 315.5 (63.8)[c] |
| | IIb [6] | 454.1 (100.0)[b] | 499.4 (110.0)[b] | 380.1 (83.7)[b] | 349.3 (76.9)[b] | 516.3 (100.0)[b] | 571.5 (110.6)[b] | 469.2 (90.8)[b] | 490.1 (94.9)[b] | 508.4 (100.0)[b] | 543.3 (106.8)[b] | 430.6 (84.6)[b] | 392.9 (77.2)[b] |

The letters a, b, and c indicates the significant differences at $P$ <0.05 based on Tukey's HSD among cluster groups. Reduction or increment rate was calculated by comparing RDW (%) in 0days.

Table 15. Classification of 25 INLs used in the nitrogen response experiments based on RDW (%) grown in hydroponics

| Cluster groups [1] | Total | Donor parent | Cluster groups [2] | | | |
|---|---|---|---|---|---|---|
| | | | I | IIa | IIb | III |
| AI | 4 | YP1 YP3 YP4 | Kasalath | – | YTH20 YTH35 YTH67 | YTH83 |
| AII | 6 | YP11 YP5 | – | YTH187 YTH174 | YTH157 YTH160 | YTH172 YTH183 |
| BII | 3 | YP5 | – | – | YTH190 YTH208 | YTH200 |
| BIII | 4 | YP7 YP8 | – | – | YTH284 | YTH271 YTH288 YTH290 IR64 |
| CIII | 4 | YP8 YP9 YP10 | – | – | YTH335 YTH339 | YTH310 YTH326 |
| CIV | 4 | YP10 | – | – | YTH362 YTH369 | YTH358 YTH367 |
| Total | 25 | | | 2 | 12 | 12 |

[1]Cluster groups classified based on agronomic traits in field in 2011 and 2012

[2]Cluster groups classified based on nitrogen uptake in hydoponics

Table 16. Variation of RDW (%) under different nitrogen forms and seeding stage in each cluster group of INLs with IR64 genetic background

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Relative dry weight (%)** | | | | | | | | |
| Treatment | Nitrogen source | $NO_3^-$ | | | $NH_4^+$ | | | $NH_4NO_3$ | | |
| | Stage (Days after seeding) | 0 | 7 | 21 | 0 | 7 | 21 | 0 | 7 | 21 |
| Cluster group (No. of lines) | I (1) | 698.40 (100.00) | 853.800 (122.25) | 378.01 (54.12) | 647.62 (100.00) | 619.25 (95.61) | 270.61 (41.78) | 693.25 (100.00) | 724.42 (104.39) | 296.49 (42.72) |
| | IIa (2) | 275.26 (100.00) | 382.13 (138.82) | 278.58 (101.18) | 267.45 (100.00) | 333.88 (124.08) | 293.55 (109.75) | 276.39 (100.00) | 326.28 (118.05) | 272.66 (98.64) |
| | IIb (12) | 329.91 (100.00) | 407.47 (123.51) | 233.79 (70.86) | 346.61 (100.00) | 434.37 (125.31) | 264.81 (76.40) | 341.30 (100.00) | 423.45 (124.04) | 243.58 (71.37) |
| | III (12) | 372.42 (100.00) | 463.12 (124.35) | 270.01 (72.50) | 403.29 (100.00) | 476.29 (118.10) | 294.80 (73.09) | 390.80 (100.00) | 455.36 (116.51) | 266.66 (68.23) |
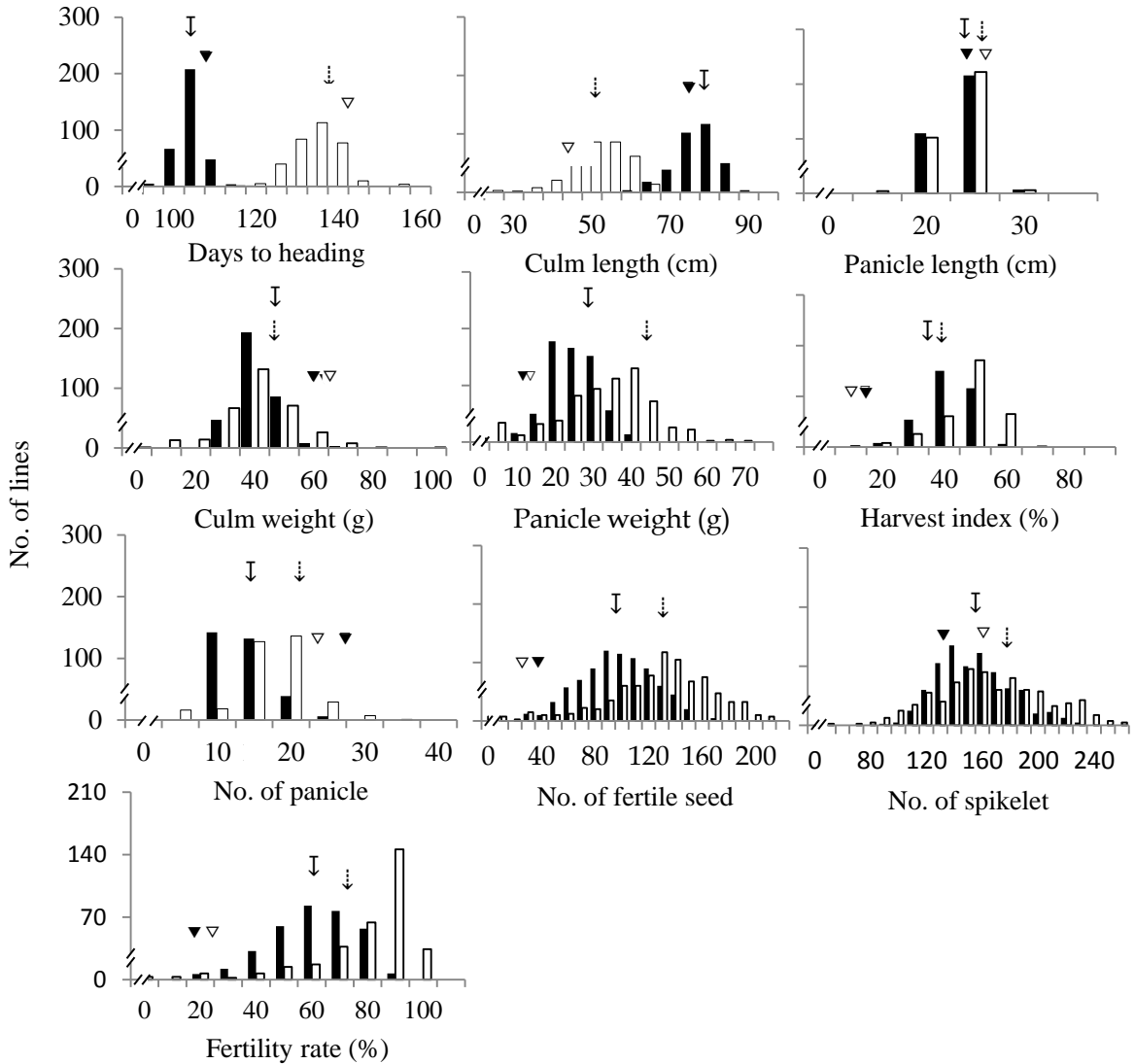
Fig. 1. Variation for agronomic traits of INLs with IR64 genetic background cultivated in 2011

A total of 333 INLs were cultivated at IL and UP, Tsukuba, Japan.
A total of 10 agronomic traits were investigated with 10 plants in each lines, and the average was calculated and used as the representative value of each line.
These distribution of IL and UP conditions are indicated by black and white histograms, respectively.

▼: Average value of IR64 in IL.
▽: Average of IR64 in UP
⬇: Average of INLs in IL.
⬇: Average of INLs in UP
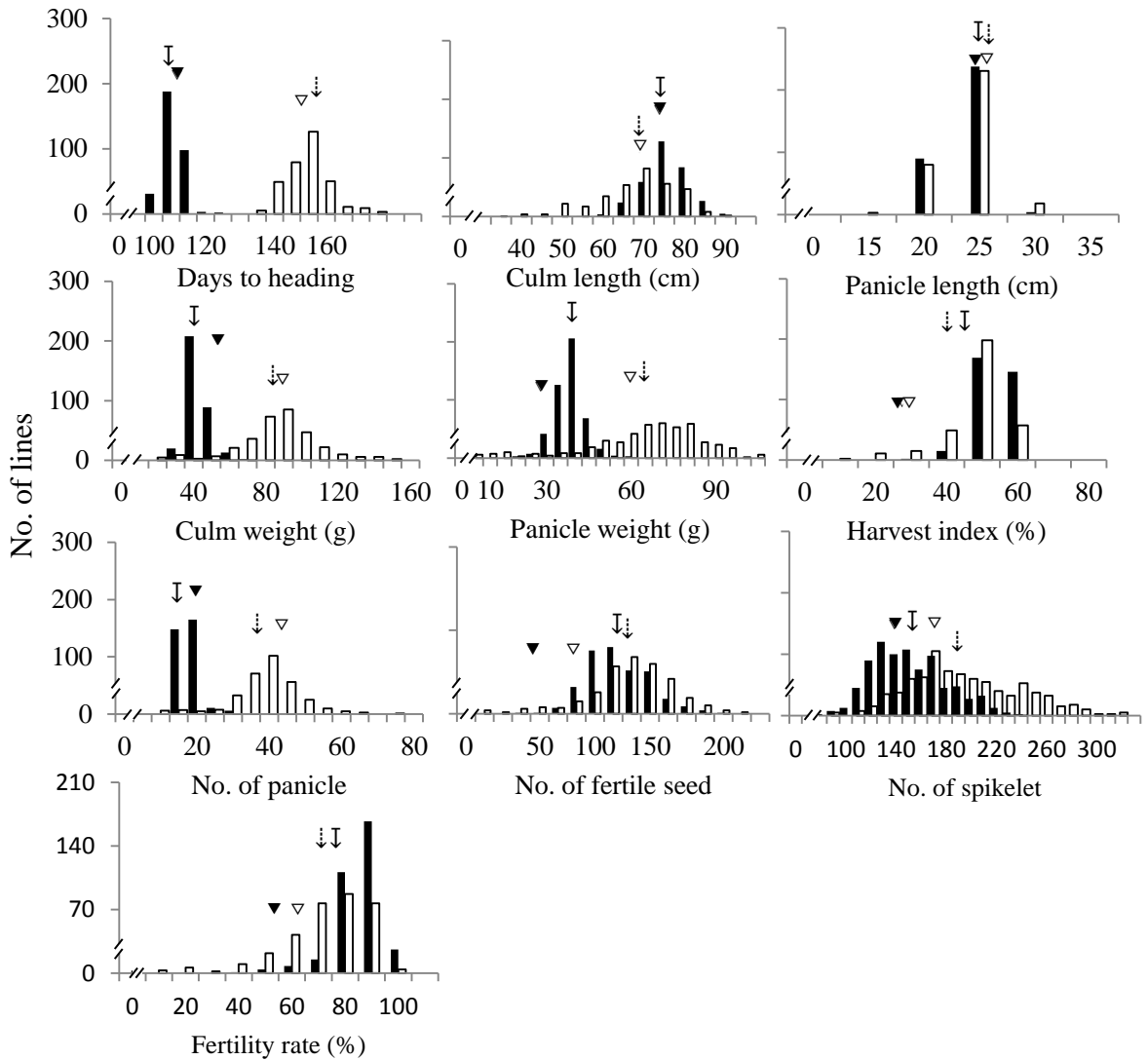
Fig. 2. Variation for agronomic traits of INLs with IR64 genetic background cultivated in 2012

A total of 333 INLs were cultivated at IL and UP, Tsukuba, Japan.
A total of 10 agronomic traits were investigated with 10 plants in each lines, and the average was calculated and used as the representative value of each line.
These distribution of IL and UP conditions are indicated by black and white histograms, respectively.

▼: Average value of IR64 in IL.
▽: Average of IR64 in UP
↧: Average of INLs in IL.
↓: Average of INLs in UP
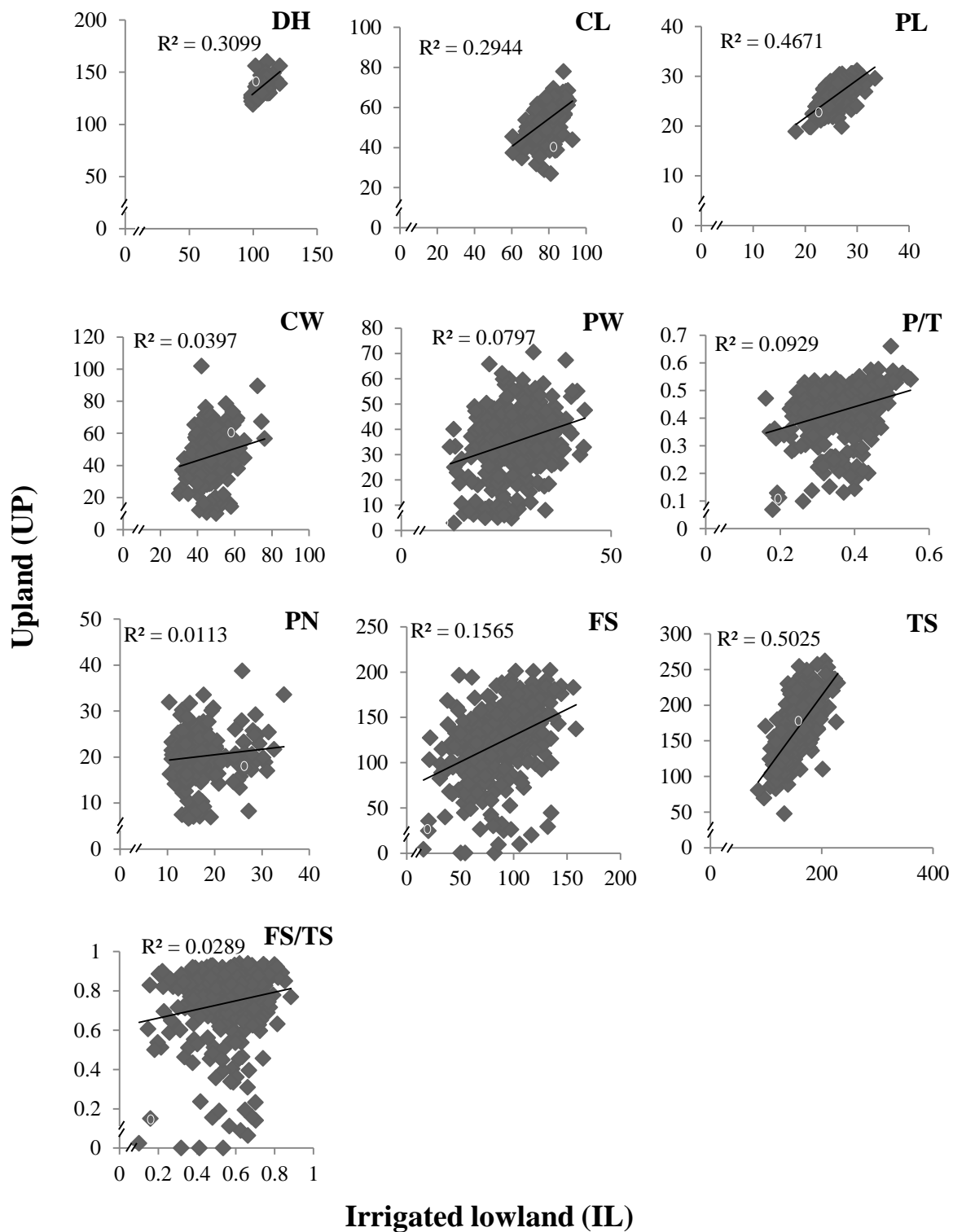
Fig. 3. Comparison of agronomic traits between IL, and UP condition grown in 2011.

A total of 333 INLs were cultivated at IL and UP, Tsukuba, Japan.
A total of 10 agronomic traits were investigated with 12 plants in each lines, and the average was calculated and used as the representative value of each line. White circle indicate the position of IR 64 for respective traits
DH; Days to heading, CL; Culm length, PL; Panicle length; CW; Culm weight, PW; Panicle weight, P/T; Harvest index, PN; Panicle No. FS; No. of Fertile seeds, TS; No. of Total spikelet's, and FS/TS; Fertility rate
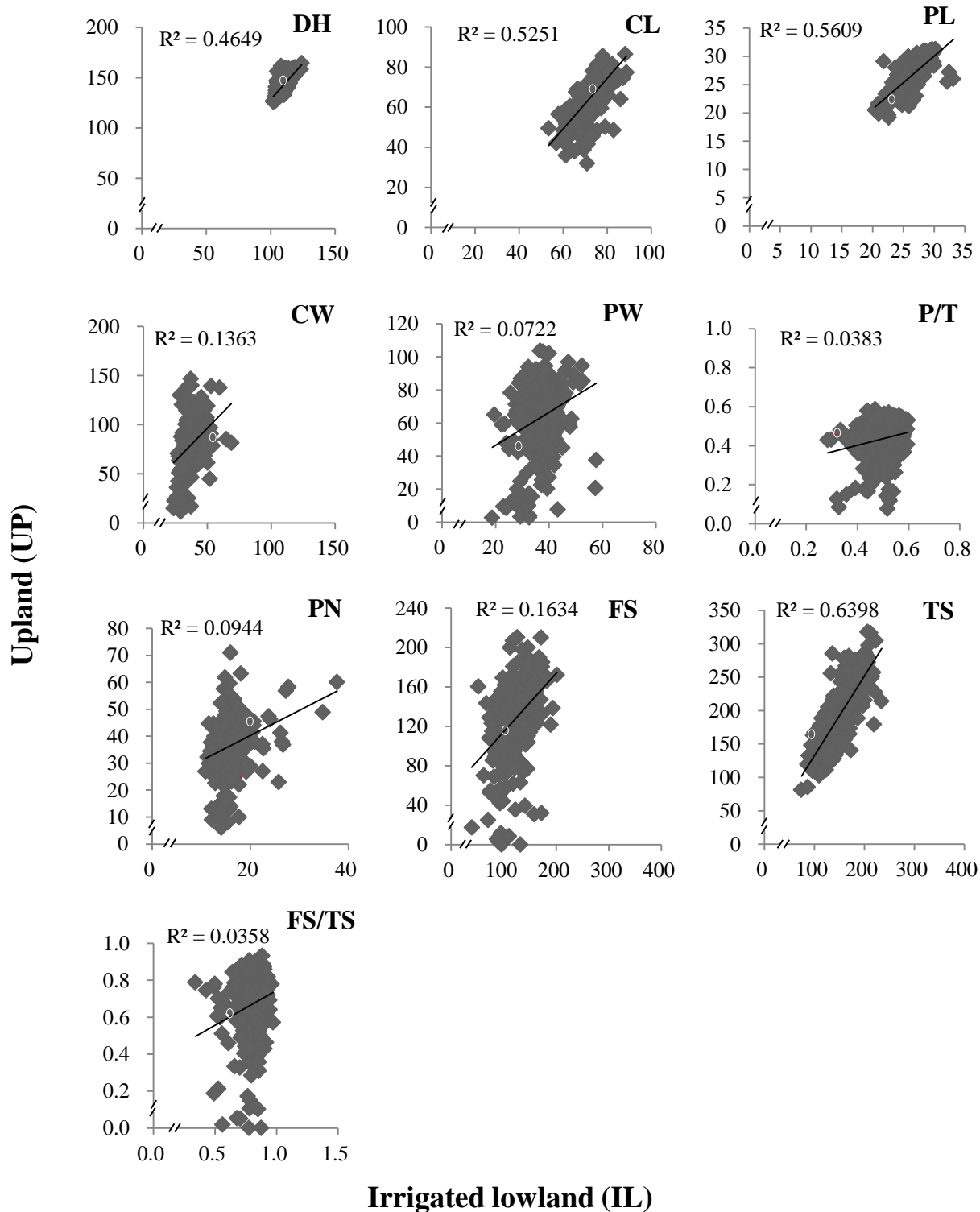
Fig. 4. Comparison of agronomic traits between IL, and UP condition grown in 2012.

A total of 333 INLs were cultivated at IL and UP, Tsukuba, Japan.
A total of 10 agronomic traits were investigated with 12 plants in each lines, and the average was calculated and used as the representative value of each line. White circle indicate the position of IR 64 for respective traits
DH; Days to heading, CL; Culm length, PL; Panicle length; CW; Culm weight, PW; Panicle weight, P/T; Harvest index, PN; Panicle No. FS; No. of Fertile seeds, TS; No. of Total spikelet's, and FS/TS; Fertility rate
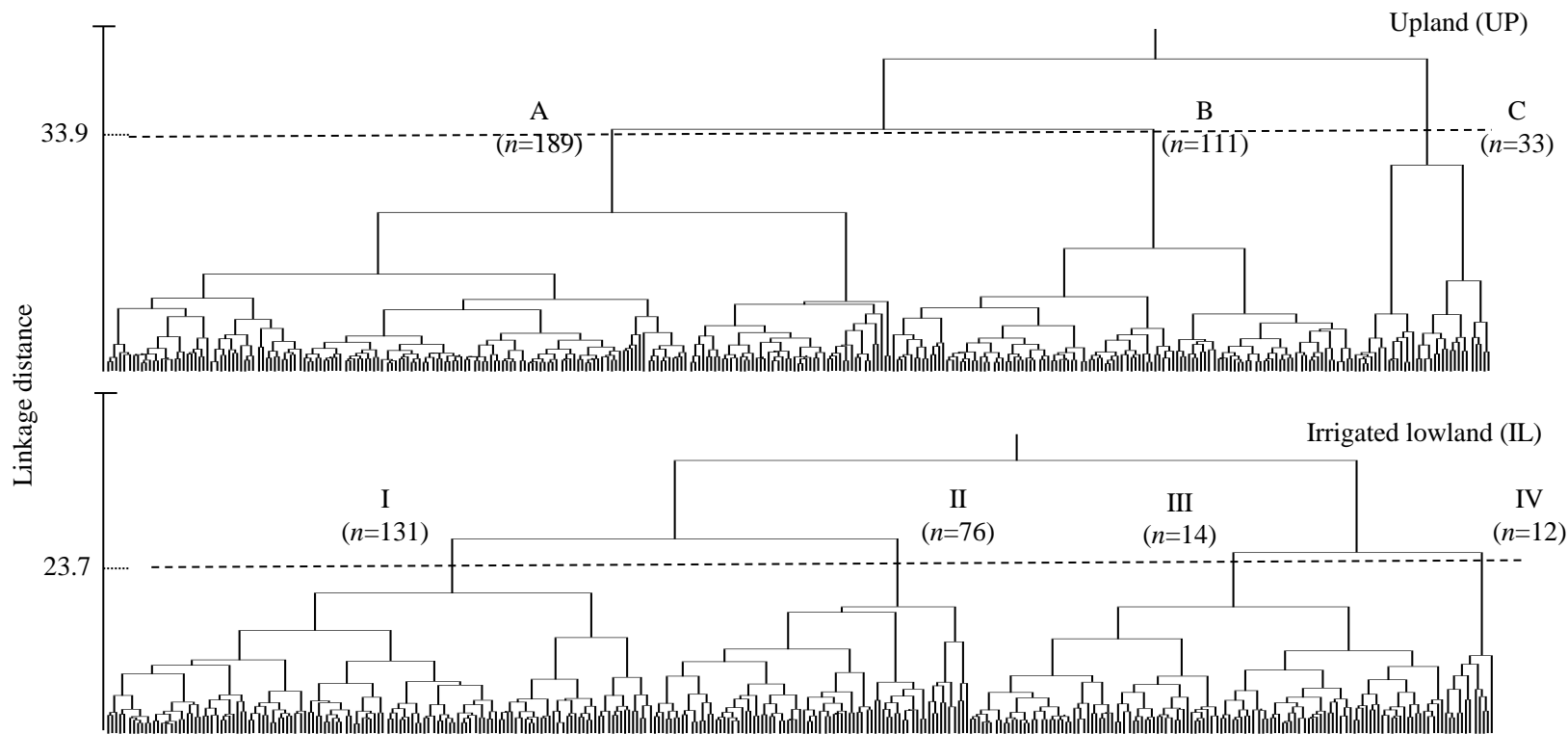
Fig. 5. Classification of 333 INLs with IR64 genetic background

The INLs were cultivated at UP and IL of Hachimandai fields of JIRCAS, Tsukuba, Ibaraki, Japan, in 2011 and 2012.

Cluster analyses were conducted out using 10 agronomic traits' data from upland and lowland in 2011 and 2012, with Ward`s hierarchical analysis (Ward 1963) using JMP7.0 (SAS institute Inc., Cary, NC, USA).

IR64 is classified into cluster groups, B in UP and III in IL.
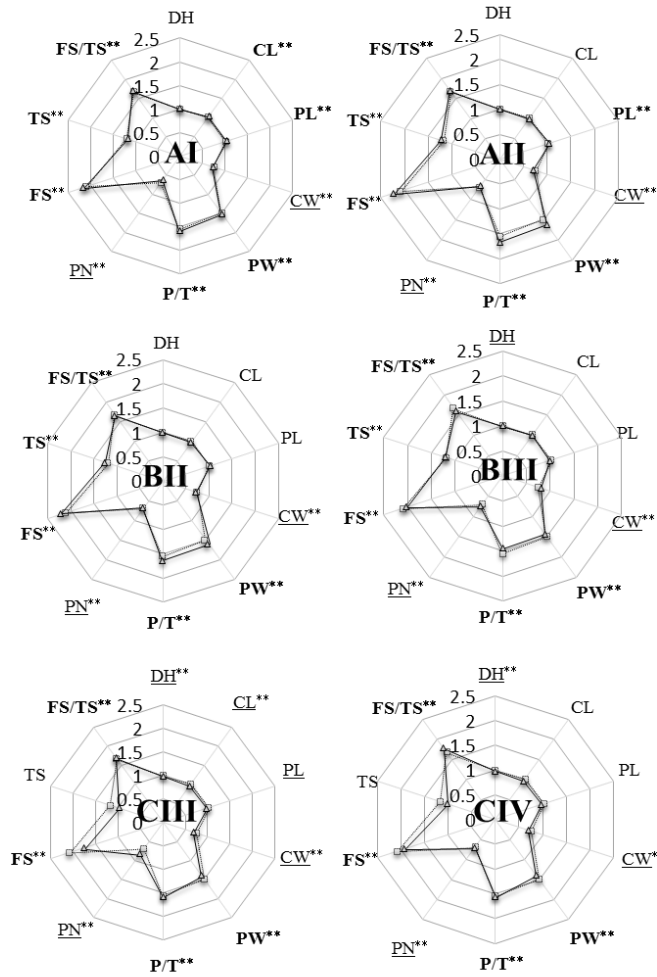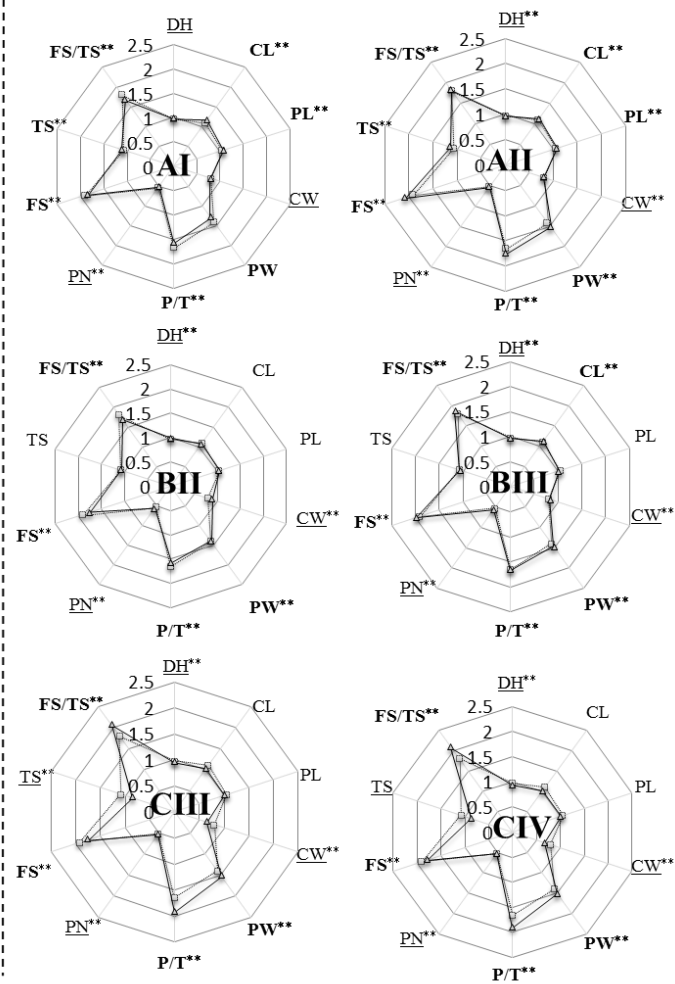
Fig. 6. Genetic variation of agronomic traits in cluster groups

A total of 333 INLs were classified into six cluster groups, A-I, A-II, B-II, B-III, C-III, and C-IV, based on data from UP and IL.

The average of agronomic trait of IR64 was indicated as relative value,1.0.

The whole mean of all INLs including IR64 and each group were shown as dotted and solid lines in each group.

Bold character indicates the increasing of value of agronomic trait, and underline is decreasing. *T*-test results are shown by double (1%) and single (5%) asterisks.

HD: Days to heading, CL: Culm length, PL: Panicle length, CW: Culm weight, PW: Panicle weight, P/T: Ratio of panicle weight per whole weight (harvest index), PN: No. of panicle per plant, FS: No. of fertility seeds per panicle, TS: Total spikelet per panicle, FS/TS: Ratio of fertility seeds per total spikelet.
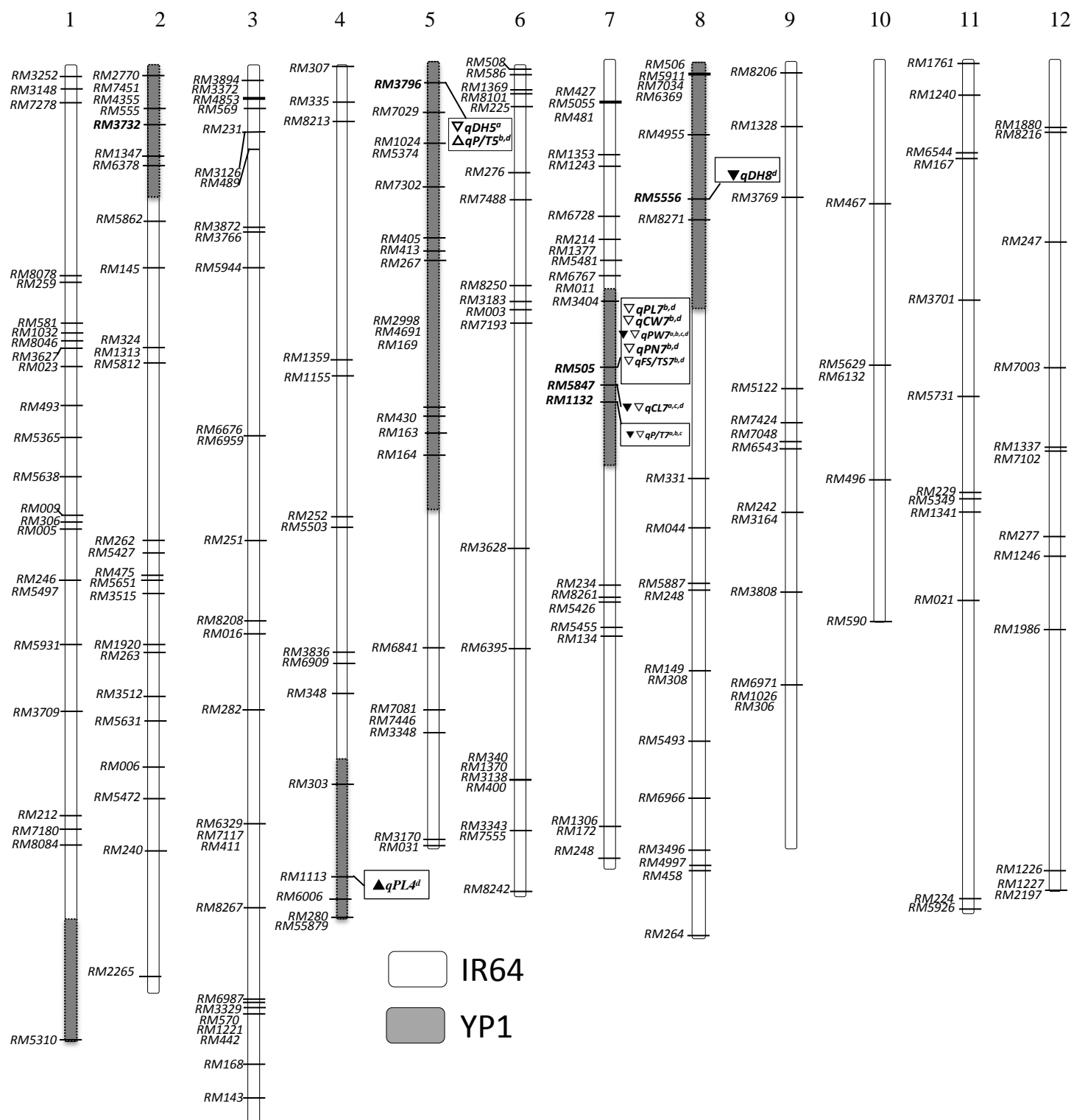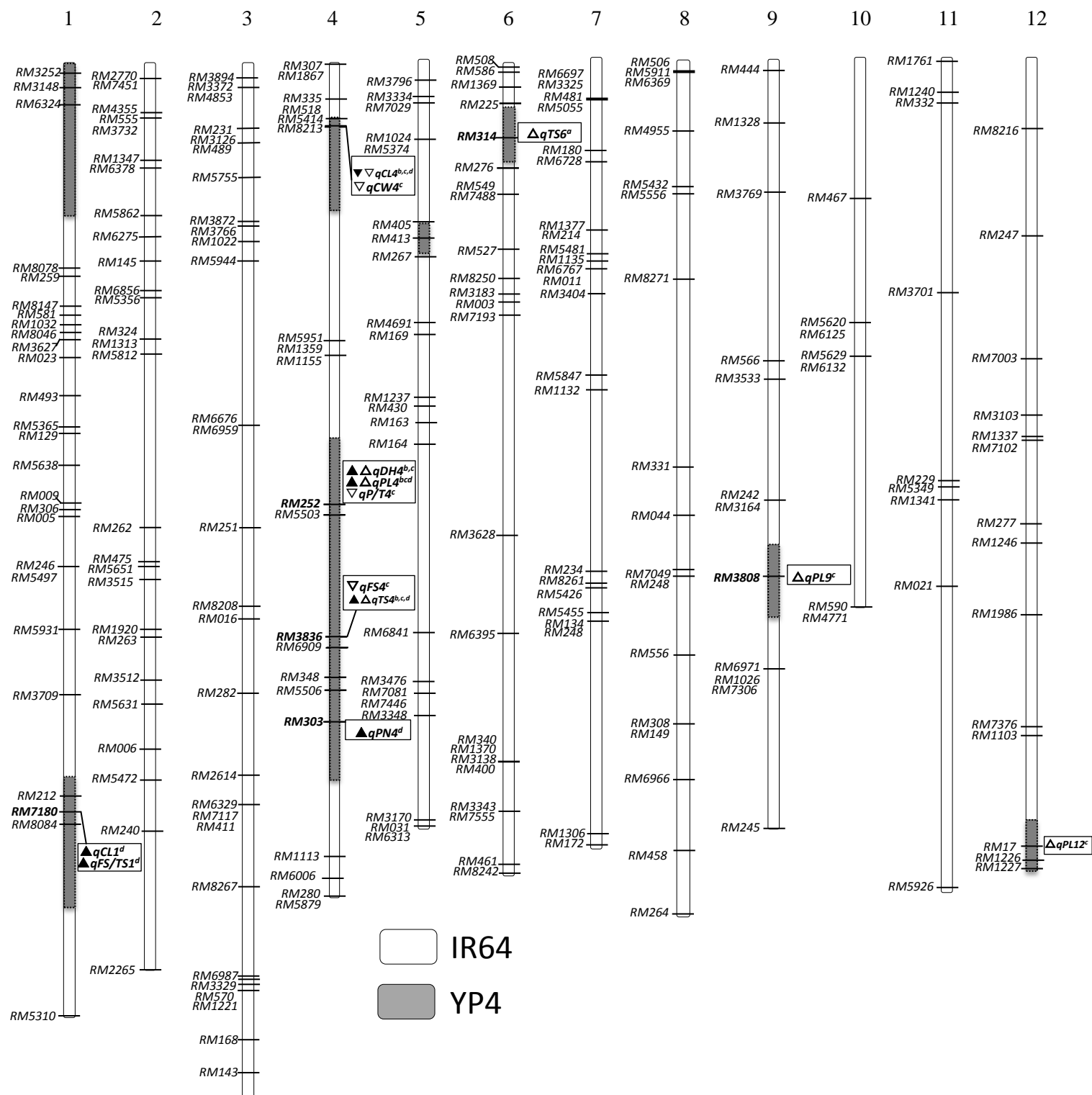
Fig. 7. Chromosomal location of introgressed segments and QTLs detected in sib-group YP1

A total of 35 introgression lines were derived from the donor parent YP1, IR 65600-87-2-2-3 (Fujita *et al.* 2009).

Chromosome No. indicate on the top of each vertical bar.

QTL detected by association analysis for agronomic traits is indicated in the right side of chromosome and SSR markers in the left.

Gray region indicates the chromosome segments introgressed from donor variety.

White region on chromosome indicates the genetic background of IR64.

Black and white triangles indicated the QTLs detected in Irrigated lowland and Upland, respectively, and

Upper and down triangles indicate the increase effect with donor parent and IR64 allele, respectively.

These superscript words on the designation of QTL indicates the conditions and years, a: Upland in 2011, b: Irrigated lowland in 2011, c: Upland in 2012, and d: irrigated lowland in 2012.
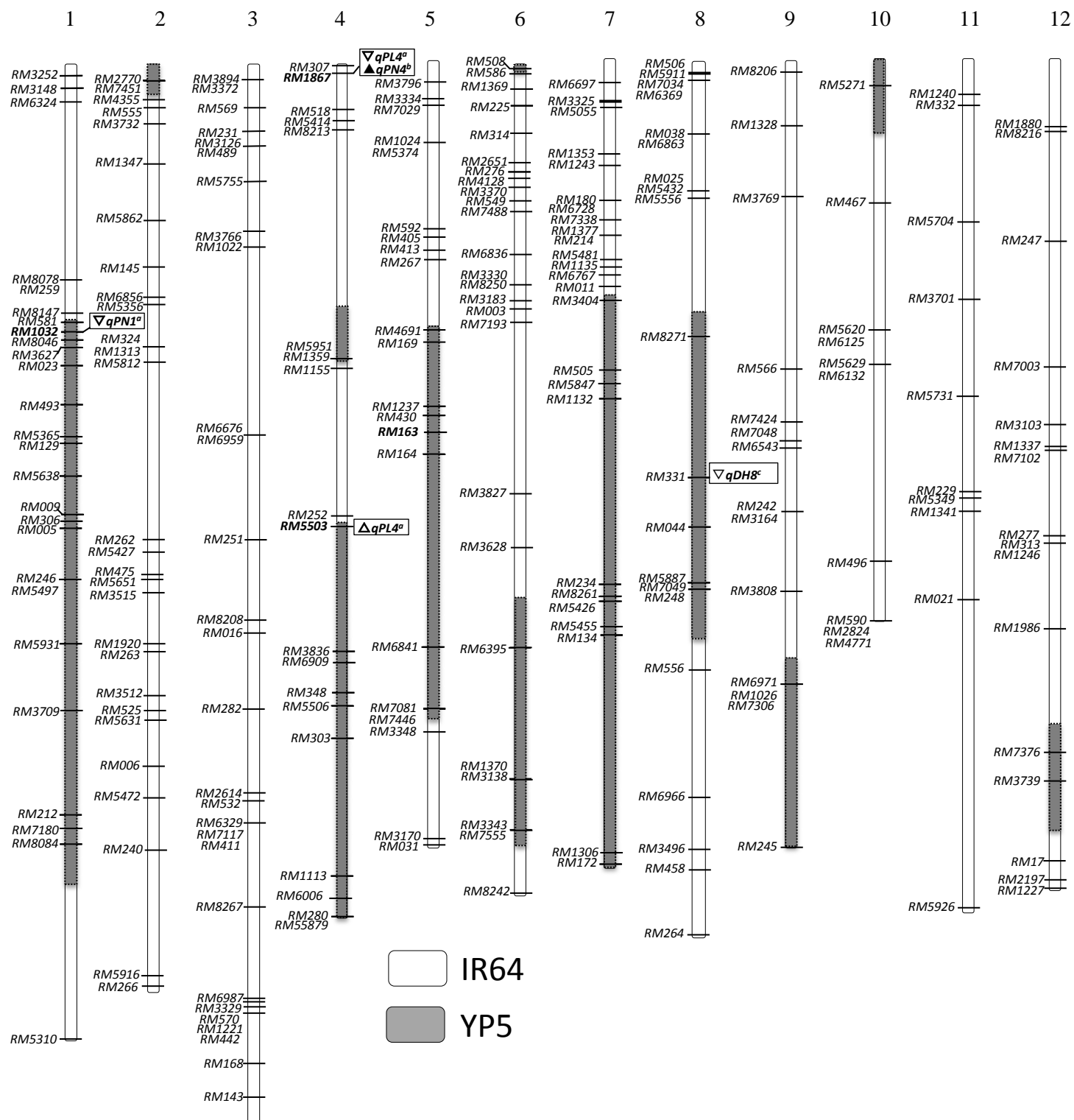
Fig. 8. Chromosomal location of introgressed segments and QTLs detected in sib-group YP3

A total of 35 introgression lines were derived from the donor parent YP3, IR 65598-112-2 (Fujita *et al.* 2009).

Chromosome No. indicate on the top of each vertical bar.

QTL detected by association analysis for agronomic traits is indicated in the right side of chromosome and SSR markers in the left.

Gray region indicates the chromosome segments introgressed from donor variety.

White region on chromosome indicates the genetic background of IR64.

Black and white triangles indicated the QTLs detected in Irrigated lowland and Upland, respectively, and

Upper and down triangles indicate the increase effect with donor parent and IR64 allele, respectively.

These superscript words on the designation of QTL indicates the conditions and years, a: Upland in 2011, b: Irrigated lowland in 2011, c: Upland in 2012, and d: irrigated lowland in 2012.
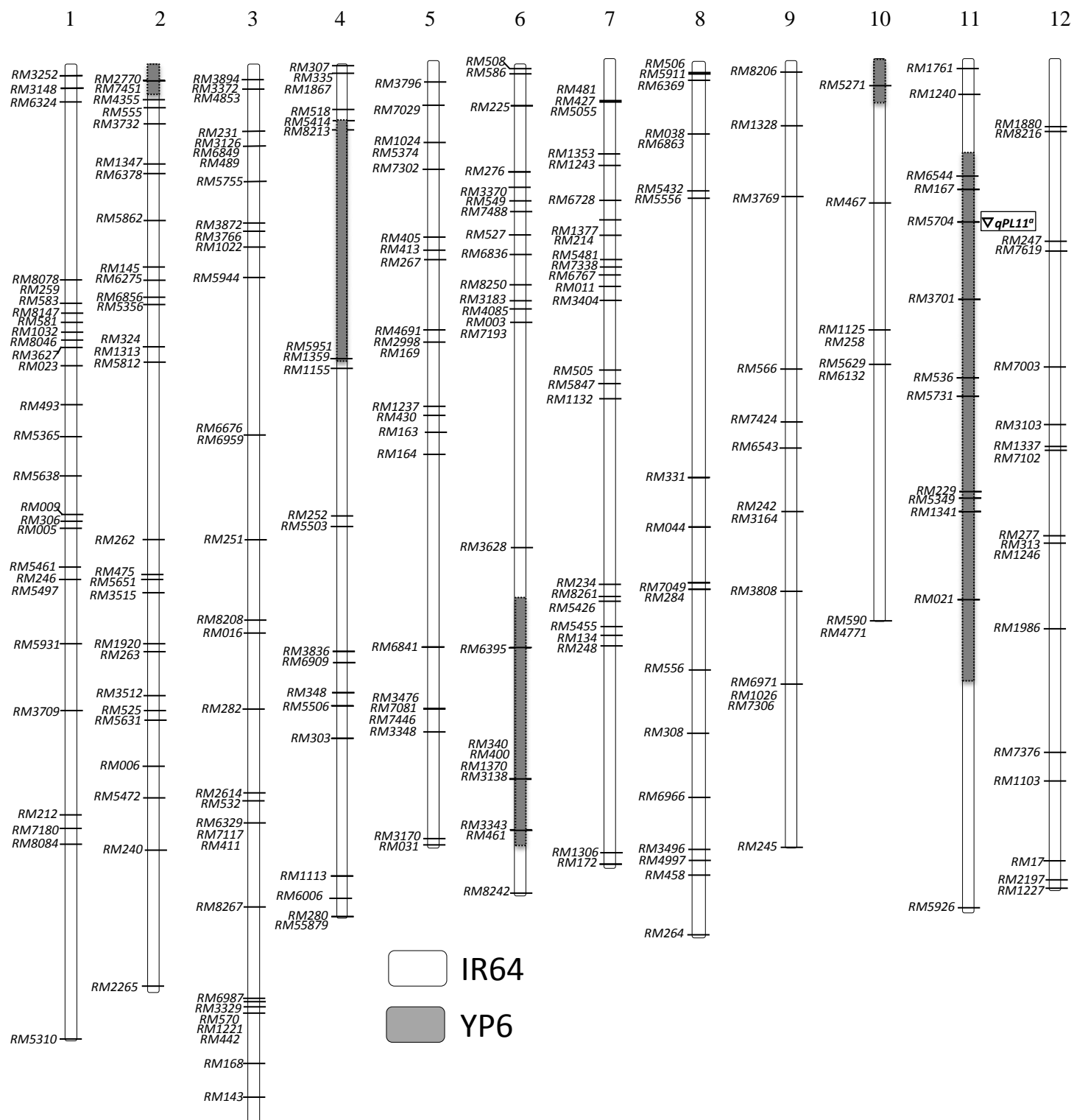
Fig. 9. Chromosomal location of introgressed segments and QTLs detected in sib-group YP4

A total of 35 introgression lines were derived from the donor parent YP4, IR 65564-2-2-3 (Fujita *et al.* 2009).

Chromosome No. indicate on the top of each vertical bar.

QTL detected by association analysis for agronomic traits is indicated in the right side of chromosome and SSR markers in the left.

Gray region indicates the chromosome segments introgressed from donor variety.

White region on chromosome indicates the genetic background of IR64.

Black and white triangles indicated the QTLs detected in Irrigated lowland and Upland, respectively, and

Upper and down triangles indicate the increase effect with donor parent and IR64 allele, respectively.

These superscript words on the designation of QTL indicates the conditions and years, a: Upland in 2011, b: Irrigated lowland in 2011, c: Upland in 2012, and d: irrigated lowland in 2012.
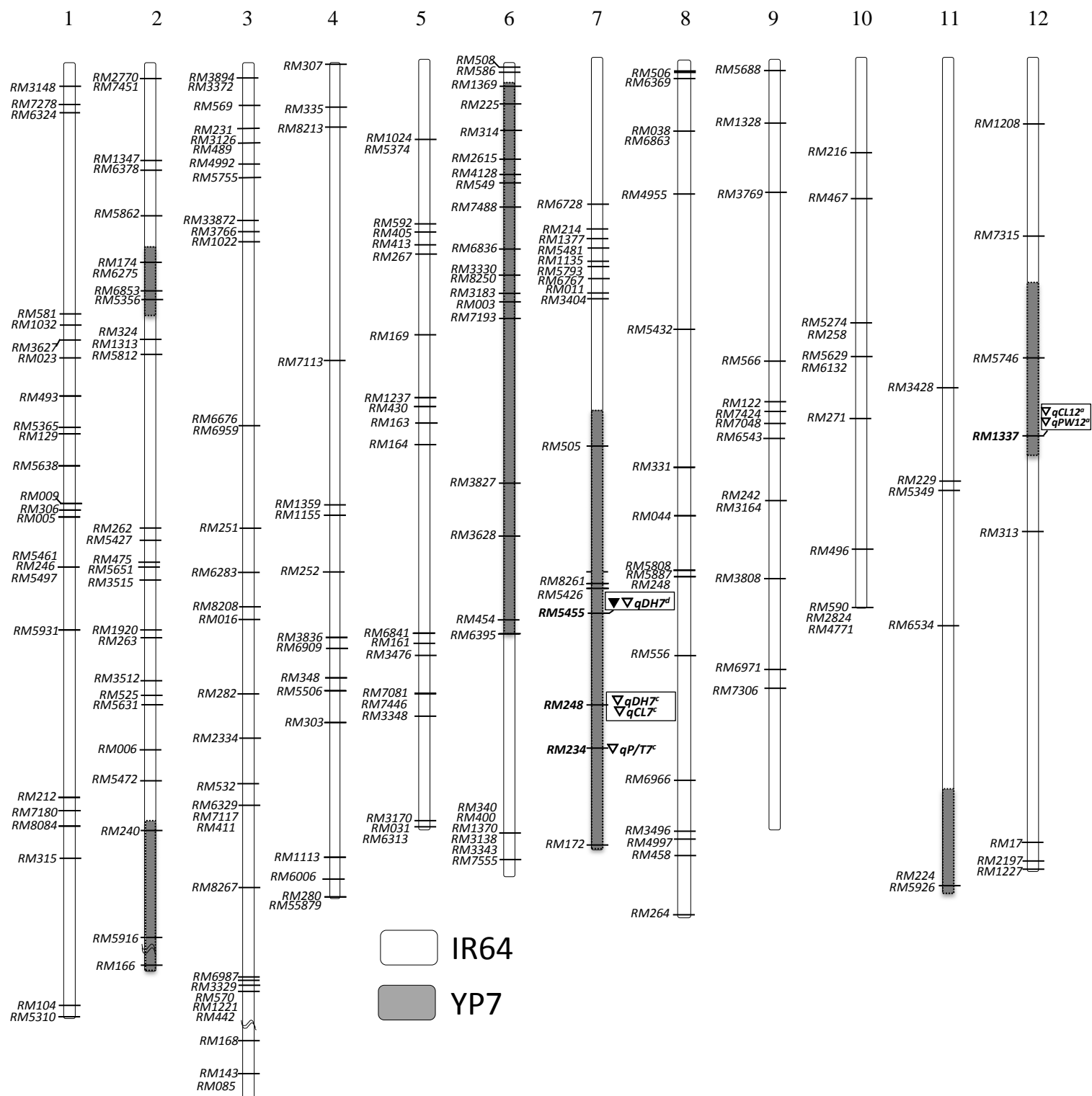
114

Fig. 10. Chromosomal location of introgressed segments and QTLs detected in sib-group YP5

A total of 35 introgression lines were derived from the donor parent YP5, IR 69093-41-2-3-2 (Fujita *et al.* 2009).

Chromosome No. indicate on the top of each vertical bar.

QTL detected by association analysis for agronomic traits is indicated in the right side of chromosome and SSR markers in the left.

Gray region indicates the chromosome segments introgressed from donor variety.

White region on chromosome indicates the genetic background of IR64.

Black and white triangles indicated the QTLs detected in Irrigated lowland and Upland, respectively, and

Upper and down triangles indicate the increase effect with donor parent and IR64 allele, respectively.

These superscript words on the designation of QTL indicates the conditions and years, a: Upland in 2011, b: Irrigated lowland in 2011, c: Upland in 2012, and d: irrigated lowland in 2012.
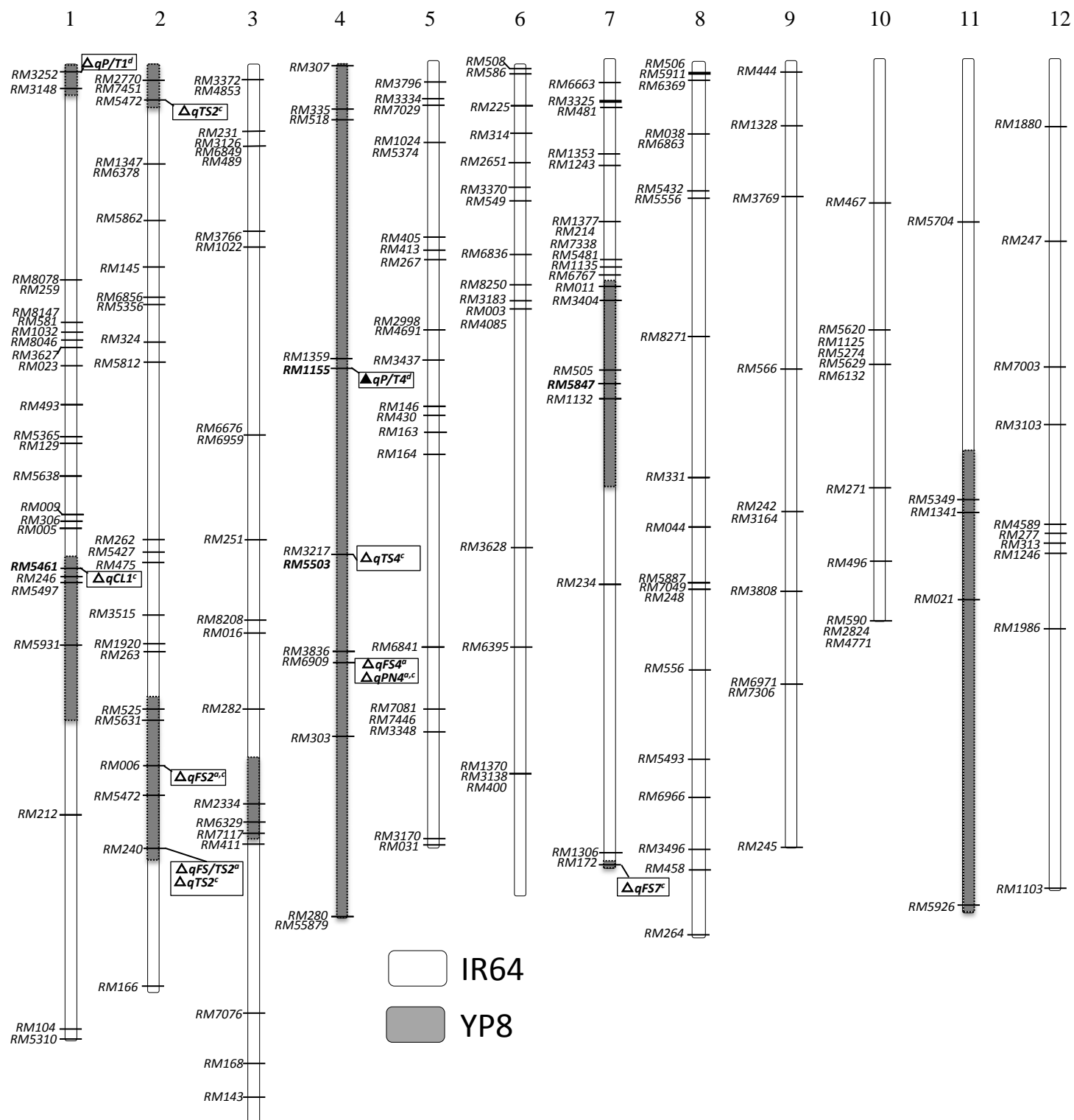
Fig.11. Chromosomal location of introgressed segments and QTLs detected in sib-group YP6

A total of 35 introgression lines were derived from the donor parent YP6, IR 69125-25-3-1-1 (Fujita *et al.* 2009).

Chromosome No. indicate on the top of each vertical bar.

QTL detected by association analysis for agronomic traits is indicated in the right side of chromosome and SSR markers in the left.

Gray region indicates the chromosome segments introgressed from donor variety.

White region on chromosome indicates the genetic background of IR64.

Black and white triangles indicated the QTLs detected in Irrigated lowland and Upland, respectively, and

Upper and down triangles indicate the increase effect with donor parent and IR64 allele, respectively.

These superscript words on the designation of QTL indicates the conditions and years, a: Upland in 2011, b: Irrigated lowland in 2011, c: Upland in 2012, and d: irrigated lowland in 2012.
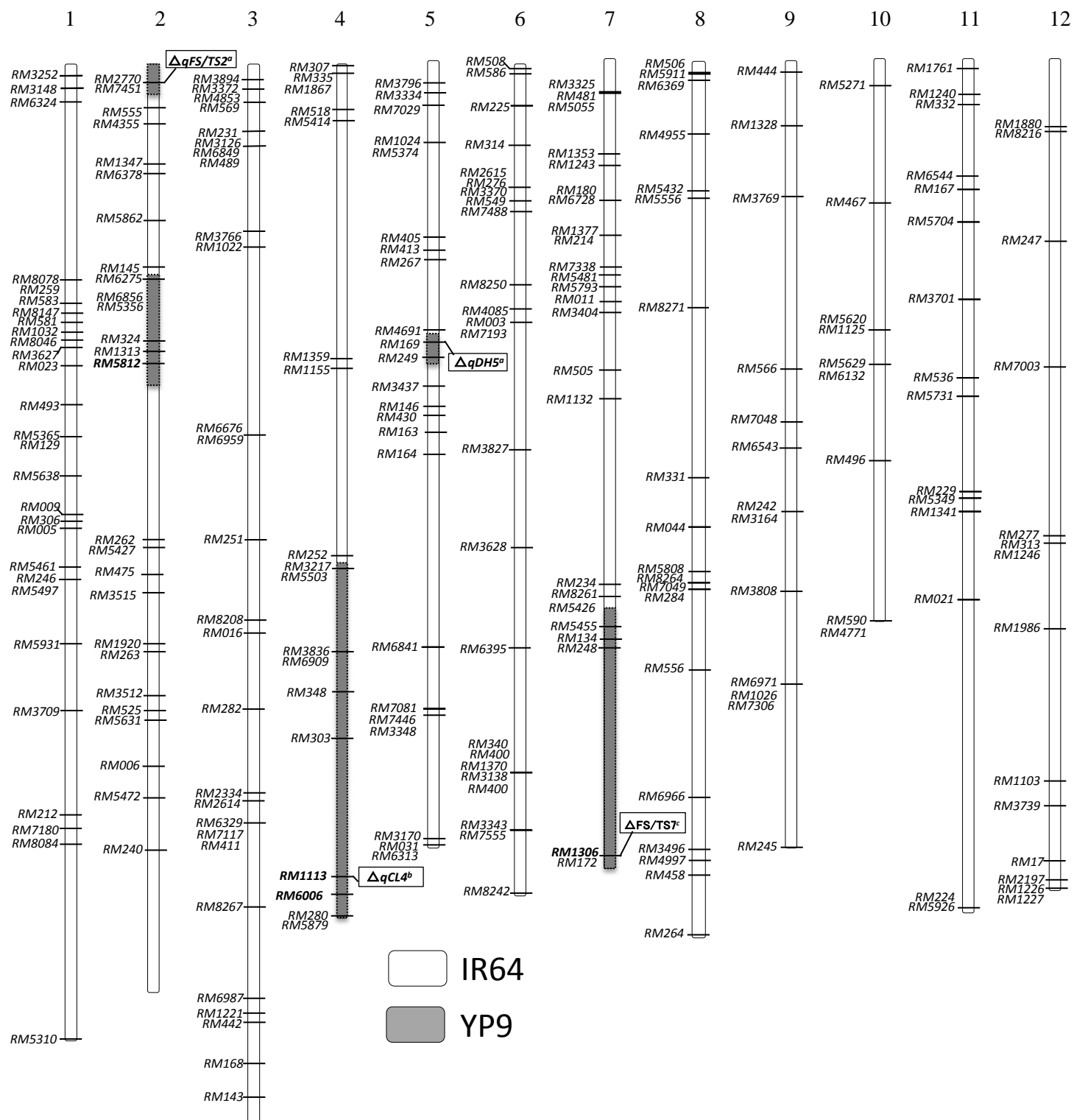
Fig. 12. Chromosomal location of introgressed segments and QTLs detected in sib-group YP7

A total of 35 introgression lines were derived from the donor parent YP7, Hoshiaoba (Fujita *et al.* 2009).

Chromosome No. indicate on the top of each vertical bar.

QTL detected by association analysis for agronomic traits is indicated in the right side of chromosome and SSR markers in the left.

Gray region indicates the chromosome segments introgressed from donor variety.

White region on chromosome indicates the genetic background of IR64.

Black and white triangles indicated the QTLs detected in Irrigated lowland and Upland, respectively, and

Upper and down triangles indicate the increase effect with donor parent and IR64 allele, respectively.

These superscript words on the designation of QTL indicates the conditions and years, a: Upland in 2011, b: Irrigated lowland in 2011, c: Upland in 2012, and d: irrigated lowland in 2012.

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Fig. 13. Chromosomal location of introgressed segments and QTLs detected in sib-group YP8

A total of 35 introgression lines were derived from the donor parent YP8, IR 66215-44-2-3 (Fujita *et al.* 2009).

Chromosome No. indicate on the top of each vertical bar.

QTL detected by association analysis for agronomic traits is indicated in the right side of chromosome and SSR markers in the left.

Gray region indicates the chromosome segments introgressed from donor variety.

White region on chromosome indicates the genetic background of IR64.

Black and white triangles indicated the QTLs detected in Irrigated lowland and Upland, respectively, and

Upper and down triangles indicate the increase effect with donor parent and IR64 allele, respectively.

These superscript words on the designation of QTL indicates the conditions and years, a: Upland in 2011, b: Irrigated lowland in 2011, c: Upland in 2012, and d: irrigated lowland in 2012.
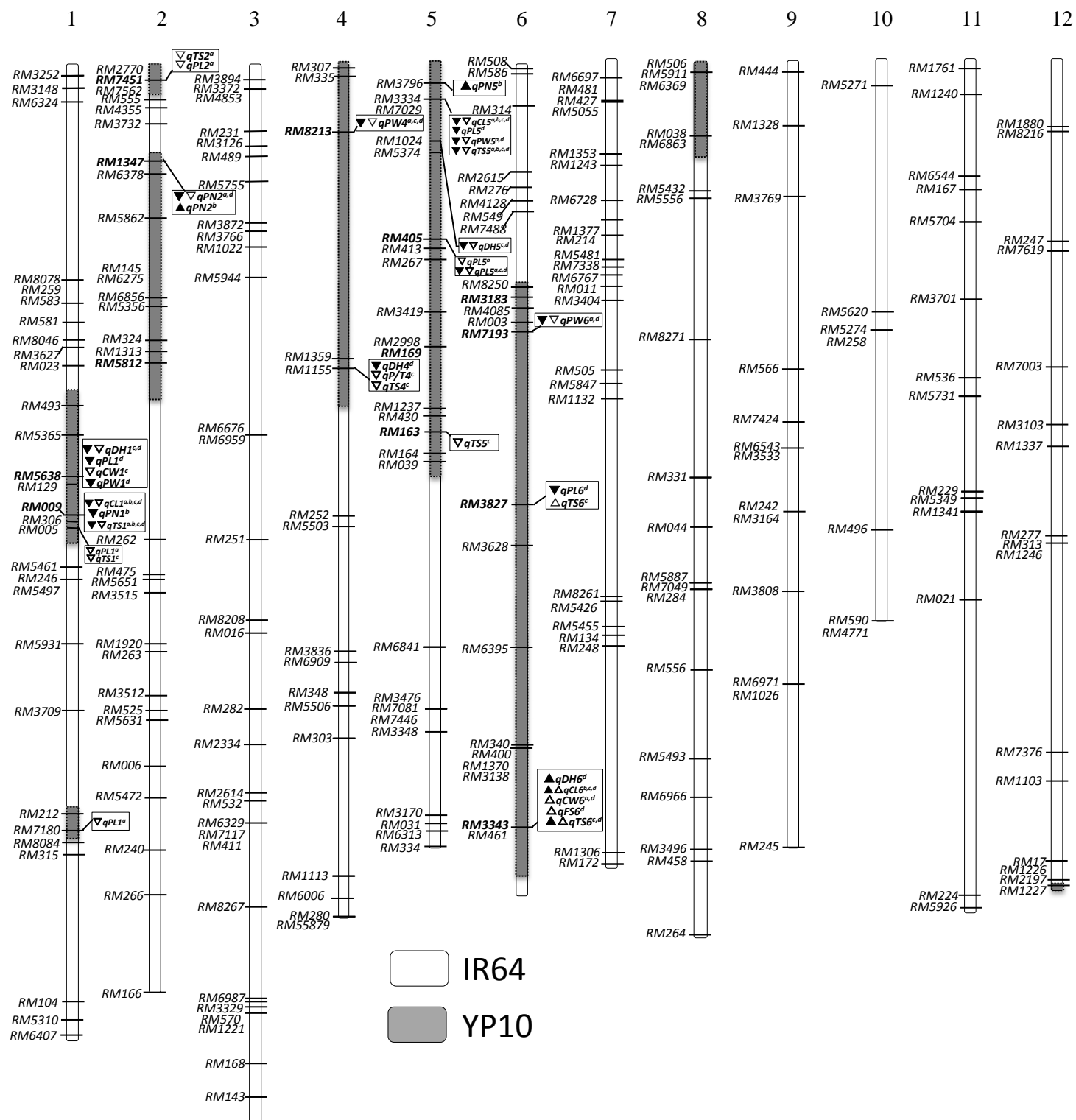
Fig. 14. Chromosomal location of introgressed segments and QTLs detected in sib-group YP9

A total of 35 introgression lines were derived from the donor parent YP9, IR 68522-10-2-2 (Fujita *et al.* 2009).

Chromosome No. indicate on the top of each vertical bar.

QTL detected by association analysis for agronomic traits is indicated in the right side of chromosome and SSR markers in the left.

Gray region indicates the chromosome segments introgressed from donor variety.

White region on chromosome indicates the genetic background of IR64.

Black and white triangles indicated the QTLs detected in Irrigated lowland and Upland, respectively, and

Upper and down triangles indicate the increase effect with donor parent and IR64 allele, respectively.

These superscript words on the designation of QTL indicates the conditions and years, a: Upland in 2011, b: Irrigated lowland in 2011, c: Upland in 2012, and d: irrigated lowland in 2012.
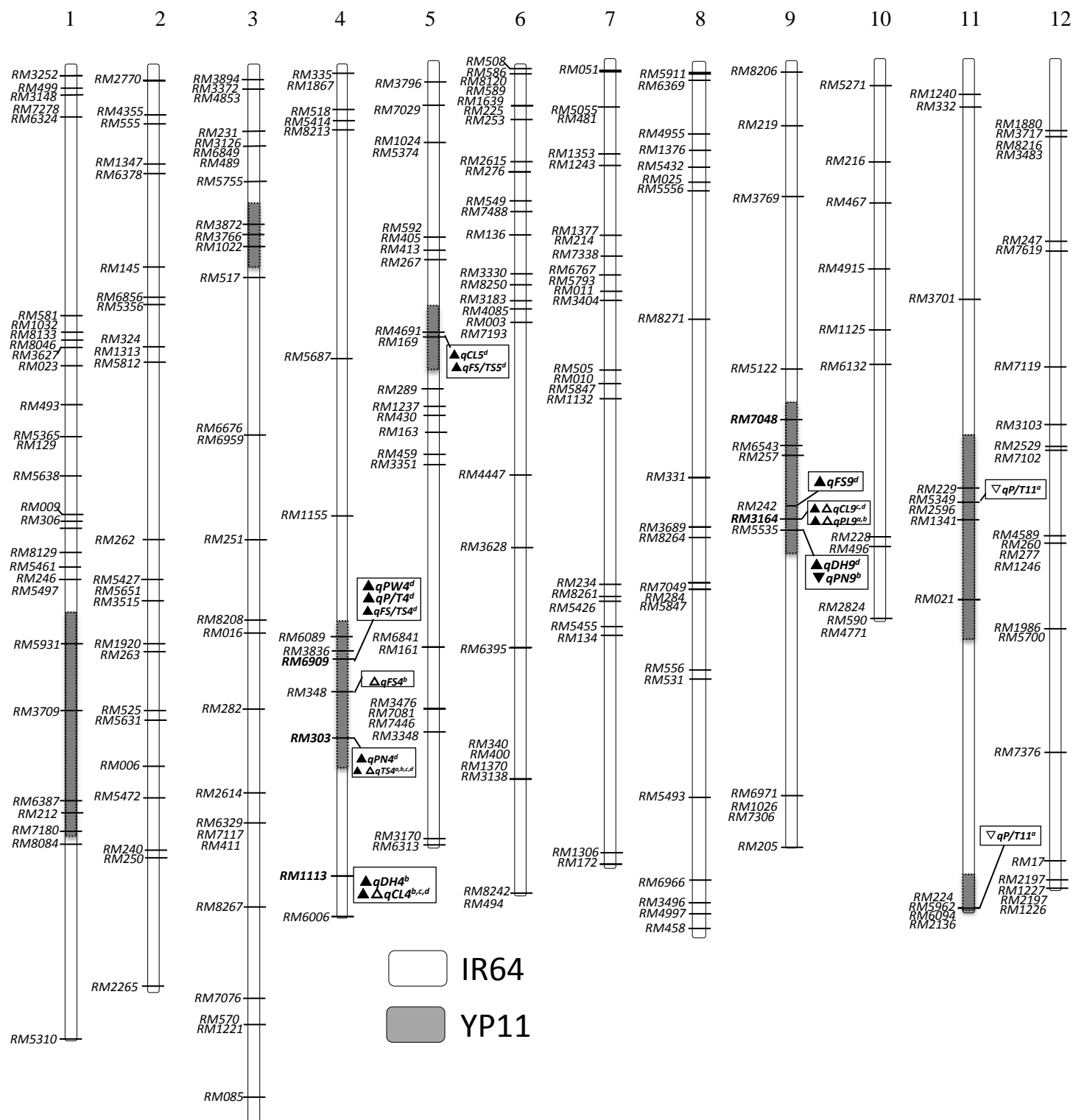
Fig. 15. Chromosomal location of introgressed segments and QTLs detected in sib-group YP10

A total of 35 introgression lines were derived from the donor parent YP10, IR 1195-AC1 (Fujita *et al.* 2009).

Chromosome No. indicate on the top of each vertical bar.

QTL detected by association analysis for agronomic traits is indicated in the right side of chromosome and SSR markers in the left.
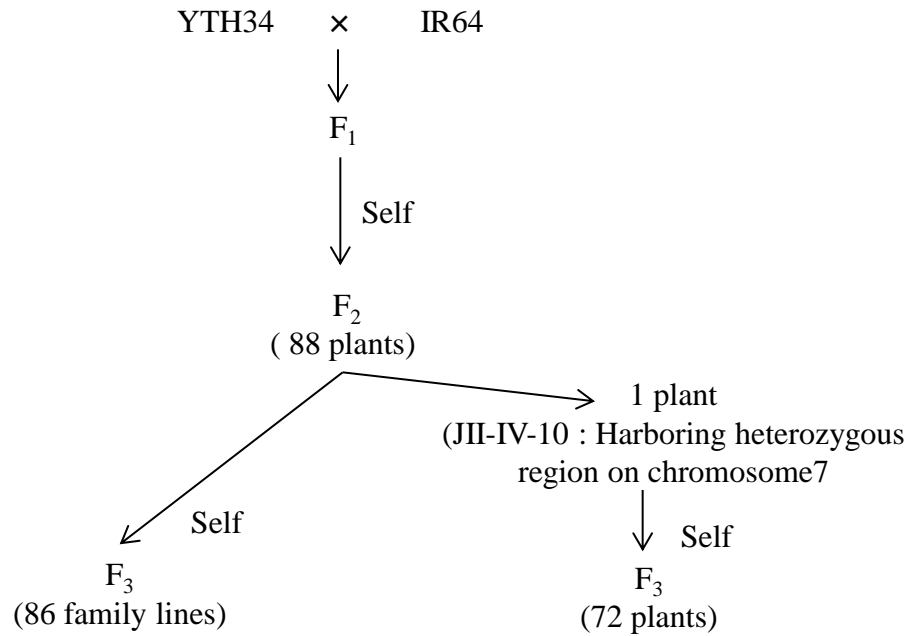
Gray region indicates the chromosome segments introgressed from donor variety.

White region on chromosome indicates the genetic background of IR64.

Black and white triangles indicated the QTLs detected in Irrigated lowland and Upland, respectively, and

Upper and down triangles indicate the increase effect with donor parent and IR64 allele, respectively.

These superscript words on the designation of QTL indicates the conditions and years, a: Upland in 2011, b: Irrigated lowland in 2011, c: Upland in 2012, and d: irrigated lowland in 2012.

Fig. 16. Chromosomal location of introgressed segments and QTLs detected in sib-group YP11

A total of 35 introgression lines were derived from the donor parent YP11, IR 66750-6-2-1 (Fujita *et al.* 2009).

Chromosome No. indicate on the top of each vertical bar.

QTL detected by association analysis for agronomic traits is indicated in the right side of chromosome and SSR markers in the left.

Gray region indicates the chromosome segments introgressed from donor variety.

White region on chromosome indicates the genetic background of IR64.

Black and white triangles indicated the QTLs detected in Irrigated lowland and Upland, respectively, and

Upper and down triangles indicate the increase effect with donor parent and IR64 allele, respectively.

These superscript words on the designation of QTL indicates the conditions and years, a: Upland in 2011, b: Irrigated lowland in 2011, c: Upland in 2012, and d: irrigated lowland in 2012.

YTH34   ×   IR64

↓

$F_1$

| Self

↓

$F_2$
( 88 plants)

→ 1 plant
(JII-IV-10 : Harboring heterozygous
region on chromosome7

Self ↓ Self

$F_3$              $F_3$
(86 family lines)      (72 plants)

These lines were cultivated at upland
and irrigated lowland fields, TARF,
JIRCAS, Ishigaki, Okinawa, Japan

These progenies were cultivated at seeding
tray in greenhouse, JIRCAS, Tsukuba,
Ibaraki, Japan, and No. of tiller and dry
matter production of each progeny were
investigated

Fig. 17. Development of hybrid populations and progenies derived from a cross between YTH34
and IR64

A total of 88 $F_3$ family lines and 72 self pollinated $F_3$ plants, were used in the QTL analyses for
tiller No. and dry matter production.

IR64          YTH 34

Fig. 18. An introgression line YTH34 with an Indica-type rice variety
IR64 genetic background

IR64 and YTH34 were cultivated in upland field at Tropical
Agricultural Research Front, JIRCAS, Ishigaki, Okonawa, Japan, in
2015.
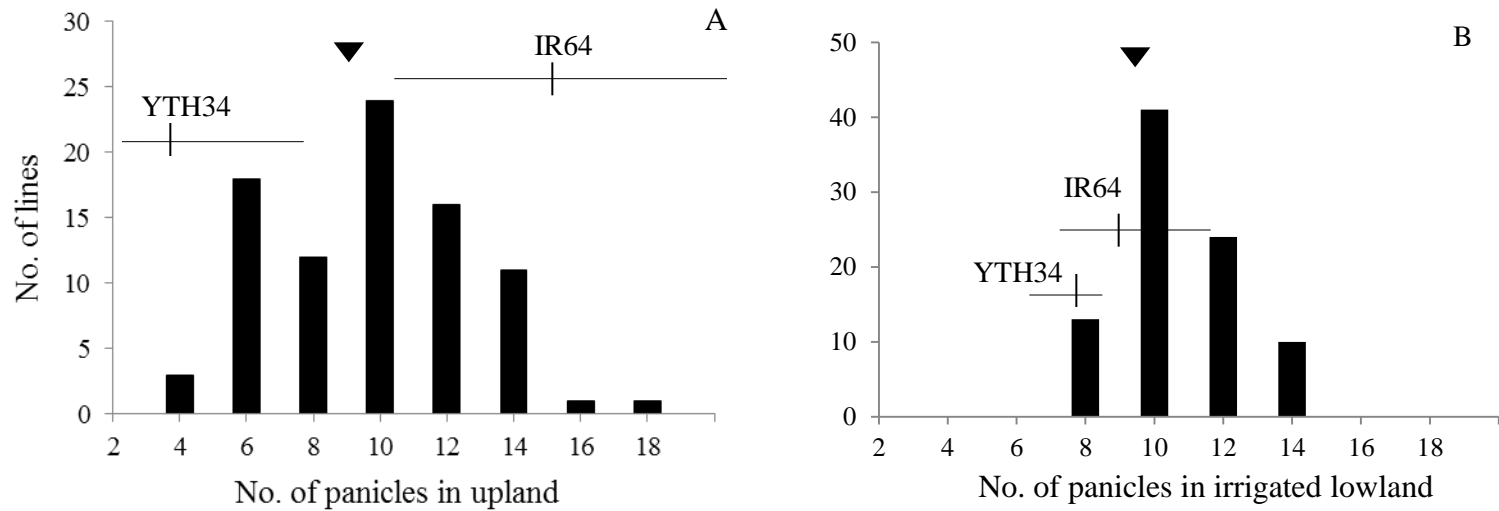
Fig. 19. Segregation of panicle number in $F_3$ family lines derived from a cross between YTH34 and IR64

A total of 86 $F_3$ family lines self pollinated in each $F_2$ plants were cultivated at upland and irrigated lowland.
A total of 18 plants in each line were investigated its panicle No. and the average was used as the representative value of each line.

▼ : Average of hybrid population ——————— : Range and average of parents varieties
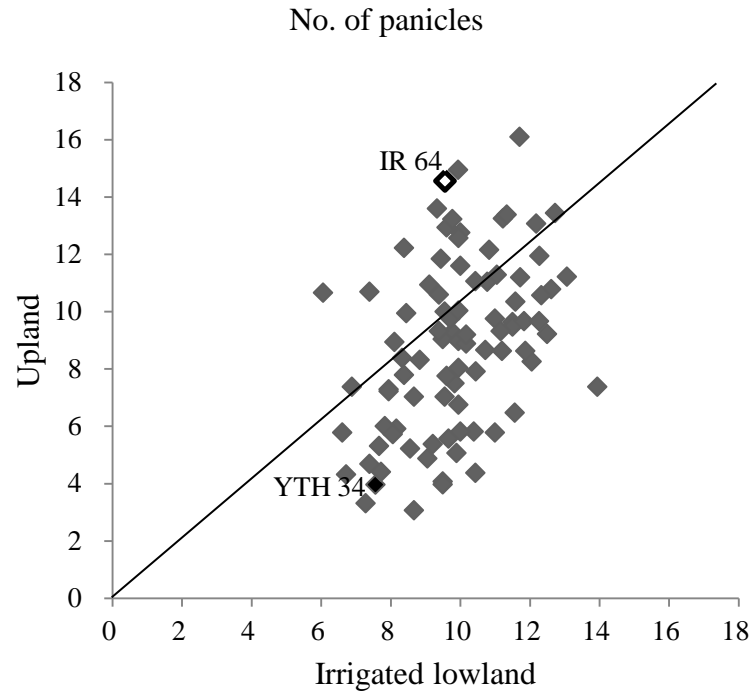
No. of panicles



Fig. 20. Relationships of panicle numbers in the 86 $F_3$ family lines between irrigated lowland and upland
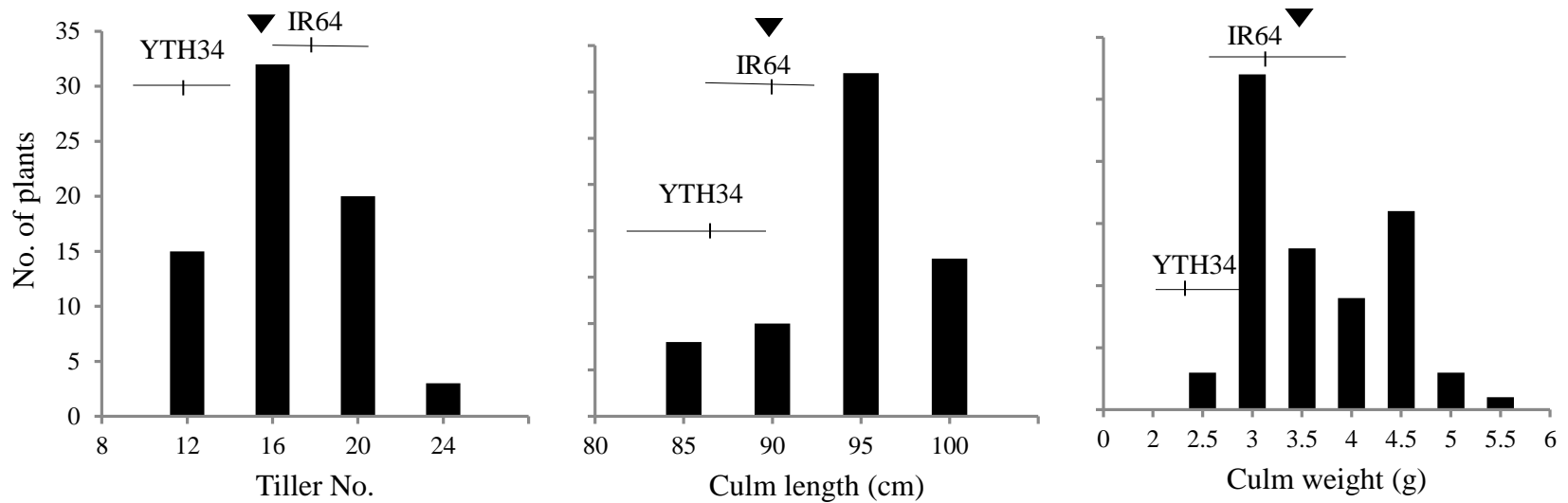
Fig. 21. Segregation of 72 $F_3$ progenies derived from a $F_2$ plant, JII-IV-10, derived from a cross between YTH34 and IR64

JII-IV-10 harbor heterozygous region on chromosome 7 where detected QTL for panicle No, by association analysis, using sib-introgression lines which have chromosome segment originated a common donor parent, in previous study (unpublished).
Plants were grown with 6 × 7 cm spacing in a seeding tray under upland condition at green house of JIRCAS, Tsukuba, Ibaraki, Japan in 2015.

▼ : Average of hybrid population        ———|——— : Range and average of parents varieties
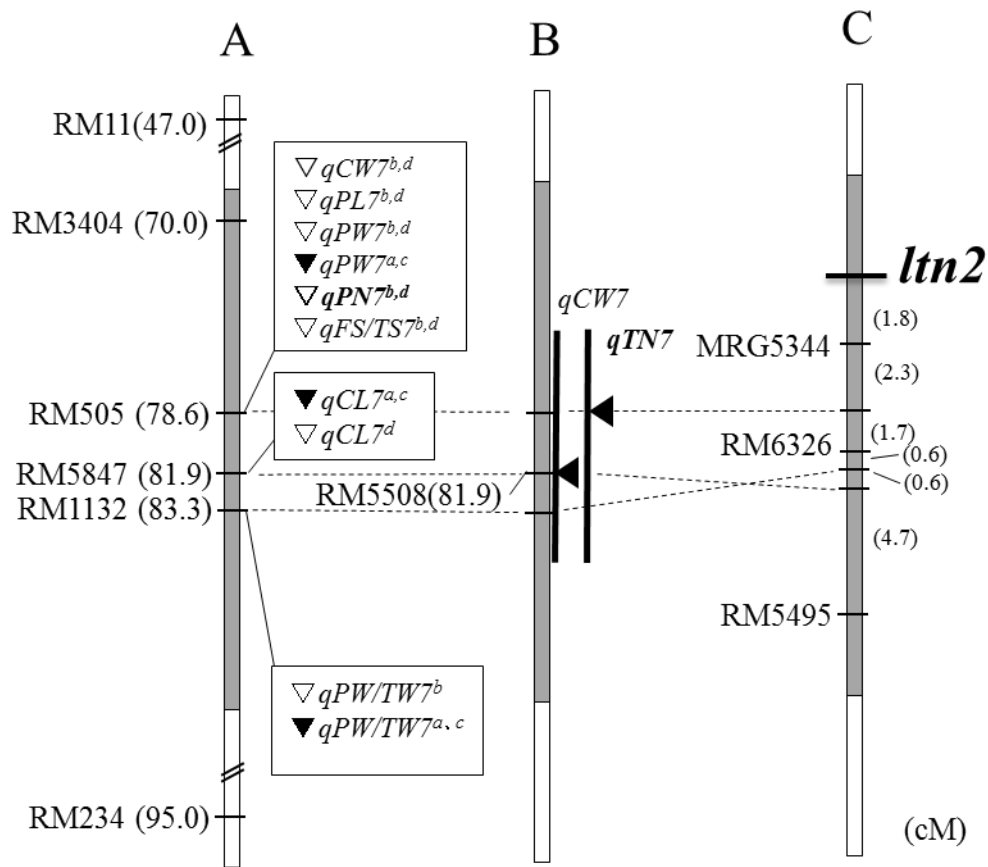
Fig. 22. Chromosomal location of a novel low tiller gene, *ltn2*

A: Association mapping for yield components using sib-introgression lines harboring chromosome segments from parent YP1 developed by Fujita *et al.* (2009)

B: QTL mapping using a 72 F3 progenies derived from a JII-F2-IV-10 plant which harboring heterozygous chromosome region on chromosome 7 detected a QTL for low panicle No. in previous association mapping.

C: Mapping of low tillering gene using 86 F3 family lines derived from F2 plants self-pollinated in each. The segregation of F3 lines were investigated under upland condition and confirmed single gene segregation.

| Seeding stage | |
| --- | --- |
| Dependent<br>(Cultivation without N) | Independent<br>(Cultivation with N) |

0 days ⊗

14 days

7 days ⊗

14 days ⊗

21 days ⊗
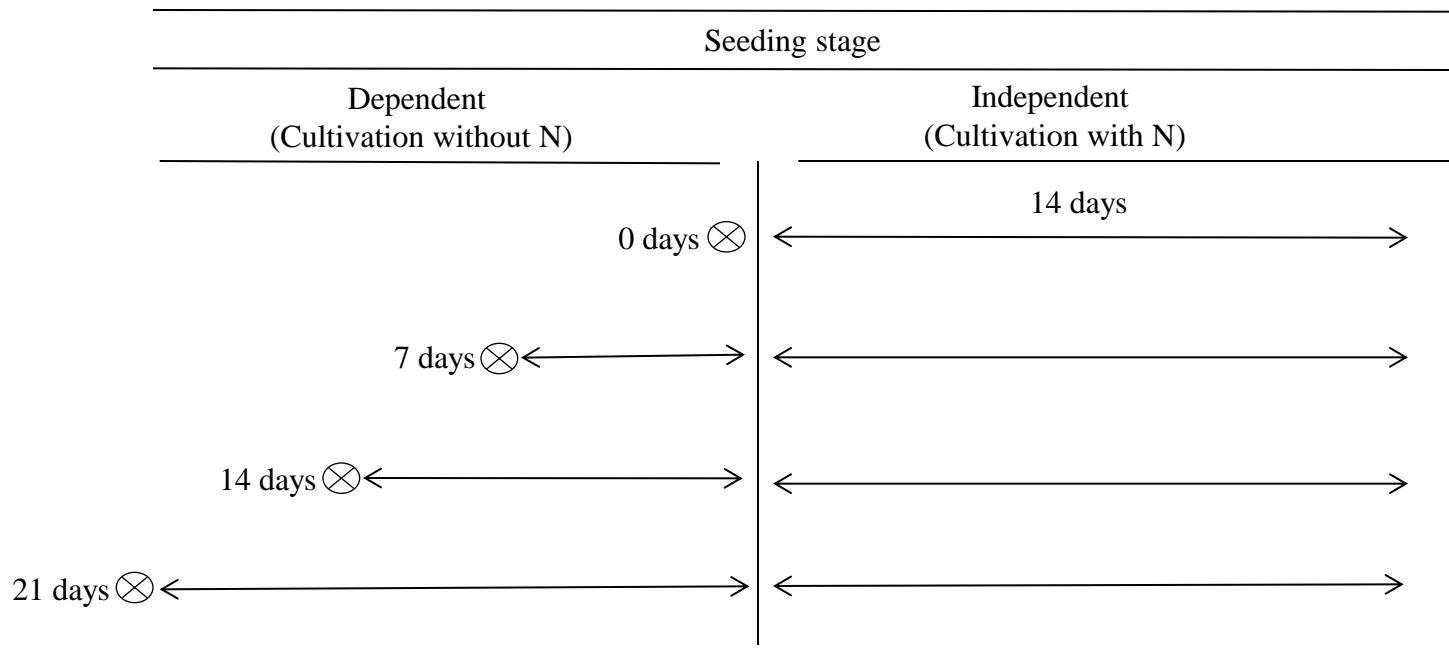
Fig. 23. Schematic representation of the nitrogen treatment experiment condition

Whole shoot of each lines were collected after 14 days of nitrogen treatment, and investigated the dry matter production. The summing junction, ⊗ , Indicate sowing day
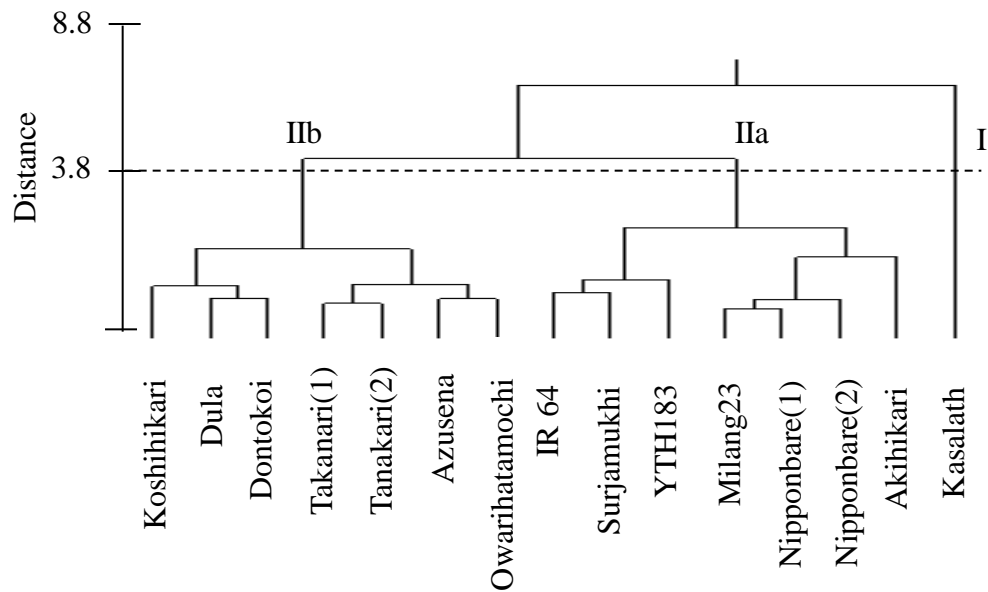
Fig. 24. Classifications of 13 rice varieties. Cluster analysis carried out by Ward`s
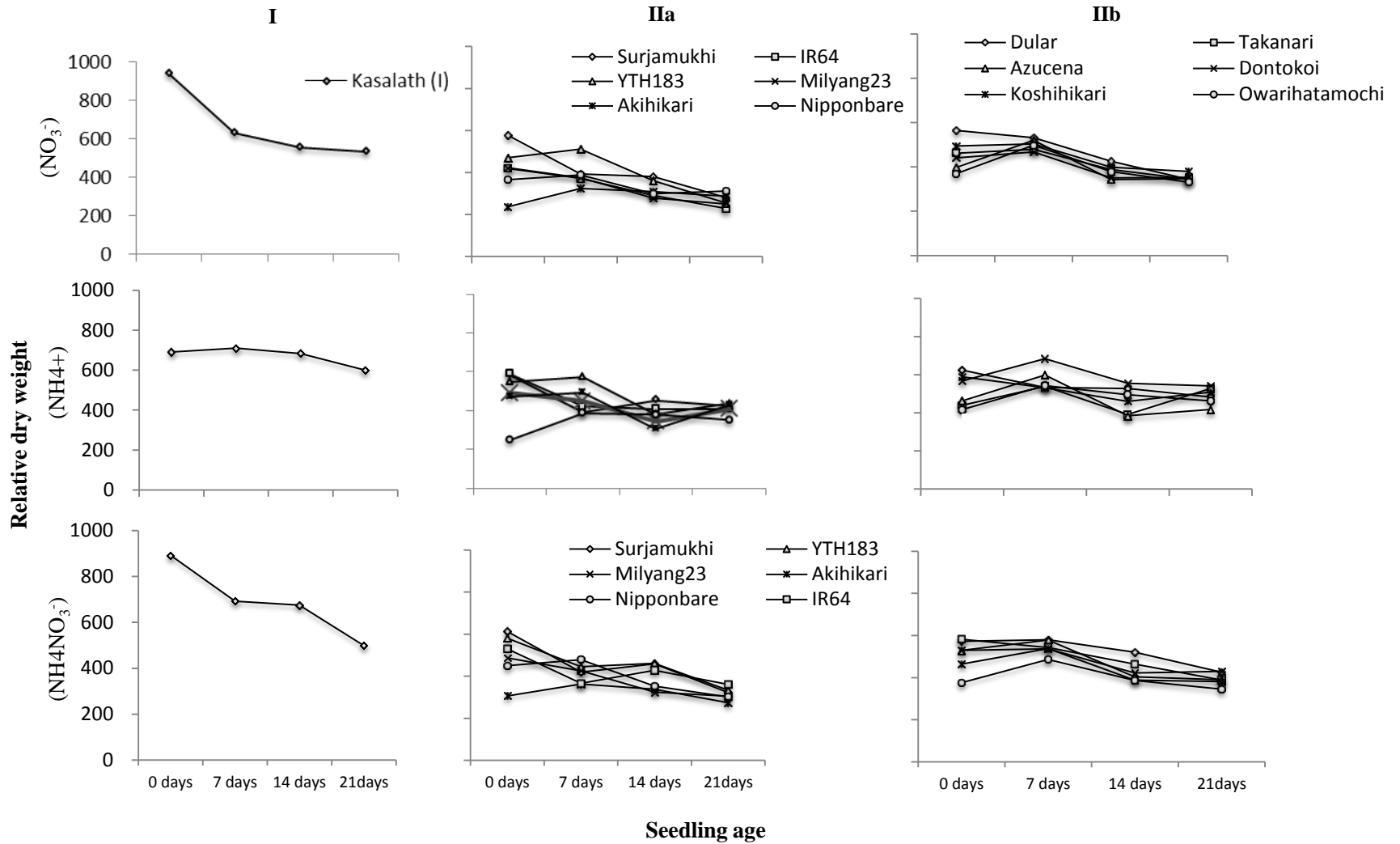Hierarchical analysis (Ward 1963) using a computer software, JMP7.0

Fig. 25. Variations of RDW (%) among the cluster groups in response to various nitrogen forms at seedling ages
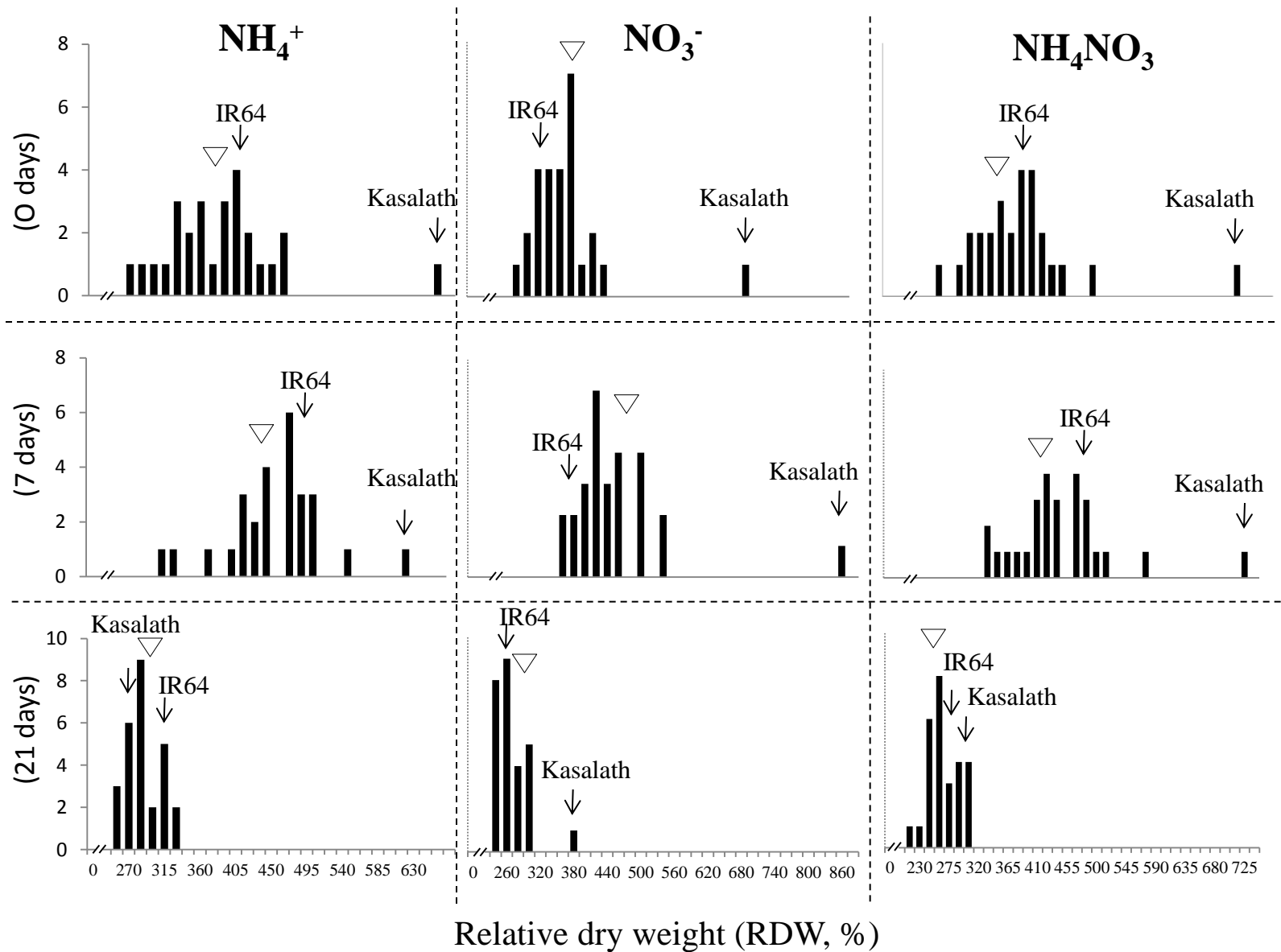
Fig. 26. Frequency distribution of the relative dry weight (RDW, %) of 25 INLs with IR64 genetic background, and two control variety, IR64 and kasalath, under three nitrogen forms, ammonia ($NH_4^+$), nitrate ($NO_3^-$), and ammonium nitrate ($NH_4NO_3$) at three seedling stage, 0, 7, and 21 days. Triangle indicate the average RDW (%) value of INLs
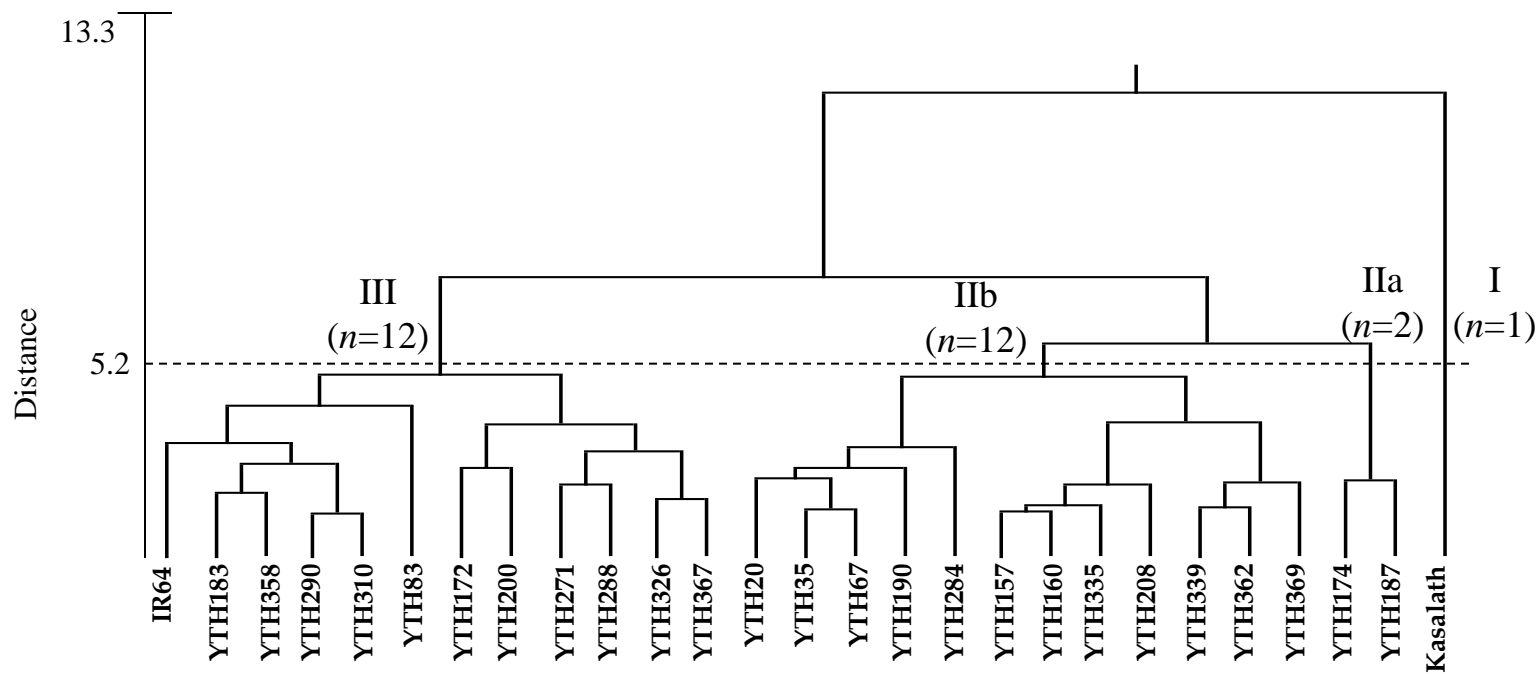
Fig. 27. Classification of 25 INLs with IR64 genetic background and, IR64 and kasalath as control variety

The 0, 7, and 21 days rice seedlings were grown for 14 days in hydroponics under $NH_4^+$, $NO_3^-$ and $NH_4NO_3$ at Tropical greenhouse of JIRCAS, Tsukuba, Ibaraki, Japan, in February and October 2014.

Cluster analyses were conducted out using the data of relative dry weight (RDW, %) of two consecutive experiments with Ward`s hierarchical analysis (Ward 1963) using JMP7.0 (SAS institute Inc., Cary, NC, USA).