# Effect of drought on morpho-physiological, yield and yield traits of chromosome segment substitution lines (CSSLs) derived from wild species of rice

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#### ABSTRACT

Eighty chromosome segment substitution lines (CSSL) developed in the background of Curinga x O. rufipogon and Curinga x O. meridionalis along with four checks (tolerant and susceptible) were subjected to vegetative and reproductive stage drought stress. At vegetative stage, drought stress significantly reduced total chlorophyll content, relative leaf water content with an increase in proline content. RUF-44, MER-13 and MER-20 were found promising with consistent performances in various morpho-physiological observations. The higher accumulation of proline, more chlorophyll retention and more relative leaf water content at vegetative stage during moisture stress were major criteria for stable yield production of drought tolerant CSSLs. At reproductive stage stress, the CSSLs with high grain yield, minimal relative yield reduction (RYR) and lowest susceptibility index (DSI) were considered as drought tolerant and the reverse as susceptible line. RYR and DSI along with high grain yield under moisture stress was observed in MER-20 and MER-13 with 81.84% and 8.35% RYR and 0.83 and 0.11 DSI values in dry and wet seasons, respectively. However, the extent of RYR was maximum with high DSI in IR 20 and Curinga in both the seasons.

Key words: CSSL, physiological traits, yield attributes, drought stress

In India, the total area under irrigated, rainfed lowland and upland rice is 22.0, 14.4 and 6.3 million ha, respectively (Singh 2009). Out of the total 20.7 million ha of rainfed rice area reported in India, approximately 16.2 million ha lie in eastern India (Singh et al. 2000), of which 6.3 million ha of upland area and 7.3 million ha of lowland area are highly drought-prone (Pandey and Bhandari 2009). As the global climate changes continue, water shortage and drought have become an increasingly serious constraint limiting rice production worldwide (Wassmann et al. 2009 a, b). Among the abiotic stresses in the rainfed systems, drought is the most important factor limiting rice productivity (Ali et al. 2008; Venuprasad et al. 2008). Rice is particularly sensitive to drought stress and even mild drought stress can result in significant yield reduction in rice (Centritto et al. 2009).

Wild species of rice (genus Oryza) contain many useful genes but a vast majority of these genes remain untapped to date. The wild rice relatives serve as a rich reservoir of novel genes or alleles that can be used for the improvement of existing rice cultivars. Chromosome segment substitution lines (CSSLs), which carry a specific donor chromosome segment in the genetic background of a recurrent cultivar, are powerful tools for enhancing the potential of genetic analysis and identifying naturally occurring favorable alleles in unadapted germplasm. To date, CSSLs derived from distant relatives of rice including O. meridionalis, O. glumepatula, O. rufipogon and O. glaberrima have been constructed (Hirabayashi et al. 2010, Shim et al. 2010, Yoshimura et al. 2010) in different institutions.

Cornell University, USA is pioneering in using wild genetic resources to improve the performance of elite rice cultivars for drought stress. Eighty chromosomal segment substitution lines developed from crosses between the tropical japonica elite cultivar, Curinga, and two wild relatives, OR44 (*O. meridionalis*) and IRGC105491 (*O. rufipogon*) have been received by National Rice Research Institute (NRRI), Cuttack from Cornell University, USA for testing under field condition to identify the best drought tolerant lines for reproductive stage stress which can be rapidly introgressed further into multiple commercial cultivars.

# MATERIALS AND METHODS

The plant material consisted of two sets of CSSLs (total eighty CSSL lines) and four checks including IR20 (drought sensitive), CR143-2-2, Azucena (drought tolerant), and Curinga (parent). These were collected from McCouch laboratory, Cornell University, USA. The two sets of CSSL was developed by backcrossing two different wild donor parents with recurrent parent Curinga (O. sativa ssp. tropical japonica) (CUR), a commercial rice variety released in 2005, developed at Brazil (de Morais et al. 2005), respectively, using marker assisted selection. The recurrent parent Curinga is a semi-early maturing, drought-tolerant cultivar with an average yield under upland conditions of 4,465 kg/ ha. In the first set the donor was O. meridionalis Ng, acc. W2112 (Oryzabase :http://www.shigen.nig.ac.jp/ rice/oryzabaseV4/), and in the second set, the donor was O. rufipogon Griff. acc. IRGC 105491 (International Rice Research Institute, IRRI; http:// www.irgcis.irri.org:81/grc/IRGCISHome.html) (Arbelaez et al, 2015). The O. meridionalis/Curinga CSSL (32 in No) is hereafter referred as MER, whereas O.rufipogon/Curinga CSSLs (48 in No) referred as RUF. The objective was to phenotype these CSSLs under field condition to identify the best drought tolerant lines for reproductive stage stress which could be rapidly introgressed further into multiple commercial cultivars.

The experiment was conducted at National Rice Research Institute Cuttack, (NRRI) Odisha during two seasons *i.e.*, dry and wet seasons of 2014 under rain out shelter condition for stress and nearby field for non stress (irrigated) trial. The plant material was consisting of eighty CSSLines and four checks IR20 (drought sensitive), CR143-2-2, Azucena (drought tolerant), and Curinga (parent).

Seeds of each CSSlines were dry direct seeded in two replications following randomized block design (RBD) under both rain out shelter (for stress) and control field conditions. Seeds were dibbled to a depth of 2 cm with 20 cm row spacing and 10 cm spacing between hills. To avoid differences in flowering time and impose uniform stress at the time of flowering in each line, staggered sowing was done in 10 days interval. Based on flowering durations, the lines were grouped into two groups: Group -1 (86-95 DFF) and Group -2 (75-85 DFF). Recommended dosage of fertilizers (N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O @ 40:20:20 kg/ha) were applied basally. The crop in rainout shelter was irrigated after sowing and then in three days interval after germination for good crop stand, while the crop grown under field condition was frequently irrigated. Weeds were controlled manually until full canopy was achieved.

# Stress treatment

Stress treatment was imposed in two cycles; one in vegetative stage and other one in reproductive stage. For vegetative stage, the stress was imposed on 21 days old seedlings for 14 days and then re-irrigated for recovery. For reproductive stage stress, when the crop attained panicle initiation stage (65 days after germination in Group-1 and 55 days after germination in Group-2), irrigation was withdrawn about 25-30 days till the soil moisture tension (SMT) reaches upto -50kPa at 30cm and -70kPa at 15cm depth with 13% and 15% soil moisture content.

# Observations

Morpho-physiological traits like early vegetative vigor (EVV), relative leaf water content (RLWC), total chlorophyll content and proline content were measured at 0, 7 and 14 days after stress for vegetative stage stress. EVV was scored following standard evaluation system (IRRI 2002), the total chlorophyll content was estimated according to method of Arnon (1949), proline content was measured by the method of Bates *et al.* (1973) and RLWC by Barrs and Weatherley (1962).

For reproductive stage stress, days to 50% flowering, plant height, effective tiller number, panicle number, stem weight, panicle weight, no. of fertile grains, no. of chaffs, total number of spikelets, grain

# Effect of drought on CSSL derived rice lines

filling percentage and maturity dates were recorded precisely.

Drought Susceptibility Index (DSI) for grain yield and Relative Yield Reduction (RYR) were calculated as follows:

1. Drought susceptibility index (DSI) was used as per Fischer and Maurer (1978):

DSI = (1 - Ys/Yc)/D

Where Ys = Grain yield of the CSSLs under stress condition

 $\label{eq:condition} Yc = Grain \ yield \ of \ the \ CSSLs \ under \ control \ condition$ 

D = 1-(Mean yield of all CSSLs under stress / Mean yield of all CSSLs under control)

2. Relative yield reduction (RYR) was estimated by the equation of Kumar *et al.* 2008: RYR% =  $100 \times [1-$  (Grain yield under moisture stress / Grain yield under control)]

All hydrological observations during stress period like soil moisture content (SMC) at 15cm and 30cm depth by gravimetric method and soil moisture tension by installing tensiometer tubes at 15cm and 30cm depth were recorded in weekly intervals. During peak stress period, low soil moisture content (SMC %) of 12.13 to 14.72% and high soil moisture tension (SMT) of -30kPa to -40 kPa for vegetative stage and SMC of 9.13 to11.12%, SMT of -50kPa to -55kPa for reproductive stage at 30cm soil depth was maintained.

# **RESULTS AND DISCUSSION**

#### (i) Morpho-physiological traits during

#### vegetative stage stress

Identification of a drought tolerant CSSLs of rice is a difficult job for several reasons. Several attributes are related to drought tolerance. It is highly impossible to have a genotype possessing all these characters responsible for drought tolerance. For the selection of such genotypes, the studies on morpho-physiological characters related to plant parts are essential (Deshmukh *et al.* 2004). Eighty CSSLs and four checks under rainout shelter condition were evaluated for drought tolerance in two seasons (Dry and wet season) of 2014.

Early vegetative vigor (EVV) of 21 days old seedlings was scored following standard evaluation system (IRRI 2002) before imposing stress. The scoring values were 1,3,5,7 and 9, which specifies extra vigorous, vigorous, normal, weak and very weak growth conditions respectively at seedling stage. This scale was used for evaluating genetic material and varieties under stress and control conditions. Among 84 lines, 32 lines had SES score '1', 23 lines had '3', 15 lines had '5', 11 lines had '7' and 3 lines had '9' score in both the seasons.

Moisture stress significantly reduced the relative leaf water content (RLWC) and total chlorophyll content with the increase in proline accumulation over the seasons. Among the lines, 14 days after moisture stress during dry and wet season, RLWC ranged from 46.04% to 75.53% and 46.67% - 70.75%, respectively. Among the best 10 lines RUF-44 (71.19% and 70.70%) and MER-20 (73.30% and 70.42%) were found promising and consistent with higher RLWC over the seasons. According to Almeselmani et al. (2011; 2006) RLWC indicates the water status of the cells and has significant association with yield and stress tolerance. This is a very important trait that indicates drought tolerance and varieties which exhibit restricted changes in relative water content per unit reduction of water potential are often considered to be relatively drought tolerant (Vurayai et al. 2011).

In the present study, plants showed a tendency to accumulate proline under severe moisture stress. In dry season MER-6 (32.62 µmol g fr wt<sup>-1</sup>) followed by MER-13 (32.20 µmol g fr wt<sup>-1</sup>) and RUF-27 (29.65 µmol g fr wt<sup>-1</sup>) recorded highest proline accumulation at 14 days after stress while in wet season RUF-44  $(21.03 \,\mu\text{mol g fr wt}^{-1})$  followed by RUF-13  $(20.92 \,\mu\text{mol})$ g fr wt<sup>-1</sup>) and MER-16 (20.89 µmol g fr wt<sup>-1</sup>) had higher values. Among the best 10 lines RUF-19 and MER-13 were observed to have highest accumulation of proline in both the seasons. The role of proline in adaptation and survival of plants had been observed by Watanabe et al. 2000 and Saruhan et al. 2006. The resistant varieties accumulate high proline content and tolerate stress for longer time than susceptible varieties (Saruhan et al. 2006).

Photosynthetic pigments plays important role in harvesting light. The content of both chlorophyll 'a', 'b' and total chlorophyll content changes under drought



Fig. 1. Soil moisture content (SMC%) and soil moisture tension (SMT) at 15cm and 30 cm depth during moisture stress period starting from vegetative to reproductive stage

stress (Farooq *et al.* 2009). Total Chlorophyll content decreased with the increase in moisture stress. In dry season highest content was recorded in RUF-32 (1.79 mg g fr wt<sup>-1</sup>), RUF-30 (1.73 mg g fr wt<sup>-1</sup>) and MER-13 (1.64 mg g fr wt<sup>-1</sup>), while in wet season RUF-5 recorded highest chlorophyll content of 2.43 mg g fr wt<sup>-1</sup> followed by RUF-32 (2.37 mg g fr wt<sup>-1</sup>) and MER-10 (2.14 mg g fr wt<sup>-1</sup>). RUF-32, MER-13 and MER-4 were found common in both the seasons among the best 10 lines under moisture stress. LI Rong-hua *et al.* (2006) reported that in barley the chlorophyll content was decreased in different genotypes with different levels under drought stress and the decrease was more prominent in sensitive genotypes than tolerant

#### genotypes.

# (ii) Yield and yield attributes under reproductive stage stress

Significant differences were observed in grain yield of CSSLs under moisture stress and control conditions. Moisture stress reduced the grain yield irrespective of rice lines. In dry season, out of 84 lines only 13 lines produced grain yield in the range of 0.22-36.96 g m<sup>-2</sup> whereas, other lines could not produce grain yield under drought condition. Among the other promising lines, highest grain yield was obtained in MER-20 (36.96 g m<sup>-2</sup>) with minimal relative yield reduction (RYR %) of 81.84% and low DSI of 0.83 followed by CR 143-2-2

Sl no.	CSSLs	Days to 50% flowering (DFF)		Plant height (cm)		Grain Yield (g m <sup>-2</sup> )		Relative Yield Reduction (RYR %)	Drought Suscept- ibility Index(DSI)	Total dry matter (g m <sup>-2</sup> )		Grain filling (%)	
		С	S	С	S	С	S			С	S	С	S
1	MER-20	72	77	103.6	96.9	203.5	36.96	81.84	0.83	815.15	592.40	92.69	19.95
2	RUF-27	75	77	70.10	67.4	95.5	4.00	95.81	0.97	667.50	443.85	82.64	9.54
3	RUF-32	76	86	97.70	86.4	423.0	3.70	99.12	1.00	769.35	645.00	96.84	7.49
4	MER-14	69	78	62.8	64.2	77.5	1.19	98.46	0.99	676.50	414.50	80.00	4.91
5	MER-30	76	81	75.80	61.9	162.8	1.18	99.28	1.00	817.75	645.00	85.62	4.88
6	RUF-7	69	76	79.60	78.3	189.0	0.53	99.72	1.01	653.00	654.00	89.13	2.57
7	RUF-16	71	74	73.40	79.5	73.0	0.38	99.48	1.00	621.50	684.50	53.64	1.21
8	RUF-19	76	79	77.40	70.8	121.0	0.34	99.72	1.01	736.00	422.00	89.09	1.46
9	RUF-1	77	78	84.30	83.9	101.0	0.24	99.76	1.01	777.50	366.00	87.66	0.86
10	MER-32	78	79	79.20	75.7	45.0	0.23	99.49	1.00	741.50	399.50	72.46	1.83
11	RUF-10	73	82	73.70	64.2	223.5	0.22	99.90	1.01	752.50	747.00	90.53	0.67
12	IR 20(CH)	76	83	87.10	81.5	195.0	0.00	100	1.01	812.50	616.45	80.32	0.00
13	AZUCENA (CH)	84	89	93.2	86.3	114.0	0.58	99.49	1.00	595.50	586.00	84.13	0.52
14	CR 143-2-2 (CH)	70	75	89.5	80.8	288.5	30.96	89.27	0.90	797.50	739.85	82.81	17.08
15	CURINGA (CH)	80	82	77.0	88.6	195.0	0.00	100	1.01	792.50	424.50	84.89	0.00
LSD (84 lines) at 5%		4.19	6.62	12.68	9.28	84.0	3.44			122.32	105.97	9.45	2.24

**Table 1.** Performance of promising CSSLines for yield and yield traits under reproductive stage drought during dry season.

C = Control, S = Stress

#### Effect of drought on CSSL derived rice lines

Sl no.	CSSLs Days to 50% flowering (DFF)		Plant height (cm)		Grain Yield (g m <sup>-2</sup> )		Relative Yield Reduction (RYR%)	RelativeDroughtYieldSuscepti-Reductionbility Index(RYR%)(DSI)		Total dry matter (g m <sup>-2</sup> )		Grain filling (%)	
-		С	S	С	S	С	S			С	S	С	S
1	RUF-44	75	85	71.75	88.45	169.0	147.68	12.51	0.17	709.25	727.2	78.50	60.85
2	RUF-14	79	81	79.90	80.18	162.0	101.83	37.11	0.51	719.80	529.8	62.00	54.32
3	MER-10	61	73	80.55	78.70	122.7	82.33	32.90	0.45	604.40	588.8	77.90	50.68
4	MER-13	78	82	79.45	89.63	86.6	79.38	8.35	0.11	596.00	717.5	61.75	59.69
5	RUF-47	69	74	82.80	82.38	139.0	74.80	46.34	0.63	665.50	563.5	58.50	49.89
6	RUF-13	68	73	72.45	83.68	125.0	73.58	40.95	0.56	770.95	547.5	61.75	44.63
7	RUF-38	68	75	81.90	86.88	98.4	68.75	30.13	0.41	683.20	535.8	55.50	51.75
8	RUF-2	64	72	94.05	81.38	134.0	67.45	50.94	0.68	852.70	531.5	86.50	45.75
9	RUF-5	83	91	70.70	78.25	78.9	61.65	21.85	0.30	791.35	447.75	73.00	55.08
10	RUF-33	65	72	94.5	107.25	261.4	51.28	80.38	1.10	610.85	580.05	95.00	63.99
11	RUF-30	80	72	76.35	61.00	85.37	47.63	44.21	0.61	799.40	430.60	69.00	45.14
12	IR 20(CH)	78	88	88.40	88.3	188.0	0.00	100.0	1.37	763.6	564.18	75.00	0.00
13	AZUCENA(CH)	85	91	99.35	97.18	246.8	60.80	75.37	1.03	788.60	628.5	91.00	30.18
14	CR 143-2-2(CH)	73	75	88.05	71.08	193.0	128.6	33.40	0.46	797.00	583.5	84.00	45.35
15	CURINGA (CH)	75	82	75.40	77.00	92.0	0.00	100.0	1.37	765.45	413.18	73.45	0.00
16	RUF-10	74	78	80.90	77.75	67.3	49.93	25.84	0.35	684.85	480.25	73.50	44.54
17	RUF-32	69	75	98.50	73.95	245.6	47.18	80.80	1.11	784.80	431.45	94.00	39.60
18	RUF-16	72	75	78.65	73.63	246.8	46.85	81.02	1.11	740.05	491.10	91.00	58.90
19	RUF-1	68	77	81.95	78.00	81.10	43.20	46.73	0.64	798.10	576.45	73.50	46.37
20	MER-20	73	77	73.60	85.70	102.0	42.88	57.97	0.79	794.45	437.13	66.95	53.51
21	MER-32	68	77	80.90	76.95	62.50	42.45	32.08	0.44	740.90	493.65	57.25	36.81
22	RUF-7	74	74	77.95	66.50	74.74	40.83	45.37	0.62	741.45	432.26	72.00	48.25
LSD	(84 lines) at 5%	3.73	2.71	8.86	7.60	57.8	19.89			101.62	75.87	5.34	11.83

Table 2. Performance of promising CSSLines during wet season for yield and yield traits under reproductive stage drought.

 $\overline{C} = Control, S = Stress$ 

(30.96 g m<sup>-2</sup>) and RUF-27 (4.00 g m<sup>-2</sup>) with 89.27%, 95.81% of RYR and 0.90, 0.97 DSI respectively. The RYR and DSI value ranged from 81.84%-100% and 0.83-1.01, respectively. Ahmad *et al.* (2003) have reported that drought susceptible varieties had higher values (DSI >1), while resistant varieties had lower



**Fig. 2.** Mean performance of relative leaf water content (RLWC) of 84 CSSLs under moisture stress and control conditions at vegetative stage in both dry and wet seasons

values (DSI<1). The yield stability in resistant varieties was due to specific adaptive feature that make it able to produce stable grain yield even in stress condition (Van Heerden and Lune 2008). Grain filling % was highest in MER-20 (19.95%) followed by, CR 143-2-2 (17.08%), RUF-27 (9.55%), and RUF- 32 (7.49 %) (Table 1).

There are some reports indicated that lower soil moisture inhibit photosynthesis and decrease translocation of assimilates to the grain which lowered grain weight (Van Heerden *et al.* 2008 and Liu *et al.* 2008). Wild relatives of rice typically have long awns, severe shattering for seed dispersal, higher dormancy, coloured pericarp, smaller grain size and open panicle (Sweeney and Mc Couch 2007). Common wild rice (*O. rufipogon*) is the wild ancestor of cultivated rice (Second 1982; Oka 1988; Wang *et al.* 1992). During the course of domestication from wild rice to cultivated rice, only 60% of the numbers of alleles of wild rice were remained in cultivated rice (Sun *et al.* 2001). To broaden the genetic variation and overcome the yield plateaus, exploitation and utilization of the favorable



**Fig. 3.** Mean performance of proline content of 84 CSSLs measured under moisture stress and control condition at vegetative stage in both dry and wet seasons

alleles of wild rice which have been lost or weakened in cultivated rice has become more important and urgent in modern breeding programs. Grain yield of wild rice genotypes is normally less compared to other cultivated rice varieties. As these CSSLs are derived from wild ancestors yield of *O. rufipogon* and *O. meridionalis* is less under favorable condition and after getting exposed to moisture stress in dry season it became more less. However, the temperature inside the rainout shelter was little higher than outside (4% during dry season, which might have played a role to reduce the grain yield more during dry season compared to wet season).

Among the 84 lines tested during wet season, highest grain yield was obtained in CSSL line RUF-44 (147.68 g m<sup>-2</sup>) with highest grain filling of 60.85% followed by the tolerant check CR 143-2-2 (128.63 g m<sup>-2</sup>), RUF-14 (101.83 g m<sup>-2</sup>), MER-10 (82.32 g m<sup>-2</sup>) and MER-13 (79.38 g m<sup>-2</sup>) under moisture stress. MER-13 recorded with minimal RYR of 8.35% and low DSI of 0.11 followed by RUF-44, RUF-5 and RUF-10 with 12.51%, 21.85% and 25.85% RYR and 0.17, 0.30 and 0.35 of DSI respectively (Table 2).

The mean value of DSI close to or below 1.0 for any trait indicates its relative tolerance to drought. However, high values for DSI represent drought susceptibility (Winter *et al.* 1988). The attributes like DSI and RLWC have a direct bearing on the ability of a genotype to withstand against water stress (Singh 2003). This is reflected in relative values of percent

reduction in yield due to water stress in comparison with control condition in both the seasons. The data on these attributes in rain out shelter and controlled condition are presented in Table 1 and Table 2.

Moisture stress is a complex mechanism. Various adaptive features of plant make it able to produce stable yield under stress regimes. Resistant/ tolerant varieties showed stable growth and grain yield due to high accumulation of osmolytes and better scavenging system. Based on the vegetative and reproductive stage stress performances, the chromosome segment substitution lines RUF-44 and MER-13 in wet season and MER-20 and RUF-32 in dry season are identified to be best drought tolerant lines among 84 lines tested. In dry season though the yield of RUF-32 (3.70 g m<sup>-2</sup>) was little less than RUF-27 (4.00 g m<sup>-2</sup>) and in wet season the yield of MER-13 (79.38 g m<sup>-2</sup>) was less than RUF-14 (101.83 g m<sup>-2</sup>) and MER-10 (82.33 g m<sup>-2</sup>), for morpho-physiological traits during vegetative stage, MER-20 and RUF-44 had high water retention capacity (RLWC), MER-13 had high chlorophyll and proline content, and RUF-32 had high chlorophyll content in both the seasons. However, RUF-10, RUF-32, RUF-16, RUF-1, MER-20, MER-32 and RUF-7 lines commonly had better drought tolerance in both the seasons though they had poor yield compared to best lines. Therefore, from the data of moisture stress (under rain out shelter) and control conditions at both the stages, it can be concluded that the tolerant lines with high RLWC, more proline accumulation, high



**Fig. 4.** Mean performance of total chlorophyll content of 84 CSSLs measured under moisture stress and control condition at vegetative stage in both dry and wet seasons

# Effect of drought on CSSL derived rice lines

chlorophyll content and high grain yield, minimal RYR and low DSI can be used further for agronomy and breeding programmes aiming at management practices under drought and variety development for drought prone areas.

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# Oryza Vol. 54 No. 1, 2017 (65-72)

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