THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Agricultural Biotechnology)

DEGREE

Agricultural Biotechnology — **FIELD**

Interdisciplinary Graduate Program — **PROGRAM**

TITLE: Development of Chromosome Segment Substitution Lines Related to Drought Tolerance in Rice (*Oryza sativa* L.)

NAME: Mr. Vaiphot Kanjoo

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR

(Mr. Theerayut Toojinda, Ph.D.)

THESIS CO-ADVISOR

(Associate Professor Apichart Vanavichit, Ph.D.)

GRADUATE COMMITTEE CHAIRMAN

(Associate Professor Pongthep Akratanakul, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON _____

DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

DEVELOPMENT OF CHROMOSOME SEGMENT SUBSTITUTION LINES RELATED TO DROUGHT TOLERANCE IN RICE (*Oryza sativa* L.)

VAIPHOT KANJOO

A Thesis Submitted in Partial Fulfillment of
The Requirements for the Degree of
Doctor of Philosophy (Agricultural Biotechnology)
Graduate School, Kasetsart University
2012

Vaiphot Kanjoo 2012: Development of Chromosome Segment Substitution Lines Related to Drought Tolerance in Rice (*Oryza sativa* L.). Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Mr. Theerayut Toojinda, Ph.D. 95 pages.

Drought tolerance (DT) in rice (*Oryza sativa* L.) is a complex quantitative trait and tolerance mechanisms are not fully understood. This study aims to develop plant materials particularly chromosome segment substitution lines (CSSLs) carrying single DT quantitative trait loci (QTL) by marker assisted backcrossing (MAB). The CSSLs can be used as genetic material to understand the genetic basis and mechanisms of DT in rice. Four genomic segments on chromosomes 1, 3, 4, 8 and 9 containing DT-QTL inherited from doubled haploid lines IR68586-F_2-CA-31 and IR68586-F_2-CA-143 are the target of introgression. Five rounds of MAB were conducted and a set of CSSLs was developed. In each CSSL, a different chromosome segment of DT donors was substituted in the genetic background of Khao Dawk Mali 105 (KDML105). The substituted chromosome segments of the 104 CSSLs covered most of DT-QTL regions located on the five target chromosomes, except for the middle region of DT-QTL on chromosome 9. A set of 131 polymorphic SSR markers were used for genome scanning to estimate the proportion of donor alleles in the non-target areas. CSSLs recover up to 96.30% of the KDML105 genome (individuals ranging from 88.50 to 100 %). The CSSLs were evaluated for agronomic performance under rainfed and irrigated conditions. Variations of agronomic traits were observed in both conditions. Some of the CSSL showed higher grain yield than the recipient parent, KDML105 under rainfed condition (mild drought stress). This result demonstrated that CSSLs can be a useful material to dissect genes underlay the DT. The CSSLs that show a good adaptation under drought stress can be used as genetic materials to improve drought tolerance in the rainfed lowland rice breeding program.

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Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

I would like to acknowledge my thesis advisor, Dr. Theerayut Toojinda, who is a good teacher for consulting, transferring skills and knowledge of plant breeding to me. He is my idol in subjects of ability to work with others and coolness to solve the problem. His kindness to me during the course and encourages me to have the enthusiasm to develop academic. I want to thank him deeply for correction of my dissertation and publications even though he has very busy.

Special thanks to Associate Dr. Apichart Vanavichit, who is my thesis co-advisor. He had compassion for me in a lot about study and advice. His facilitate equipments and place is useful for my research. I wish to express my sincere thanks to all staffs of Rice Science Center, Kasetsart University, Kampaeng Saen campus, for a good friendship during my studies, especially Mr. Sumon Hoymala, Mr. Pawat Vijarat, Miss Siriporn Kor-insak, Miss Kanchana Punyawaew and MAS staffs.

This research was supported by National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand and was partially funded by Center for Agricultural Biotechnology (CAB) from Subproject Graduate Study and Research in Agricultural Biotechnology under the Higher Education Development Project, Commission on the Higher Education, and The Ministry of education. And also, I thank University of Phayao for funding Ph.D. scholarships.

Finally, I would like to respectfully grateful remembrance of my father and mother who gave birth to me. Although they are farmers, they went on to provide the highest education to me. I do not have any words to say than the words “I love them both”. I gratefully acknowledge all members of my family and grandmother “Yay Laun” , who gave me the take care.

Vaiphot Kanjoo

January 2012

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LIST OF ABBREVIATIONS

ABA	=	abscisic acid
BADH	=	betaine aldehyde dehydrogenase
BC	=	backcrossing
Chr	=	chromosome
cM	=	centimorgan
CSSL	=	chromosome segment substitution line
CPA	=	Chumpae Rice Research Center
DAS	=	days after sowing
DF	=	flowering date
DHL	=	double haploid line
DNA	=	deoxyribonucleic acid
dNTP	=	deoxynucleotide-5'-triphosphate
DT	=	drought tolerance
DTF	=	days to flowering
FG	=	fine genotyping
FGN	=	filled spikelet number
FGW	=	filled grain weight
GS	=	genome scanning
GY	=	grain yield per rai (1,600 m^2)
IRRI	=	International Rice Research Institution
KDML105	=	Khao Dawk Mali 105
KPS	=	Kampaeng Saen campus
LEA	=	late embryogenesis abundant proteins
LSD	=	Least significant difference
m	=	meter
MAB	=	marker assisted backcrossing
MAS	=	marker assisted selection
$MgCl_2$	=	magnesium chloride
mRNA	=	messenger ribonucleic acid

LIST OF ABBREVIATIONS (Continued)

NaCl	=	sodium chloride
OA	=	osmotic adjustment
Os2AP	=	*Oryza sativa* 2-acetyl-1- pyroline
PCR	=	polymerase chain reaction
PH	=	plant height
PN	=	panicle number per plant
PSS	=	percentage of spikelet sterility
QTL	=	quantitative trait locus
RP	=	recurrent parent
RCBD	=	randomly complete block design
SIS	=	salt injury scores
SSR	=	simple sequence repeat
ST	=	salinity tolerance
TGW	=	total grain weight
TN	=	tiller number per plant
TSN	=	total spikelet number per panicle
mm	=	millimeter
UFGN	=	unfilled spikelet number
UFGW	=	unfilled grain weight
µm	=	micrometer
°C	=	degree celsius
1000GW	=	1,000-grain weight

DEVELOPMENT OF CHROMOSOME SEGMENT SUBSTITUTION LINES RELATED TO DROUGHT TOLERANCE IN RICE (*Oryza sativa* L.)

INTRODUCTION

Drought is the most important constraint limiting grain yield of rice in rainfed lowland and insufficient irrigation areas of the North and Northeast of Thailand since its effect or damage on rice may range from minor reduction to complete loss in yield. The using of drought tolerance (DT) rice variety is a strategy for reducing negative influence of unpredictable drought. The genetically and physiologically complex of DT inherited in a quantitative manner and typically controlled by many genes or quantitative trait loci (QTLs) that strongly influenced by environmental factors (Fukai and Cooper, 1995). Consequently, improvement and selection for rice tolerant varieties are difficult and give little progress.

DT in rice is a complex trait which concerned many traits. Quantitative trait loci (QTL)/ gene clusters for DT in rice were discovered on chromosomes 1, 3, 4, 8 and 9 (Babu *et al.*, 2003; Kamoshita *et al.*, 2008). These common QTL are the key genomic regions for DT-QTLs because QTLs were found in consensuses in various populations (Zeng *et al.*, 2006). Owing that all QTLs on each chromosome are very large (Lanceras *et al.*, 2004) and are locality of other QTLs related with morpho-physiological traits such as large vascular bundle number (Cui *et al.*, 2002) chlorophyll content (Yang *et al.*, 2003) and days to heading (Wang *et al.*, 2002). The QTL introgressed segments tended to cluster in the same genomic region has effects on other agronomic traits through pleiotropy or linkage drag (Steele *et al.*, 2006), such as the QTLs of root system affected on grain yield under drought (Shen *et al.*, 2001). These constraints which controlled by polygenes with minor effects, linkage drag is more difficult to be solved by using classical genetic and traditional breeding method (Liu *et al.*, 2009). To minimize the linkage drag, it must be fine-mapped the large segment to a small. To understand genetic mechanisms for tolerant ability, the QTL

regions on five target chromosomes are highlight in this study need to be dissected DT mechanism.

Chromosome segment substitution lines (CSSLs) as novel mapping populations which carry a particular chromosome segment from a donor line in the genetic background of recurrent line were developed for genetic studies. CSSLs can be use for fine mapping and cloning of QTLs such as grain size (Kubo *et al.*, 2002) eating quality (Wan *et al.*, 2004), flowering date (Takai *et al.*, 2007). Although, development of these materials is laborious and time consuming but this approach will facilitate for many researchers to perform QTL map-based cloning (Ebitani *et al.*, 2005). In rice, CSSLs were constructed in many populations with various genetic backgrounds (Doi *et al.*, 1997; Ebitani *et al.*, 2005; Kubo *et al.*, 2002; Takai *et al.*, 2007). Therefore, CSSLs will be candidate materials for further study to identify more accurately genetic regions for a complex trait such as DT. In this paper, we attempted to develop CSSLs which carrying the overlapping DT-QTL segments in Khao Dawk Mali 105 (KDML105) genetic background through marker assisted selection (MAS). These CSSLs can be used as near isogenic lines for evaluation of phenotypes under rainfed environment for finding association with their genotypes to re-analyze DT-QTLs and compare with previous map. This strategy will lead to understanding DT genetic mechanisms with different resistant abilities and can be identify tightly linked markers or candidate genes regulating DT in rice.

OBJECTIVES

1. To develop chromosome segment substitution lines (CSSLs) in a genetic background of KDML105 via marker-assisted selection (MAS).

2. To evaluate agronomic performance and analyze genetic association of these CSSLs under drought and irrigated conditions, and to construct the DT-QTL mapping.

LITERATURE REVIEW

Rainfed lowland rice production and drought problem in Northeast Thailand

Approximately 76% of rice growing areas in Thailand are under rainfed condition. The smallest area is covered with irrigated rice. Rainfed rice ecosystems are classified into three patterns: upland, rainfed lowland, deep water and floating rice. The majority of the rainfed lowland areas are found in Northeast and North of Thailand. Paddy yield in different rice ecosystems in different parts of the country is naturally limited by several constraints.

Rainfed lowland areas in Thailand have several constraints such as rainfall variability, flooding, diseases and low soil fertility. Drought is a factor limiting rice production in rainfed lowland and poor irrigation areas in Northeast and North of Thailand since its effect or damage on rice may range from minor reduction to complete loss in yield. In Thailand, most areas of rainfed lowland rice are classified as shallow favorable and shallow drought prone (Jongdee *et al.*, 2002). Seasonal rainfall is bimodal, usually beginning in May and ending around mid October, but is highly variable. Drought may develop at any time during the growing season.

Rainfed lowland areas in Northeast and North of Thailand are rice-growing locations of popular aromatic varieties such as KDML105 and RD6. These varieties are responsive to photoperiod, and can only be planted once a year during the rainy season. If this period has water deficit or no rainfall problems, paddy yield is reduced or impossible to harvest. The production failure by drought does not only result in starvation of poor farmers but also has many consequences on the farmer community such as unemployment and migration problems. Therefore, improving drought resistance of rice is an approach for reducing negative influence of drought situation.

Types of drought stress

Drought can be classified in three categories (Figure 1) based on the duration of the wet season and severity of water stress (Fukai and Cooper, 1995).

- An early-season drought that occurs during vegetative growth.
- An intermittent midseason drought that occurs between tilling and mid-grain filling.
- A late-season drought that occurs during flowering and grain filling.

Drought is a major factor limiting grain yield of rainfed lowland rice in Northeastern region, especially late-season drought, which develops at the end of the monsoon and has large effects on grain yield and yield components (Pantuwan *et al.*, 2002)

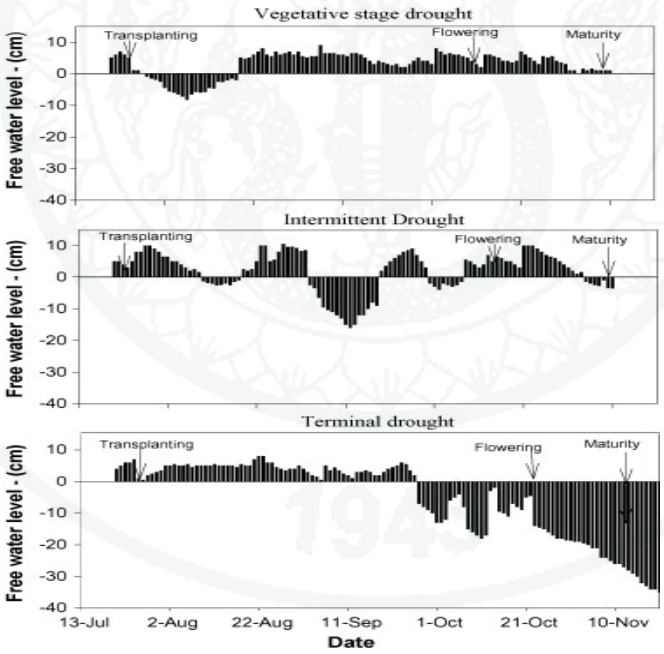

Figure 1 Types of drought stress were indentified by free water level above and below soil surface.

Source: Fukai and Cooper (1995); Kamoshita *et al*. (2008)

Effects of drought stress

Water stress can occurs under flooding and drought conditions. Plant responses to water deficit depend on the amount of water lost, the rate of loss and the duration of the stress. Water loss from plant tissues under drought results in growth inhibition and various biochemical and physiological changes. These include abscisic acid (ABA) accumulation, stomatal closure, and changes in leaf water potential, decreased photosynthesis and solute accumulation.

The drought stress signal is mediated through both ABA-dependent and ABA-independent pathway to regulate expression of various genes that are involved in drought tolerance and responses. These gene products are thought to function with the accumulation of osmoprotectants; detoxification protection of the cells; protein turnover; stress signalling pathways; transcriptional regulation and so on (Shinozaki and Yamaguchi-Shinozaki, 1997; Xiong and Zhu, 2002)

Growth stages of rice can be classified in vegetative, reproductive and grain filling periods (Yoshida, 1981). Water deficit affects grain yield of rice in various ways depending on the intensity, duration and timing (O'Toole and Moya, 1978). It is less damaging during the vegetative than the reproductive stage, as young plants are able to recover when stress is relieved. The most sensitive period is during the reproductive stage especially during flowering one (Cruz and O'Toole, 1984).

Drought stress during reproductive stage, which is particularly damaging during booting and flowering, desiccates spikelets and anthers, reduces pollen shedding, inhibit panicles exertion, and increase sterility rate. If drought occurs soon after panicle initiation, the number of primordial spikelet developed is reduced while drought at pollen or anthesis development increases spikelets sterility. This results in decreasing in grain yield that associated with reduction of grain number per panicle because of a decrease in spikelet number per panicle, and perhaps also with grain size. During mid to late panicle development or booting, which coincides with pollen

development, particularly at meiosis in pollen mother cells, causes pollen sterility. Unlike the effect of drought at heading and anthesis, sterility occurred because of failure in pollination.

Drought during grain filling period is however less damaging than at the panicle initiation or flowering stage. Reduction in grain yield may be due to reduction in individual grain weight, but the effect may be small due to increased translocation of stored nutrients assimilate from vegetative parts to grain (O'Toole and Chang., 1979).

Plant adaptation mechanisms to drought stress

There are several mechanisms by which plants can adapt to drought. In field crop production, survival alone during a drought not sufficient; the crop needs to produce a reasonable yield for subsistence requirements or for economic reasons. The four common adaptation mechanisms in crop are drought escape, dehydration avoidance, dehydration tolerance and drought recovery. Each mechanism may be the result of a number of traits.

1. Drought escape

The most effective method of minimizing the adverse effect of drought is for the crop to grow during the period of high rainfall and high soil water availability, to escape the drought period. Crop duration is important in determining grain yield because early maturing cultivars often escape a terminal stress while late-maturity cultivars may be affected. Timing of drought development in relation to phenology is also important for determination of grain yield. It is well known that the stage from panicle development to anthesis is the most sensitive to water stress in rice (Boonjung and Fukai, 1996; O'Toole, 1982). Boonjung and Fukai (1996) have shown that grain yield decreases with the rate of 2% per day when plant exposed to stress for 15 days during panicle development. Assuming a reduction of 2% grain yield per day during a

15-day stress, a 20-day difference in flowering time between two cultivars of equal yield potential could cause a grain yield difference about 40%. Thus it is likely that cultivars with different phenology will react differently to a drought, depending on the timing of the stress (Maurya and O'Toole, 1986). These results suggest that genotypes should be compared for drought resistance or susceptibility within the same phenology group, or at least genotypic variation in phenology should be corrected in some way before differences in drought resistance are estimated. Alternatively, it is possible in some experiments to implement a strategy of staggered planting of lines so that they flower approximately at the same time (Lilley and Fukai, 1994)

2. Dehydration avoidance

This is mechanism is the ability of plant to maintain relative high leaf water potential. One hence is to extract more water from the soil, while another is to use soil water slowly during early drought period to make it more available in later period. These two methods are useful in some crops including upland rice, but may have more limited use in rainfed lowland rice where the development of a hard pan often inhibits deep root system development and water is lost mostly through deep percolation, seepage, and soil or surface water evaporation. Since transpiration is a small proportion of total water loss, saving water by reduced transpiration is usually insignificant. However dehydration avoidance can also be achieved in both lowland and upland conditions by reducing evaporative demand or heat load on the shoot. Plant water status is determined by the balance of water uptake through the root system and water demand by the shoot. Thus high water potential may be maintained by shoot mechanisms, which reduce the demand (Fukai and Cooper, 1995).

3. Dehydration tolerance

This is a plant mechanism to maintain metabolism even at lower leaf water potential. Arrandeau (1989) consider translocation of assimilate is a trait associated with dehydration tolerance in rice. When stress develops during grain filling,

dehydration tolerance may allow the plants to maintain metabolic activity for a few additional days, and continue the translocation of previously produced assimilates to fill grain. Compared with other cereal crops, rice is known to rely more on stored assimilates for grain filling (Weng *et al.*, 1982) and dehydration tolerance appears important for rice under terminal stress.

4. Drought recovery

This is an important mechanism when drought occurs early in crop development. Some genotypes are able to produce more tillers upon drought relief, and these tillers are productive if the remaining growing season is long enough to complete grain filling. Recovery of a genotype from drought is related to its ability to retain green leaves during that period. Leaf retention may be particularly important when stress develops around panicle initiation, because lines with good leaf retention can supply more assimilate to the developing panicle during subsequent recovery. This results in smaller reduction of spikelet numbers (Lilley and Fukai, 1994).

Improving drought tolerance in rice

Most improved cultivars grown in drought prone areas were originally bred for irrigated condition, and were never selected for drought tolerance. These cultivars have high yield potential, but are often highly prone to yield reduction under drought. On the other hand, some traditional rice cultivars grown in rainfed areas are highly drought tolerance but have low yield potential. In order to assure high yield in non-drought years and acceptable yield in drought years, farmers require cultivars that combine high yield potential with improved tolerance of drought.

Earlier efforts to improve rice yield under drought mainly focused on improving secondary traits such as root architecture, leaf water potential, osmotic adjustment and relative water content. (Fukai *et al.*, 1999; Jongdee *et al.*, 2002; Pantuwan *et al.*, 2002; Price and Courtois, 1999; Toorchi *et al.*, 2003). However,

broad sense heritability of these traits were assumed to be low and often not highly correlated with grain yield under drought stress. Drought tolerance traits including primary traits, secondary traits and integrative traits are often considered with plant type traits and phenology for selecting tolerant rice varieties. Thus, associations among them were showed in Figure 2.

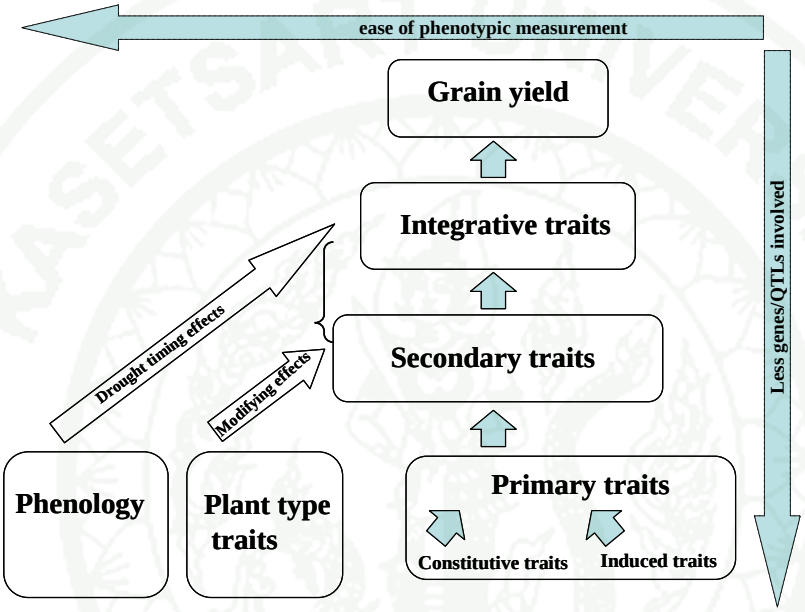

Figure 2 Associations between primary, secondary, integrative drought tolerance traits, plant type traits and phenology.

Source: Kamoshita *et al.* (2008)

Several putative traits contributing to drought resistance in rice have been suggested (Fukai and Cooper, 1995). Root characteristics such as thickness, depth of rooting, root length density, root pulling resistance, and root penetration ability have been associated with drought avoidance in rice (Nguyen *et al.*, 1997). Osmotic adjustment (OA) capacity is an important, shoot-related component of drought tolerance in crop plants. OA, defined as the active accumulation of solutes during the development of water stress in plants, allows maintenance of higher turgor potential at a given leaf water potential. OA delays leaf rolling, tissue death, and leaf senescence

under water stress in rice and has been shown to enhance grain yield under water limited conditions in several other crops (Zhang *et al.*, 1999). However, a yield benefit due to OA is yet to be demonstrated in rice. Despite our increased understanding of the role of putative traits in drought tolerance, these traits are rarely selected in crop improvement programs because phenotypic selection for most root traits and OA is laboratory difficult. Considering these limitations to efficient selection, molecular marker technology is a powerful tool for selecting such traits. QTLs have been detected for several root-related traits and OA in rice (Ali *et al.*, 2000; Champoux *et al.*, 1995; Lilley *et al.*, 1996; Price and Tomos, 1997; Price *et al.*, 2000; Yadav *et al.*, 1997; Zhang *et al.*, 1999; Zheng *et al.*, 2000). A significant proportion of the phenotypic variability of several putative drought tolerance traits is explained by the segregation of relatively few genetic loci, thus leading to the possibility of indirect selection of these complex traits by means of MAS strategy.

QTL mapping for drought tolerance

QTLs linked to drought tolerance have been mapped in at least 15 different populations (Kamoshita et al., 2008). Most of the mapping populations were derived from indica × japonica parents, and it is often the case that favorable alleles for drought-tolerance traits are contributed by japonica lines. Considering that indica and japonica ecotypes are grown in different environments and that most breeding programs involve locally adapted rice accessions, the results for traits such as yield in indica × japonica populations have to be interpreted with care. Hence, it is desirable to look for genetic variation among indica ecotypes (IR58821/IR52561) as well as among japonica ecotypes (Akihikari/IRAT109) QTL for adventitious and lateral roots in rice and to map QTLs using populations derived from lines adapted to target environments. CT9993/IR62266 population is most widely studied mapping population that was used to construct QTL map for DT in rice, in which both of the parents are well adapted to rainfed rice-growing environments. Kamoshita et al. (2008) identified 34 genomic regions with multiple QTLs for putative drought-tolerance traits including primary, secondary, integrative, phenology, and plant-type

traits. In this study, QTLs that were mapped from this population were introgressed to KDML105 and were dissected DT mechanisms.

QTL/gene clusters for drought related traits in rice were studied and identified on various populations. Almost all of them are located on chromosomes 1, 2, 3, 4, 8 and 9 (Lanceras *et al.*, 2004; Zeng *et al.*, 2006). Although previous analysis indicated the map positions of QTL associated with drought tolerant traits, the effects of those traits on plant production under drought has not yet been established. Thus there is a need to determine whether the QTL linked to drought tolerant traits also affect yield under stress. By comparing the coincidence of QTL for specific traits and QTL for plant production under drought, it is possible to test whether a particular constitutive or adaptive response to drought stress is significant in improving field level drought resistance (Lebreton *et al.*, 1995). Such associations would also improve the efficacy of MAS in breeding for drought tolerance in rice. Thus, DHLs developed from two rice lines, differing in root traits and OA, were used in this study to identify the QTL linked to rice performance under drought and to genetically dissect the nature of association between drought tolerant traits and yield under drought in the field.

Information of DT-QTLs for dissection of DT trait

Five genomic DT-QTLs on chromosomes 1, 3, 4, 8 and 9 carrying a cluster of DT-QTL identified in the CT9993-5-10-1-M (CT9993) and IR62266-42-6-2 (IR62266) doubled-haploid population (Babu *et al.*, 2003; Lanceras *et al.*, 2004) were chosen as the targets of introgression in this study. This population is most widely studied mapping populations, and suggests ways to develop cultivars that will perform well in drought prone ecosystem (Kamoshita *et al.*, 2008). All of five QTL segments cover the genetic distances of 49, 14.8, 53, 60 and 30 cM, respectively (Lanceras *et al.*, 2004). In the region of these DT-QTL segments are carrying QTLs of GY and important agronomic traits that related with drought stress (Kamoshita *et al.*, 2008). Future more, these regions are containing QTLs for other traits involving morphology and physiology.

Co-location of QTLs for plant-type traits, integrative traits, primary and secondary drought-tolerant traits.

Individual DT-QTL region also contained other QTLs for morpho-physiology traits. Zhang *et al*. (2001) reported that the marker interval R2417–RZ909 (64 cM) on chromosome 1 of rice has frequently been associated with grain yield, various drought tolerant traits, and plant types. Many QTLs for primary drought tolerant traits were identified under stress conditions in this region, including cell membrane stability (Tripathy *et al.*, 2000), osmotic adjustment (Lilley *et al.*, 1996; Robin *et al.*, 2003), and various root traits such as root thickness, maximum depth and penetration (Price *et al.*, 2002). Under well water conditions, QTLs for various deep and thick root traits were identified across different genetic backgrounds in this region (Ali *et al.*, 2000; Li *et al.*, 2005; Yadav *et al.*, 1997). This region also contains QTLs for secondary traits such as plant water status under stress; LWP and RWC (Babu *et al.*, 2003; Courtois and Lafitte, 2003; Jearakongman, 2005), as well as leaf rolling and leaf drying (Courtois and Lafitte, 2003; Price *et al.*, 2002). The positive effects of maintaining higher plant water status under stress came from the deeper rooting parents (such as Azucena, CT9993). QTLs for integrated traits such as delay in flowering time (Yue *et al.*, 2005), panicle exsertion rate, drought response index (DRI) (Jearakongman, 2005), and yield components such as PSS, spikelet fertility, weight per grain (Jearakongman, 2005; Yue *et al.*, 2005), as well as for GY (Kumar *et al.*, 2007), all under drought stress in the field, have been mapped in this region. This region is linked to plant-type QTLs with maximum of 46% variability in the data set, such as PH, panicle length, TN and PN, under both control and stress conditions across several mapping populations. This segment contains the position of *sd-1*, a major gene that controls semi-dwarfism that is widely used by IRRI because of its strong association with harvest index and responsiveness to fertilizer under well-watered conditions (Courtois and Lafitte, 2003).

Candidate genes and gene expression related to drought tolerance in rice

Many drought inducible genes with various functions have been identified by molecular and genomic analyses in rice and other plants, including a number of transcription factors that regulated stress inducible gene expression. The products of stress inducible genes may function in stress response and tolerance at the cellular level. Many genes via gene transfer resulted in improving plant stress tolerance (Garg *et al.*, 2002). Hazen *et al*. (2005) identified a number of drought inducible gene expressions using microarray analysis in susceptible and tolerant rice parents and found 622 transcripts were differentially expressed. Gorantla *et al*. (2007) identified expression profiles of drought responsive genes in leaf and panicle of rice. They have found a total of 125 genes expressed under drought stress in both organs. The functional classification of these 125 genes showed that a majority of them are associated with cellular metabolism, signal transduction, and transcriptional regulation.

The products of drought inducible genes identified through the recent microarray analyses could be classified into two groups (Shinozaki and Yamaguchi-Shinozaki, 1997).

1. The group of functional proteins; this group comprises proteins that most probably have functions in abiotic stress tolerance. These include molecules such as chaperones, late embryogenesis abundant (LEA) proteins, osmotin, antifreeze proteins, mRNA binding proteins, key enzymes for osmolyte biosynthesis, water channel proteins, sugar and proline transporters, detoxification enzymes and various proteases.

2. The group of regulatory proteins, this group comprises regulatory proteins such as protein factors involved in further regulation of signal transduction and stress responsive gene expression. These include various transcription factors, protein

kinases, protein phosphatases, enzymes involved in phospholipids metabolism, and other signalling molecules such as calmodulin binding protein.

Salinity tolerance in rice

Salinity tolerance (ST) is also a complex trait governed by polygenes, and has high heritability values (Gregorio and Senadhira, 1993). The inheritance of salt tolerance is co-inherited with undesirable agronomic characters (Heu and Koh, 1991). The QTL for ST were founded distributing on chromosomes 1, 3, 5, 6 and 7 (Gong *et al.*, 1999; Koyama *et al.*, 2001; Lee *et al.*, 2007). Many genes respond to drought and salinity stress were identified in many plants such as wheat (Peng *et al.*, 2009) and rice (Rabbani *et al.*, 2003). More than fifty genes were activated and expressed under both stress conditions, more than half of these genes were response to ABA hormone.

However, it is difficult to breed the ST rice varieties for several reasons. First, donors of salt tolerance are very poor because most rice varieties are sensitive to salt except for some traditional indica varieties such as Pokkali, Nona Bokra and Kararata (Lee *et al.*, 2007). Second, salt tolerance is a complex trait governed by QTLs making it difficult to understand mechanisms underlying ST. Finally, it is very difficult to evaluate physiological traits because some of them related to drought after responses

Chromosome segment substitution lines (CSSLs)

Chromosome segment substitution lines (CSSLs) as novel mapping populations which carry a particular chromosome segment from a donor line in the genetic background of recurrent line were developed for genetic studies. CSSLs can be used in genetic analysis to associate QTL with particular chromosome region and to quickly develop NILs of target regions containing QTLs of interests. Many researchers were used CSSLs for QTL mapping in various traits such as grain size (Kubo *et al.*, 2002), eating quality (Wan *et al.*, 2004), flowering date (Takai *et al.*, 2007). Although, development of these materials is laborious and time consuming but

this approach will facilitate for many researchers to perform QTL map-based cloning (Ebitani *et al.*, 2005). In rice, CSSLs were constructed in many populations with various genetic backgrounds (Doi *et al.*, 1997; Ebitani *et al.*, 2005; Kubo *et al.*, 2002; Takai *et al.*, 2007). Therefore, CSSLs will be candidate materials for future study to identify more accurately genetic regions for a complex trait such as DT. In this study, a set of CSSLs carrying the overlapping DT-QTL segments on five target chromosomes in KDML105 genetic background were developed through marker assisted selection (MAS). All CSSLs were used as genetic material to evaluate phenotypes under rainfed environment for finding association with their genotypes to re-analyze DT-QTLs and compare with previous map. This strategy will lead to understand DT genetic mechanisms with different resistance abilities and can be identified tightly linked markers or candidate genes regulating DT in rice.

In addition, we evaluated DT-QTL on five target chromosomes for evaluating the performance of ST using CSSLs that contained a different size of DT-QTL segments on chromosomes 1, 3, 4, 8 and 9. CSSLs that showed high performances under both drought and salinity stresses will be use as a new source of breeding materials in Thai rainfed lowland areas.

MATERIALS AND METHODS

Materials

Plant materials

1. For backcrossing

Twenty-eight introgression lines of BC_3F_2 displaying homozygous allele at three SSR loci on DT-QTLs which developed by Lanceras *et al.* (2007) were continued backcrossing to KDML105 for producing BC_4F_1 population. There were included 9, 7, 4, 3 and 5 lines which contained a single large DT-QTL segment on chromosomes 1, 3, 4, 8 and 9, respectively. Rice seeds of KDML105 were obtained from Ubon Ratchathani Rice Research Center. Seed of selected BC_3F_2 were obtained from Rice Gene Discovery unit (RGDU), Kasetsart University, KPS Campus.

2. For genome scanning

There were included two sets of plant material that were scanned on the whole genome. Set-1 is a set of 83 BC_5F_1 lines that were randomly selected. These lines displayed heterozygous genotype at three SSR loci on DT-QTL. All of lines in set-1 were scanned on the genome by 60 SSR markers. For set-2 included 90 CSSLs carrying a different size of DT-QTL segment. These materials were scanned on the genome using 131 polymorphic SSR markers which covered most of the whole genome.

3. For phenotyping under rainfed and irrigated conditions

A total of 90 CSSLs were evaluated agronomic phenotypes under rainfed and irrigated conditions at CPA and KPS in 2009 that there are the same materials in set-2. Individual lines were carried a different single DT-QTL segment cover 5 target chromosomes including 22, 15, 23, 20 and 10 lines for DT-QTLs on chromosomes 1,

3, 4, 8 and 9, respectively. These materials indentified as 83 haplotypes which were genotyped in BC_5F_2 and BC_5F_3. Five standard varieties were also evaluated too, including KDML105, CT9993, IR62266, DHL103 and DHL212.

4. For screening salinity tolerance

A set of 96 CSSLs indentify as 85 haplotypes carrying a different DT-QTL segment on five targeted chromosomes including chromosomes 1, 3, 4, 8 and 9 was evaluated salinity tolerance in this study. In each targeted chromosome were included 28, 15, 23, 20 and 10 lines of CSSLs, respectively. Nine standard varieties which were used to screen salinity tolerance were showed on Table 1.

Table 1 Nine rice cultivars were used as a standard check in salinity screening.

Cultivars	**Information**
KDML105	Genetic background of CSSLs, susceptible to drought and salt
CT9993	Drought tolerance cultivar
IR62266	Drought susceptible cultivar
DH103	Donor of drought tolerance
DH212	Donor of drought tolerance
IR29	Salt susceptible
Pokkali	Salt tolerance
FL496	Donor of salinity tolerance
FL530	Donor of salinity tolerance

Methods

Development of the CSSL population

The backcrossing MAS process for the CSSLs is shown in Figure 3. A famous Thai aromatic rice cultivar KDML105 used as the recipient parent were crossed with two donor DHLs, IR68586-F_2-CA-31 (DHL103) and IR68586-F_2-CA-143 (DHL212). These two donors were derived from a cross between CT9993 and IR62266. These adaptive donors carried CT9993 alleles at the QTL locations on chromosomes 1, 3, 4 and 9, and also IR62266 alleles on chromosome 8. The development of F_1 to BC_3F_1 generations was provided by Lanceras *et al*. (2007). The continued backcrossing was conducted in this study until BC_5F_1 generation. In each backcrossing generation, two to five heterozygous plants in which the target chromosome segment (as male parent) were randomly selected and then back crossed to KDML105 (as female parent). Approximately 100 plants per target chromosome in each generation were generated. The criteria for selection in backcross generations were as follow: a single, large chromosome segment of donors was substituted in the target chromosome which will be display heterozygous at selectable markers on its target region. Fifty BC_5F_1 plants carrying completely single target DT-QTLs and displaying highly KDML105 genetic background will be self-pollinated to generate BC_5F_2 to BC_5F_4 populations. In each selfing generation, a total of 2000 plants will be selected for genotyping.

In segregated BC_5F_2, one set of population was grown under drought stress condition at CPA in 2007. The plants displaying segregated phenotypes were collected for genotyping to increase the chance to get different genotypes. This strategy can limit the number of genotyping plants and largely sped up genotype analysis for population development, reduced workload and cut down genotyping expanse.

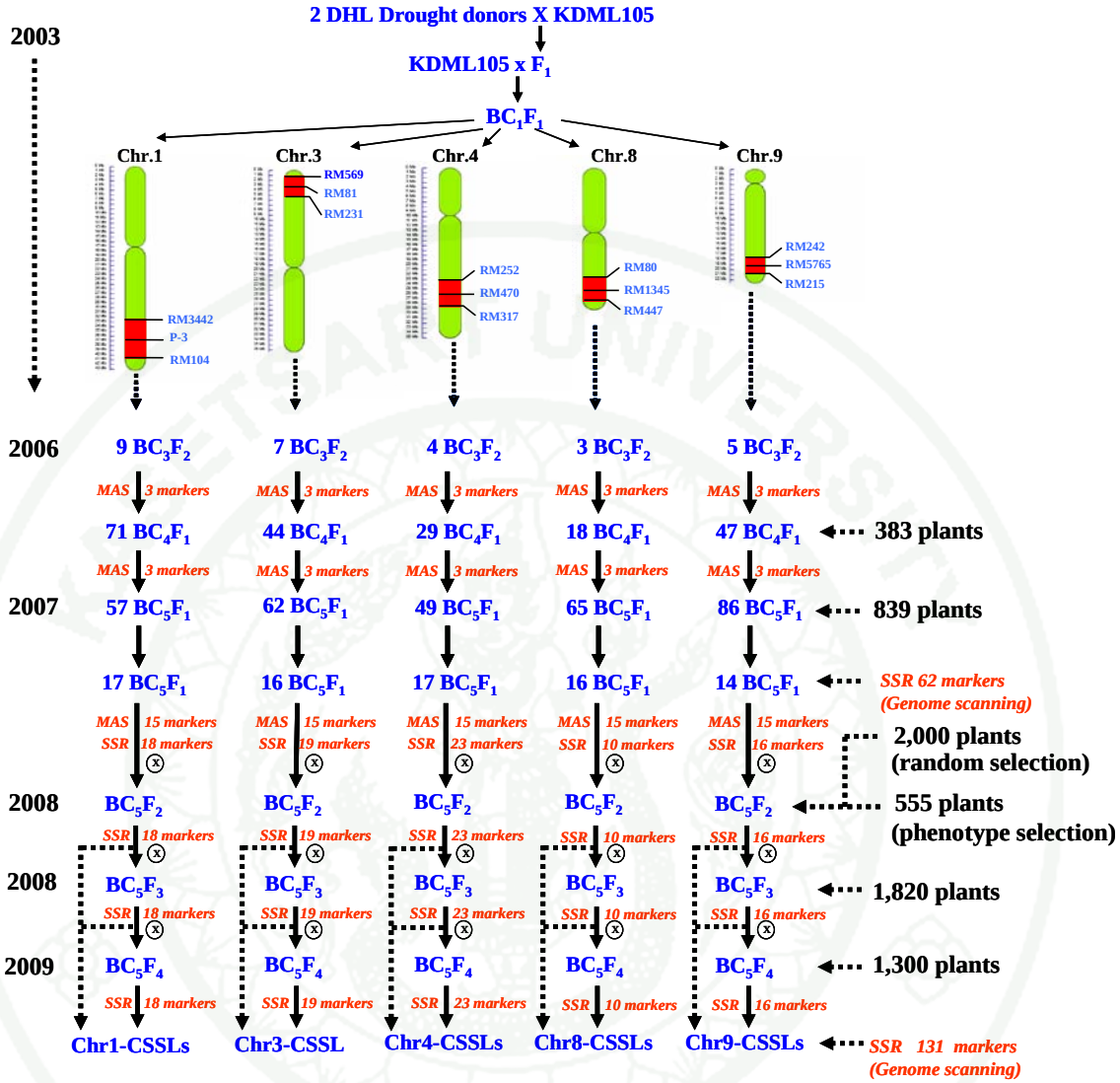

Figure 3 Backcross breeding scheme for developing chromosome segment substitution lines (CSSLs) of DT-QTLs in KDML105 genetic background.

Genotyping

The genomic DNA from the young leave tissue of backcross and selfed progenies in each generation was extracted by DNA-Trap® method. Genomic DNA of progenies was amplified by PCR for production of target markers. PCR was performed in a 10 ul reaction mixture containing 50 ng template DNA, 2.5 µM of each primer, and 200 µM of each dNTP, 2.5 mM $MgCl_2$, 1 unit *Taq* polymerase and 1

µl of × 10 PCR reaction buffer. Amplification was performed for 35 cycles (30 sec at 94°C, 30 sec at 55°C and 2 min at 72°C) followed by 5 min at 72°C. Each target chromosomes from all these selected lines were genotyped with 3 polymorphic SSR markers and electrophoresis on 4.5% denaturing polyacrylamide gel were detected by the silver staining method.

1. Initial target survey

Three loci of the anchor SSR markers/target chromosome were used genotyping in the backcrossing progenies in BC_4F_1 and BC_5F_1 generations for selecting the lines carrying the interested QTL (Table 3).

2. Fine target survey

The genotypes within target region of selfing progenies was assayed by 18, 19, 23, 10 and 16 SSR markers for the target 1, 3, 4, 8 and 9, respectively (Figure 4). The position and its information of these SSR markers have reported in Gramene database (http://www.gramene.org). The genotype selection in these generations will be emphasized on overlapping a small DT-QTL segment in isogenic lines of KDML105.

Table 2 SSR markers were used genotyping on backcross progenies of F_1 to BC_5F_1 generations.

Targeted Chr.	Polymorphic markers			Genetic distance (cM)		Size (position)
Chr 1-QTL	RM3442	P-3	RM104	49	9.99	(33.38-43.37) Mbp
Chr 3-QTL	RM569	RM81	RM231	14.8	3.46	(0.35-3.81) Mbp
Chr 4-QTL	RM252	RM317	RM470	53	14.62	(20.50-35.12) Mbp
Chr 8-QTL	RM515	RM80	RM447	60	7.06	(21.01-28.07) Mbp
Chr 9-QTL	RM215	RM5765	RM242	30	5.00	(17.72-22.72) Mbp

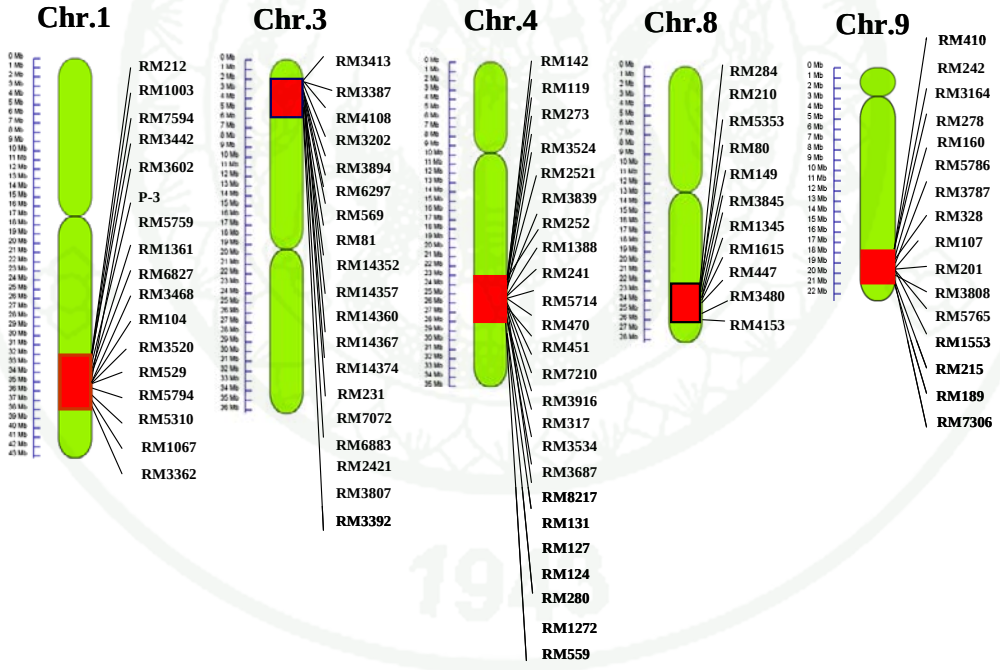

Figure 4 86 SSR markers on DT-QTL regions that were used to genotyping in BC_5F_1 to BC_5F_4 generations

3. Genome scanning

Two sets of SSR markers outside the target regions and displaying polymorphisms were used to scan on the whole genome.

First, a total of 60 SSR markers outside the target regions and displaying polymorphisms were selected to identify the recurrent parent background of 80 selected BC_5F_1 progenies. Lines with high identity to KDML105 and displaying heterozygous at 3 flanking markers on each DT-QTL fragment were selected to generate BC_5F_2 progenies.

Second, a total of 131 well-distributed polymorphic SSR markers were chosen for genome scan. These markers were used in the whole-genome survey of a set of 90 representative genotype lines for estimate the proportion of CT9993 and IR62266 genomes which remaining in the non-target areas (Figure 5).

For the market value in the future, cooking qualities is important to select although it was not associated with DT. Fragrance, softness and gelatinization temperature were screened in CSSL population. The selection criteria for cooking qualities based on KDML105 qualities. Aromarker used to separate aromatic rice from non-aromatic rice. It was developed based on an eight bps deletion in the exon7 of the *Os2AP* or *BADH2* gene which encodes a putative betain aldehyde dehydrogenase 2 (Vanavichit, 2007). WAXY and GT11 on chromosome 6 are specific gene markers developed by RGDU. There are specific to amylose content (AC) and gelatinization temperature (GT), respectively and were used to select starch properties like KDML105 in progeny population (Table 3).

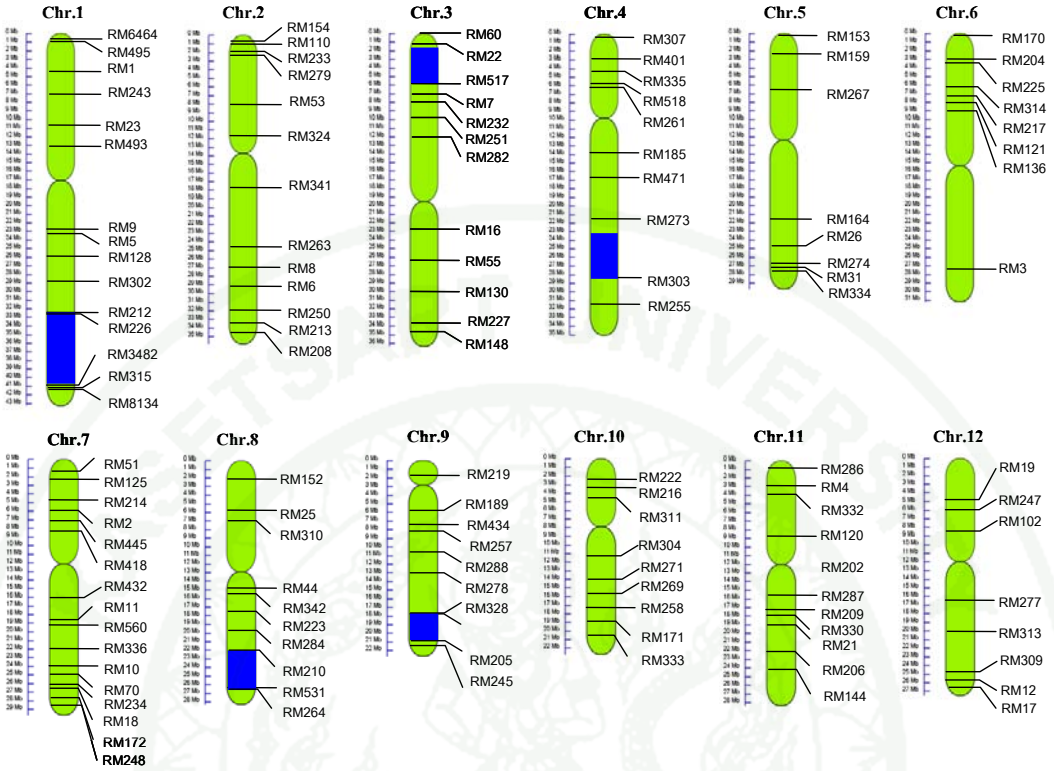

Figure 5 131 well-distributed polymorphic SSR markers were used in the whole-genome survey of a set of 90 representative genotype lines.

Table 3 Molecular markers for selection of cooking qualities.

Markers	Types	Chr.	Traits	Primer sequence (5'→3')
Aromarker	INDEL	8	Fragrance	F-TGCTCCTTTGTCATCACACC
				R-TTTCCACCAAGTTCCAGTGA
WAXY	SSR	6	AC	F-GTAAAATGTGTTGCGGAGG
				R-GGAAAAACGAGCAATGAAA
GT11	SSR	6	GT	F-CGAGCGAGGGTTTACTGTTC
				R-GGAGGAAACAGCAGCAACTC

Preliminary evaluation of phenotypes in CSSL population

A set of 90 CSSLs carrying partial/complete DT-QTLs in KDML105 genetic background were evaluated using phenotypic variation under irrigated (non stress) and rainfed (stress) conditions in 2009. The experiment was conducted at two different locations: Trial 1 at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom (KPS, Central of Thailand) and Trial 2 at Chumpae Rice Research Center (CPA, Northeast of Thailand). The experiment design was a randomized complete block design (RCBD) with four replications. Experimental plot sizes in Trial1 and Trial2 were 1.25 x 2.25 and 2 x 2.50 m^2, respectively. The spacing between plants was 0.25 and 0.20 m, respectively. For non stress condition, all plots received sufficient water by irrigation to maintain soil moisture until maturity. In contrary, stress condition, water was drained out from the field at 84 days after sowing (DAS) in Trial 1 and 77 DAS in Trial 2. These incidents were induced to start water stress until harverst. Time schedules of rainfed condition in both of experiments were presented in Figure 6.

Phenotypic data were collected from CSSLs in both trials. Flowering date (DF) was the days when panicles were visible on half of the hills in the plot or 50 % flowering plant in each plot. Delay in flowering (DeF) was determined as the difference between flowering dates under stress and those under irrigated condition. At harvest, yield and its components including plant height (PH), tiller number per hill (TN), panicle number per hill (PN), filled grain weight (FGW), unfilled grain weight (UFGW) and total grain weight (TGW) from two plants under well-watered and two plants under stress condition were collected follow by Zou *et al.* (2005). Grains were threshed from the sampled plants, dried in a oven at 80^oC, and then weighed to determine 1,000-grain weight (1,000GW) and grain yield/plot which was converted to grain yield/rai (1,600 m^2). Filled and unfilled spikelets (FGN and UFGN) were measured from five panicles to determine the total spikelet number per panicle (TSN). Percentage of spikelet sterility (PSS) was calculated from the FGN and TSN.

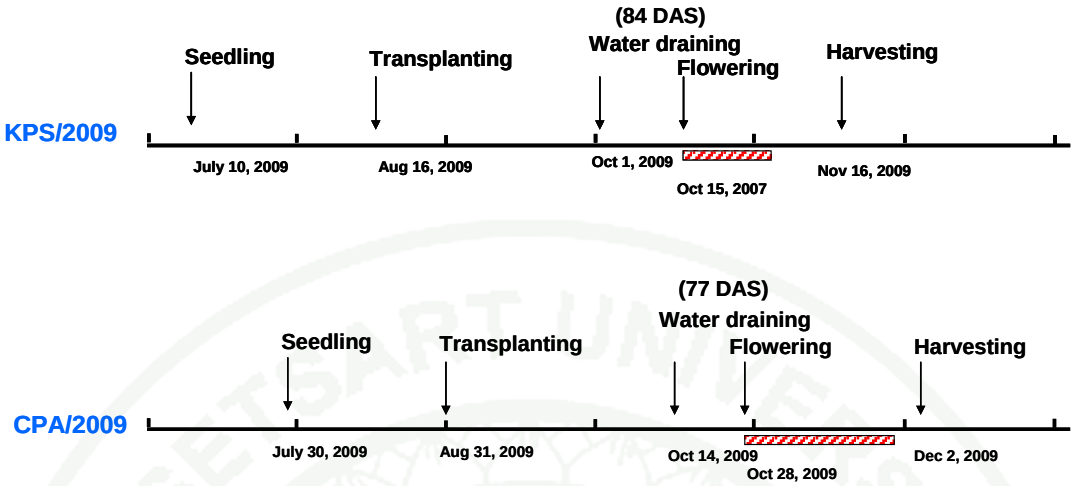

Figure 6 Time schedules of rainfed condition in both of experiments. Horizontal shade bars indicates flowering time of all genotypes.

Data and QTL analysis

All data were analyzed genetic association based on ANOVA by STATGRAPHIC plus 3 program. Least Significant Difference (LSD) in STATGRAPHIC plus 3 was used to compare mean of measured traits among the CSSLs. QTLs for drought related traits were constructed by single marker analysis and confirmed with R^2 from regression analysis. The new DT-QTLs map was compared with a previous map from CT9993/IR62266 population

Screening for salinity tolerant ability in CSSL population

A total of 105 CSSLs carrying a different size of DT-QTL fragment were evaluated for salt tolerant ability to prove the shared mechanism between DT and ST. The experiment was conducted using RCBD design with 4 blocks. The plant materials were directly germinated in artificial soil culture. One five-day old seedling was grown in plastic tray about 6 well. This plastic tray is present 6x12 well; each well containing wet soil 1.5 $inch^3$. After 19 days of germination, seedlings were exposed to

salinity stress (150 mM NaCl) two plant nutrient solutions were applied with a ratio 1:200 follow by Bangsai Agricultural Center Co. Ltd. After 16 days of salt treatment, salt injury symptom was scored from 6 plants per one experimental unit. Salt injury scores (SIS) were following as IRRI (2002).

1 = Growth and tillering nearly normal

2 = Growth nearly normal but there is some reduction in tillering and some leaves discolored (alkali)/ whitish and rolled (salt)

5 = Growth and tillering reduced; most discolored (alkali)/ whitish and rolled (salt); only a few elongating

7 = Growth completely ceases; most leaves dry; some plants drying

9 = Almost all plants dead or drying

SIS data was scored from 6 plants per one experimental unit. LSD in STATGRAPHIC plus 3 was used to compare mean of measured traits among the CSSLs. QTLs for ST traits were constructed by single marker analysis.

RESULTS AND DISCUSSION

Results

Development and characteristic of CSSLs

A total of 196 heterozygous lines were detected in a population of 383 BC_4F_1 representing to 51.2% which was close to the theoretical value**.** These heterozygous lines were sufficiency selected for backcrossing to KDML105 for generated BC_5F_1 progenies. In BC_5F_1, 839 backcross progenies were produced and genotyped by SSR markers similar with previous generation. A total of 319 heterozygous lines were found in this generation, representing to 38%. This was less than the expected value which should be theoretically value of 50% (Table 4).

Table 4 Summary of backcrossing progenies that were genotyped in BC_4F_1 and BC_5F_1 generations and percent of discovery target genotype by MAS.

Generation	No. of progenies	No. of homozygous lines	Discovery of target genotype by MAS (%)	No of heterozygous for crossing
BC_4F_1				
Chr.1	111	71	64.0	24
Chr.3	105	44	41.9	9
Chr.4	70	29	41.4	5
Chr.8	34	11	32.4	5
Chr.9	63	41	65.1	13
Total	383	196	51.2	56
BC_5F_1				
Chr.1	154	57	37.0	10
Chr.3	169	62	36.7	10
Chr.4	123	49	39.8	10
Chr.8	181	65	35.9	10
Chr.9	212	86	40.6	10
Total	839	319	38.0	50

A total of 944 CSSLs which were identified as 104 haplotypes were developed in this study. Individual haplotypes carried different single DT-QTL segments spanning 5 target chromosomes including 23, 16, 33, 21 and 11 haplotypes for DT-QTLs on chromosomes 1, 3, 4, 8 and 9, respectively. These haplotypes displayed distingue different graphical genotypes which were determined by using 86 SSR markers located on DT-QTLs. Most of individual CSSLs for chromosomes 1, 3, 4 and 9, only one chromosome segment of DHL212 donor were substituted in the KDMl105 genetic background. For chromosome 8, substituted chromosome segment was DHL103 replacing on its DT-QTLs. The substituted chromosome segments in the CSSLs covered most of DT-QTL region on 5 target chromosomes, excluding middle region of DT-QTLs on chromosome 9 that displaying homozygous KDML105 allele between RM107 and RM108 markers (Figure 7). In addition, some small region on DT-QTLs of chromosomes 1 and 4 remained homozygous KDML105 allele in their CSSLs (RM341 on chromosome 1 and RM317 on chromosome 4). This is because recombination had occurred within each target region during the process of MAS. Additionally, some CSSLs carried homozygous donor allele not only at the target region but also undesirable region such as CSSL-11 favoring chromosome 1 was exposed homozygous donor allele RM529 and RM81 on chromosome 1 and 3, respectively.

Number of selecting plant for MAS and homozygous genotype in each selfing generation are shown in Table 5. In the segregating BC_5F_2 generation, the chance to have the desirable genotypes was increased by selection for segregating phenotypes under rainfed condition. Seventy five lines that display homozygous allele at target DT-QTL were found out of 555 selected plants, representing 13.51%. For BC_5F_2 of 2,000 randomly selected plants showed the homozygous genotypes of 172 lines, representing 8.6%. Genotype patterns of these two populations can be identified as 25 and 27 haplotypes, respectively, which were similar (Figure 8). Almost of homozygous donor lines that were found in BC_5F_2 contained a large DT-QTL segment. The lines which carried small DT-QTL segment were found to a small number. This result may be affected from the incidence of low recombination.

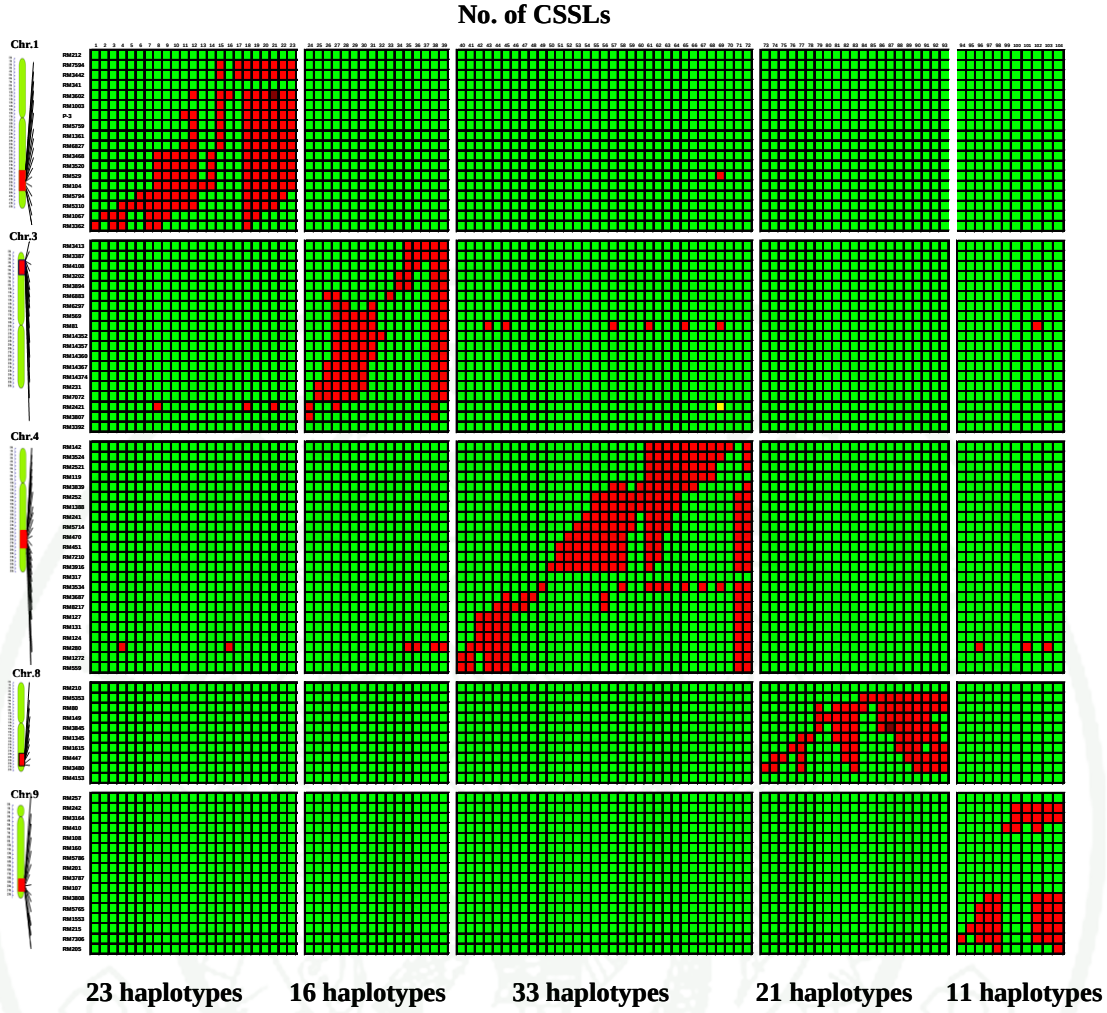

Figure 7 Graphical genotypes of 104 CSSLs carrying DT-QTLs on chromosomes 1, 3, 4, 8 and 9. The red regions indicate the regions homozygous of donor alleles; the green regions indicate the regions homozygous KDML105 allele, yellow indicate the regions heterozygous and brown indicates the regions off types.

In BC_5F_3 and BC_5F_4, lines displaying homozygous genotype of donor allele at DT-QTL were found which size of DT-QTL segment in these lines are smaller than previous generation. Selections in these generations were used twenty plants for genotyping per one target genotype, which there were enough for receiving the homozygous genotypes.

Genotypic patterns	Genotypes of Chr.1 Markers																	No. of homozygous lines		
	RM212	RM1003	RM7594	RM3442	RM3602	P-3	RM5759	RM1361	RM6827	RM3468	RM104	RM3520	RM529	RM5794	RM5310	RM1067	RM3362	Selected	Randomized	Total
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	1	1	2
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	3	-	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	-	1	1
4	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	-	2	2
5	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	7
6	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	1	1	2
7	1	3	3	3	3*	3	3	3	3	3	3	3	3	3	3	1	1	-	1	1
8	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1
																Total		6	11	17

Genotypic patterns	Genotypes of Chr.3 Markers																	No. of homozygous lines		
	RM3413	RM3387	RM4108	RM3202	RM3894	RM6297	RM569	RM81	RM14352	RM14357	RM14360	RM14367	RM14374	RM231	RM7072	RM6883	RM2421	RM3807	RM3392	Selected	Randomized	Total
9	1	1	1	1	1	3	3	5	3	3	3	3	3	3	3	1	1	1	1	2	9	11
10	1	1	1	1	1	3	3	5	3	3	3	3	3	3	3	3	1	1	1	7	25	32
11	3	3	3	3	3	3	3	5	3	3	3	3	3	3	3	3	1	1	1	2	-	2
12	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3	12	15
																		Total		14	46	60

Genotypic patterns	Genotypes of Chr.4 Markers																			No. of homozygous lines		
	RM142	RM119	RM3524	RM2521	RM3839	RM252	RM1388	RM241	RM5714	RM470	RM451	RM7210	RM3916	RM317	RM3534	RM3687	RM8217	RM131	RM127	RM124	Selected	Randomized	Total
13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	3	-	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	-	1
15	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	-	1	1
16	1	1	1	1	1	3	3	5	5	3	3	5	5	1	3	3	-	3	3	3	-	1	1
17	1	1	1	1	1	5	5	5	5	3	3	5	5	1	3	5	5	3	3	3	1	-	1
18	1	1	1	1	1	5	5	5	5	3	3	5	5	1	3	5	5	1	1	1	1	-	1
19	1	1	1	1	5	5	5	5	5	3	3	5	5	1	3	1	1	1	1	1	-	1	1
20	1	1	5	1	5	5	5	5	5	3	3	5	5	1	1	5	5	3	3	3	1	-	1
21	3	3	3	3	3	3	3	3	3	3	3	3	3	1	3	1	1	1	1	1	15	14	29
22	3	1	5	1	1	5	5	5	5	3	3	5	5	1	3	1	1	1	1	1	1	-	1
23	3	1	5	1	1	5	5	5	5	3	3	5	5	1	3	5	1	1	1	1	6	-	6
24	3	1	5	5	5	5	5	5	5	3	3	5	5	1	1	1	1	1	1	1	1	1	2
25	3	1	5	5	5	5	5	5	5	3	3	5	5	1	1	5	1	1	1	1	1	-	1
26	3	1	5	5	5	5	5	5	5	2	3	5	5	1	0	5	1	1	1	1	1	-	1
27	3	1	5	5	5	5	5	3	5	3	3	5	5	1	3	3	5	3	3	3	-	3	3
																			Total		29	22	51

Genotypic patterns	Genotypes of Chr.8 Markers									No. of homozygous lines		
	RM210	RM5353	RM80	RM149	RM3845	RM1345	RM1615	RM447	RM3480	RM4153	Selected	Randomized	Total
28	1	1	1	1	1	1	5	1	1	1	-	3	3
29	1	1	1	1	1	5	1	1	1	1	1	-	1
30	1	1	1	5	1	1	1	1	1	1	-	1	1
31	1	1	5	5	5	5	5	5	5	1	5	3	8
32	1	5	5	1	5	5	5	5	5	1	1	-	1
33	1	5	5	5	5	5	5	5	5	1	4	-	4
34	1	5	5	5	5	5	1	5	5	1	-	36	36
									Total		11	43	54

Genotypic patterns	Genotypes of Chr.9 Markers															No. of homozygous lines		
	RM257	RM410	RM242	RM3164	RM108	RM160	RM5786	RM3787	RM107	RM201	RM3808	RM5765	RM1553	RM215	RM7306	RM205	Selected	Randomized	Total
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	-	1
36	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	-	1	1
37	1	1	1	1	1	1	1	1	1	1	3	3	3	3	3	1	3	12	15
38	1	1	3	3	1	1	1	1	1	1	3	3	3	3	3	1	1	-	1
39	1	3	3	3	1	1	1	1	1	1	3	3	3	3	3	1	10	35	45
40	1	3	3	3	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1
41	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1	1
																Total	15	50	50

Figure 8 Summary of graphical homozygous genotypes of selected and randomized BC_5F_2 lines carrying DT-QTLs on chromosomes 1, 3, 4, 8 and 9.

Table 5 Summary of selfing progenies in BC_5F_2 to BC_5F_4 generations that were genotyped by SSR markers located on DT-QTL regions and percent of homozygosity.

Generation	No. of progenies	No. of homozygous	No. of haplotypes	No of heterozygous for selfing	%homo-zygosity
BC_5F_2					
Chr.1	400	11	8	28	
Chr.3	400	46	3	15	
Chr.4	400	22	7	20	
Chr.8	400	43	4	22	
Chr.9	400	50	5	7	
Total	2,000	172	27	92	8.6
Selected BC_5F_2					
Chr.1	109	6	3	-	
Chr.3	137	14	4	-	
Chr.4	135	29	10	-	
Chr.8	75	11	4	-	
Chr.9	99	15	4	-	
Total	555	75	25	-	13.51
BC_5F_3					
Chr.1	560	56	16	14	
Chr.3	300	44	10	8	
Chr.4	400	82	19	26	
Chr.8	420	94	16	13	
Chr.9	140	21	5	4	
Total	1,820	297	66	65	16.32
BC_5F_4					
Chr.1	280	104	16	-	
Chr.3	160	58	7	-	
Chr.4	520	211	25	-	
Chr.8	260	138	11	-	
Chr.9	80	31	7	-	
Total	1,300	542	66	-	41.69

Genome coverage and uniformity of genetic background in CSSL population

A total of 90 CSSLs (82 haplotypes) were scanned with 131 polymorphic SSR markers covered the whole genome for estimating the proportion of recipient alleles in the non-target areas. Individual CSSL was shown the recovering of KDML105 genome ranging from 88.50 to 100% with an average of 96.30% (Table 6) after five backcross generations. This was non significant to the expected value, in which the recipient genome is theoretically recovered to 98% in BC_5. The proportion of donor alleles remaining in the non target areas is very low. The frequency of donor alleles in the non target regions of each chromosome ranged from 0.09% on chromosome 10 to 10.72% on chromosome 4, with an average of 3.70% (Table 7) which was in the range expected for the backcross stage reached. For cooking qualities, all of CSSLs displayed homozygous KDML105 alleles at Aromarker, WAXY and GT11 loci. This result indicated that all CSSLs are fragrance and soft rice like KDML105.

Table 6 The percentage of KDML105 recurrent parent of 90 CSSLs (82 haplotypes) that derived from the genome scanning using SSR markers located on non-target QTL and distribute on rice genome.

Chr.	No. of CSSLs	SSR markers	% KDML105 genetic background		
			Min	Max	Average
Chr.1	22	113	93.81	100.00	97.12
Chr.3	15	113	95.58	100.00	98.02
Chr.4	23	113	92.04	100.00	96.34
Chr.8	20	115	89.57	100.00	95.65
Chr.9	10	113	88.50	99.12	93.09
	90	**131**	**88.50**	**100.00**	**96.30**

Table 7 The proportion of donor alleles at non DT-QTL region.

Chr.	No. of SSR markers for genome scanning	Proportion of Donor alleles (%)
Chr.1	15	5.93
Chr.2	13	1.24
Chr.3	12	0.69
Chr.4	10	10.72
Chr.5	8	3.19
Chr.6	9	0.48
Chr.7	17	4.64
Chr.8	10	1.60
Chr.9	9	4.00
Chr.10	9	0.09
Chr.11	11	0.80
Chr.12	8	3.00
Total	**131**	**average = 3.7 %**

Comparison of the genetic background between CSSL and BC_5F_1 populations.

The frequency of KDML105 alleles at the non target loci for the individual BC5F1 ranged from 91.51 to 100% with an average of 96.92% and these lines also displayed heterozygous genotypes at three SSR loci on DT-QTL region. The mean values of BC_5F_1 carrying DT-QTLs on chromosomes 1, 3, 4, 8 and 9 are 97.98% 97.33% 96.48% 96.46% and 96.37%, respectively. And when compared with the mean of CSSLs which had an average of about 96.30%, the mean value was not significantly different. It might be because the donor allele was found outside the target QTL were homozygous donor allele is fixed already. When CSSLs were scanned on the genome by 131 SSR markers, donor alleles were found again. Therefore, averages of percent genetic background in BC_5F_1 and CSSL populations is non different.

Drought severity of experiments

Severity of drought at reproductive stage in both of experiments including KPS and CPA were measured by the decrease in yield under stress compared to well water condition. Soil moisture content was measured (Figure 9). The results showed that drought stress did not happen at KPS. Grain yield of KDML105 under drought conditions decreased 3.21% compared with a complete water treatment. When measuring the moisture content in the soil of the plot, the data showed that the KPS has higher moisture content in soil during the flowering stage due to high rainfall, and moisture content in the soil began to decrease at the beginning of a grain filling. Because it is not affected by drought that occurred late season, grain yield are not much lower.

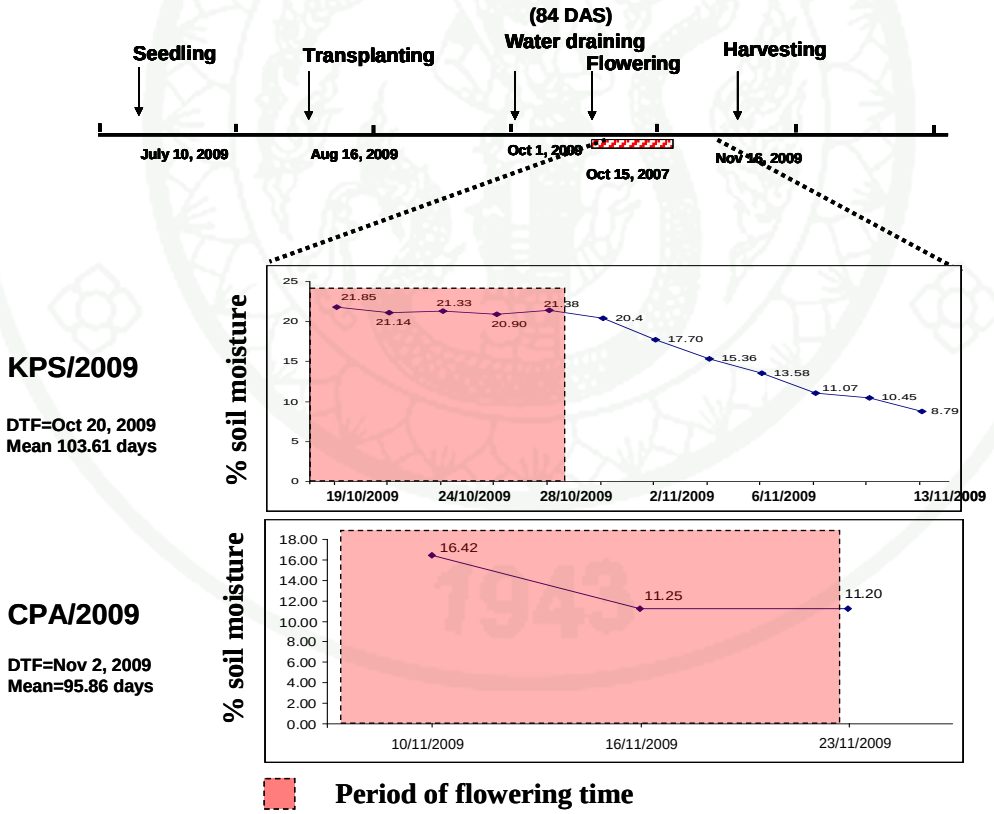

Figure 9 Soil moisture content in both of stressed field and the flowering date of CSSLs.

At CPA, severity of drought stress in field is medium severity. The yields of KDML105 under drought decreased 30.10% when compared with well water condition. The soil moisture content in stressed field was relatively low during the flowering stage. Grain yield was affected by drought that occurred. Effects of drought resulted in delayed flowering more in CSSL population. The number of unfilled grains and the percentage of spikelet sterility increase.

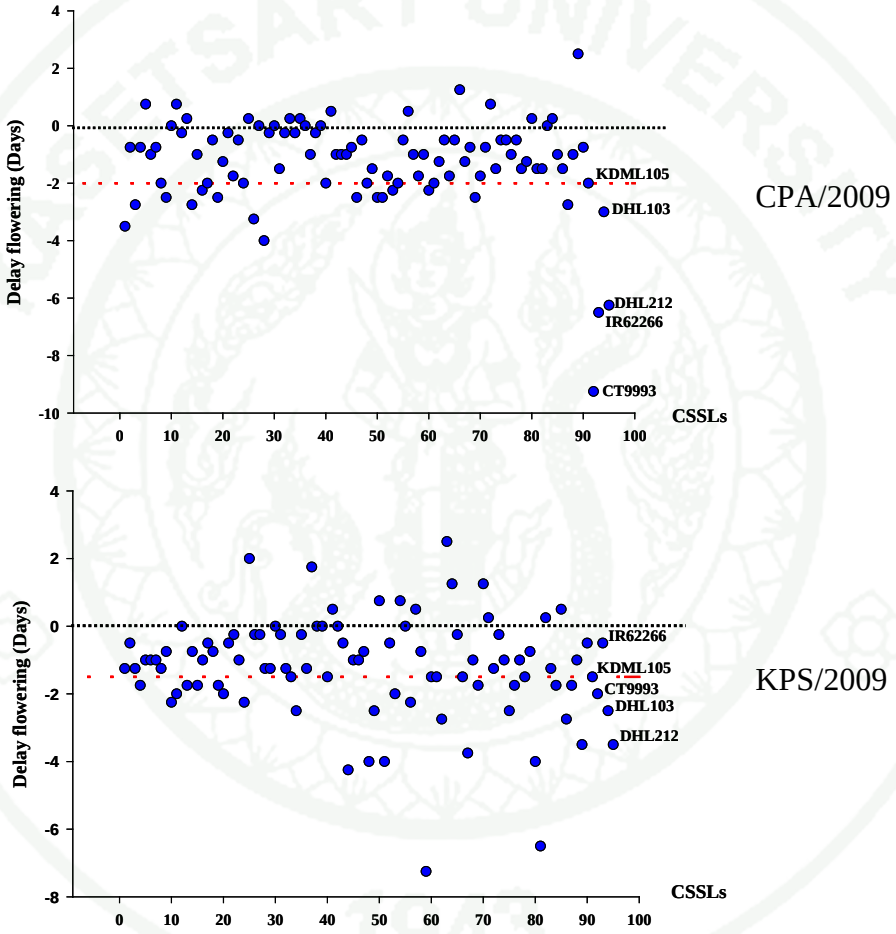

Figure 10 Delay flowering of 90 CSSLs under rainfed condition at CPA and KPS in 2009.

Delay flowerings (DeF) of 90 CSSLs and their parents were analyzed for measuring the effect of drought on the flowering date. In the experiment at CPA, DeF was found ranging from -4 (no delay) to 2.5 days in the CSSLs while KDML105

showed 2 days early in flowering. For KPS experiment, DeF was found ranging from -7.25 (no delay) to 2.5 days in the CSSLs while KDML105 showed 1.5 days early in flowering. As mentioned above**,** DeF due to stress was not observed in both experiments, but most of lines displayed early flowering to escape drought. This will be determined from the average of DeF showing minus values (Figure 10). Another theme, it is possible that the mind stress at CPA and like aerobic condition at KPS, induced an early of flowering in CSSLs. And it is difficult to know whether the target chromosome relating to delay flowering in the CSSL population.

Phenotypes variation of drought related traits in the CSSL population

ANOVA analysis showed the variations in grain yield and yield components in both of stressed and well watered conditions of 90 CSSLs carrying a DT-QTL segment with different sizes. The significant difference was detected among CSSLs and the recurrent parent KDML105 (Table 8 and 9). Mean grain yield and its components of the parents and the population are presented in Table 10. The average of traits such as GY, FGW, TGW, FGN and TSN were higher than it of KDML105 under well water condition (Figure 11). Under stress condition, The TSN value was 146.9 spikelets in DHL212 and 118.8 spikelets in KDML105. It ranged from 93.6 to 180.9 among the 90 CSSLs (Figture 12). Especially 23 CSSLs carrying DT-QTL on chromosome 4, showed higher TSN than KDML105 ranging from 98.8 to 180.9 spikelets with average of 130.2 spikelets. The TSN value was lower than that of KDML105 in lines CSSLs-39, -40, -41, -44, -45, -48 and -51, but higher in lines CSSLs-38, -42, -43, -49, -56, -57, -58 and -60. It also showed the TSN value is higher in lines CSSLs-46, -47, -50, -52 -53 -54, -55 and -59 than both of parents. In addition, we founded that undesirable traits such as UFGW, FGN and PSS were average higher than KDML105 (Figure 13). This indicates that the DT-QTL segment with different sizes, which is introgressed into KDML105 affect grain yield and yield components differently and carried desirable and undesirable traits.

Table 8 ANOVA results estimated for agronomic traits of 90 CSSLs that were evaluated under rainfed and irrigated conditions at CPA.

Source of variance	df	DF	PH	TN	PN	GY	FGW	UFGW	TGW	FGN	UFGN	TSN	PSS	1000GW
Rainfed condition														
Replication	3	ns	**	ns	ns	**	**	ns	**	ns	ns	ns	ns	**
Entry	94	**	**	**	**	**	**	**	*	ns	**	**	**	**
Between DT-QTL	6	**	**	ns	ns	**	**	**	**	*	**	**	**	**
Within DT-QTL	89													
Between entry in chr.1	21	**	**	**	**	ns	ns	**	ns	ns	**	*	ns	**
Between entry in chr.3	14	ns	**	ns	ns	ns	ns	**	ns	ns	**	ns	*	**
Between entry in chr.4	22	**	**	ns	ns	ns	ns	**	ns	ns	ns	*	**	ns
Between entry in chr.8	19	*	*	ns	ns	ns	ns	*	ns	*	**	**	*	**
Between entry in chr.9	9	ns	**	*	*	ns	ns	**	ns	ns	**	**	**	**
Between parental lines	4	**	**	ns	ns	**	**	ns	**	ns	**	**	**	**
Irrigated condition														
Replication	3	**	**	ns	ns	**	**	*	**	**	ns	**	**	ns
Entry	94	**	**	**	**	**	**	**	**	**	**	**	**	**
Between DT-QTL	6	**	**	**	**	**	**	**	**	**	**	**	**	ns
Within DT-QTL	89													
Between entry in chr.1	21	**	**	ns	ns	**	ns	**	ns	ns	**	*	**	**
Between entry in chr.3	14	**	**	*	*	**	ns	ns	ns	ns	**	ns	**	**
Between entry in chr.4	22	**	**	ns	ns	**	ns	**	ns	*	**	**	**	**
Between entry in chr.8	19	ns	**	ns	ns	**	ns	*	ns	ns	ns	**	ns	**
Between entry in chr.9	9	**	**	ns	ns	ns	ns	ns	ns	**	*	**	*	ns
Between parental lines	4	**	**	ns	ns	**	ns	*	ns	**	**	**	**	**

* and ** represent the significance levels of P = 0.05 and 0.01.

Table 9 ANOVA results estimated for agronomic traits of 90 CSSLs that were evaluated under rainfed and irrigated conditions at KPS.

Source of variance	df	DF	PH	TN	PN	GY	FGW	UFGW	TGW	FGN	UFGN	TSN	PSS	1000GW
Rainfed condition														
Replication	3	**	ns	**	**	ns	ns	*	ns	**	*	**	*	**
Entry	94	**	**	**	**	**	**	**	**	**	**	**	**	**
Between DT-QTL	6	**	**	**	**	**	**	**	**	**	**	**	**	**
Within DT-QTL	89													
Between entry in chr.1	21	**	**	**	**	**	**	**	**	**	**	ns	**	**
Between entry in chr.3	14	**	**	ns	ns	ns	ns	**	ns	ns	**	**	ns	**
Between entry in chr.4	22	**	**	**	**	**	**	**	*	ns	**	**	**	**
Between entry in chr.8	19	**	**	ns	ns	*	ns	**	ns	**	**	**	**	**
Between entry in chr.9	9	**	**	ns	ns	*	*	ns	ns	ns	ns	*	ns	**
Between parental lines	4	**	**	**	**	**	**	ns	**	ns	**	**	*	**
Irrigated condition														
Replication	3	**	**	**	**	**	ns	**	ns	**	**	**	**	**
Entry	94	**	**	**	**	**	**	**	**	**	**	**	**	**
Between DT-QTL	6	**	**	**	**	**	**	**	**	**	**	**	**	**
Within DT-QTL	89													
Between entry in chr.1	21	**	**	ns	ns	**	*	**	*	ns	**	**	ns	**
Between entry in chr.3	14	**	**	ns	ns	*	ns	ns	ns	ns	*	**	ns	**
Between entry in chr.4	22	**	**	*	**	**	**	**	**	**	**	**	**	**
Between entry in chr.8	19	*	**	ns	ns	ns	ns	*	ns	ns	**	**	**	**
Between entry in chr.9	9	**	**	**	**	ns	ns	ns	ns	*	**	**	ns	**
Between parental lines	4	**	**	**	**	**	**	ns	**	ns	*	*	ns	**

* and ** represent the significance levels of P = 0.05 and 0.01.

Table 10 Summary statistics of phenotypic performance of the CSSL population and its parent across two water levels in 2 locations.

Traits/ Locations/ coditions	Variation									
	Parents			The CSSL population						
	KDML105	DH103	DH212	Min	Max	Mean	SD	h^2	LSD	CV(%)
DF(day)										
KPS-IR	103.25	112.00	111.25	100.25	115.50	104.63	2.77	0.83	0.57	2.65
KPS-DS	101.75	109.50	107.75	99.00	112.00	103.46	2.63	0.79	0.54	2.54
CPA-IR	97.25	120.00	112.75	92.75	102.75	96.29	2.25	0.83	0.46	2.33
CPA-DS	95.25	117.00	106.50	85.75	109.75	95.34	2.93	0.39	0.60	3.07
PH(cm)										
KPS-IR	147.50	131.25	124.63	116.88	174.88	147.18	12.17	0.84	2.51	8.27
KPS-DS	150.00	131.63	121.75	121.63	181.00	149.49	12.62	0.85	2.60	8.44
CPA-IR	98.25	86.75	83.88	75.13	121.25	97.33	8.68	0.78	1.79	8.92
CPA-DS	100.88	85.25	85.38	83.13	122.38	104.63	8.49	0.74	1.75	8.11
TN(tiller)										
KPS-IR	11.50	10.50	9.25	8.13	16.63	12.00	1.48	0.27	0.30	12.33
KPS-DS	14.25	11.25	8.13	8.25	15.88	11.68	1.49	0.29	0.31	12.73
CPA-IR	7.38	6.88	5.75	5.50	9.13	7.24	0.83	0.12	0.17	11.45
CPA-DS	8.25	6.88	7.25	6.25	10.88	7.87	0.92	0.14	0.19	11.67
PN(panicle)										
KPS-IR	11.50	10.50	9.13	8.13	16.50	11.89	1.49	0.27	0.31	12.56
KPS-DS	14.25	11.13	8.00	7.88	15.75	11.58	1.50	0.29	0.31	12.96
CPA-IR	6.75	6.38	4.88	4.50	8.88	6.56	0.85	0.15	0.18	12.95
CPA-DS	7.38	6.00	6.00	4.88	9.50	6.87	0.91	0.12	0.19	13.29

Table 10 (Continued)

Traits/ Locations/ coditions	Variation									
	Parents			The CSSLs population						
	KDML105	DH103	DH212	Min	Max	Mean	SD	h^2	LSD	CV(%)
GY/rai(Kg)										
KPS-IR	628.86	365.38	320.92	329.86	786.33	554.92	100.42	0.47	20.68	18.10
KPS-DS	606.68	295.71	358.68	363.84	794.43	589.88	97.39	0.60	20.06	16.51
CPA-IR	256.16	143.16	221.70	185.78	353.28	271.70	38.70	0.51	7.97	14.25
CPA-DS	179.06	58.60	110.94	151.84	261.48	215.00	23.31	0.25	4.80	10.84
FGW/hill(g)										
KPS-IR	30.10	19.27	19.64	16.74	41.37	30.26	5.64	0.26	1.16	18.63
KPS-DS	38.05	19.49	23.27	14.72	41.16	30.02	5.11	0.23	1.05	17.03
CPA-IR	7.28	6.29	6.86	5.60	10.54	7.92	1.02	0.13	0.21	12.88
CPA-DS	8.69	2.77	5.55	6.68	12.44	9.14	1.31	0.11	0.27	14.36
UFGW/hill(g)										
KPS-IR	3.42	2.62	2.89	1.98	7.70	4.11	1.33	0.35	0.27	32.46
KPS-DS	3.91	2.73	3.61	1.49	9.40	4.47	1.59	0.38	0.33	35.60
CPA-IR	0.50	0.65	0.86	0.28	1.37	0.65	0.20	0.36	0.04	31.45
CPA-DS	1.32	0.74	1.81	0.70	2.45	1.33	0.37	0.44	0.08	28.14
TGW/hill(g)										
KPS-IR	33.53	21.89	22.53	21.90	44.19	34.37	5.17	0.24	1.06	15.03
KPS-DS	41.95	22.21	26.88	21.66	46.92	34.49	4.45	0.17	0.92	12.91
CPA-IR	7.78	6.94	7.72	5.93	11.12	8.57	1.05	0.12	0.22	12.26
CPA-DS	10.01	3.50	7.36	7.78	13.74	10.47	1.28	0.09	0.26	12.19

Table 10 (Continued)

Traits/ Locations/ coditions	Variation									
	Parents			The CSSLs population						
	KDML105	DH103	DH212	Min	Max	Mean	SD	h^2	LSD	CV(%)
FGN(spikelet)										
KPS-IR	132.55	108.20	110.05	82.15	185.30	124.52	19.45	0.28	4.00	15.62
KPS-DS	145.75	115.75	129.45	78.70	184.45	135.62	19.73	0.27	4.06	14.55
CPA-IR	75.85	70.95	87.95	59.10	106.05	81.57	10.21	0.27	2.10	12.52
CPA-DS	81.05	59.90	63.90	62.40	118.40	82.94	11.29	0.03	2.32	13.61
UFGN(spikelet)										
KPS-IR	27.95	55.50	59.25	20.45	116.05	50.19	22.24	0.50	4.58	44.31
KPS-DS	35.85	74.65	82.00	14.85	148.35	53.10	23.47	0.46	4.83	44.21
CPA-IR	18.35	32.85	49.95	11.30	58.50	24.94	9.54	0.61	1.97	38.27
CPA-DS	37.75	67.45	83.00	15.60	94.75	36.91	14.76	0.22	3.04	39.99
TSN(spikelet)										
KPS-IR	160.50	163.70	169.30	127.75	257.65	174.71	27.83	0.55	5.73	15.93
KPS-DS	181.60	190.40	211.45	147.05	299.80	188.71	26.09	0.51	5.37	13.83
CPA-IR	94.20	103.80	137.90	84.30	162.10	106.51	14.88	0.51	3.06	13.97
CPA-DS	118.80	127.35	146.90	93.55	180.90	119.85	18.11	0.14	3.73	15.11
PSS(%)										
KPS-IR	17.24	35.40	35.02	12.46	50.16	28.16	9.22	0.34	1.90	32.74
KPS-DS	19.89	40.20	38.21	9.15	55.00	27.55	9.69	0.38	2.00	35.18
CPA-IR	19.97	31.59	36.72	11.93	42.70	23.25	6.39	0.48	1.32	27.50
CPA-DS	37.13	35.85	30.03	23.05	52.07	37.82	5.34	0.37	1.10	14.13
1000GW(g)										

Table 10 (Continued)

Traits/ Locations/ coditions	Variation									
	Parents			The CSSLs population						
	KDML105	DH103	DH212	Min	Max	Mean	SD	h^2	LSD	CV(%)
1000GW(g)										
KPS-IR	24.78	20.88	24.30	21.15	27.54	24.55	1.16	0.78	0.24	4.73
KPS-DS	24.62	21.02	24.46	21.25	26.79	24.24	1.12	0.82	0.23	4.63
CPA-IR	24.27	21.03	24.16	18.62	26.75	24.43	1.22	0.25	0.25	5.01
CPA-DS	21.49	17.31	22.00	16.49	24.12	22.21	1.09	0.30	0.22	4.91

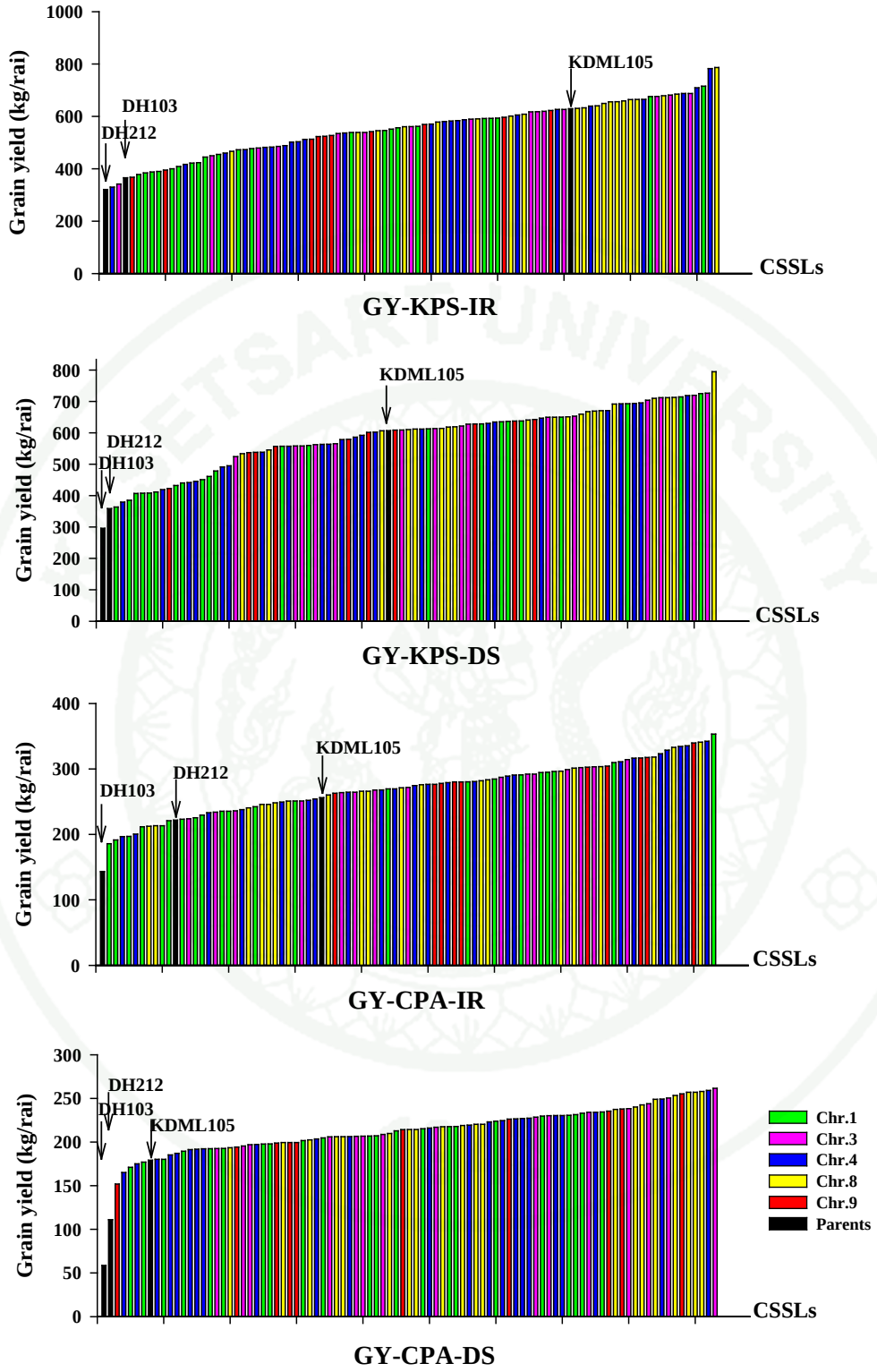

Figure 11 The mean grain yield (kg/ rai; 1,600 m^2) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.

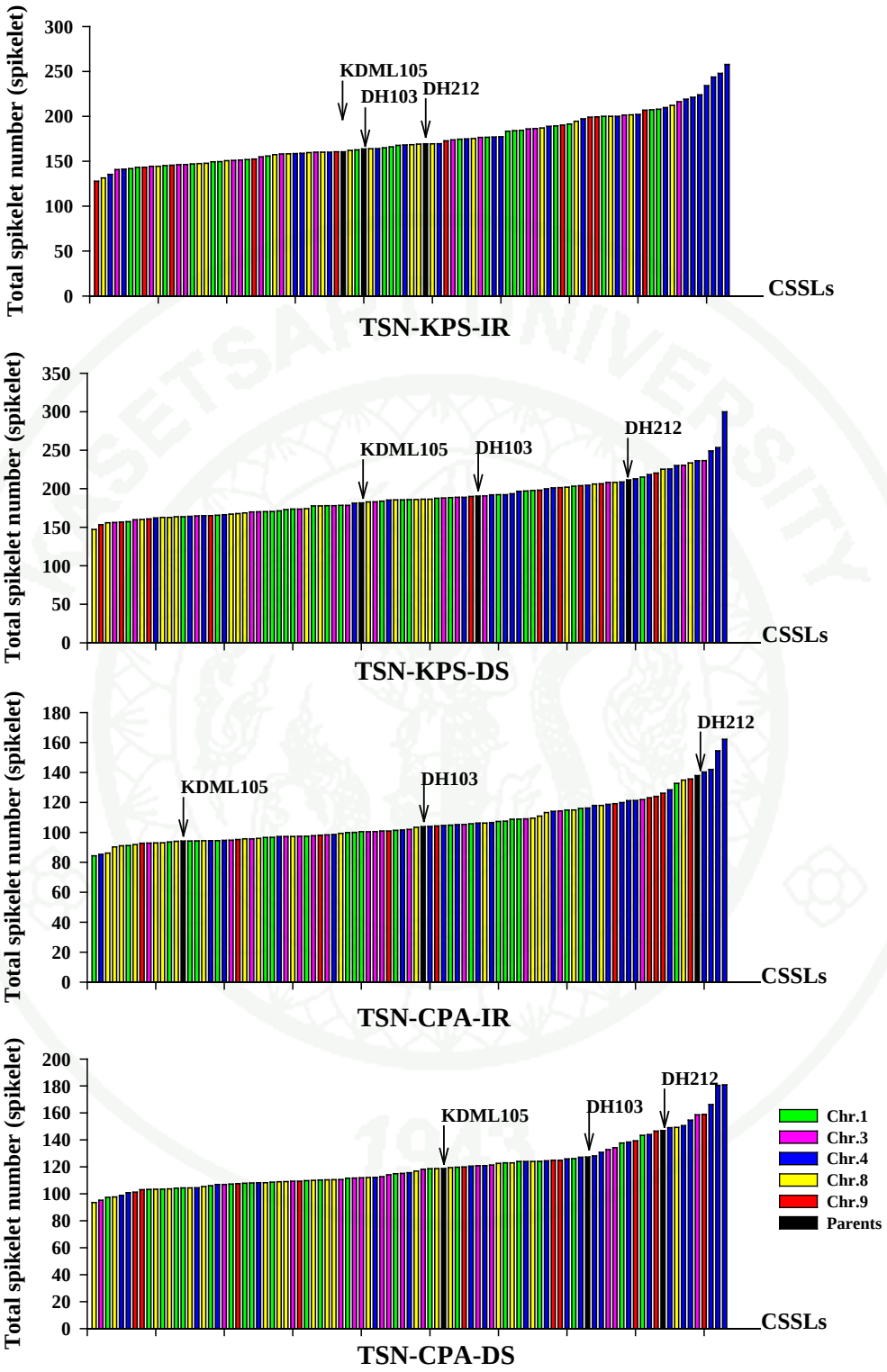

Figure 12 The mean value of total spikelet number per panicle (TSN) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.

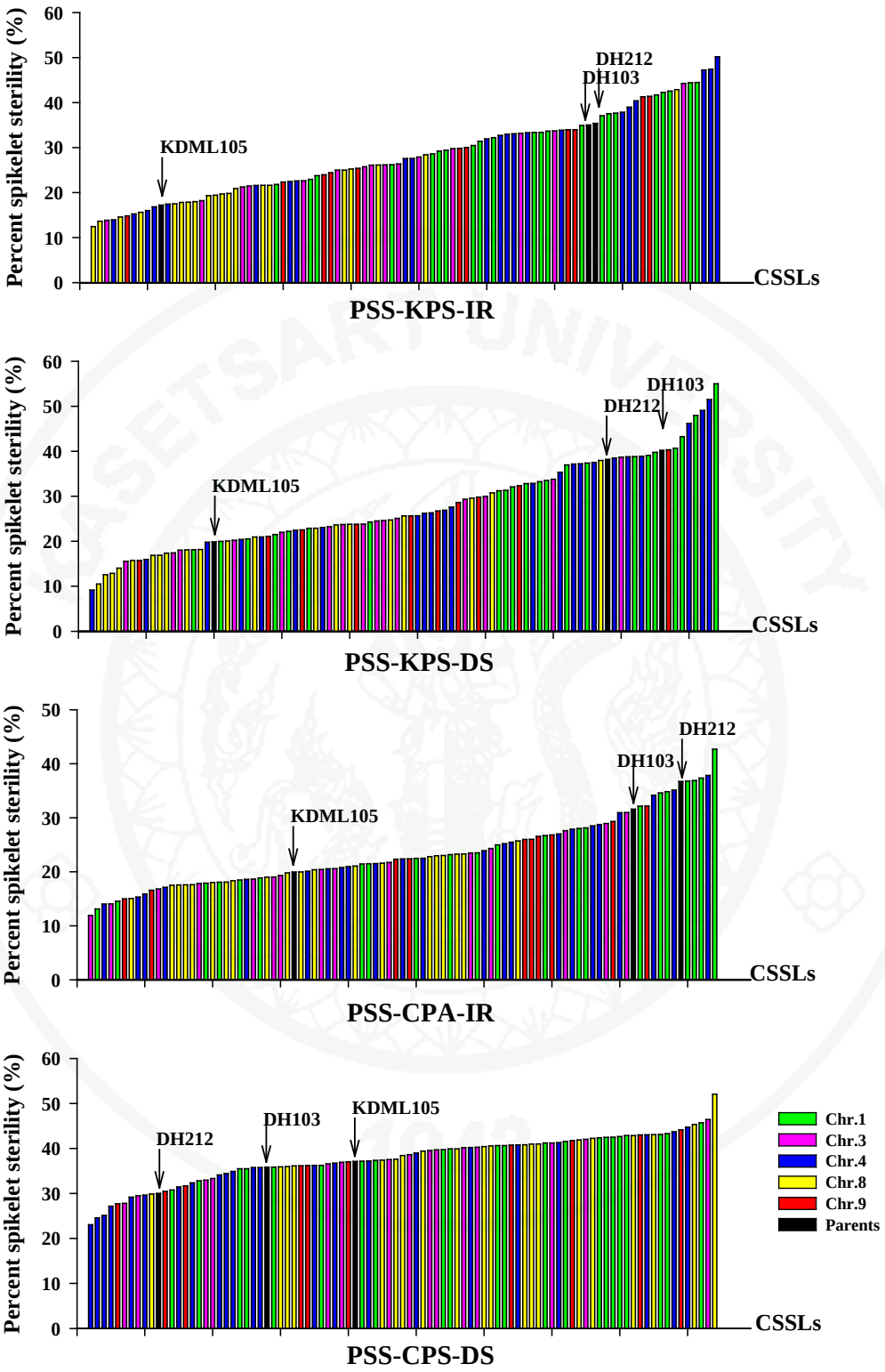

Figure 13 The mean value of percent spikelet sterility (PSS) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.

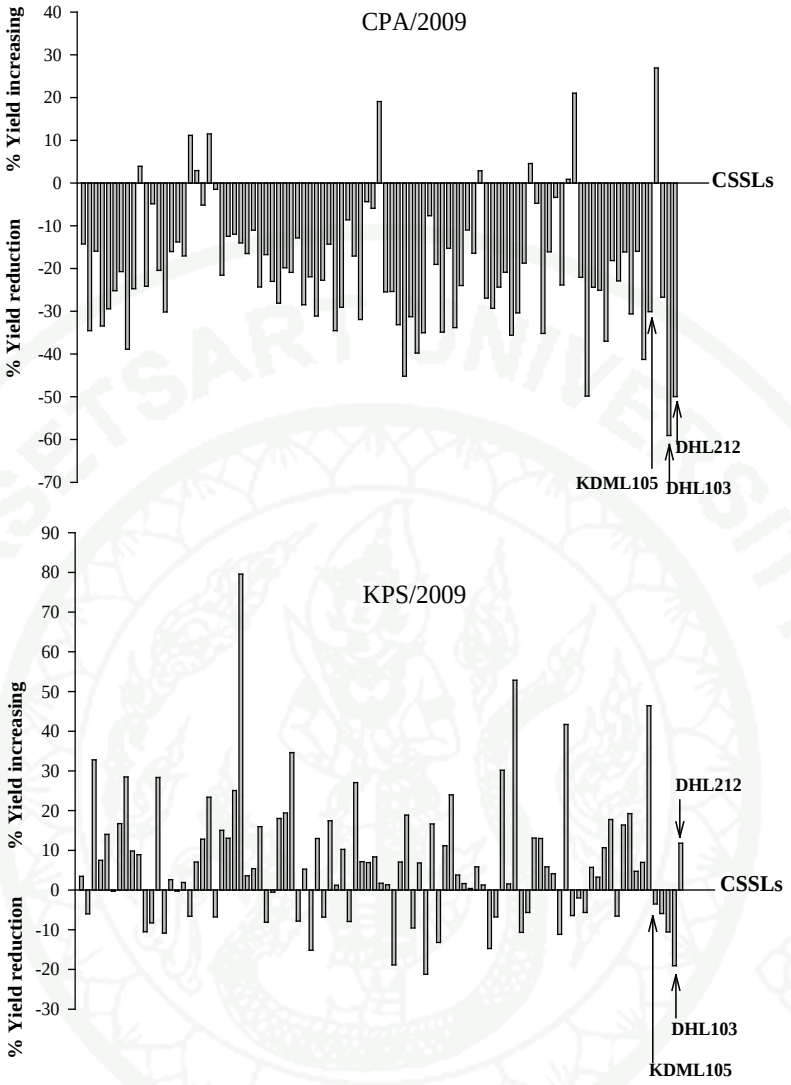

Figure 14 Percent yield reduction of 90 CSSLs under rainfed condition at CPA and KPS in 2009.

The percentage of yield reduction under rainfed condition in CSSL population was showed in Figure 14. Almost of CSSLs displayed GY decreasing under mind stress at CPA. The amount of percent yield reduction in CSSL population has variation. Percent yield reduction at CPA was found ranging from 1.47 to 49.83% in the CSSLs while KDML105 showed 30.10% in yield reduction. Some lines showed yield increasing ranged 0.86 to 21.02%. For KPS experiment, percent yield reduction was found ranging from 0.27 to 21.26% in the CSSLs while KDML105 showed

3.53% in yield reduction. More than half of all lines showed yield increasing ranged 0.36 to 79.54% under like aerobic condition. The results suggest that CSSLs carrying DT-QTL segment were adapted to like aerobic condition.

Genotype-phenotype association

The trait with high heritability, plant height, displayed distinguishes association with its genotype. The region that should be relevant to plant height is between RM1003 and RM3442, where it is close to the location of the gene semidwarf (*sd1*), a gene that acts as a *OsGA20ox2* genes responsible for plant height in rice (Spielmeyer *et al.*, 2002). Genotypes containing donor allele at RM1003 to RM3442 on chromosome 1 were showed a plant height ranging 116.88 to 131.13 cm with average of 122.58 cm which was close to DHL212 donor at approximately 124.63 cm. For lines which were carried KDML105 allele in this region expressed plant height ranging 141.75 to 155.75 cm with average of 150.47 cm which is close to KDML105 (Figure 15).

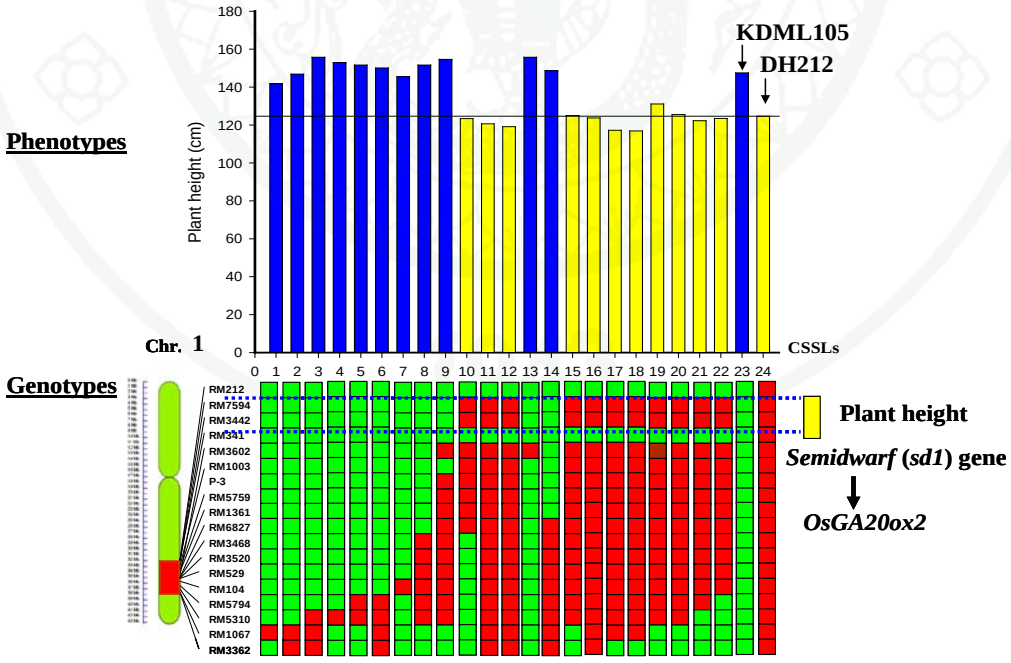

Figure 15 Association analyses of plant height variations with genotypes in a CSSL population that containing DT-QTL segment on chromosome 1.

QTLs for drought-related traits were created using genotypic and phenotypic data from the assessment process CSSLs under drought and well watered in both of experiments. We founded that 182 QTLs were detected on five target chromosomes including 40 QTLs for chromosome 1, 38 QTLs for chromosome 3, 70 QTLs for chromosome 4, 21 QTLs for chromosome 8 and 13 QTLs were founded on chromosome 9. Among them 102 and 80 QTLs for GY and its components were found in well watered and drought stress, respectively (Table 11). Location of QTLs for traits which have been high heritability such as *qph* was detected in the same position at RM1003 to RM529. It also found that the high R^2 values are the same in both conditions of two experiments. The grain yield, which was relatively low heritability were detected the position of the QTL, but may change when the environment varies. In addition, *qgy* was detected in all 5 chromosomes. The average at peak marker of the individual traits indicates that almost of traits was affected by donor allele. QTLs affected by KDML105 allele were founded such as TN and PN on chromosome 3 and 4 (*qtn3*, *qtn4*, *qpn3* and *qpn4*), which was higher than the donor allele (Figure 16).

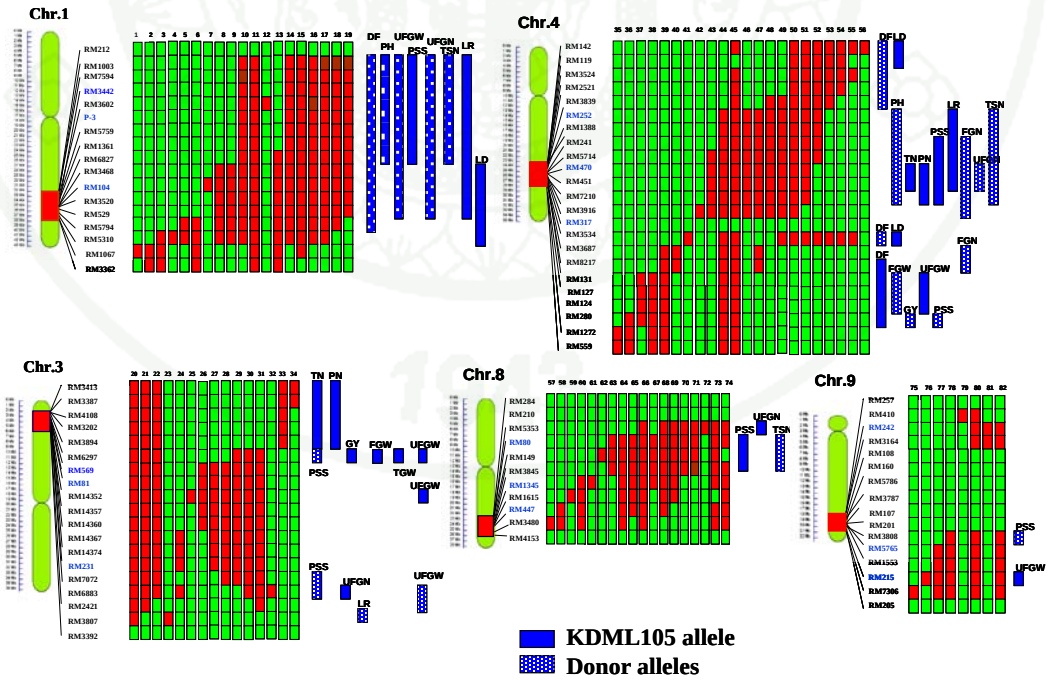

Figure 16 Location of QTLs for grain yield and yield components from the evaluation of CSSLs under drought condition at CPA.

Table 11 QTL mapping for drought related traits of CSSL population that was evaluated phenotypes under rainfed and irrigated conditions at CPA and KPS in 2009.

Traits	Location/ Treatment	Chr.	QTL name	Flanking markers	Peak marker	Mean		R^2
						KDML105	Donor	
DF	CPA-DS	1	*qdf1.1*	RM1003 - RM5794	RM3520	93.94	96.40	72.28
		4	*qdf4.1*	RM142 - RM3839	RM119	94.87	98.71	41.82
		4	*qdf4.2*	-	RM3534	94.65	97.20	22.00
		4	*qdf4.3*	RM8217 - RM280	RM280	96.62	93.75	21.21
	CPA-IR	1	*qdf1.2*	RM3468 - RM5794	RM3520	94.89	97.81	52.08
		3	*qdf3.1*	-	RM6883	96.69	94.61	44.08
		4	*qdf4.2*	RM142 - RM3839	RM119	96.16	99.88	31.66
		4	*qdf4.4*	RM131 - RM559	RM280	98.03	94.58	26.61
PH	CPA-DS	1	*qph1.1*	RM1003 - RM6827	RM3442	105.78	88.83	83.75
		4	*qph4.1*	RM252 - RM7210	RM470	103.28	113.39	31.83
	CPA-IR	1	*qph1.2*	RM1003 - RM529	RM3442	100.27	80.51	90.32
		3	*qph3.1*	RM3413 - RM3387	RM3413	96.37	102.88	36.29
		8	*qph8.1*	-	RM3480	101.40	96.68	27.64
		8	*qph8.2*	-	RM5353	100.98	97.20	15.80
		9	*qph9.1*	-	RM410	101.82	94.83	36.65
TN	CPA-DS	3	*qtn3.1*	RM3413 - RM3894	RM3413	8.17	7.00	39.06
		4	*qtn4.1*	RM470 - RM451	RM470	8.13	7.29	17.78
	CPA-IR	4	*qtn4.2*	RM252 - RM3916	RM470	7.61	6.25	63.15
		8	*qtn8.1*	-	RM1615	6.90	7.50	21.14

Table 11 (Continued)

Traits	Location/ Treatment	Chr.	QTL name	Flanking markers	Peak marker	Mean		R^2
						KDML105	Donor	
PN	CPA-DS	**3**	*qpn3.1*	RM3413 - RM3894	RM3413	7.20	6.05	39.31
		4	*qpn4.1*	RM470 - RM7210	RM470	7.18	6.25	23.56
	CPA-IR	**4**	*qpn4.2*	RM252 - RM3916	RM470	6.84	5.62	56.07
		8	*qpn8.1*	-	RM1615	6.27	7.03	33.56
GY	CPA-DS	**3**	*qgy3.1*	-	RM6297	229.83	207.93	20.94
		4	*qgy4.1*	-	RM280	204.34	227.85	14.68
	CPA-IR	**1**	*qgy1.1*	RM1003 - RM6827	RM3442	287.24	215.97	67.09
		3	*qgy3.2*	-	RM3807	278.99	237.43	21.29
FGW	CPA-DS	**3**	*qfgw3.1*	-	RM6297	9.85	8.24	27.18
		4	*qfgw4.1*	RM131 -RM124	RM127	8.67	10.07	14.99
	CPA-IR	**1**	*qfgw1.1*	RM1003 - RM529	RM3442	8.13	6.76	41.68
		3	*qfgw3.2*	RM7072 - RM2421	RM2421	8.32	7.10	28.47
		3	*qfgw3.1*	-	RM6297	8.43	7.54	21.64
		8	*qfgw8.1*	-	RM149	6.75	7.91	26.29
UFGW	CPA-DS	**1**	*qufgw1.1*	RM1003 - RM529	RM1003	1.20	1.59	37.78
		3	*qufgw3.1*	RM6883 - RM2421	RM6883	1.03	1.37	42.02
		3	*qufgw3.2*	-	RM6297	1.31	1.04	22.30
		3	*qufgw3.3*	-	RM14352	1.34	1.12	20.89
		4	*qufgw4.1*	RM131 -RM124	RM127	1.63	1.05	22.83
		9	*qufgw9.1*	-	RM215	1.86	1.27	50.36

Table 11 (Continued)

Traits	Location/ Treatment	Chr.	QTL name	Flanking markers	Peak marker	Mean		R^2
						KDML105	Donor	
	CPA-IR	1	*qufgw1.1*	RM1003 - RM529	RM1003	0.58	0.96	58.40
		3	*qufgw3.4*	-	RM6883	0.60	0.47	32.85
		4	*qufgw4.2*	RM142 - RM3839	RM119	0.59	0.90	39.17
		4	*qufgw4.3*	RM131 - RM280	RM131	0.73	0.47	22.34
		8	*qufgw8.1*	-	RM149	0.48	0.61	19.67
TGW	CPA-DS	3	*qtgw3.1*	-	RM6297	11.17	9.28	34.41
	CPA-IR	1	*qtgw1.1*	RM1003 - RM6827	RM1003	8.71	7.73	23.41
		3	*qtgw3.2*	RM7072 - RM2421	RM2421	8.85	7.63	26.30
		3	*qtgw3.3*	-	RM6297	9.00	8.02	24.92
		8	*qtgw8.1*	-	RM149	7.23	8.51	27.28
		9	*qtgw9.1*	-	RM215	9.79	9.00	33.46
PSS	CPA-DS	1	*qpss1.1*	RM1003 - RM6827	RM1003	41.59	37.47	26.75
		3	*qpss3.1*	RM7072 - RM6883	RM7072	34.80	40.60	32.55
		3	*qpss3.2*	-	RM6297	35.39	40.67	24.41
		4	*qpss4.1*	RM241 - RM7210	RM241	37.49	31.31	21.61
		4	*qpss4.2*	-	RM280	33.19	39.37	16.00
		8	*qpss8.1*	RM80 - RM3845	RM80	43.09	38.49	22.06
		9	*qpss9.1*	-	RM3808	33.53	40.26	32.62
	CPA-IR	1	*qpss1.2*	RM1003 - RM5794	RM1003	19.36	33.01	69.51
		4	*qpss4.3*	RM8217 - RM124	RM8217	25.51	19.10	13.67

Table 11 (Continued)

Traits	Location/ Treatment	Chr.	QTL name	Flanking markers	Peak marker	Mean		R^2
						KDML105	Donor	
FGN	CPA-DS	**4**	*qfgn4.1*	RM241 - RM3916	RM241	82.78	92.71	17.34
		4	*qfgn4.2*	RM3687 - RM8217	RM3687	83.87	98.70	28.91
	CPA-IR	**1**	*qfgn1.1*	RM3468 - RM529	RM104	82.31	71.42	34.85
		1	*qfgn1.2*	-	RM3442	79.46	71.30	19.14
		4	*qfgn4.3*	RM252 - RMRM7210	RM241	82.09	93.48	25.68
UFGN	CPA-DS	**1**	*qufgn1.1*	RM1003 - RM529	RM1003	31.81	43.02	33.74
		3	*qufgn3.1*	-	RM6883	38.69	25.69	50.59
		4	*qufgn4.1*	RM470 - RM451	RM470	35.97	68.77	16.53
		8	*qufgn8.1*	-	RM5353	31.99	24.98	19.30
	CPA-IR	**1**	*qufgn1.1*	RM1003 - RM529	RM1003	18.50	35.35	64.34
		3	*qufgn3.1*	-	RM6883	24.98	17.46	26.70
		4	*qufgn4.2*	RM470 - RM7210	RM470	24.07	34.00	17.18
		4	*qufgn4.3*	-	RM280	31.55	19.41	16.35
TSN	CPA-DS	**1**	*qtsn1.1*	RM1003 - RM6827	RM1003	107.88	120.59	27.83
		4	*qtsn4.1*	RM252 - RM7210	RM241	120.44	159.28	18.86
		8	*qtsn8.1*	RM80 - RM3845	RM80	105.06	116.57	17.92
	CPA-IR	**1**	*qtsn1.2*	RM1003 - RM3602	RM1003	97.96	106.67	14.51
		4	*qtsn4.2*	RM3524 - RM 119	RM119	110.44	129.56	15.48
		9	*qtsn9.1*	-	RM410	118.15	97.42	35.73

Table 11 (Continued)

Traits	Location/ Treatment	Chr.	QTL name	Flanking markers	Peak marker	Mean		R^2
						KDML105	Donor	
1000GW	CPA-IR	**1**	*q1000gw1.1*	RM1003 - RM6827	RM3442	25.03	23.56	48.15
		1	*q1000gw1.2*	-	RM3362	23.91	25.11	25.09
		3	*q1000gw3.1*	RM569 - RM14360	RM569	25.25	24.65	22.29
		4	*q1000gw4.1*	-	RM3687	24.62	23.39	22.71
		8	*q1000gw8.1*	-	RM1345	25.26	23.66	39.25
		8	*q1000gw8.2*	-	RM80	25.07	23.88	18.04
		8	*q1000gw8.3*	-	RM447	24.84	23.76	15.79

Evaluation of salinity tolerance in CSSL population.

ANOVA results showed that variation of salinity tolerance in the CSSL population is highest after 16 days after salt treatment with concentration of 150 mM NaCl. The damage symptom of salt injury was found in CSSLs, consequences to separate the salt tolerant lines from susceptible. The mean value of SIS in CSSL was ranged 5.71-8.29 with an average of 7.01 that lower than 7.52 score in KDML105 SIS (Figure17). For drought tolerant cultivars and donors including CT9993, DHL103 and DHL212 showed low SIS near Pokkali tolerance cultivar. In addition, drought susceptible IR62266 cultivar was also showed salinity tolerance. While SIS of 10 days after salt treatment (DAST) displayed non significant among genotypes because appearance of the damage symptom was bagan. And SIS of 21 DAST can not use as a standard for genotypic screening because almost of plant nearly die (Figure 18).

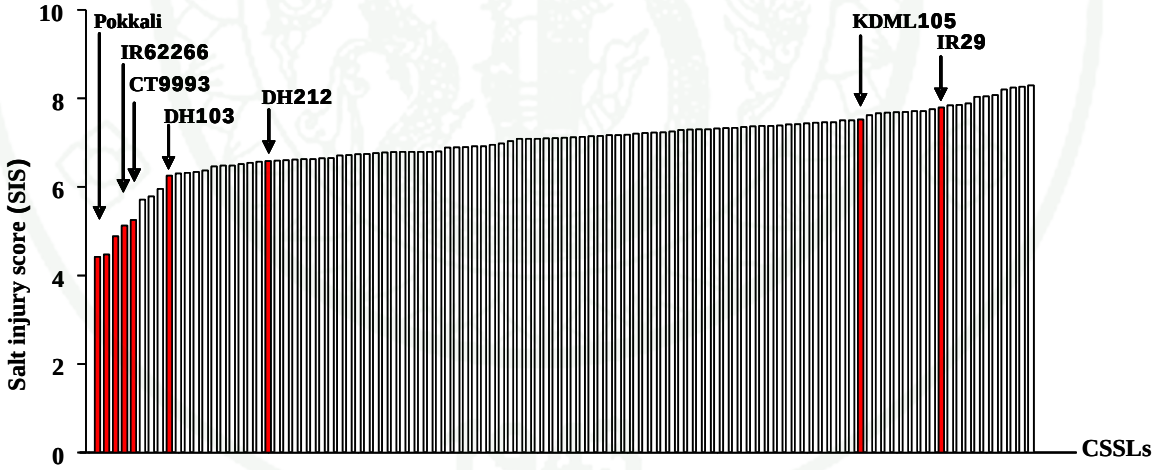

Figure 17 The mean value of SIS of 96 CSSLs and nine standard cultivars that were exposed in 150 mM NaCl, 16 days.

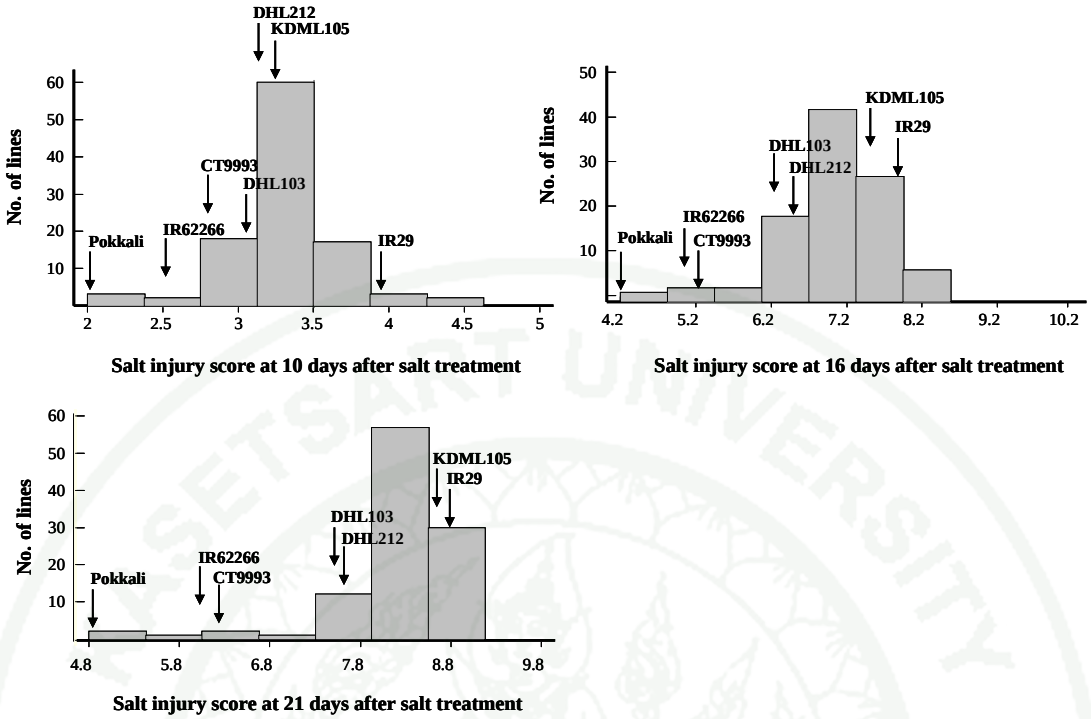

Figure 18 Salt injury score of CSSL population at 10, 16 and 21 days after salt treatment.

Significant difference (P<0.001) in the SIS value were detected between seven CSSLs and KDML105 (Figure 19). These seven CSSLs carried DT-QTL segment on chromosomes 1, 4 and 8. The SIS value was lower than KDML105 in lines CSSLs-11, -15, -16, -59, -73, -79, and -80, but higher than CT9993 and IR62266. The transgressive segregation in this population was observed for salinity tolerance. Broad-sense heritability of salinity was low (0.37). The coefficient variation of the CSSL remained largely consistent for salinity tolerance trait.

The graphical genotypes of seven CSSLs that showed higher salinity tolerance than KDML105 were presented on Figure 19. There were consisted CSSLs-11, -15 and -16 carrying DT-QTL in the region of RM1003-RM3362 on chromosome 1, CSSLs-73, -79 and -80 carrying the region of RM5353-RM3480 on chromosome 8, for CSSL-59 displayed donor allele at RM142, RM3524 and RM3534 on chromosome 4.

Lines	SIS at 16 days after salt treatment	SSR loci in DT-QTL segments
Chr.1		RM212 RM1003 RM7594 RM3442 RM3602 P-3 RM5759 RM1361 RM6827 RM3468 RM104 RM3520 RM529 RM5794 RM5310 RM1067 RM3362
CSSL-11	5.71 bcd	1 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
CSSL-15	6.34 cdefgh	1 3 3 3 3 3 3 3 3 3 3 3 3 3 3 1 1
CSSL-16	6.32 cdefg	1 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
Chr.4		RM142 RM119 RM273 RM3524 RM2521 RM3839 RM252 RM1388 RM241 RM5714 RM470 RM451 RM7210 RM303 RM3916 RM317 RM255 RM3534 RM3687 RM8217 RM131 RM127 RM124 RM280 RM1272 RM559
CSSL-59	5.78 bcd	3 1 1 3 1 1 1 1 1 1 1 1 1 1 1 1 1 5 1 1 1 1 1 1 1 1
Chr.8		RM210 RM5353 RM80 RM149 RM3845 RM1345 RM1615 RM447 RM3480 RM4153
CSSL-73	5.95 bcde	1 5 5 5 5 5 5 5 5 1
CSSL-79	6.37 defghi	1 5 5 5 5 5 1 5 5 1
CSSL-80	6.30 cdefg	1 5 5 1 5 5 5 5 5 1
KDML105	7.52 j	Recipient
CT9993	5.25 abc	Drought tolerance
IR62266	5.13 ab	Drought susceptible
DHL103	6.25 cdef	Drought donor
DHL212	6.58 defghij	Drought donor
Pokkali	4.42 a	Salinity tolerance
FL496	4.88 ab	Salinity donor
FL530	4.48 a	Salinity donor
IR29	7.79 j	Salinity susceptible
Min	4.42	
Max	8.29	1 Homozygous KDML105 allele
average	7.01	
SD	0.72	3 Homozygous DHL212 allele
h^2	0.37	
LSD	0.14	5 Homozygous DHL103 allele
%CV	10.28	

Figure 19 SIS mean value and graphical genotypes of 7 CSSLs expressed salinity tolerance after salt treatment 16 days compared with their parents and standard cultivars.

QTL analysis for ST

Individual CSSL was shown the recovering of KDML105 genome ranging from 88.50 to 100 % with an average of 96.30%. The proportion of donor alleles remaining in the non target areas is very low. The frequency of donor alleles in the non target regions of each chromosome ranged from 0.09% on chromosome 10 to 10.72% on chromosome 4, with an average of 3.70% which was in the range expected for the backcross stage reached.

The results of genotyping and SIS value were analyzed the QTL mapping for salinity tolerance. Four main effect ST-QTLs distributing at chromosomes 1, 3, 8 and 9, were mapped on DT-QTL regions (Figure 20). The large ST-QTL segment was found on chromosome 1 at the region RM1003-RM5794 (9.6 Mbp) with high R^2 approximately 35.11 at peak marker RM104. In addition, we founded small segments of ST-QTL on chromosomes 3, 8 and 9 that display significant at markers RM6883 (R^2=39.60), RM5353 (R^2=26.93), and RM215 (R^2=54.13), respectively.

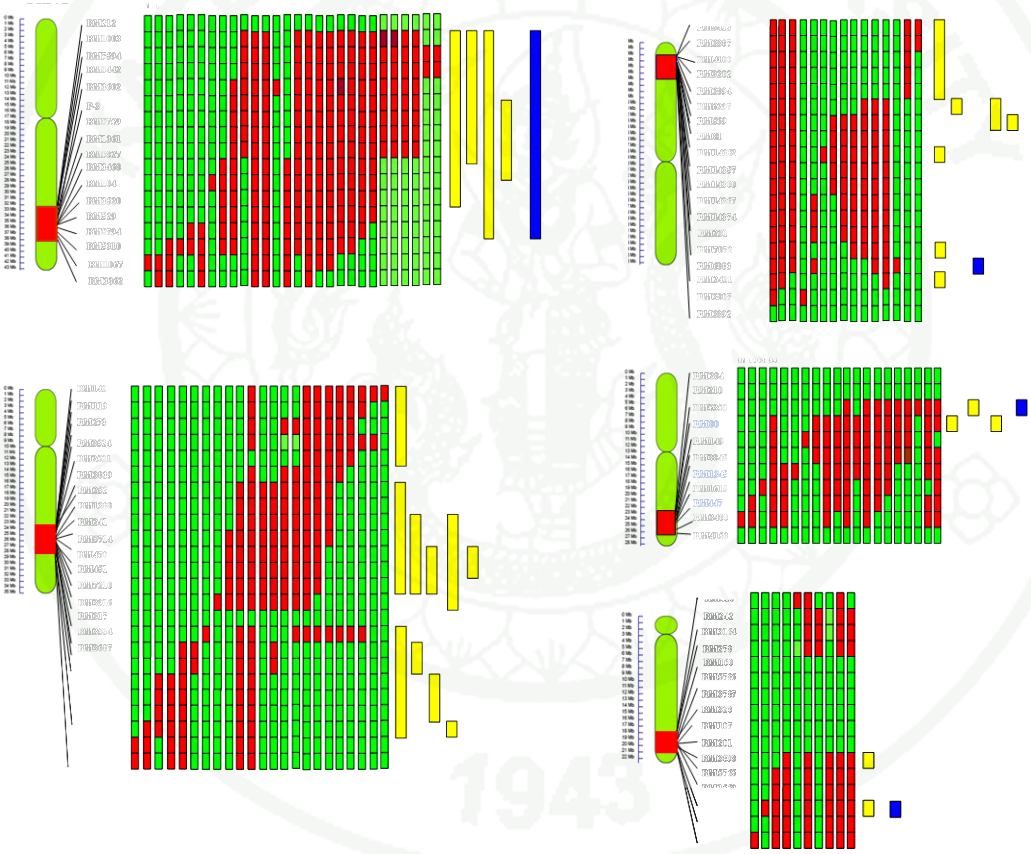

Figure 20 Coincidence of QTL mapping of salinity and drought tolerances in CSSL population. Drought related traits included flowering date (DF), plant height (PH), tiller number per hill (TN), panicle number per hill (PN), filled grain weight (FGW), unfilled grain weight (UFGW) and total grain weight (TGW), grain yield (GY) filled and unfilled spikelets (FGN and UFGN), total spikelet number per panicle (TSN) and percentage of spikelet sterility (PSS).

Genotypic data from the genome survey were analyzed the association with SIS data. We founded, many marker showed weak association with ST. There included markers around DT-QTL region on chromosomes 1, 4 and 9. Future more, small region on chromosomes 7, 10 and 12 displayed an association to ST. All of these were affected from donor allele except RM335 on chromosome 4 displaying effective KDML105 allele (Table 12).

Table 12 QTL position of salinity tolerant trait of CSSL population.

Chr.	Flanking markers	Peak marker	Salt injury score		P-value	R^2	Location
			KDML105	Donor			
1	RM1003-RM5794	RM104	7.53	6.77	0.0037	35.11	DT-QTLs
1	RM302	RM302	7.18	6.75	0.0107	9.57	Near DT-QTLs
3	RM6883	RM6883	7.23	6.73	0.0169	39.60	DT-QTLs
4	RM335	RM335	7.06	7.49	0.0161	6.4	Near DT-QTLs
7	RM70-RM234	RM70	7.1	6.48	0.0131	8.83	-
8	RM5353	RM5353	7.31	6.73	0.0191	26.93	DT-QTLs
9	RM215	RM215	7.81	7.17	0.0152	54.13	DT-QTLs
9	RM245	RM245	7.11	5.78	0.0097	1.40	Near DT-QTLs
10	RM304	RM304	7.11	5.78	0.0097	1.12	-
12	RM102	RM102	7.19	6.68	0.0052	13.69	-

Discussion

Development of CSSL population

Advanced mapping population using for locating QTLs and discovering genes will be well diversified and display detailed position QTL. It can be used for evaluating QTLs as single Mendelian factor and can be applied to MAS in a breeding program. Many set of advanced mapping population including introgression lines (Ahn *et al.*, 2002; Aida *et al.*, 1997) , near-isogenic lines (Bernacchi *et al.*, 1998) and CSSLs (Doi *et al.*, 1997; Ebitani *et al.*, 2005; Kubo *et al.*, 2002; Takai *et al.*, 2007) have been developed and can be used for genetic analysis of associated QTL with particular chromosome region and quickly to develop NILs of target regions containing QTLs of interests. Each CSSL carries either one or more donor segments in the high percentage background of the recurrent parent. However, this strategy is laborious and time-consuming, but it prevents many researchers to perform map based cloning.

Many QTLs for drought tolerant traits were located as a large region on many chromosomes, they may be affecting with coincidence QTLs by pleiotropy effects or close linkage (Kamoshita *et al.*, 2008; Lanceras *et al.*, 2004). In order to understand mechanisms of drought tolerance, the primary objective of our study was to develop a set of CSSLs with substituted chromosome segment of DHL103 on chromosome 8 and DHL212 on chromosomes 1, 3, 4 and 9 in the genetic background of KDML105. Although, the middle region of DT-QTL region on chromosome 9 could not be covered by donor segments. This study was successful for developing 104 haplotypes of 994 CSSLs in which the substituted donor chromosome segments covered most of the DT-QTL regions on five targeted chromosomes. There are no less certain haplotypes show a small segment of DT-QTLs.

The selection for phenotypic segregation in the segregated BC_5F_2 population was done for increasing the chance to get different genotypes. Number of haplotypes

in this experiment (25 haplotypes) did not significant from haplotype number of randomly selected BC_5F_2 (27 haplotypes). The results of these experiments may interpret in two hypothesizes. The first hypothesis, the selection for different phenotype because of genetic segregation under the target environment has the chance to get desirable genotype more than a random selection. Second hypothesis, haplotypes in this population is the same. Although plant number for genotyping is too much, but the number of haplotype pattern do not vary according to the plant number.

Potential of CSSLs for QTL analysis for drought-related traits

To demonstrate the potential power of CSSLs for QTL analysis, we used a set of 90 CSSLs to detected QTLs for drought tolerant traits. Because the study of physiology under drought must require an accurate phenotype method, in this study we have made measured agronomic traits such as yield and yield components to evaluate the potential of introgressed donor segments. In our study, variability of GY and its components were detected in the CSSL population. Differences mean among CSSLs as result of the DT-QTL differences that were introgressed to KDML105. Some lines show higher yield under drought and well watered than KDML105 especially CSSLs for chromosomes 4 and 8 display high TSN that correlated to GY. In all, 80 QTLs were detected for drought tolerant traits and 109 QTLs were detected under well watered. The number of those QTLs are rather greater than that has been previously reported (Lanceras *et al.*, 2004). Most of these QTLs appear to coincide with those of loci presented in the previous map. The resolution of QTL mapping in CSSLs can be indentified the candidate genes for PH that affect by major gene and high heritability (h^2 = 0.7 to 0.85 at CPA and KPS, respectively). Analysis of genotype and phenotype association was able to locate the region of *sd1* gene controlling PH in rice (Spielmeyer *et al.*, 2002) which located between RM1003 to RM3442 on chromosome 1. But the use of CSSLs for discovering the candidate genes may be disadvantage in the complex trait is controlled by multiple factors with epistatic interaction such as GY, PN, TN, and UFGN. Because the single substituted

segment has little effect, it would be very difficult to observe phenotypic effects generated by the interaction of two or more chromosome segments from donor parent.

At reproductive stage, drought will be affected in the delay of the rice flowering. However, these experiment results showed that almost of CSSLs displayed earlier DF under drought than irrigated condition, especially like aerobic condition at KPS. It is possible that drought stress may occur after panicle initiation, which drought may be stimulated panicle exertion. However, low soil moisture might inhibit decrease translocation of assimilates to the grain which lower grain weight (Katoa *et al.*, 2008) as can be found at 1000GW.

Coincidence of QTLs for DT and ST

Drought and salinity are two of the most important stress limiting plant growth and productivity. In natural paddy field that explores salinity, rice plant will experience to salinity stress after drought occurs due to salt move to soil surface by capillary force. Because of this, the DT-plant should be display tolerance to salinity. From this hypothesis, CSSLs carrying DT-QTL segment were evaluated performance of ST at seedling stage for confirm that DT and ST QTLs were coinciding.

In this study, QTL for salinity tolerance located on chromosomes 1, 3, 8 and 9 affected form DHL212 allele at chromosomes 1, 3, 8 and 9, and DHL103 allele at chromosome 8. The mean value of SIS indicated that 6 lines of CSSL population carrying DT-QTLs on chromosomes 1 and 8 showed higher ST than KDML105. The result of genome survey indicated that CSSL-59 containing DT-QTL on chromosome 4 displaying ST, it also contained DT-QTL on chromosome 1. Therefore, ST was observed in this line. Surprisingly, QTLs for major traits related with ST have been frequently detected on chromosome 1 (Gong *et al.*, 1999; Koyama *et al.*, 2001; Lee *et al.*, 2007; Lin *et al.*, 2004) and were also located on the similar region, which consistent with our study.

The primary effect of both drought and salinity is to impose osmotic stress. One mechanism of ST in rice is osmotic adjustment that the plant will use this mechanism by solute accumulation for preventing water loss from the cell by the osmosis process. Several solutes can be accumulated for osmoprotectance such as sugar, glycine betaine and proline that there solutes were used to balancing osmotic pressure in the plant cell. It should be noticed that the varieties of rice IR62266, which is susceptible to drought, it also expressed tolerance to salinity. The high performance of ST than KDML105, it might be because IR62266 rice cultivar has higher osmotic adjustment. Rabbani *et al*. (2003) reported that the amount of expressed genes response to salinity and drought conditions approximately 57 and 62 genes, respectively, of these 56 genes show the same gene that are responsive to the hormone ABA of 39 genes. From the study it is possible that one mechanism that has the salinity and drought tolerant in rice is osmotic adjustment. However mechanism for DT which is more complex than ST trait may be used more mechanisms. Therefore, osmotic adjustment must measure in CSSL population and study more.

The application of CSSLs for rice breeding

Presently, the knowledge of the markers was applied to integrate with plant breeding, MAS is one which used the information of markers specific to genes or targeted traits. The efficiency of this strategy depends on selectable markers must be closely linked to genes or QTL controlling target traits. Understanding controlled mechanism for a complex trait of interest is necessary for MAS application in breeding program.

In our study, novel plant materials of CSSLs were developed for dissecting DT mechanism. Evidences of this study indicating that not all of five target chromosomes of DT-QTLs were introgressed into KDML105 for improving high GY under water deficit condition. The result shows that DT-QTLs on chromosomes 4 and 8 are importance for improving DT. For other chromosomes, there are also expressed undesirable traits such as high spikelet sterility. Some CSSLs showed good adaptation

and high GY under rainfed condition bester than KDML105, there can be used as plant materials for advanced line selection, or genetic materials for QTL pyramiding. Future more, we founded the trait that contributed by KDML105 allele such as *qgy* on chromosome 1 and 3. KDML105 was used drought escape mechanism by expressed early flowering before drought appears in the paddy field. Therefore KDML105 may be classified as moderated tolerance cultivar. It can adapt to drought prone rainfed lowland ecosystem because it was grow in rainfed lowland of North and Northeast Thailand more than fifty years ago.

Interestingly, the end region on the long arm of rice chromosome 1 (RM212 to RM5794) is importance for stress tolerance. Because QTLs for many stress tolerance were found on this region including drought tolerance (Kamoshita *et al.*, 2008; Lanceras *et al.*, 2004; Zeng *et al.*, 2006), salinity tolerance (Ammar *et al.*, 2007; Kim *et al.*, 2009; Lee *et al.*, 2007), submergence tolerance (Fukao *et al.*, 2011; Septiningsih *et al.*, 2009; Toojinda *et al.*, 2003), ultraviolet-b resistance (Sato *et al.*, 2003), rice yellow mottle virus resistance (Pressoir *et al.*, 1998), brown planthopper resistance (Xu *et al.*, 2002), blast disease resistance (Chen *et al.*, 2003) and bacterial leaf blight (Wisser *et al.*, 2005). It might be a candidate target for molecular characterization or engineering to improve stress tolerance rice cultivar.

CONCLUSION AND RECOMMENDATION

Conclusion

In this study, 944 CSSLs identified as 104 haplotypes which carrying the overlapping DT-QTL segments in Khao Dawk Mali 105 (KDML105) genetic background were developed through marker assisted backcrossing (MAB). Individual haplotype were carried a different single DT-QTL segment cover 5 target chromosomes including 23, 16, 33, 21 and 11 haplotypes for DT-QTL on chromosomes 1, 3, 4, 8 and 9, respectively. A set of 131 polymorphic SSR markers were used for genome scaning to estimate the proportion of donor alleles in the non-target areas. Individual CSSL was shown the recovering of KDML105 genome ranging from 88.50 to 100 % with an average of 96.30%.

MAB was used as a methodology of developing CSSLs carrying DT-QTL segments. In order to obtained CSSLs carrying single DT-QTL segment in KDML105 genetic background, the MAB was done reaching to BC_5 generation to introgressed DT-QTLs from donors to KDML105 recipient parent. In each generation, three SSR markers were used to select the target progenies. After that, many SSR markers were increased for genotyping on DT-QTL region since BC_5F_2 to BC_5F_4 to find the homozygous lines containing DT-QTL with different sizes. Although, this strategy is time-consuming and required a lot of progenies for genotyping, but it quickly produced NILs of target regions containing QTLs of interests. And these materials are powerful genetic materials for QTL analysis.

The performance of CSSLs was evaluated under rainfed condition compared with irrigated condition. Variation of agronomic traits was observed in CSSL population. Some of CSSLs showed higher grain yield than recipient parent under drought stress. The mean value of GY, FGW, TGW, FGN and TSN were higher than it of KDML105. In addition, we founded that undesirable traits such as UFGW, FGN and PSS were average higher than KDML105. This indicates that the DT-QTL

segment with different sizes, which is introgressed into KDML105 affect grain yield and yield components differently and carried desirable and undesirable traits. In addition, they also found that the variation in plant morphology in the CSSL population, such as leaf size, panicle shape and root traits. This indicated that CSSLs can be useful materials to dissect genes underlay drought tolerance and observed variation trait.

A total of 182 QTLs for drought-related traits were detected on five target chromosomes including 40 QTLs for chromosome 1, 38 QTLs for chromosome 3, 70 QTLs for chromosome 4, 21 QTLs for chromosome 8 and 13 QTLs were founded on chromosome 9. Among them 102 and 80 QTLs for GY and its components were found in well water and drought stress, respectively. The average of the individual traits indicating that almost of traits was affected by donor allele. QTLs affected by KDML105 allele were founded such as TN and PN on chromosome 3 and 4, which was higher than the donor allele.

QTL for salinity tolerance located on chromosomes 1, 3, 8 and 9 affected form DHL212 allele at chromosomes 1, 3, 8 and 9, and DHL103 allele at chromosome 8, which major ST-QTL located on chromosome 1. The mean value of SIS indicated that 6 lines of CSSL population carrying DT-QTLs on chromosomes 1 and 8 showed higher ST than KDML105. The results showed that the QTLs of DT and ST are some common positions and some mechanisms may be used together.

Recommendation

The method for development of CSSL materials is laborious and time consuming. Therefore, the researcher should be adding the step of phenotype selection in segregating population to increase the chance to get homozygous genotypes and different size DT-QTL segment.

Time lost for genotyping in the MAS process was reduced by multiplex PCR and loading technique of two layer of PCR products in one PAGE.

LITERATURE CITED

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APPENDIX

Appendix Table 1 Allele frequency and percentage of KDML105 genetic background of 90 CSSLs that were scanned on the genome by 131 SSR markers.

No.	Plant No.	Generation	Pedigree	Chr.	No. SSR markers	Genotypes			Total	% RP	% Average
						homo KD	Hetero (KDxDonor)	Homo donor			
1	49	BC_5F_3	G45-2-75-13-9	1	113	112	1	0	113	99.56	
2	194	BC_5F_2	G45-2-10-24	1	113	112	1	0	113	99.56	
3	14	BC_5F_2	D12-2-2-14	1	113	112	0	1	113	99.12	
4	336	BC_5F_2	G45-2-67-16	1	113	113	0	0	113	100.00	
5	119	BC_5F_3	G45-2-16-3-19	1	113	112	0	1	113	99.12	
6	121	BC_5F_3	G45-2-10-36-1	1	113	111	0	2	113	98.23	
7	246	BC_5F_2	G45-2-16-20	1	113	113	0	0	113	100.00	
8	190	BC_5F_3	G45-2-16-19-10	1	113	113	0	0	113	100.00	
9	185	BC_5F_3	G45-2-16-19-5	1	113	110	0	3	113	97.35	
10	548	BC_5F_3	G45-5-7-9-8	1	113	107	0	6	113	94.69	
11	404	Selected BC_5F_2	G45-2-31-39	1	113	107	1	5	113	95.13	97.12
12	285	BC_5F_2	G45-2-31-25	1	113	113	0	0	113	100.00	
13	21	BC_5F_2	D12-2-2-21	1	113	109	0	4	113	96.46	
14	415	BC_5F_3	G45-2-31-17-15	1	113	112	0	1	113	99.12	
15	349	BC_5F_2	G45-2-67-29	1	113	106	0	7	113	93.81	
16	330	BC_5F_2	G45-2-67-10	1	113	108	0	5	113	95.58	
17	532	Selected BC_5F_2	G45-5-7-35	1	113	107	0	6	113	94.69	
18	368	BC_5F_2	G45-2-75-18	1	113	107	0	6	113	94.69	
19	90	BC_5F_2	P23-5-2-20	1	113	108	4	1	113	97.35	
20	513	BC_5F_3	G45-2-9-31-13	1	113	106	0	7	113	93.81	
21	484	BC_5F_3	G45-2-10-8-4	1	113	106	1	6	113	94.25	
22	501	BC_5F_3	G45-2-9-31-1	1	113	106	1	6	113	94.25	

Appendix Table 1 (Continued)

No.	Plant No.	Generation	Pedigree	Chr.	No. SSR markers	Genotypes			Total	% RP	% Average
						homo KD	Hetero (KDxDonor)	Homo donor			
23	762	BC_5F_2	G70-17-1-3	3	113	109	0	4	113	96.46	
24	437	Selected BC_5F_2	B97-4-9-56	3	113	112	0	1	113	99.12	
25	139	Selected BC_5F_2	B97-4-9-44	3	113	110	2	1	113	98.23	
26	581	BC_5F_3	G70-17-1-12-1	3	113	109	1	3	113	96.90	
27	622	BC_5F_3	B97-3-6-35-2	3	113	109	1	3	113	96.90	
28	723	BC_5F_3	B97-3-6-7-3	3	113	111	0	2	113	98.23	
29	675	BC_5F_3	B97-3-27-28-4	3	113	112	0	1	113	99.12	
30	703	BC_5F_3	B97-3-64-41-3	3	113	111	0	2	113	98.23	98.02
31	711	BC_5F_3	B97-3-64-41-11	3	113	112	0	1	113	99.12	
32	579	BC_5F_2	B97-3-20-30	3	113	110	0	3	113	97.35	
33	432	BC_5F_2	B97-3-6-8	3	113	110	1	2	113	97.79	
34	445	Selected BC_5F_2	B97-4-29-41	3	113	111	0	2	113	98.23	
35	747	BC_5F_3	B97-3-20-11-7	3	113	113	0	0	113	100.00	
36	788	BC_5F_3	G70-43-4-41-8	3	113	112	0	1	113	99.12	
37	821	BC_5F_3	G70-17-1-29-1	3	113	108	0	5	113	95.58	

Appendix Table 1 (Continued)

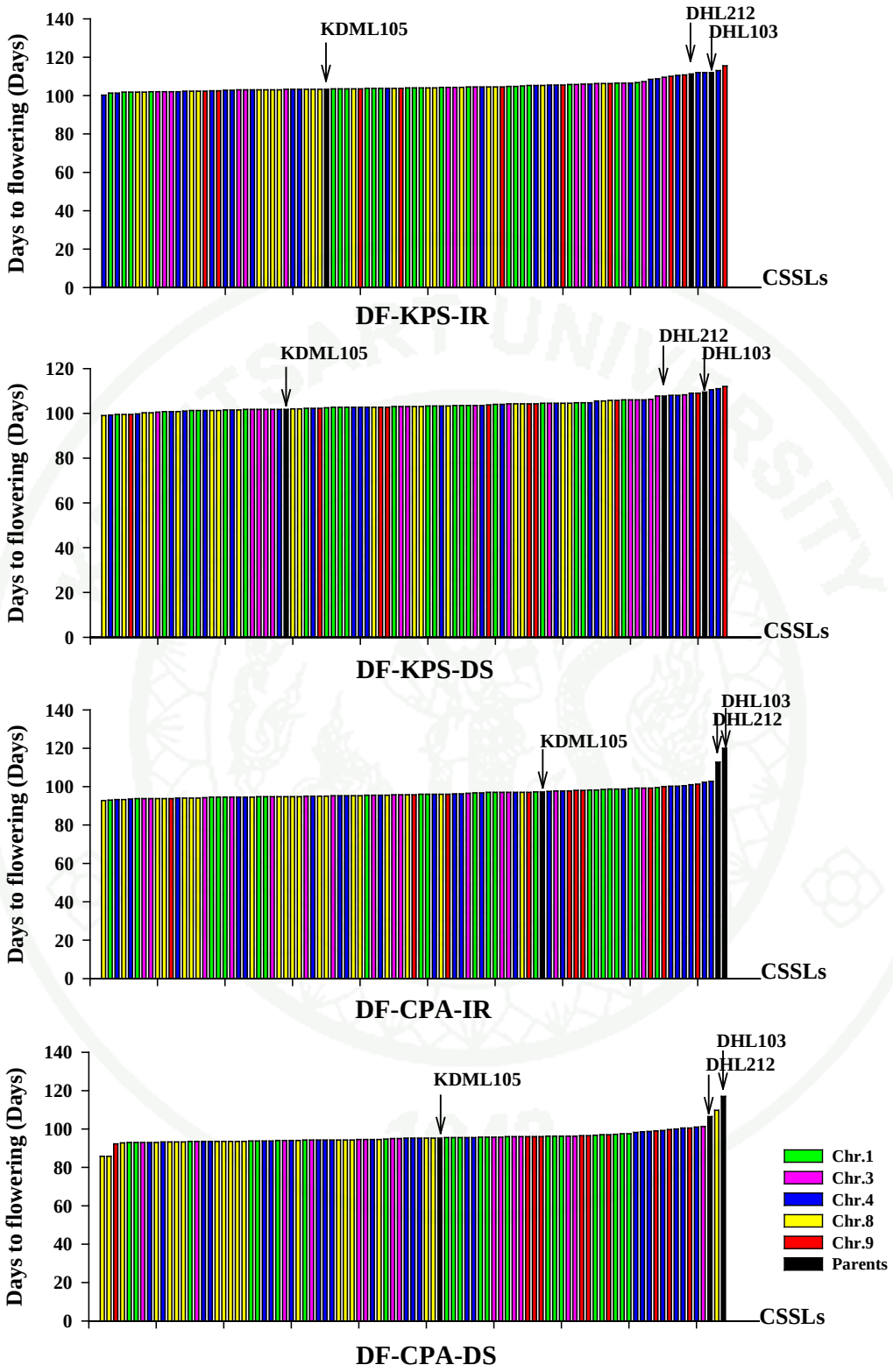
No.	Plant No.	Generation	Pedigree	Chr.	No. SSR markers	Genotypes			Total	% RP	% Average
						homo KD	Hetero (KDxDonor)	Homo donor			
38	881	BC_5F_3	G67-3-2-15-1	4	113	105	6	2	113	95.58	
39	910	BC_5F_3	P41-18-2-34-10	4	113	112	0	1	113	99.12	
40	922	BC_5F_3	P41-16-14-1-2	4	113	113	0	0	113	100.00	
41	1117	BC5F2	P41-18-2-37	4	113	110	1	2	113	97.79	
42	1131	BC_5F_3	P41-16-14-26-11	4	113	111	0	2	113	98.23	
43	941	BC_5F_3	P41-16-14-16-1	4	113	113	0	0	113	100.00	
44	452	Selected BC_5F_2	G67-1-9-60	4	113	107	0	6	113	94.69	
45	846	BC_5F_2	G67-1-9-46	4	113	112	0	1	113	99.12	
46	998	BC_5F_3	G67-1-9-22-18	4	113	107	0	6	113	94.69	
47	1039	BC_5F_2	P41-16-14-39	4	113	111	0	2	113	98.23	
48	1045	BC_5F_2	P41-16-16-5	4	113	109	1	3	113	96.90	
49	1018	BC_5F_3	P41-16-14-31-18	4	113	109	2	2	113	97.35	96.34
50	1148	BC_5F_3	P41-16-14-3-8	4	113	107	1	5	113	95.13	
51	863	BC_5F_3	G67-1-17-3-3	4	113	107	1	5	113	95.13	
52	114	BC_5F_2	P41-18-50-13	4	113	109	1	3	113	96.90	
53	892	BC_5F_2	G67-1-17-12	4	113	107	1	5	113	95.13	
54	1216	BC_5F_3	G67-1-9-31-16	4	113	104	0	9	113	92.04	
55	895	BC_5F_2	G67-1-9-40-11	4	113	107	1	5	113	95.13	
56	1201	BC_5F_3	G67-1-9-31-1	4	113	105	0	8	113	92.92	
57	1225	BC_5F_3	G67-1-17-21-5	4	113	108	0	5	113	95.58	
58	1247	BC_5F_3	G67-3-2-37-7	4	113	109	1	3	113	96.90	
59	1070	BC_5F_3	P41-18-50-6-1	4	113	105	0	8	113	92.92	
60	1094	BC_5F_3	P41-18-50-6-5	4	113	108	2	3	113	96.46	

Appendix Table 1 (Continued)

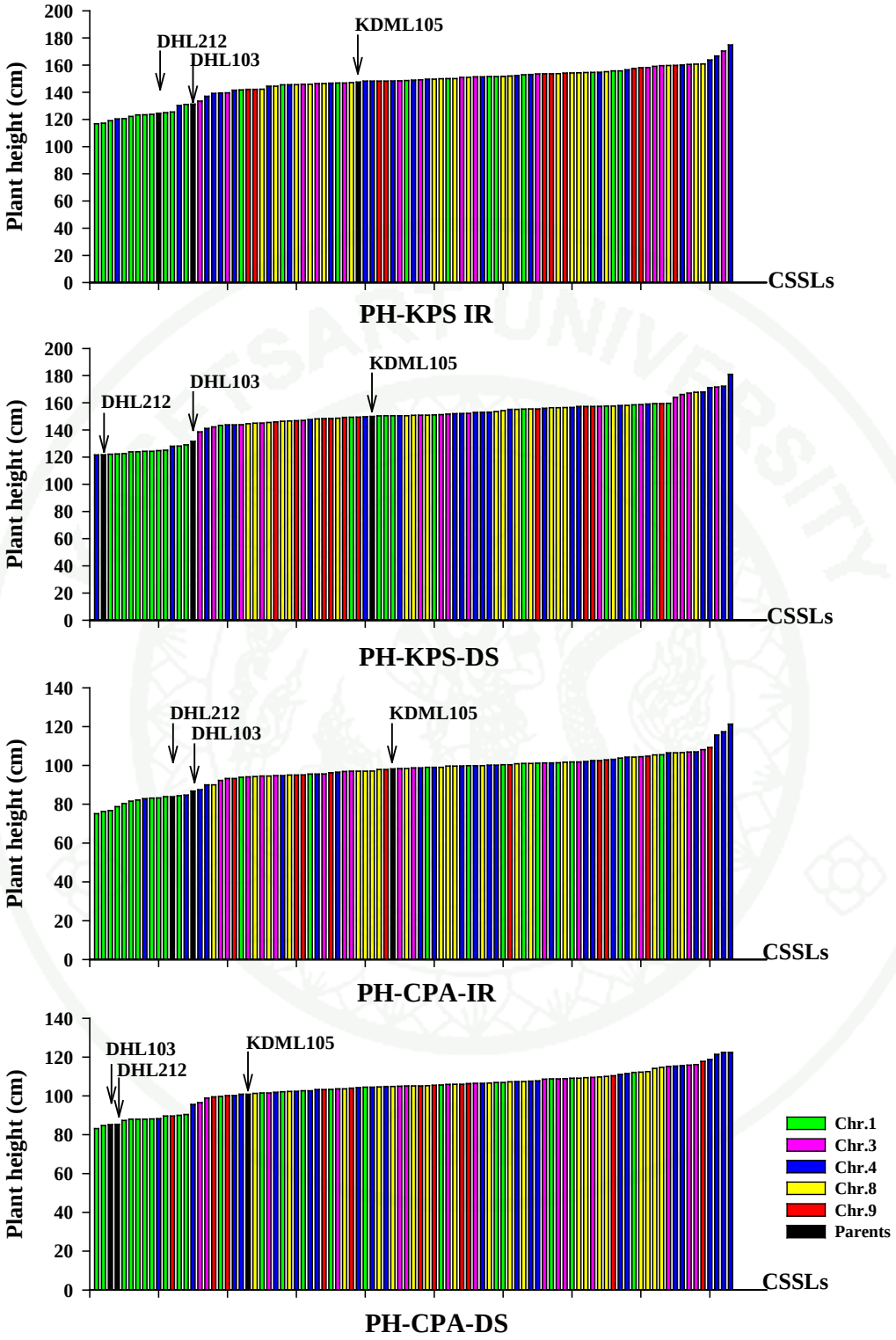
No.	Plant No.	Generation	Pedigree	Chr.	No. SSR markers	Genotypes			Total	% RP	% Average
						homo KD	Hetero (KDxDonor)	Homo donor			
61	1261	BC_5F_3	O5-1-7-10-1	8	115	111	0	4	115	96.52	
62	1301	BC_5F_3	O5-1-7-39-1	8	115	110	1	4	115	96.09	
63	1513	BC_5F_2	O145-1-3-3	8	115	104	0	11	115	90.43	
64	1564	BC_5F_2	O145-1-69-24	8	115	103		12	115	89.57	
65	1398	BC_5F_3	O145-1-69-8-18	8	115	103	2	10	115	90.43	
66	483	Selected BC_5F_2	O5-1-26-58	8	115	114	0	1	115	99.13	
67	1481	BC_5F_2	O5-1-64-1	8	115	109	1	5	115	95.22	
68	1423	BC_5F_3	O5-1-65-19-3	8	115	110	2	3	115	96.52	
69	1555	BC_5F_2	O145-1-69-15	8	115	104	2	9	115	91.30	
70	1585	BC_5F_3	O5-1-65-8-5	8	115	109	1	5	115	95.22	95.65
71	278	Selected BC_5F_2	O5-1-6-52	8	115	114	0	1	115	99.13	
72	1602	BC_5F_3	O145-1-3-25-2	8	115	104	0	11	115	90.43	
73	1622	BC_5F_3	O5-1-6-16-2	8	115	108	1	6	115	94.35	
74	1468	BC_5F_3	O5-1-26-45-8	8	115	114	0	1	115	99.13	
75	1656	BC_5F_3	O5-1-42-39-16	8	115	115	0	0	115	100.00	
76	1448	BC_5F_3	O5-1-42-4-8	8	115	114	1	0	115	99.57	
77	1524	BC_5F_3	O5-1-6-48-4	8	115	115	0	0	115	100.00	
78	1481	BC_5F_3	O5-1-64-25-1	8	115	109	0	6	115	94.78	
79	1226	BC_5F_2	O5-1-6-26	8	115	112	1	2	115	97.83	
80	488	Selected BC_5F_2	O5-1-44-47	8	115	112	0	3	115	97.39	

Appendix Table 1 (Continued)

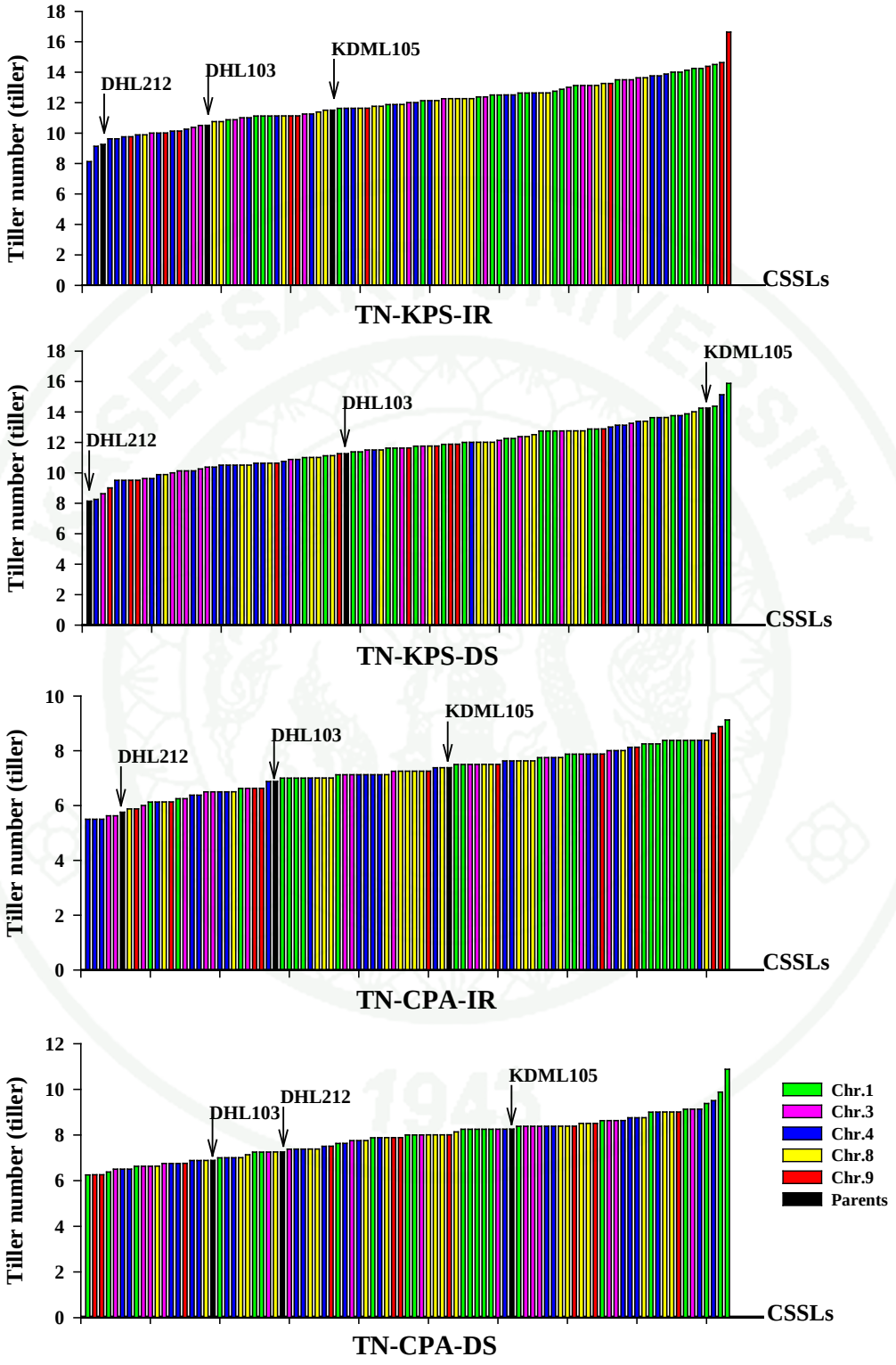
No.	Plant No.	Generation	Pedigree	Chr.	No. SSR markers	Genotypes			Total	% RP	% Average
						homo KD	Hetero (KDxDonor)	Homo donor			
81	1685	BC_5F_3	B23-1-13-19-5	9	113	101	1	11	113	89.82	93.09
82	1860	BC_5F_2	B26-29-43-10	9	113	112	0	1	113	99.12	
83	1728	BC_5F_3	B28-5-12-36-8	9	113	103	2	8	113	92.04	
84	1615	BC_5F_2	B5-5-5-15	9	113	108	0	4	112	96.43	
85	1762	BC_5F_3	B5-5-5-21-2	9	113	108	0	4	112	96.43	
86	1729	BC_5F_2	B5-5-16-29	9	113	100	0	13	113	88.50	
87	1808	BC_5F_3	B28-5-12-22-8	9	113	108	1	4	113	96.02	
88	1959	BC_5F_2	B28-5-13-9	9	113	101	2	10	113	90.27	
89	1738	BC_5F_2	B23-1-13-8	9	113	100	2	11	113	89.38	
90	374	Selected BC_5F_2	B28-5-12-56	9	113	105	0	8	113	92.92	
								Range	min	88.50	
									max	100.00	
								average		96.30	



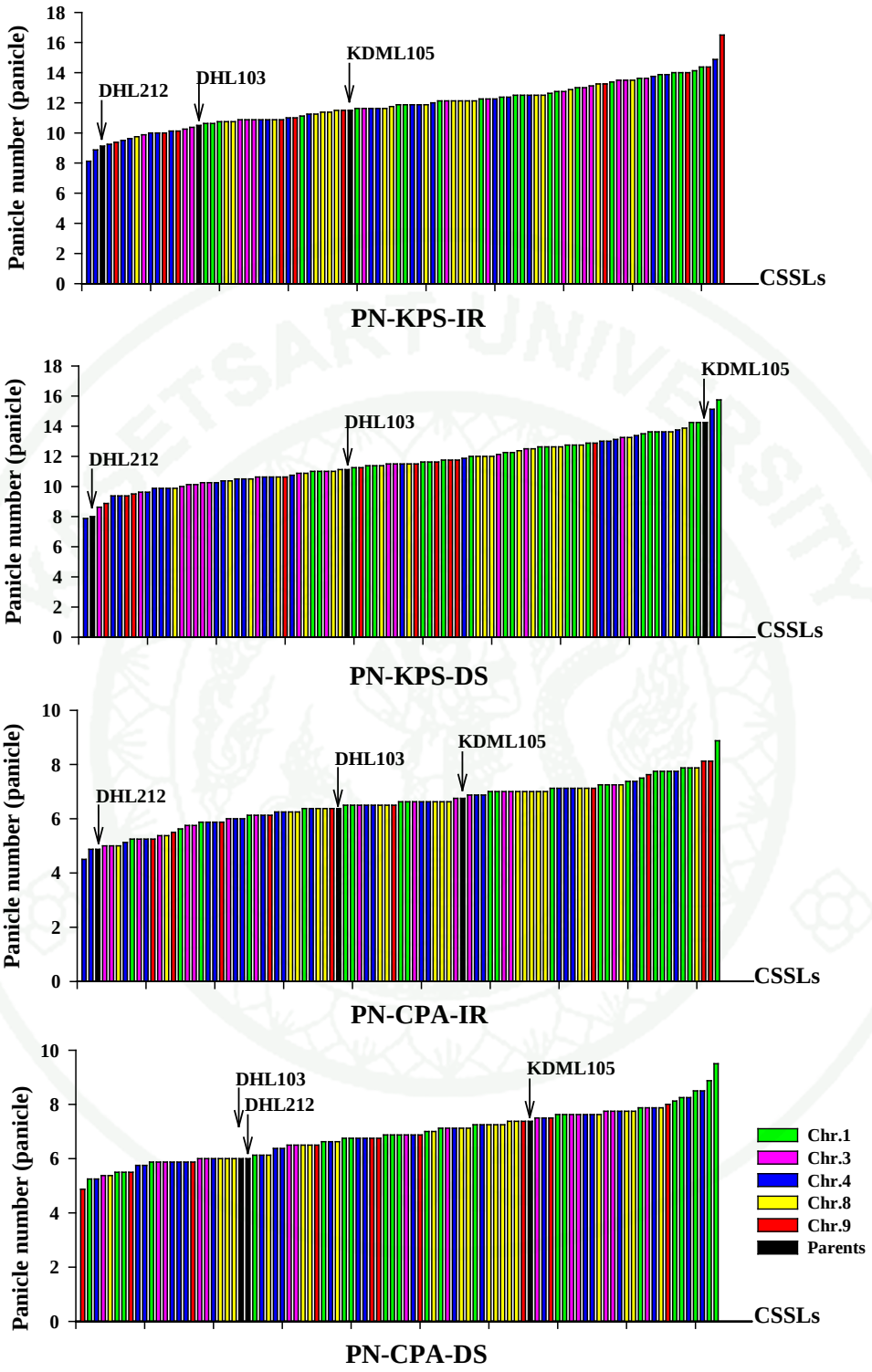
Appendix Figure 1 The mean value of days to flowering (DF) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.



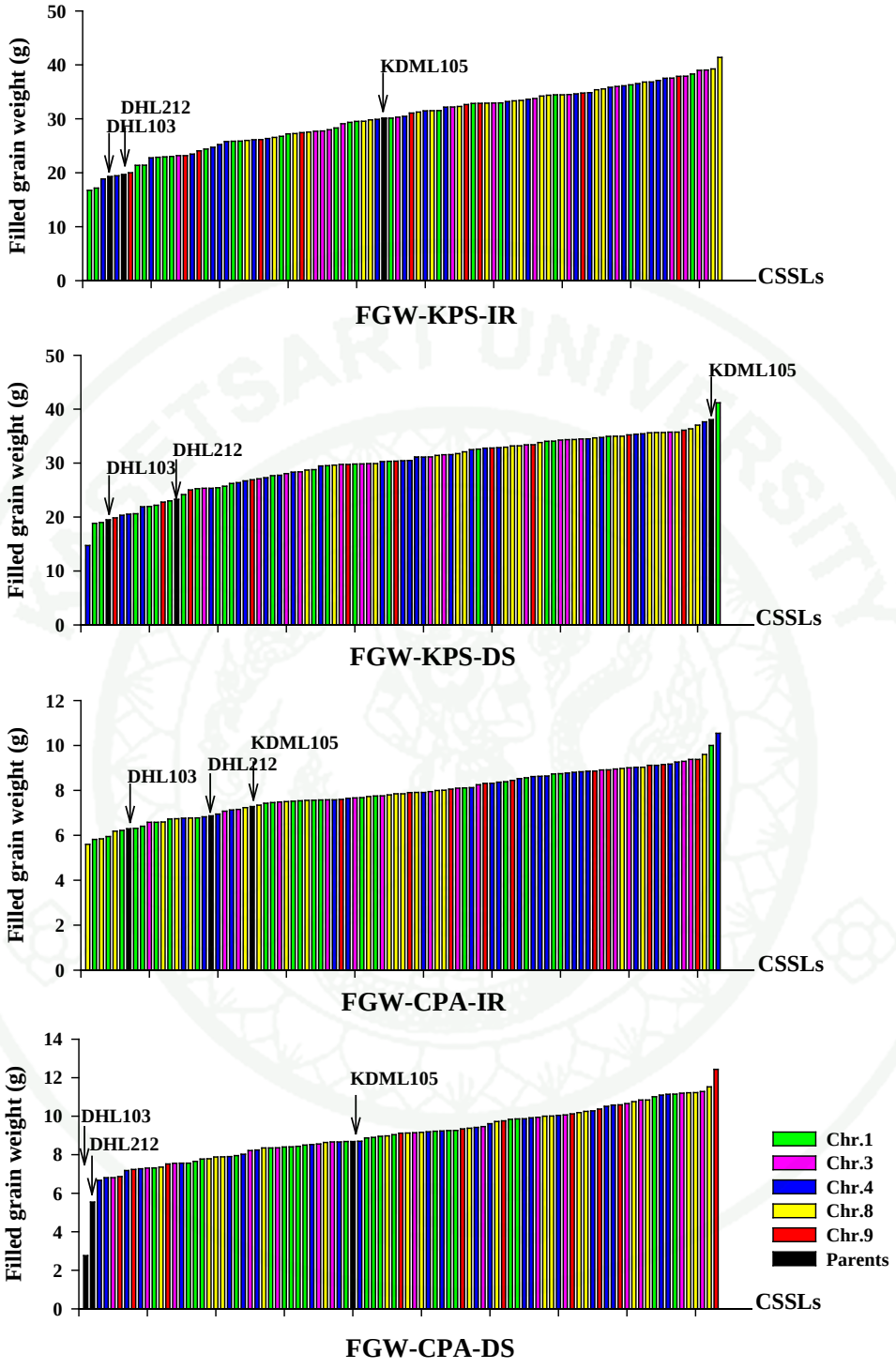
Appendix Figure 2 The mean value of plant height (PH) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.



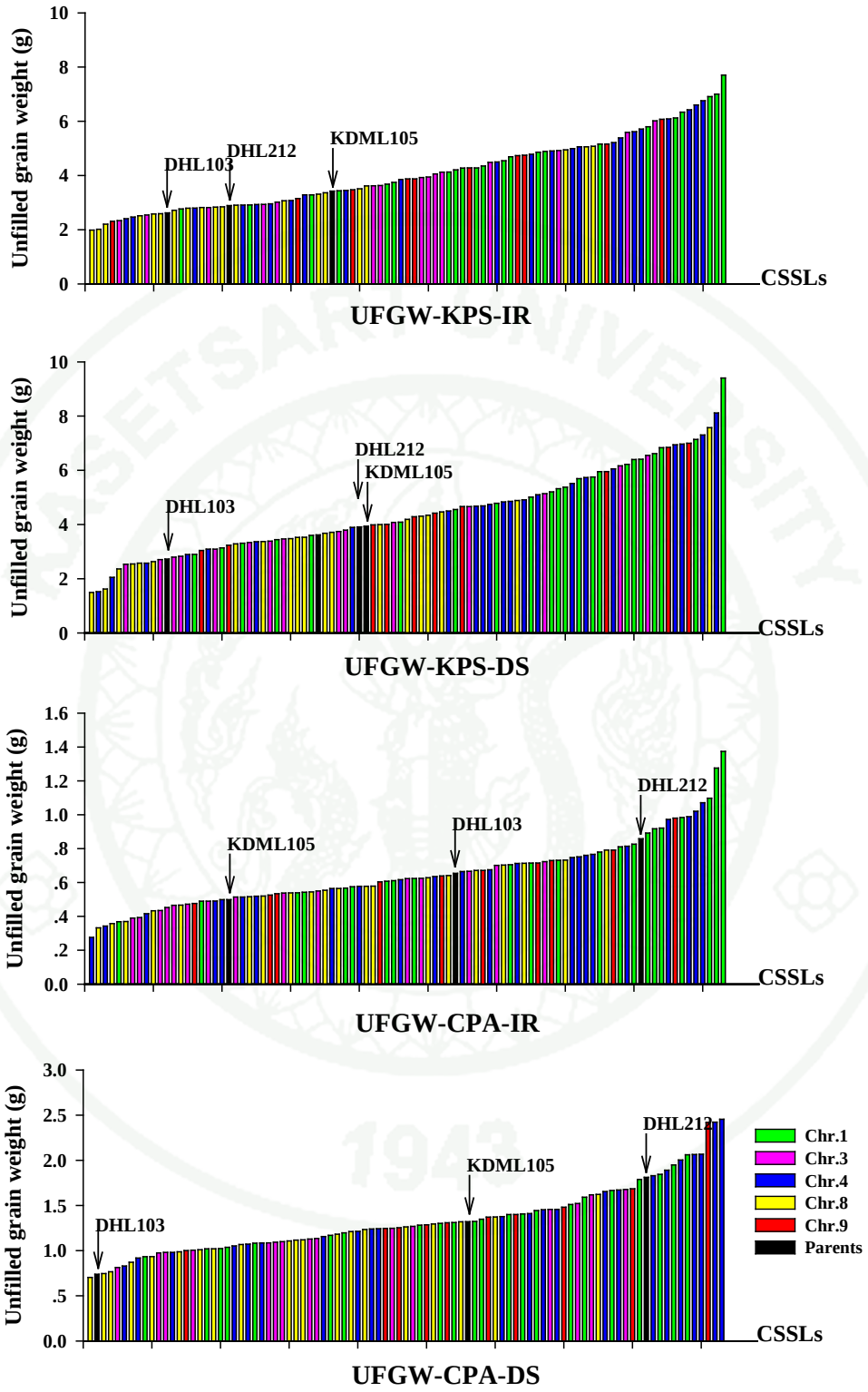
Appendix Figure 3 The mean value of tiller numer (TN) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.



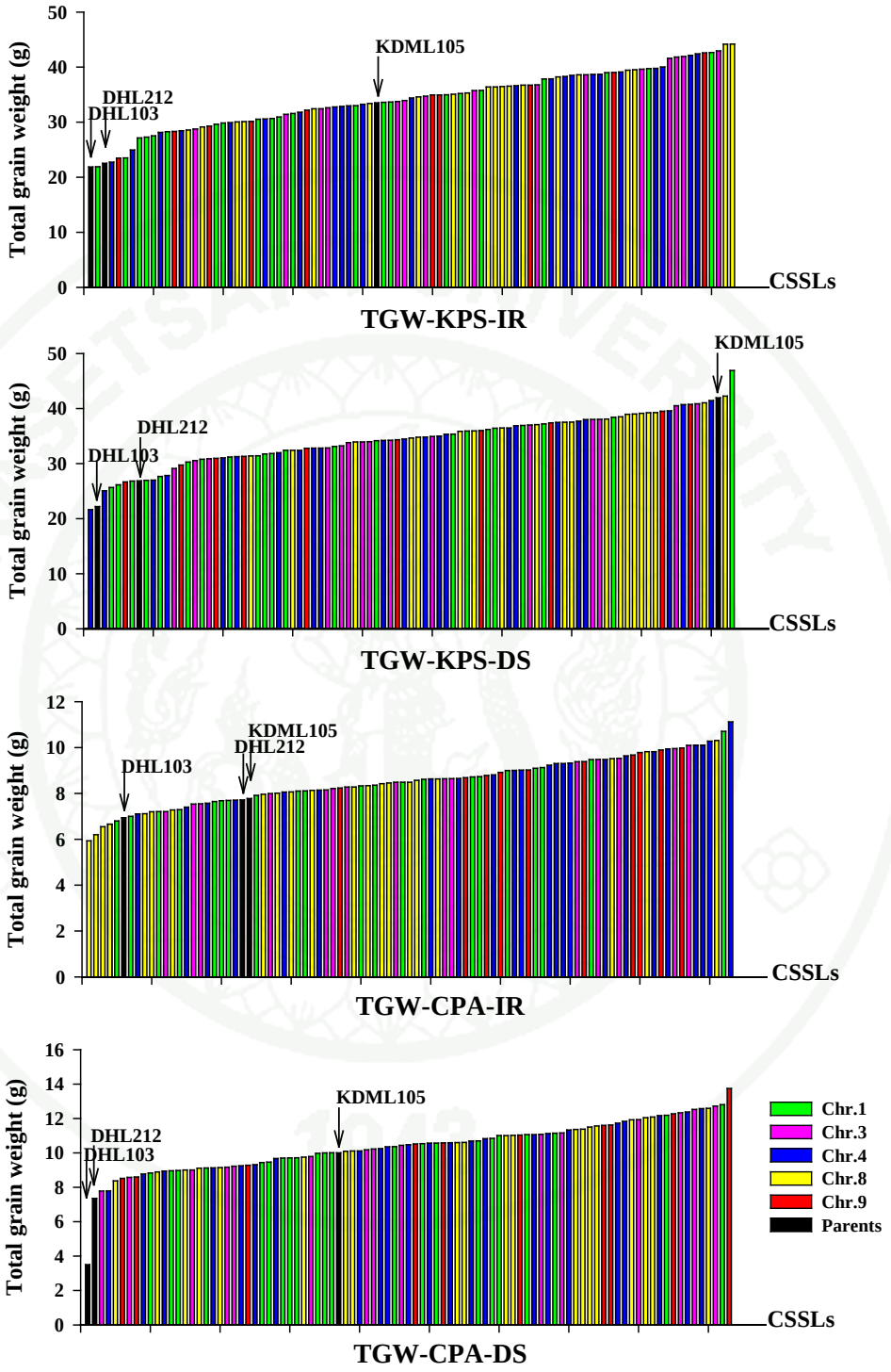
Appendix Figure 4 The mean value of panicle number (PN) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.



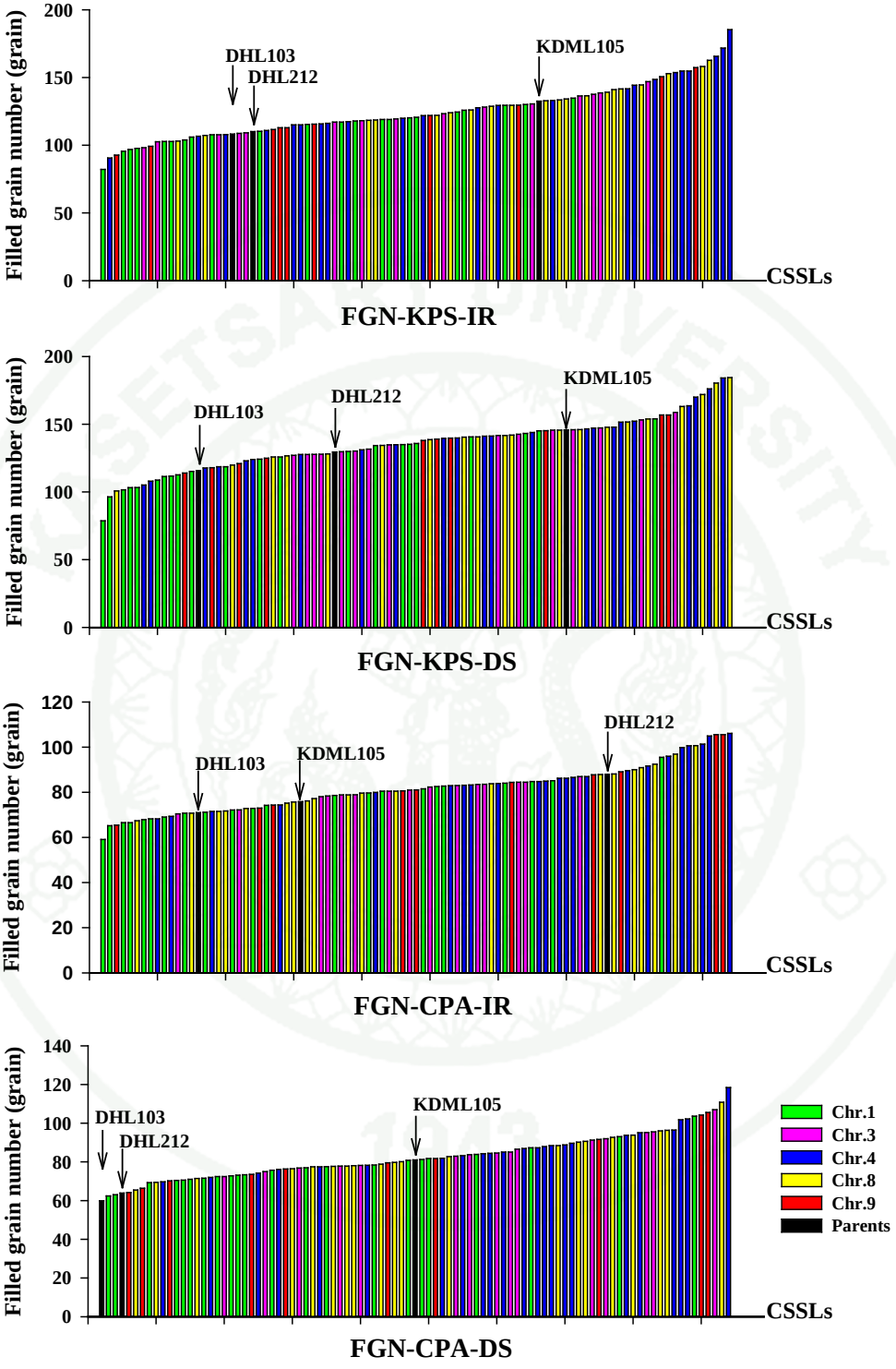
Appendix Figure 5 The mean value of filled grain weight (FGW) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.



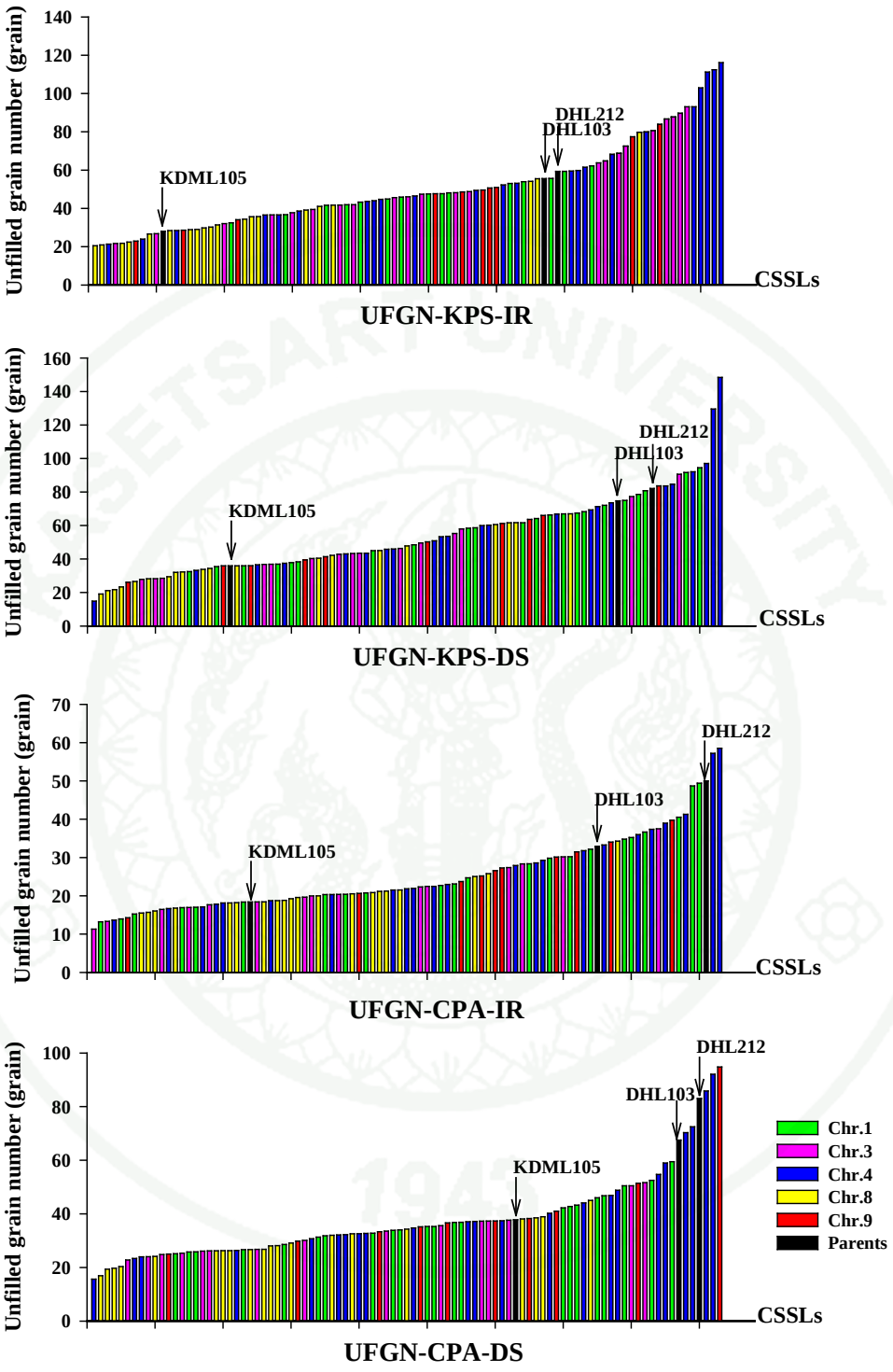
Appendix Figure 6 The mean value of unfilled grain weight (UFGW) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.



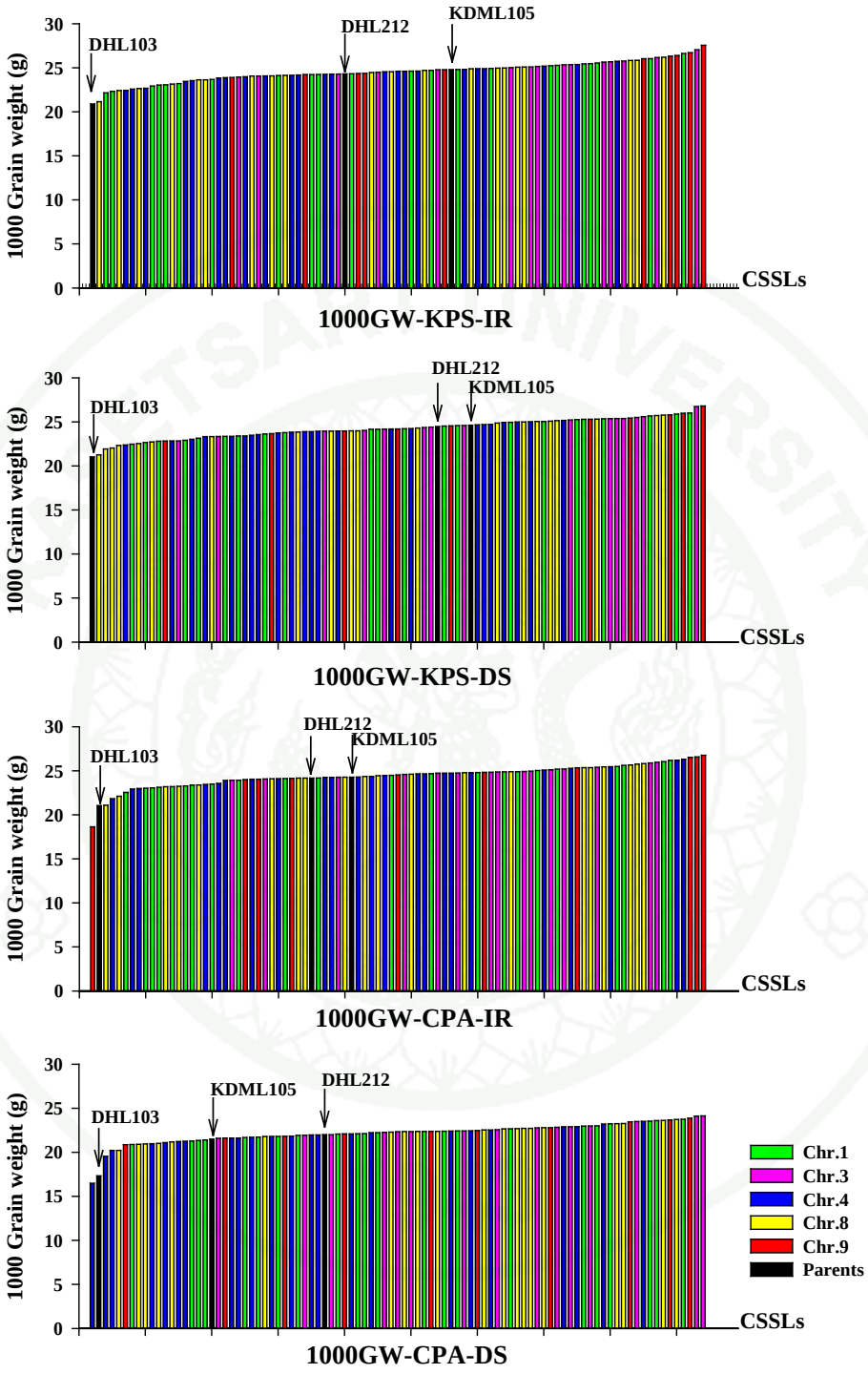
Appendix Figure 7 The mean value of total grain weight (TGW) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.



Appendix Figure 8 The mean value of filled spikelet number (FGN) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.



Appendix Figure 9 The mean value of unfilled spikelet number (UFGN) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.



Appendix Figure 10 The mean value of 1,000 grain weight (1000 GW) of 90 CSSLs under rainfed and irrigated conditions at CPA and KPS.

CIRRICULUM VITAE

NAME Mr. Vaiphot Kanjoo

BIRTH DATE July 9, 1979

BIRTH PLACE Phichit, Thailand

EDUCATION

YEAR	INSTITUTE	DEGREE/DIPLOMA
2001	Naresuan Univ.	B.Sc. (Agriculture)
2004	Kasetsart Univ.	M.S. (Genetics)
2011	Kasetsart Univ.	Ph.D. (Agricultural Biotechnology)

POSITION/TITLE Lecturer

WORK PLACE School of Agriculture and Natural Resources, University of Phayao, Phayao, Thailand

SCHOLARSHIP/AWARDS Lecturer Development Scholarship, (2001-2008) From Office of the Higher Education Commission