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## Study of quantitative traits locis for grain characters in double haploid population of Rice

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### Abstract

A double haploid mapping population consisting of 75 lines of a cross between the irrigated *indica* variety IR64 and the upland *japonica* variety Azucena was used in the present experiment. The complete set of double haploid lines along with parents was evaluated under two sets of conditions, 1. Transplanted with water stress and 2. Transplanted with no stress over two years. Water stress was imposed at flowering and observations were recorded for two traits. A total of 10 putative QTLs for 100 grain weight, grain length, grain breadth and length and breadth ratio were detected, which were found to be explaining a minimum of 9.8 per cent to maximum of 19.3 per cent of phenotypic variation, individually. These QTLs were mapped on the rice genome and linked DNA markers have been validated for use in the marker assisted breeding program.

**Keywords:** Rice, Drought, LB ratio, 100 grain weight, QTL mapping

### Introduction

Rice, wheat, and maize are the three major food crops in the world. Among them, rice is cultivated in 148 million ha and 85% of its total production is used for human consumption (IRRI, 1993) [5]. With the rapidly increasing population in these areas and the limited ground water available for rice cultivation, there is an urgent need to improve the rice plant by increasing its tolerance for biotic and abiotic stresses. Drought is a major abiotic stress, which limits plant growth and productivity, and is a major cause of yield instability. However, most measures of agricultural productivity, such as size, shape, yield and quality are influenced by many genes (polygenes), so that traits in a population do not fall into discrete classes, but show a continuous range of phenotypes. Quantitative variation in phenotype can be explained by the combined action of many discrete genetic factors, each having a rather small effect on the overall phenotype, and the influence of environments. As a result, breeding for quantitative traits tends to be a less efficient and time-consuming process. These genes cannot be studied individually using the methods of classical Mendelian genetics because their effects are lost in the statistical fog of all other background variation. Recently QTL mapping studies in rice revealed two important results on the genetic basis of quantitatively inherited traits. First, identification of few QTLs each having relatively large phenotypic effect and second, the complex phenotype tend to show greater QTL X E interaction, which makes marker-aided selection (MAS) for QTL to genetic improvement of complex trait difficult. Considering this aspect in view, a study of identification and molecular mapping of QTLs for 100 grain weight, grain length, grain breadth and length and breadth ratio in rice was undertaken.

### Materials and Methods

A population of 75 Double Haploid (DH) lines derived from a cross between the irrigated *indica* variety IR64 and the upland *japonica* variety Azucena (Guiderdoni *et al.* 1992) [3] developed at IRRI, was used in the present investigation. IR64 is high yielding improved semidwarf *indica* rice variety suitable for irrigated habitats, were as Azucena is a more drought tolerant *japonica* rice variety. Populations of 75 Double Haploid (DH) lines were grown in randomized complete block design with two replications under two different environmental conditions *viz.*, 1. Transplanted with water stress (TD) and 2. Transplanted with irrigated conditions (TI). In both conditions, the seeds were sown in a bed and seedlings were transplanted to a paddy field 31 days latter, with single plant<sup>-1</sup> hill spaced at 15 X 20 cm. Each plot included three lines with ten plants per line. All normal packages of practices were followed to raise a good crop.

The drought condition was imposed by stopping irrigation at 50 per cent flowering stage. Observations were recorded on in each replication. 100 random grain samples were used for obtaining 100 grain weight data, twenty grains were taken randomly and average breadth was recorded in millimeter, twenty random grain samples were taken randomly and average length was recorded in millimeter and the ratio of grain length to grain breadth was calculated. The mean values over ten plants were considered for analysis. The mean data for two replications under transplanted with water stress, transplanted with irrigated condition were analyzed for QTLs identification. MAPMAKER / QTL 1.1 was used for interval mapping (Locating the QTLs between flanking molecular marker by maximum – likelihood estimation) (Lander *et al.*, 1987) [23], and to estimate the percentage of the total phenotypic variance explained by each QTL. A threshold of LOD > 1.5 was used<sup>-1</sup> test to claim the presence of a QTL.

### Results and Discussion

A total number of QTLs detected under different environmental condition and different season is presented in Table 1 and Fig 3. In general identified QTL can be classified in two types, first type represent major gene that affect quantitative traits, which are detected with large LOD score (>10). The second types include most of QTL identified in rice, which have relative small effect. In this study, 3 QTLs were identified and had small phenotypic effect and LOD score of <10 this is expected as a different characters are under different genetic control and depend on its complexity along with variable level of QTL X E interaction. This is in agreement with the Li *et al.*, (1999) [8] who reported that more than 80 per cent of loci identified in rice are of this type. Number of QTLs identified in this study has been reported by other workers to be present on the same chromosome. e.g. Subramaniam and Madhava (1973) [17]; Yan *et al.* (1998a); Jinhua *et al.* (1998) [6]; Wu-WeiRen *et al.* (1999) [18] reported the QTLs on same chromosome for said traits. The identification of QTLs at same position by different workers in different populations has significant implication and can be inferred as real. These QTLs, which are common, stable and if their relative contribution is also high can be the real candidate for MAS and even the map based cloning (Fig. 3). Apart from these QTLs numbers of other QTLs were also detected in the present study, which are not reported by the previous workers. The difference in location of QTLs for various traits may be because of different cross combination used in this analysis, as the detection of QTL is based on allelic differences between parental lines, different population size, statistical threshold for detecting putative loci, the number of markers used in the analysis (Yano and Sasaki, 1997) [21].

### Frequency distribution of DH lines

The field performance of double haploid lines were graphically presented in Fig. 1. Observations were recorded for 4 quantitative traits on five randomly selected plants in each plot of individual genotypes, grown over two seasons and two different environmental conditions under the Randomized Complete Block Design with two replications. The distribution of individual lines for each character was graphically plotted to assist the symmetry and continuity of the distribution. The character wise graph of DH lines along with parents for each trait is presented in (Fig. 2). The

segregation pattern of both the traits the characters did not followed near normal distribution. Since the characters studied in the present investigation are known to be quantitative in nature. The skewed distribution of many plant showing values close to zero was expected in *indica X japonica* crosses, which typically show high level of sterility. The population cannot be classified into discrete classes, this indicated the polygenic nature of inheritance therefore its inheritance cannot be followed through the simple Mendelian inheritance (Rao 1953) [13]. The perfect normal distribution can be distorted by various factors like environmental interaction and different types of gene (QTL) interactions. For both traits studied in the present investigation, transgressive segregation was observed, thus the DH population exhibited greater amount of variability compared to parents Fig. 2. This also indicated that both the parents differ for their allelic composition for all the traits under study. Different types of gene interactions have been reported (Singh *et al.*, 1980; Rohman *et al.*, 1981; Haque *et al.*, 1981; Cheema, 1988; Manuel and Palanisamy, 1989 and Mohanty *et al.*, 1995) [16, 12, 4, 2, 10, 11] for said traits.

### QTLs Analysis

QTL is segment of chromosome affecting the trait, not necessarily a single locus. The idea of using genetic markers to locate the individual quantitative trait locus (QTL) responsible for variation in quantitative traits goes back nearly to the beginning of modern genetics (Sax, 1923) [15]. Recent progress in DNA markers and development of high density molecular maps of rice (Causse *et al.*, 1994) [1] has allowed the localization of QTL and determination of relative magnitudes of their effect on the trait in rice. In the present investigation the DH population was used to detect QTL for four grain yield characters (Fig. 3).

### Identification of QTLs for 100 Grain Weight

Two QTLs were detected under water stress on chromosome number 1 and 2. One was found on chromosome number 1 with LOD score of 1.7 between marker RG472 - K5 having negative additive effect and explained 11.7% of phenotypic variation. Second QTL was detected on chromosome number 2 with LOD score 1.5 between marker RG437 - RG171 having positive additive effect and explained 10.2% of phenotypic variation. However, one QTL was detected under transplanted with irrigated condition on chromosome number 2 with LOD of 1.5 between marker PaII - RM221 having positive additive effect and explained 10.4% of phenotypic variation.

### Identification of QTLs for Grain Length

Two QTLs were detected under water stress one QTL on chromosome number 1 with LOD score of 1.9 between marker RM34 - RZ19 having negative additive effect and explained 12.2% of phenotypic effect. Second QTL was detected on chromosome number 7 with LOD score of 1.8 between marker RZ488 - PGM0.7 having negative additive effect and explained 11.9% of phenotypic variation. However one QTL was detected under irrigated condition on chromosome number 10 with LOD 1.6 between marker RG257 - CDO98 having negative additive effect and explained 9.8% of phenotypic variation. Zhang *et al.*, (1999) [22], Yan-JuQiang *et al.*, (1999a) [19], Maheswaran *et al.*, (2000) [24], reported the QTLs for grain trait on chromosome # 4 and 6. The difference in location of QTLs for various

traits may be because of different cross combination used, as the detection of QTL is based on different criteria e.g. allelic differences between parental lines, different population size, statistical threshold for detecting putative loci and the number of markers used in the analysis (Yano and Sasaki, 1997) [21].

**Identification of QTLs for Grain Breadth**

One QTL was detected under water stress condition on chromosome number 10 with LOD score of 2.9 between marker RM216 - RG241 having positive additive effect and explained 19.3% of phenotypic variation. However, two QTLs were detected under irrigated condition, one on chromosome number 2 with LOD score of 2.1 between marker CDO686 - RG256 having positive additive effect and explained 14% of phenotypic variation. Second QTL

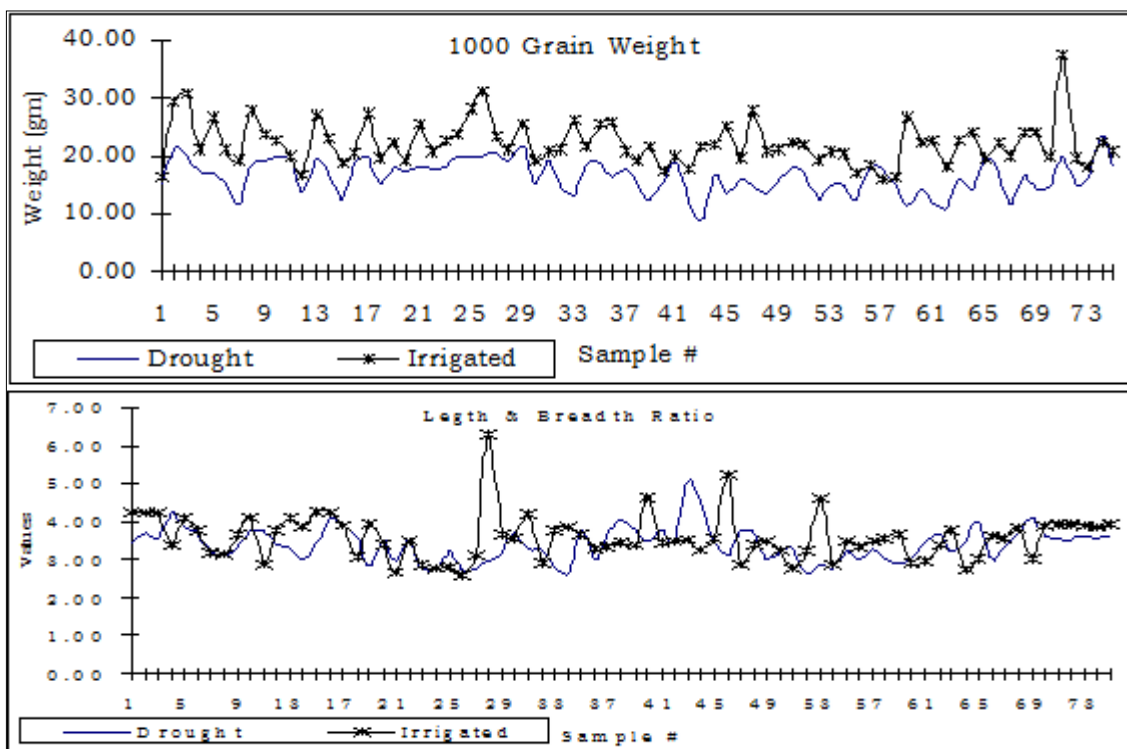
was found on chromosome number 8 with LOD score of 2.6 between marker RM210 - TGMS1.2 having positive additive effect and explained 18.1% of phenotypic effect. Redofia and Mackill (1998) [14] reported the presence of QTLs on chromosome # 10 for grain length and for grain breadth on chromosome # 2, as in this study.

**Identification of QTLs for Grain Length Breadth Ratio**

For this character one QTL was detected under water stress condition on chromosome number 6 with LOD score of 1.5 between marker RM204 - RM50 having negative additive effect and explained 10.4% of phenotypic variation. However, one QTL was detected under irrigated condition on chromosome number 8 with LOD score of 1.8 between marker RM223 - RG8\_Aro having negative additive effect and explained 11.8% of phenotypic variation.

**Table 1:** Putative QTLs detected using interval mapping

Character	Regions between DNA markers	Chr. #	Additive effect	% Variation explained	LOD
<b>100 Grain Weight</b>					
Irrigated	Pall - RM221	2	6.3	10.4	1.5
Drought	RG472 - K5	1	-5.3	11.7	1.7
	RG437 - RG171	2	4.2	10.2	1.5
<b>Grain Length</b>					
Irrigated	RG257 - CDO98	10	-4.7	9.8	1.6
Drought	RH34 - RZ19	1	-5.1	12.2	1.9
	RZ488 - PGM0.7	7	-5.0	11.9	1.8
<b>Grain Breadth</b>					
Irrigated	CDO686 RG256	2	4.0	14.0	2.1
	RM210 TGM1.2	8	4.9	18.1	2.6
Drought	RM216 - RG241	10	0.6	19.3	2.9
<b>Length / Breadth Ratio</b>					
Irrigated	RM223 - RG8_Aro	8	-5.1	11.8	1.8
Drought	RM204 - RM50	6	-3.4	10.4	1.5



**Fig 1:** Fields performance of DH lines

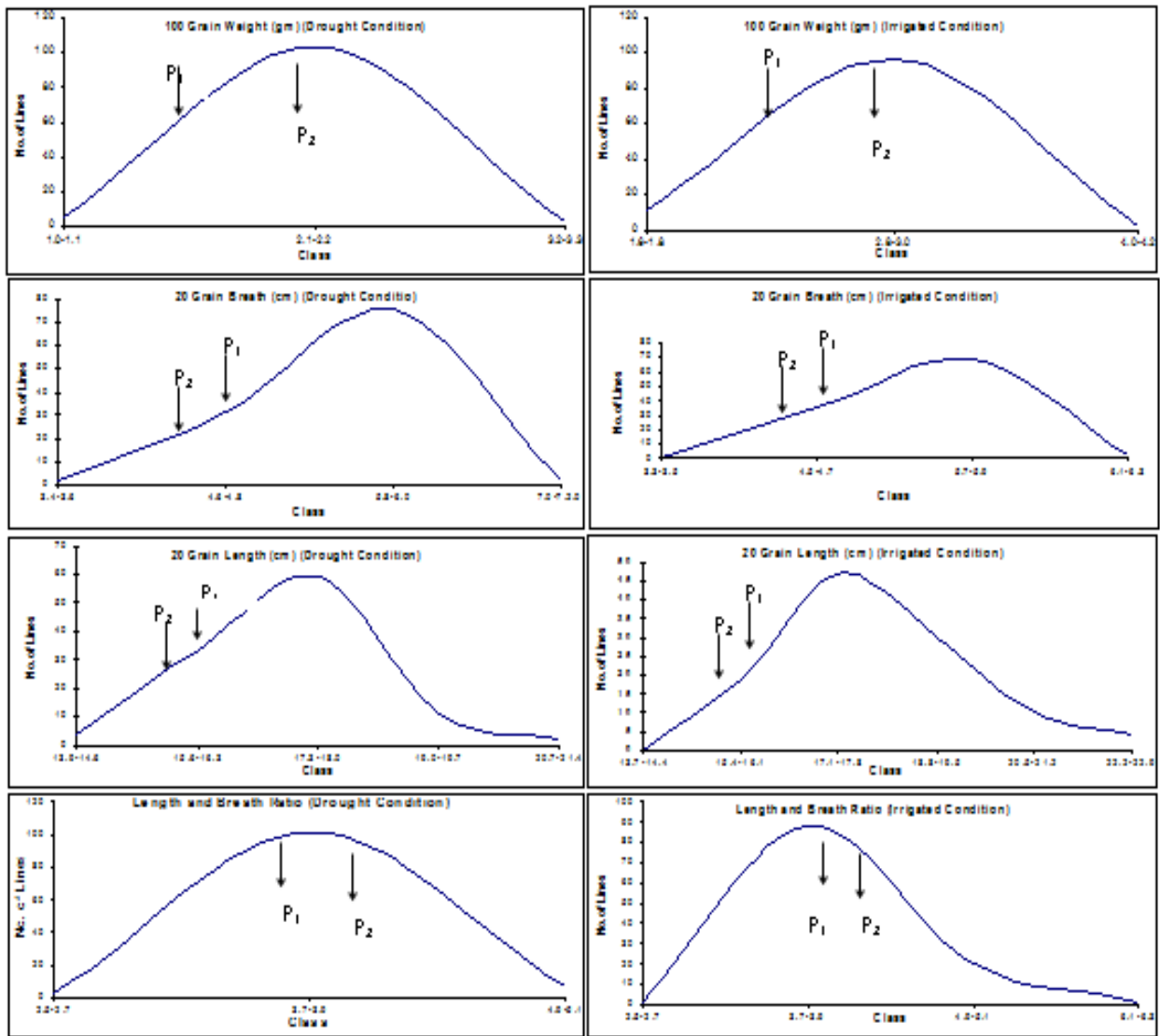


Fig 2: The Frequency Distribution of DH Lines

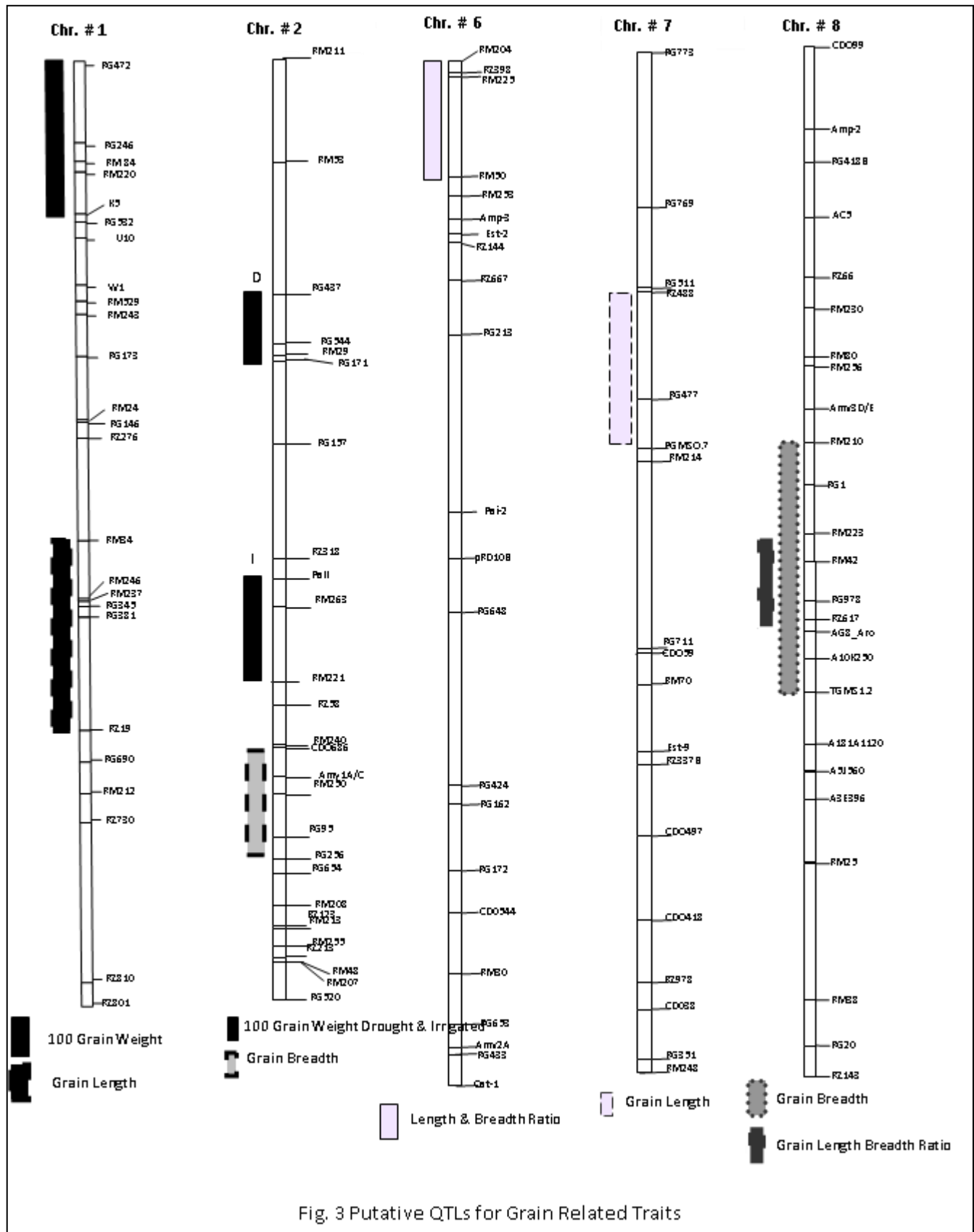


Fig 3: Putative QTLs for Grain Related Traits

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