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Evaluation of Rice Recombinant Inbred Lines Developed from the Cross Rasi × Improved Samba Mahsuri for Drought Tolerance

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Drought has been one of the most important limiting factors for rice productivity, particularly in the rainfed ecosystem. It is important to understand the genetic basis of drought tolerance in donor lines and develop drought tolerant rice cultivars, based on this information. We earlier identified the rice line, Rasi as tolerant to drought and developed a recombinant inbred line (RIL) mapping

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population consisting of 209 lines. These lines along with their parents were grown under well-watered and drought stress conditions in a two-year experiment (wet season 2020 and 2021) with drought stress imposed during reproductive stage. The study revealed high genetic variability for 12 key agro morphological traits associated with drought tolerance among the RILs. Of the 209 RILs, 59 showed superior performance over the checks and even the tolerant parent, Rasi under severe drought condition. Two RILs, RIL-33 and RIL-58 showed exceptional drought tolerance along with greater plot yield and grain weight under drought condition and they possessed medium slender grain type. These two lines can be a new novel genetic resource for drought tolerance in breeding programmes.

Keywords: Drought tolerance; Rasi; Improved Samba Mahsuri; Recombinant Inbred Lines.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the world's second most important cereal crop following corn. Traditionally, countries in Asia have the largest share in world rice production. Global consumption of rice has seen a slight increase over the last several years. In, 2021-22 crop year, about 509.87 million metric tons of rice was consumed worldwide. Rice is an important source of calories along with few proteins and essential nutrients, making it a very useful food for more than half of the world's population [1,2].

Drought stress is one of the major abiotic constraints which causes remarkable losses in rice crop yield. The intensity of drought is increasing with changing environmental conditions and water scarcity. Rice production is heavily affected by drought which results in reduced germination, plant height, biomass, numbers of tillers, chlorophyll content, leaf number and size at various growth stages [3]. Drought not only reduces rice yield but also affects the potential beneficial effects of improved crop management practices such as fertilizer application, pest and disease management. Plant responses to drought stress are very complex as the stress itself involves various climatic, soil and agronomic factors. Rice plants have a variety of strategies to combat drought; however tolerance to drought enables them to yield more, even in conditions of low water availability. Drought tolerance of rice is a complex trait and involves complex morpho-physiological mechanisms [4]. As the changing environment has a strong effect on drought tolerance response, different physio and morphological traits and the use of molecular markers helps to make a strategy for study of drought tolerance in rice under particular conditions. Thus, development of drought tolerant varieties is the most needed approach to tackle the problems imposed by a rapidly changing climate.

Selection of rice lines with improved grain yield under drought can be obtained by direct or indirect selection of traits that contribute to drought tolerance under water deficit condition [5,6,7]. Traditional breeding methods for developing drought-tolerant rice varieties have become less effective due to low heritability and genotype selection being hampered by environmental and genetic interactions. This can be addressed by adopting cutting-edge high throughput genotyping and phenotyping methods [8].

In the current study, a rice cultivar, Rasi which was earlier identified and released as a drought tolerant rice variety was used to develop a recombinant inbred line (RIL) mapping population. This population was further screened under well-watered and drought stress conditions to identify highly drought tolerant RILs with desirable characters. These can be exploited as the recurrent parents in future rice breeding programs for drought tolerance.

2. MATERIALS AND METHODS

2.1 Plant Material

Rasi is a drought-tolerant variety developed and released in the year 1977 by the ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, India. It has resistance to leaf blast, brown spot and tolerance to low soil phosphorus [9,10] and it can also grow well in aerobic conditions, saving 40-50% of the water consumption. Improved Samba Mahsuri (ISM) was developed through marker-assisted pyramiding of three bacterial blight resistance genes, *Xa21*, *xa13*, and *xa5* in the genetic background of the elite mega-variety of rice, Samba Mahsuri, and has broad spectrum resistance against bacterial blight disease [11]. For drought tolerance screening under field conditions, the varieties, Sahbagidhan, DRR

Dhan-42 and Vandana were used as positive checks, while Gangavathi Sona and BPT5204 were used as negative checks. When screened for drought at ICAR- IIRR, Hyderabad, ISM was found to be very sensitive while Rasi showed excellent tolerance to drought.

2.2 Experimental Site

The experiments were conducted in upland irrigated conditions of ICAR-National Rice Research Institute-Central Rainfed Upland Rice Research Station (ICAR-NRRI-CRURRS), Hazaribagh, Jharkhand, India (23°56′34″ N and 85°21′46″ E) during Kharif 2020 (i.e. wet season 2020) and Kharif 2021 (i.e. wet season 2021). The RIL mapping population was subjected to drought stress and non-stress (control) conditions. Drought stress was artificially imposed by draining out water from the field during the reproductive stage which was referred to as stress trials [12].

2.3 Screening of the RIL Population for Drought Tolerance

A cross was made between Rasi and Improved Samba Mashuri (ISM) and a RIL population was developed using a single seed descent method [10] These RILs (n=209) were used for phenotypic evaluation of drought tolerance at ICAR-NRRI-CRURRS, Hazaribagh. Wet bed nursery was being raised and 21 days old seedlings of the mapping population were transplanted into the drought screening plot along with the parents. The experiment was conducted in Alpha lattice design consisting of two replications with spacing of 15 × 20 cm (in three rows, constituting of 10 hills per row). The RILs were screened for reproductive stage drought tolerance by draining out water one month after transplanting (52 DAS), and the drain was kept open, till maturity. The crop was strictly maintained as rain fed and was not irrigated even once and the soil moisture was recorded constantly. The various agro-morphological parameters were recorded for the RILs along with the donor and recurrent parents. Days to 50% flowering, Plant Height (cm), Grain yield per plot (g/plot), Grain yield per hectare (kg/ha), Harvest index (HI), Biomass (g)/0.5 m, Panicle length (cm), No. of Panicles per 0.5 m sample, Grain weight (g) of the sample for harvest index harvested from 0.5-meter length, No. of filled grains per panicle (average of 5 panicles), No. of unfilled grains per panicle (average of 5 panicles) and Sterility percentage (%). Three plants were selected randomly which represent the entire line and mean data were subjected to statistical analysis using open-source statistical software ’R’ version 3.6.3 (R Core Team, 2016).

3. RESULTS AND DISCUSSION

Rice is the world's most important stable food crop, supplying more than half of the world's population [13]. Drought is the single most significant constraint to rice productivity in both upland and lowland ecosystems. It affects 14 million ha of upland rice production and 19 million ha of lowland rice production worldwide [14]. A little effort has been put for developing drought tolerant rice cultivars. Many of the varieties grown in rainfed ecosystems were developed for irrigated ecosystems without drought tolerance screening. Farmers prefer these varieties due to their high yielding potential and good grain quality in rainfed regions of South and South East Asia [15]. However, because of their susceptibility to moisture stress, these varieties are subjected to significant yield loss due to unanticipated drought stress during the growing season. Traditional rice cultivars grown in rainfed areas, on the other hand, are highly drought tolerant but have low yield potential. Farmers need cultivars that combine high yield potential with improved drought tolerance to ensure high yield in non-drought years and acceptable yield in drought years, further development of drought tolerant varieties in genetic background (Fisher *et al*., 2003). Thus breeding of drought tolerant varieties with high yield potential is highly challenging because of its complex physiological and molecular mechanisms. Thus, mapping populations are developed by combining parents with large differences in target traits, which enables the identification of QTLs for the trait of interest [16]. In the present study, two varieties contrasting in their response to drought stress, viz., Rasi and ISM were crossed for the development of the RILs [17] at ICAR-IIRR, Hyderabad, India. Single seed descent (SSD) method was adopted for advancing the progenies in order to develop the RILs [18].

A total of 209 RILs along with the parents, tolerant and sensitive checks were screened for drought tolerance under drought stress condition (NPK @ 80:40:40) at ICAR –NRRI –CRURRS, Hazaribagh and the weather factors were recorded for the period of drought screening (Table 1).

Table 1. Weather data recorded at drought screening plot across the two seasons

Season	Month	Max temp (°C)	Min. temp (°C)	RH (%)	RAIN FALL (mm)	WIND VELOCITY (Km/hr)
WS 2020	June	36.8	24.3	65.5	100.1	7.3
	July	30.8	22.1	83.3	302.0	5.9
	August	29.9	21.9	82.8	169.3	4.9
	September	29.5	21.2	85.4	267.7	6.2
	October	27.8	17.5	83.4	174.2	2.3
	November	26.3	11.1	81.8	0.0	2.3
	December	21.8	7.2	86.1	14.2	3.0
WS 2021	June	31.23	21.87	81.03	380.8	2.59
	July	31.18	22.45	82.48	344.4	2.24
	August	30.61	21.84	85.65	313.8	1.78
	September	30.07	21.05	84.6	289.4	2.17
	October	29.02	17.82	77.35	146.4	1.21
	November	25.98	10.65	75.83	7.2	0.37
	December	22.45	7.64	77.74	48.4	0.99

Table 2. Descriptive statistic of 12 traits under drought stress

S. No	Trait	Rasi	ISM	Min	Max	Mean	S.E	CD @ 5%	CD @1%	CV%	Skewness	Kurtosis
1	Days to 50% flowering (Nos)	86.00	110.30	77.00	113.00	96.53	1.48	4.14	5.47	18.89	0.13	2.61
2	Plant Height (cm)	68.70	61.80	34.80	100.40	62.05	4.72	13.18	17.39	10.03	-0.09	2.80
3	Grain Yield per Hectare (kg/ha)	1326.60	550.00	0.00	3500.00	1198.32	170.22	474.79	626.26	19.84	0.47	2.46
4	Harvest Index	0.17	0.04	0.00	0.37	0.13	0.02	0.04	0.05	16.46	0.55	2.73
5	Biomass (g)	220.00	210.00	65.00	325.00	190.28	18.15	50.63	66.79	12.81	-0.40	3.68
6	Plot Yield (g/plot)	261.60	110.00	0.00	700.00	239.66	34.04	94.95	125.25	19.84	0.47	2.46
7	Panicle Length (cm)	18.50	24.60	13.50	24.60	18.42	0.96	2.70	3.56	6.76	-0.13	2.75
8	Panicle Number (Nos)	37.60	20.00	0.00	85.00	46.45	5.84	16.29	21.49	19.37	-0.21	2.81
9	Grain Weigth (g)	35.00	11.00	0.00	120.00	27.23	4.93	13.75	18.14	15.02	0.86	3.24
10	Number of grains per panicle (Nos)	55.30	23.00	0.00	125.40	50.15	6.49	18.11	23.89	16.78	-0.20	2.26
11	Chaffy grains per panicle (Nos)	19.50	93.60	10.00	108.00	46.73	5.30	14.79	19.51	16.33	0.42	2.81
12	Sterility Percentage (%)	26.50	81.07	10.10	100.00	49.49	4.47	12.49	16.48	11.50	0.45	2.29

Data were recorded and the average of two seasons is considered for a total of 12 agro-morphological traits i.e., Days to 50% flowering, Plant Height, Plot yield, Grain yield per hectare, Harvest index, Biomass, Panicle length, No. of Panicles per 0.5 m sample, Grain weight, Grain number per panicle, Chaffs number per panicle, Sterility percentage. Descriptive statistics of RILs along with parents under stress condition has been carried out and represented in Table 2.

The days to fifty percent flowering (DFF) under drought stress ranged from 77 ± 0.146 days (RIL-8) to 113 (RIL-82) ± 0.097 days, with an overall mean of 96.53 ± 1.48 days, while mean values of 86 ± 0.9 days and 110 ± 0.14 days were recorded in Rasi and ISM, respectively. Among the population, the late flowering genotypes suffered higher yield reduction than early maturing ones on an average among population. This is mainly because in early flowering genotypes water deficit occurs after the completion of pollination and fertilization and coincides with early grain filling period. Whereas in late flowering genotypes drought stress at this period leads to increase in embryo abortion or reduce grain weight [19]. The spikelet fertility was found to decrease with decrease in panicle excretion under drought stress, due to entrapment of spikelets in leaf sheath [19]. Similarly, delay in DFF was reported across several studies in rice under drought stress previously [20-25,15,26,27].

The plant height (PH) under drought stress ranged from 34.8 ± 0.47 cm (RIL-118) to 100.4 ± 0.12 (RIL-54) with an overall mean of 62.05 ± 4.72. While 68.7 ± 0.029 and 61.8 ± 0.024 were the mean values of plant height recorded in Rasi and ISM, respectively. Similarly, [28] reported a reduction in plant height in several field experiments under different stress levels (mild, moderate, and severe stress). The PH reduction was much greater in dry season experiments than in wet season experiments under stress. Drought reduces plant height, leaf area, and crop growth due to impaired mitosis, cell elongation, and expansion [29]. Because of the decrease in turgor pressure, cell growth is the most drought-sensitive [30]. The HI (Harvest Index) under drought stress ranged from 0 (RIL-154) to 0.37 ± 0.0014 (RIL- 33) with an overall mean of 0.13 ± 0.0159 with HI values of 0.17 ± 0.0015 and 0.04 ± 0.00011 respectively recorded for Rasi and ISM. The Biomass under drought stress ranged from 65 ± 0.97 (RIL-83) to 325 ± 0.97 (RIL- 33) with an overall mean of 190.28 ± 18.15. The average biomass values for Rasi and ISM were recorded as 220 ± 0.097 and 210 ± 0.048, respectively. Grain yield under drought has been reported to be a function of biomass production and harvest index at the vegetative and reproductive stage, respectively [31]. Severe reproductive stage stress has significant effect on harvest index. Atlin *et al*., [31] reported that a large portion of variation in yield was explained by variation in HI under reproductive stress conditions. A reduction in yield was reported due to a reduction in biomass, even when there was no significant reduction in HI, thereby producing a further reduction in yield. Genotypes with greater capacity to produce dry matter in a short growth cycle had the advantage in grain yield. The GYKGPHA (grain yield per Hectare) under drought stress ranged from 0 (RIL-154) to 3500 ± 12.2 (RIL-33), with an overall mean of 1198.32 ± 170.22. While average GYKGPHA values of 1326.6 ± 2.54 and 550 ± 0.097 were recorded for Rasi and ISM, respectively. The PLOTYLD (Plot yield) under drought stress ranged from 0 (RIL-154) to 700 ± .2.44 (RIL-33) with 261.6 ± .097 and 110 ± .048 for Rasi and ISM, respectively. The overall mean value of PLOTYLD was 239.66 ± 34.04. The panicle length (PL) under drought stress ranged from 13.5 ± 0.11 (RIL-40) to 24.6 ± 0.07 (RIL-66) with an overall mean of 18.42 ± 0.96. The Rasi and ISM exhibited PL values18.5 ± .03 and 24.6 ± 0.01, respectively. The values of PANNO (No of panicles per 0.5m) under drought stress for Rasi and ISM were 37.6 ± 0.04 and 20 ± .048, respectively. The overall range of PANNO was recorded from 0 (RIL-155) to 85 ± 0.97 (RIL-12) with the mean of 46.45 ± 5.84. The range of GRAINWT (grain weight) values under drought stress ranged from 0 (RIL-154) to 120 ± 1.71 (RIL-33) with 35 ± 0.04 and 11 ± 0.14 values recorded for Rasi and ISM, respectively. Significant reduction in grain yield was observed in many drought sensitive genotypes, when drought coincides with reproductive stage in earlier reports [20,21,23,24,15,26,32,27]. In reproductive drought stress condition, there was a reduction in grain yield among all the genotypes which flowered late, thus affecting the floral development and panicle elongation [33,34].

The GNOPPAN (grain no. per panicle) under drought stress ranged from 0 (RIL-154) to 125.4 ± 1.56 (RIL-152) with an overall mean of 50.15 ± 6.49. The parents, Rasi and ISM showed values of 55.3±0.092 and 23±0.097, respectively. The CNOPPAN (chaffy no. per panicle) under drought stress ranged from 10 ± 0.37 (RIL-112) to 108 ± 0.42 (RIL-155) with an overall mean of 46.73 ± 5.3, whereas for Rasi and ISM the CNOPPAN recorded values were 19.5 ± 0.019 and 93.6 ± 0.007, respectively. The STERILITYP (Sterility percentage) under drought stress ranged from 10.1 ± 0.43 (RIL-112) to 100 ± .004 (RIL-154) with an overall mean of 49.49 ± 4.47. The parents, Rasi and ISM showed values of 26.5 ± 0.01 and 81.07 ± 0.04, respectively. The onset of drought stress at flowering, the most sensitive growth stage, adversely affects the percentage of fertile spikelets and thus the number of grains will be reduced. This trait, is critical for rice under water stress [34]. At reproductive phase, drought results in barrenness, spikelet sterility, reduction in photosynthesis, early senescence, shortening of grain filling period and decreases remobilization of assimilates to grains leading to reduction in grain number and grain weight collectively [35,36]. The failure of panicle exertion alone accounts for approximately 25–30% of spikelet sterility because the unexserted spikelets cannot complete anthesis and shed pollens, even when development is otherwise normal [37].

A continuous distribution of phenotypic values for various agro morphological traits was recorded for drought tolerance indicating quantitative inheritance of these traits. In the histogram, the Y-axis (vertical axis) represents the frequency count i.e., number of RILs, while the X-axis (horizontal axis) represents the trait measured (Fig. 1).

In the present study, it was found that the distribution of RILs for plant height, biomass, panicle length, number of panicles and grain number per panicle show negative skewness. In contrast the distribution of days to 50% flowering, grain yield per hectare, harvest index, plot yield, grain weight, chaffy number per panicle and sterility percent showed positive skewness. Maximum skewness was observed for grain weight (0.86) and minimum for plant height (-0.09). Positive skewness towards tolerance is related to complementary gene interaction whereas negative skewness indicates duplicate gene interaction. Kurtosis is a statistical measure which shows the degree of the score cluster in peak or tail of a variable frequency distribution. Peak is the tallest part of the distribution curve whereas tails are the ends of the curve. Kurtosis is divided into three categories –a normal distribution with kurtosis three is termed as mesokurtic (kurtosis = 3), a normal distribution with kurtosis more than three (kurtosis > 3) is termed as leptokurtic and a normal distribution with kurtosis less than three (kurtosis < 3) is termed as platykurtic [38]. In the present study, day to 50% flowering (2.61), plant height (2.8), grain yield per hectare (2.46), harvest index (2.73), plot yield (2.46), panicle length (2.75), panicle number (2.81), grain number per panicle (2.26), chaffy number per panicle (2.81), sterility percent (2.29) showed kurtosis value below three, thus platykurtic distribution. While the rest of the traits such as biomass (3.68) and grain weight (3.24) exhibited values above three which means that they are showing leptokurtic distribution. Additionally, a positive kurtosis shows the presence of gene interaction, whereas a negative value denotes the absence of gene interaction [39].

The study has identified some promising RILs that have a good level of tolerance to drought. A total of 59 genotypes exhibited a very good level of tolerance to drought with reasonable yield levels under drought stress, as compared to the sensitive parent, ISM. Two RILs, RIL-33 and RIL-58 also possessed the highly desirable characters with a medium slender grain type (Fig. 2). Both the lines showed better yield-related parameters and biomass in comparison to ISM, indicating a key role for these traits with respect to drought tolerance (Table.3).

Table 3. Comparison between parents and RILs for different trait under drought stress

Entry	DFF	PH	GYKH	HI	BM	PLY	PANNO	GW	GNOPPAN	SP
RIL-33	85	84.1	3500	0.37	325	700	73	120	75.6	29.7
RIL-58	94	80.8	2600	0.27	255	520	42	70	66.6	35.6
ISM	110	61.5	550	0.05	210	110	20	10	22	81.0
Rasi	87	68.4	1300	0.16	220	260	38	35	54.4	26.5

DFF-days to 50%flowering, PH-plant height, GYKH-grain yield per hectare, HI-Harvest index, BM-Biomass, PLY-plot yield, PANNO-number of panicles, GW-grain weight, GNOPPAN-grain number per panicle, SP-sterility percent.

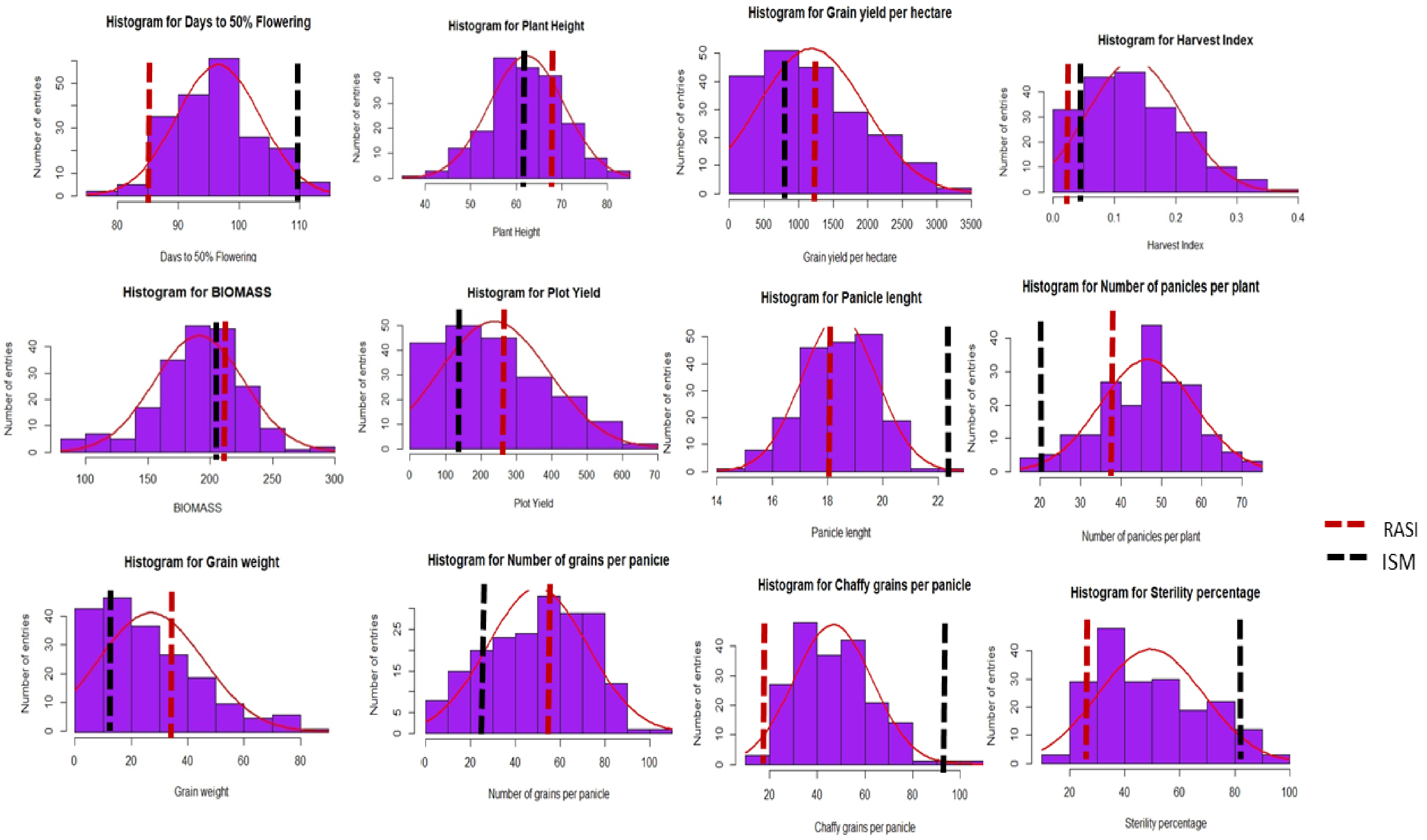

Fig. 1. Frequency distribution of recombinant inbred lines for 12 traits, based on screening for drought tolerance under stress condition

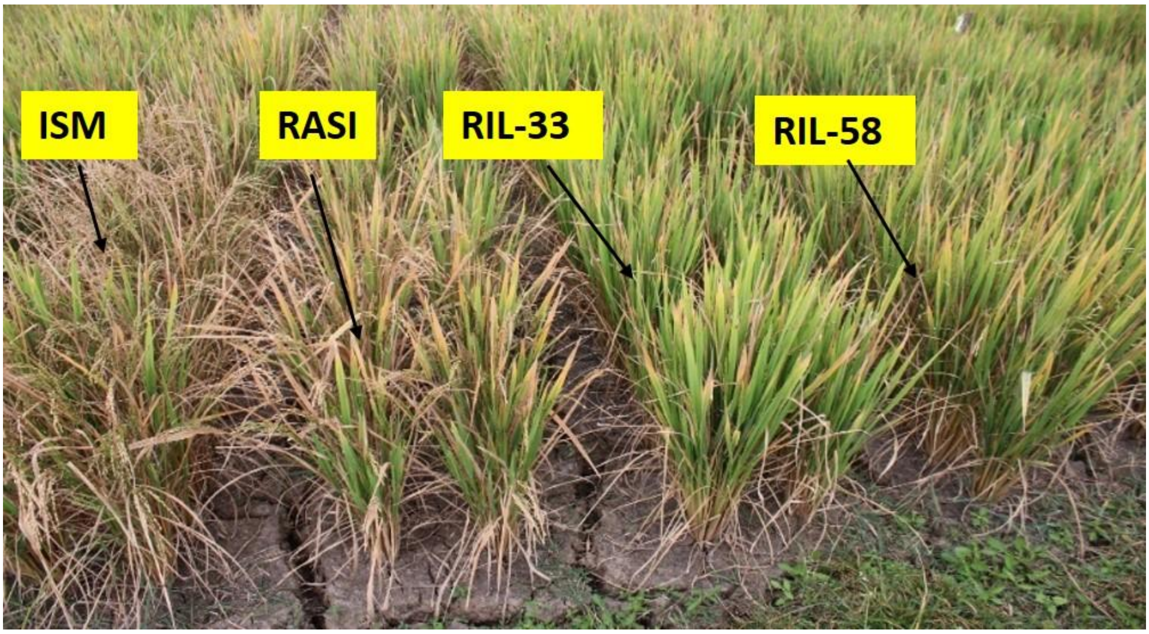

Fig. 2. Performance evaluation of selected RILs under drought stress
Performance of the selected RILs (RIL- 33 and RIL-58) along with the parents- Rasi and ISM under drought stress

4. CONCLUSION

In conclusion, the two RILs (RIL-33 and RIL-58) have highly desirable characters such as, high yield under drought, early maturing, medium-slender grain type and better plant height along with tolerance to drought in comparison to both the parents. We are advancing these two genotypes along with other RILs possessing high yield under drought stress for evaluation in larger plots. Cultivation of such genotypes possessing tolerance to drought can enhance the productivity of Samba Mahsuri farmers. The present study also concluded the skewness of the genotypes were towards tolerance. It suggests a possibility of identifying one or more main QTLs related to drought tolerance according to analysis of a few important phenotypic metrics as well as yield data. The population will soon be genotyped using a set of molecular markers for possible identification and characterization of the component loci associated with tolerance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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