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USE OF CARBON ISOTOPE DISCRIMINATION TECHNIQUE TO SUSTAIN RICE PRODUCTIVITY UNDER STRESSED AND LOW LAND IRRIGATED ECOSYSTEM OF PAKISTAN

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Abstract

Carbon Isotope Discrimination (Δ) is an indirect screening tool for breeding cultivars against water stressed conditions. Previous studies exhibited that leaf and straw Δ, under water stress, have positive and significant association with grain yield in rice genotypes. Current study was performed to assess the efficiency of Δ technique at farmer's field level. Five rice genotypes (RSP-1, RSP-2, RSP-3, RSP-4 and RSP-5), grown in lysimeters under well, medium and low water conditions, were subjected to Δ analysis for selection of high yielding water use efficient genotypes. RSP-1 and RSP-2 were identified as the high yielding (4.22 and 4.23 tons ha^{-1} respectively) and water use efficient genotypes (9.48 and 8.87 $gm/m^2/mm$ respectively) under lysimeters. For field study, three yield trials were conducted. In preliminary yield trial, under curtained water supply of 900mm for a complete crop cycle), RSP-1 and RSP-2 produced 6.73 and 7.27 tons ha^{-1} paddy yields respectively. A multi location yield trial, to test the yield stability, indicated above average and average stability of RSP-1 and RSP-2 respectively. Yield performance of RSP-2 (4.1 tons ha^{-1}) under highly saline field conditions (EC: $10dSm^{-1}$) opened the prospects for simultaneous selection of rice genotypes against water limited and saline conditions. According to our knowledge, this is the first report depicting the utility of Δ technique to select the rice cultivar for water limited and saline soil conditions of low land irrigated rice ecology of Pakistan.

Introduction

Water scarcity and salinity limit the rice productivity (Pandey *et al.,* 2007; Muns, 2002). Climate change and ever increasing population are additional future complications (Centritto *et al.,* 2009; Hongbo *et al.,* 2005). Selection of rice germplasm with high water use efficincy under limited water conditions would be a priority area for plant breeders (Centritto *et al.,* 2009). Low land irrigated ecosystem of rice production, like Bhawalnagar, Bhalwal, sargodha, Layah and Bhakar areas, of Punjab province, are marked with uncertain supply of irrigation water and unpredictable low rainfall. Mostly such areas receive 250-350mm annual rainfall and about 75% of it is received during July and August. Dry spells are not uncommon in these areas and result in water stress. Rice is very sensitive to drought and salinity, therefore, yield improvement and sustainability are serious concerns (Tao *et al.,* 2006; Yang *et al.,* 2008; Sadras and Milroy, 1996; Aslam *et al.,* 2006). It has been estimated that 30-40% yield losses in rice occur during the terminal water stress i.e., flowering stage, usually in September, in Pakistan. In Pakistan, rice is cultivated under four production zones-zone-I, zone-II, zone-III and zone-IV. Zone-I exhibits sub-humid monsoon climate with 750 to 1000 mm average rainfall, mostly received in summer. Cold susceptibility, causing leaf yellowing, stunting of seedlings, delayed heading and sterility, are the major issues in this zone. Only photoperiod insensitive varieties, like Kashmir basmati, are adapted in this region. Zone-II is situated in the broad strip of land between rivers Ravi and Chenab where both canal and sub-soil water is used for irrigation. The climate is sub-humid, sub-tropical type with 400 to 700 mm of rainfall mostly in July-August. Rice growing season is fairly long and suitable for cultivating fine aromatic as well as some IRRI varieties. The world famous basmati rice production strip, the "Kalar" tract is located in this zone. Zone-III consists of the large tract of land on the west bank of river Indus. It has an arid sub-tropical climate with 100 mm of average rainfall and maximum temperature higher than zone-I and II. The poor drainage and extreme water application to rice, in this zone, has resulted in high water table. The coarse rice varieties, due to extra hot long summer, are well adapted in this region. Zone-IV is the Indus delta which consists of vast spill flats and basins; the latter are mostly irrigated. The arid tropical marine climate of this region, with no marked season, is appropriate for coarse rice production.

Complex genetics of the drought mechanism (Hongbo *et al.,* 2005) and lack of sophisticated screening methodologies are the major constraints to address the issues. Carbon, the major building block of carbohydrates and proteins in plant tissues contains both light and heavy carbon stable isotopes (^{12}C and ^{13}C). The measurement of natural variations in the abundance of ^{13}C and ^{12}C in plant materials is increasingly being used to select and evaluate plant cultivars that can withstand drought. This technique obviates the need for measurements of the water budgets of a large number of plants during a large scale screening for WUE characteristics. Under drought, less carbon (in the form of carbon dioxide), particularly ^{13}C from the atmosphere is taken up by plants for growth because of plant stress, thus creating a major variation in the natural isotopic ratios of ^{13}C and ^{12}C (Δ) in plant materials. A plant cultivar, which is resistant to water scarcity should display less depletion in ^{13}C compared with a susceptible cultivar. Such discrimination against ^{13}C (i.e. difference between ^{13}C and ^{12}C, expressed as Δ) in plant tissues (leaves and grains) has been successfully used to develop cultivars for limited water conditions.

Farquhar *et al.,* (1982) were the first to establish the negative correlation between grain yield and Δ and since then it has been used for the selection of drought tolerant cultivars in bread wheat (Condon *et al.,* 1990; Ehdaie *et al.,* 1991), barley (Hubick & Farquhar, 1989), peanut (*Arachis* spp.) (Hubick *et al.,* 1986), common bean (*Phaseolus vulgaris* L.) (Ehleringer *et al.,* 1991), cowpea (*Vigna unguiculata* L.) (Ismail and Hall, 1992), sun flower (*Helianthus annuus* L.) (Virgona *et al.,* 1990), and chickpea (*Cicer arietinum* L.) (Udayakumar *et al.,* 1998). Under water limiting conditions, negative correlations between Δ and yield has been reported (Hall *et al.,* 1994; Brugnoli & Farquhar, 2000), nevertheless, many studies comparing different genotypes of crop species have shown that yield is often positively correlated with Δ (measured on grain or other late-formed tissue), both in the absence and in the presence of water stress (Condon *et al.,* 1987; Craufurd *et al.,* 1991; Ehdaie *et al.,* 1991; Hall *et al.,* 1994; Sayre *et al.,* 1995; Ngugi *et al.,* 1996).

Ultimate goal of plant breeding is to develop high yielding genotypes with stable performance over the range of environments (Berger *et al.,* 2007). Analysis, of genotype x environment interactions, helps to identify the cultivars, with stable performance over the range of environments. First systematic approach towards such studies was put forth by Finlay and Wilkinson in 1963, Eberhart & Russel in 1966). This model proposed three parameters to assess the phenotypic stability of the genotype - (i) high mean yield of the genotype over the environments (ii) regression coefficient (b=1) and (iii) deviation from regression ($S^2d=0$). Phenotypic stability of various crop plants, for specific and general environment, has been tested efficiently by using this model (Acikgoz *et al.,* 2009; Fikere *et al*., 2009; Parkash, 2006; Kumar *et al.,* 2010; De *et al.,* 1990; Das *et al.,* 2010; Malik *et al.,* 1988).

The present study was planned to compare the efficiency of Δ technique, under controlled water conditions in lysimeters and mega water regimes under field conditions, for the selection of high yielding, water use efficient genotypes with stable performance. Additionally, avenues for the selection of salt tolerant rice, by using Δ technique, were also explored.

Materials and Methods

Lysimeters experiment: Five, low land coarse rice mutants - RSP-1, RSP-2, RSP-3, RSP-4 and RSP-5 were evaluated for their yield performance under three water regimes i.e., well water, medium water and low water, meant for 100%, 75% and 50% of the field capacity respectively. Experiment was designed in lysimeter structures of 1m x 1m x 1m dimensions filled with sandy loam soil of NIAB farm having electrical conductivity (EC) 0.5 dSm^{-1}, pH 7.6 and sodium adsorption ratio (SAR) 1.36. Lysimeter structures were located under the climatic conditions of Faisalabad (31°2´ N, 73°05´ E) which is characterized with average monthly temperature of 5-18°C during winter and 20-48°C during summer.

Thirty five days old seedlings were planted in 5 rows with 20 cm plant to plant and row to row spacing. Plants were supplemented with measured amount of irrigation water having EC: 0.76 $dSm^{-1,}$ pH 7.5 and SAR 2.0. Maintenance of soil moisture and measurements of the total water applied, at the end of crop cycle, were made as explained in the Akhtar *et al.,* 2010. Nitrogen and P_2O_5 were applied @150 and 130 kg ha^{-1} respectively. Nitrogen was applied in three splits from sowing to pollination.

Carbon isotope discrimination: The carbon isotope composition, in the grain samples, was determined by employing the mass spectrometry (GD 150, MAT, Germany) of the carbon dioxide produced during the combustion of small sub-samples (about 10gm) from each replication of the genotype. The analysis was carried out at Pakistan Institute of Nuclear Sciences, Islamabad and results were expressed in relation to the international standard, Pee Dee Belemnite "PDB" formation in South Carolina, USA. The $\delta^{13}C$ values were converted to carbon isotope discrimination (Δ) values using the relationship established by Farquhar *et al.,* (1989):

$$\Delta\ (‰) = (\delta^{13}C_a - \delta^{13}C_p)\ /(1 - \delta^{13}C_p/1000)$$

where 'a' and 'p' represent air and plant, respectively. To convert $\delta^{13}C$ values to Δ values, -8.00 ‰ for air (Keeling *et al.,* 1979) was substituted in these calculations. Values of Δ are positive and expressed as $^{0}/_{00}$ (parts per thousand).

Field trials: Preliminary yield trial, consisting of RSP-1, RSP-2, RSP-3, RSP-4, RSP-5, KS-282 and Niab-Irri-9 (check), was conducted during 2006 under limited water conditions in the open field. Experiment was laid out in Randomized Complete Block Design (RCBD) with three replications of 2x3 meter plot size. Irrigation water supply was limited to 12 irrigations (900 mm) as compared to the general water requirement of 25 irrigations (2500 mm) for a complete cycle of rice crop. Amount of rain fall received (276mm) and irrigation water applied (624mm), during the whole crop cycle, were recorded to estimate the total amount of water applied i.e. 900mm for a complete crop cycle. At maturity crop was harvested and data were recorded on yield and some associated attributes. Data were also recorded on temperature, relative humidity and total sunshine hours to observe any extreme variation in climatic conditions during the crop cycle (Fig. 1).

Yield stability trials: Field trials, of high yielding and water use efficient genotypes selected on the basis of lysimeter studies, were conducted at seven locations of zone III (Shikarpur, Larkana, Jacobabad, Dokri and Tando Jam, Osta Muhammad), two locations of zone IV (Tando Jam, Tando Muhammad Khan), five locations of zone II (Kala Shah Kaku, Gujranwala, Farooqabad, Bhawalpur, DI Khan) and three locations in zone I (Islamabad, Bhimber, Mingora). Objective was to test the performance of selected genotypes under mega soil and climatic conditions.

Soil conditions within certain production zones have remarkable diversity. During 2007, multi location yield trials were conducted in Randomized Complete Block Design with three replications. Thirty days old seedlings were transplanted using single seedling per hill in 15 m^2 plots and maintaining 20cm plant to row spacing. Fertilizers NP were applied @ 150, 130 kg/ha. Nitrogen, urea having 46% nitrogen, was applied in three equal splits *i.e.* a starter dose, 25-30 days after transplanting (DAT) and 45-50 DAT. Inter-cultural operations and pest control measures were done as and when required. At maturity plants were harvested and data, on paddy yield, were recorded.

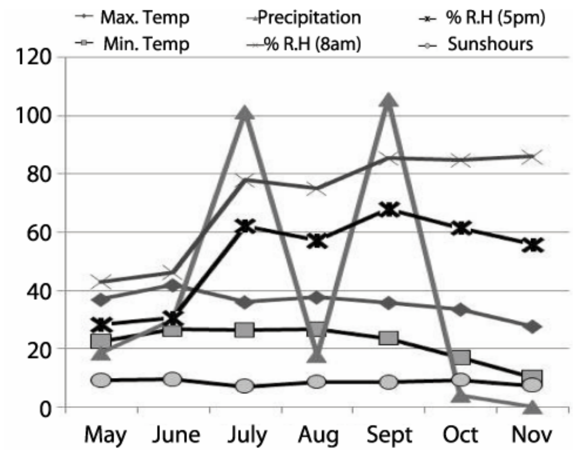

Fig. 1. Average monthly temperature, precipitation and relative humidity and sunshine hours during crop cycle in 2005.

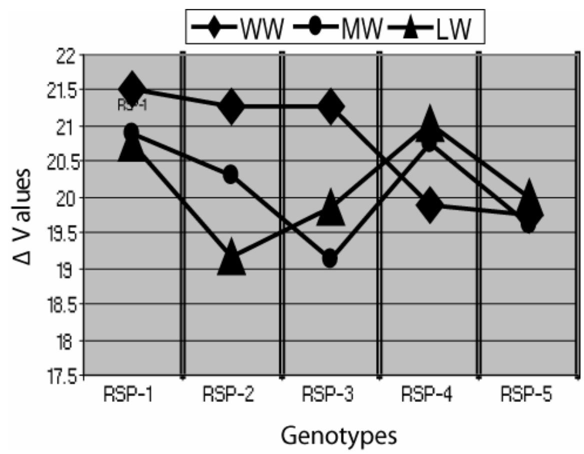

Fig. 2 Δ Values of five rice genotypes under different water regimes in lysimeter studies.

Salinity yield trial: During crop year 2011, salinity tolerance of genotype RSP-2 was estimated in a yield trial conducted under highly saline soil conditions (EC: 12-16 dS/m) of Pindi Bhattian (31° 53′ 44.81″ N, 73° 16′ 33.82″ E). Thirty five days old seedlings of RSP-2 and check variety Niab-Irri-9 (a cultivar with salt tolerant up to EC: 8dS/m) were transplanted on 1.25 acre blocks. Agronomic practices were followed as mentioned in the yield stability trial. To assess the level of salinity, 12 soil samples (500 gm each) were obtained from different locations and variable soil depths, 0-60cm by using basal-type auger. In the laboratory, samples of both depths were composite to 6 to reduce analytical work. Saturated soil pastes of all samples were prepared in 500ml beakers by adding distilled water and stirring with spatula until a characteristic end point was reached. Pastes were transferred to a set of funnels with filter paper placed in vacuum pump assembly. Extracts were collected in test tubes and EC (dSm^{-1}) was measured by using the EC meter (WTW LF-197, Germany). At maturity data on paddy yield, of RSP-2 and Niab-Irri-9, was recorded from each block of $5000m^2$.

Primary statistical analysis was performed by analysis of variance of each trial separately. Stability parameters like linear regression coefficient (b value), and deviation from regression (SEb) of genotype means over environment index, were computed as proposed by Finlay & Wilkinson in 1963 and modified by Eberthart & Russell in 1966.

Results

Lysimeter studies: Our lysimeter data suggested significant differences, between five genotypes, for grain yield (tons ha^{-1}), water use efficiency (WUE) and Δ values (carbon isotope discrimination) of grain (Table 1). RSP-1 and RSP-2 exhibited the highest grain yield and WUE. Lowest mean grain yield and WUE were exhibited by RSP-3 and RSP-4. Although RSP-1 and RSP-2 produced the highest grain yield under three water regimes, however, except RSP-3, all the genotypes showed non significant differences from each other. RSP-1 and RSP-2 were similar with respect to their water use efficiency but significantly different from RSP-3, RSP-4 and RSP-5. RSP-1 and RSP-2 showed a clear decreasing trend of grain Δ values with changing from well water to low water regimes (Table 1). Regarding the RSP-3, RSP-4 and RSP-5 no clear trend was observed in their Δ values indicating a poor adaptation of these genotypes under variable water regimes. Leaf and strawΔ, under water stress conditions, exhibited a positive and significant association with grain yield (Table 1a) Our Δ values, grain yield data and grain water use efficiency indicated that RSP-1 and RSP-2 have better performance than remaining three genotypes under different water regimes; therefore a preliminary yield trial under field conditions with limited water supply was conducted to see the utility of the technique at field level (Fig. 2).

Table 1. Grain yield (tons/ha), water use efficiency and Δ values (carbon isotope discrimination) of grain under three water regimes.

Genotype	Yield (tons/ha)				Water use efficiency				Δ value of grain			
	WW	MW	LW	Mean	WW	MW	LW	Mean	WW	MW	LW	Mean
RSP-1	7.73	7.30	6.05	4.22^{A}	8.5	9.7	10.3	9.48^{A}	21.53	20.90	20.75	21.06^{A}
RSP-2	8.87	7.25	5.04	4.23^{A}	9.2	9.3	9.1	9.2^{A}	21.27	20.29	19.15	20.24^{BC}
RSP-3	6.42	5.74	4.13	3.25^{B}	7.0	7.0	7.6	7.21^{BC}	21.29	19.14	19.86	20.10^{BC}
RSP-4	6.99	6.03	5.39	3.68^{AB}	7.2	7.8	8.7	7.90^{B}	19.90	20.76	21.03	20.56^{AB}
RSP-5	7.14	5.53	4.70	3.47^{AB}	7.4	7.1	7.6	7.34^{BC}	19.76	19.61	20.00	19.79^{C}
Mean	7.35	6.14	4.82	3.78^{AB}	6.85A	6.81A	6.74A	8.16^{BC}	20.78^{A}	20.21^{A}	20.31^{A}	20.35^{AB}

WW: well watered, MW: medium watered, LW: low watered

Table 1a. Association analysis of grain yield Vs leaf and straw Δ under different water regimes.

Plant part	Grain yield		
	WW	MW	LW
Leaf Δ	0.570	0.641	0.890**
StrawΔ	0.481	0.432	0.732*

*, **, Significant at 0.05 and 0.01 levels, respectively.
NS, Non-significant

Yield trial under limited water conditions: Analysis of variance indicated highly significant differences among the genotypes for yield and some associated traits like 1000 grain weight (gm), primary branches/plant, panicle length and days to 50% heading. Genotypes showed non significant differences for productive tillers per plant and plant height (Table 2). Mean performance, for yield and some associated traits, of test genotypes and check varieties is presented in Table 3. Genotypes RSP-1, RSP-2 and RSP-3 showed non significant differences for grain yield and were ranked as top yielder among the genotypes (6.73, 7.27 and 7.17 tons ha^{-1} respectively). Minimum grain yields were produced by the check varieties KS-282, 5.93 tons ha^{-1}, and Niab-Irri-9 with 6.09 tons ha^{-1}. Test genotypes showed considerable variation for 1000 grain weight (22.83 to 28 gm). RSP-2 and Niab Irri-9 produced minimum 1000 grain weight (22.50 to 22.83gm). The highest 1000 grain weight was exhibited by RSP-3 (28gm). Panicle length ranged from 24 to 29cm. RSP-1, RSP-4 and RSP-5 exhibited non significant variation for panicle length. Shortest panicle length was observed in Niab-Irri-9, RSP-3 and KS-282. Considerable variation was also detected for number of primary branches per plant (11.33 to 14.67). RSP-2 differed significantly from all other varieties, including checks, by producing maximum number of primary branches (14.67). Minimum numbers of primary branches were produced by Niab-Irri-9 (11.33). Genotype RSP-2 showed the highest number of days to 50% flowering (87.67 days). RSP-3 and RSP-4 showed minimum days to flowering (68.33 to 70.00 days).

Table 2. Analysis of variance of yield and some associated traits in preliminary yield trial of seven rice genotypes under Faisalabad conditions.

Sources	d.f.	Mean squares						
		Yield (tons/ha)	1000 grain weight (gm)	Panicle length (cm)	Primary branches/plant	Days to heading (50%)	Productive tillers/plant	Plant height
Genotypes	6	0.85★★	12.8★★	9.10★★	3.30★★	135.65★★	7.32^{ns}	50.96^{ns}
Replication	2	0.32	1.50	0.62	0.62	0.76	0.43	13.00
Error	12	0.11	0.90	0.67	0.40	1.32	2.98	23.61

n.s: Non-significant, ★★: Significant at 1% probability

Table 3. Pair wise comparison of genotypes for mean yield (tons/ha) and some associated traits.

Genotypes	Yield (tons/ha)	1000 grain weight (gm)	Panicle length(cm)	Primary branches / plant	Days to 50% heading
RSP-1	6.73 ^{AB}	26.07 ^{BC}	29 ^{A}	13.00 ^{B}	76.67 ^{C}
RSP-2	7.27 ^{A}	22.83 ^{E}	27 ^{BC}	14.67 ^{A}	87.67^{A}
RSP-3	7.17 ^{A}	28.00 ^{A}	25 ^{D}	12.00 ^{BC}	70.00 ^{E}
RSP-4	6.46 ^{BC}	26.87 ^{AB}	28 ^{AB}	12.33 ^{BC}	68.33 ^{E}
RSP-5	6.19 ^{BC}	24.00 ^{DE}	28 ^{A}	13.00 ^{B}	80.67 ^{B}
Niab-Irri-9	6.09 ^{C}	22.50 ^{E}	24 ^{D}	11.33 ^{C}	80.33 ^{B}
KS-282	5.93 ^{C}	24.83 ^{CD}	26 ^{CD}	13.00 ^{B}	74.00 ^{D}

Mean values sharing the same letter (s) do not differ significantly at 5% level of significance

Yield stability trial: Based on the better performance, in lysimeter and preliminary yield trial, genotypes RSP-1 and RSP-2 were selected for further yield stability studies. Analysis of variance for mean grain yield of test genotypes, at individual locations, exhibited significant to highly significant differences at all locations (Table 4). Mean paddy yield of test genotypes at individual locations is presented in Table 5. Maximum yield (8.22 tons ha^{-1}) was produced by RSP-2 at Faisalabad location. The highest mean paddy yield, over the locations, was also produced by RSP-2 (5.40 tons ha^{-1}) followed by the check variety KS-282 (4.92 tons ha^{-1}) and genotype RSP-1 (4.45 tons ha-1). Among the locations maximum yield was produced at NIAB Faisalabad (6.98 tons ha^{-1}) followed by Jamra (6.79 tons ha^{-1}), Islamabad (6.15 tons ha^{-1}), Dokri (6.03 tons ha^{-1}), TandoJam (5.51 tons ha^{-1}), Swat (4.73 tons ha^{-1}), Bhawalpur (4.68 tons ha^{-1}), Larkana (4.27 tons ha^{-1}), Gujranwala (4.25 tons ha^{-1}), Bhimber (4.19 tons ha^{-1}), Kala Shah kaku (3.99 tons ha^{-1}), Usta Muhammad (3.83 tons ha^{-1}), Farooqabad (3.79 tons ha^{-1}) and Dera Ismail khan (3.74 tons ha^{-1}).

Significant GXE interactions (Table 6) encouraged to partition the pooled analysis of variance (Table 7). Partitioning displayed highly significant results for locations, Environment + Varieties x Environments (Linear), pooled deviation and pooled error. Significant results were obtained for Varieties and Varieties x Environment. Non significant results were displayed for Varieties x Environment (Linear). The stability parameters, as described by Finlay and Wilkinson in 1963 and improved by Ebberhart & Russel in 1966, are presented in Table 8. Genotype RSP-2 produced 9.44%

and 10.74% higher yield over the grand mean across the locations and check variety KS-282. Moreover this genotype ranked first, in yield performance, among three tested genotypes. All three genotypes exhibited non significant deviations from regression. The regression coefficient (b value) of genotype RSP-2 was relatively low (0.811) than other genotypes. The highest regression coefficient was exhibited by the check genotype (1.141) followed by the genotype RSP-1(1.048).

Table 4. Analysis of variance of three rice genotypes at 14 different locations of four rice production zones in Pakistan.

Sources	d.f.	Mean squares													
		Islamabad	Kala Shah Kaku	Gujranwala	Farooqabad	Faisalabad	Bhawalpur	D.I Khan	Swat	Bhimber	Dokri	Larkana	Jamra	Tando jam	Osta Mohamad
Replications	2	0.001	0.305	0.10	0.10	0.03	0.41	0.24	0.001	0.06	0.08	0.03	0.36	0.01	0.23
Genotypes	2	0.25^{**}	1.89^{*}	0.44^{*}	1.46^{*}	0.51^{*}	2.57^{**}	30.91^{**}	4.02^{*}	2.02^{*}	1.75^{**}	0.06^{ns}	4.83^{**}	0.66^{**}	0.42^{*}
Error	4	0.005	0.24	0.01	0.09	0.02	0.05	0.12	0.27	0.14	0.04	0.07	0.07	0.03	0.05
		0.16	1.14	0.23	0.66	0.31	0.52	0.80	1.17	0.86	0.48	0.61	0.62	0.37	0.50

Table 5. Grain yield (tons/ha) of three rice genotypes at 14 locations of four rice production zones in Pakistan.

Genotype	Islamabad	Kala Shah Kaku	Gujranwala	Farooqabad	Faisalabad	Bhawalpur	D.I Khan	Swat	Bhimber	Dokri	Larkana	Jamra	Tando jam	Osta Mohamad	Mean
KS-282	6.48^{AB}	3.82^{AB}	4.64^{A}	4.4^{A}	6.95^{B}	4.43^{B}	2.61^{B}	5.40^{A}	3.81^{B}	6.87^{A}	3.67^{B}	6.37^{B}	6.04^{A}	3.40^{B}	4.82^{A}
RSP-1	6.03^{B}	3.39^{B}	4.2^{AB}	3.03^{B}	6.58^{C}	3.90^{C}	1.25^{C}	5.39^{A}	3.60^{B}	5.38^{B}	4.34^{A}	5.79^{B}	5.33^{B}	4.09^{A}	4.45^{B}
RSP-2	5.95^{B}	4.76^{A}	3.9^{B}	3.94^{A}	7.41^{A}	5.70^{A}	7.36^{A}	3.39^{B}	5.17^{A}	5.83^{B}	4.80^{A}	8.22^{A}	5.15^{B}	4.00^{A}	5.40^{A}
Mean	6.15^{B}	3.99^{A}	4.25^{A}	3.79^{A}	6.98^{B}	4.68^{B}	3.74^{D}	4.73^{A}	4.19^{B}	6.03^{B}	4.27^{A}	6.79^{B}	5.51^{B}	3.83^{A}	4.89
LSD (p<5%)	0.16	1.14	0.23	0.66	0.31	0.52	0.80	1.17	0.86	0.48	0.61	0.62	0.37	0.50	0.602

Table 6. Pooled Analysis of variance of paddy yield (tons/ha) over the locations.

Sources	d.f	Sum square	Mean square	F. value
Replication	2	0.087	0.043	0.385
Genotype	2	18.37	9.18^{**}	81.488
Environments	13	154.65	11.89^{**}	105.54
Genotype X Environment	26	82.85	3.186^{**}	28.27
Error	82	9.243	0.113	
Total	125	265.2		

Table 7. Analysis of variance for stability performance of paddy yield of rice genotypes over 14 locations of four rice production zones.

Sources	d.f	Mean square	F. value
Environments	13	3.94^{**}	6.81
Varieties	2	3.07^{*}	5.29
Var X Env.	26	1.11^{*}	1.91
Env. + Var X Env	39	2.05^{**}	3.54
Env. (Lin.)	1	51.32^{**}	88.48
Var. X Env. (Lin.)	2	0.49^{NS}	0.852
Pooled deviation	36	0.58^{**}	5.370
Pooled error	84	0.11^{**}	
Total	41	2.10	

Table 8. Estimates of stability parameters for paddy yield (tons/ha).

Genotype	Paddy yield (tons/ha)	% difference from mean	% difference from check (KS-282)	b	SE (b)
RSP-1	4.45	-9.89	-8.31	1.048	0.187 ^{ns}
RSP-2	5.40	+9.44	+10.74	0.811	0.285 ^{ns}
KS-282 (check)	4.82	-1.45	0.00	1.141	0.138 ^{ns}
Mean	4.89				

Salinity tolerance studies: Salt analysis, of the soil samples from different locations of four soil depths (0-60cm), indicated wide variation in the salinity level (8.3–12 dSm^{-1}) of the experimental area (Table 9). Under the prevailing salinity conditions, genotype RSP-2 produced 4.1 tons ha^{-1} paddy yield as compared to 3.48 tons ha^{-1} of Niab-Irri-9 check variety. Our data suggested that salinity level decreased with increasing the depth of the soil samples. Minimum salinity level was observed at 60cm soil depth.

Table 9. Yield performance of RSP-2 under saline field conditions (1.25 acre block) of Soil Salinity Research Institute, Pindi Bhattian.

Sr. No.	Depth (cm)	No. of samples	E.C. (dS/m)	Yield tons/ha		
1	0-15	12	16.00	RSP-2	Niab-Irri-9	% difference
2	16-30	12	15.30			
3	31-45	12	12.09			
4	46-60	12	8.30			
Average	0-60	12	12.92	4.1	3.48	15%

Discussions

Our lysimeter data, from well water to low water conditions, exhibited gradual reduction in grain Δ values and paddy yield, for RSP-1 and RSP-2, which indicated a linear relationship between Δ and grain yield among these genotypes. However, trend, in the reduction of Δ values in RSP-3, RSP-4 and RSP-5, was not as linear as for RSP-1 and RSP-2. A possible explanation about the reduction in Δ, under low water conditions, is less carbon fixation, particularly C^{13}, by the plant tissues which results in low photosynthetic assimilation along with significant variation in the C^{13} to C^{12} isotopic ratio. Breeding implications of this variation suggest that a drought resistant cultivar should exhaust less C^{13} as compared to susceptible one. Such carbon isotopic discrimination (Δ) in plant tissues has been successfully exploited as an indirect selection tool to select the genotypes, in various crops, for water limited conditions (Hubick *et al.,* 1989; Rebetzeki *et al.,* 2002; Wright *et al.,* 1994; Dingkuhn *et al.,* 1991; Condon *et al.,* 2002). Positive correlation between grain yield and grain Δ, depending upon the crop species and water regime in the root zone, has been reported variously (Kirda *et al.,* 1992; Condon & Richards 1993; Sayre *et al.,* 1995; Monneveux *et al.,* 2004; 2005). However, many studies comparing different genotypes of crop species have shown that yield is often positively correlated with Δ (measured on grain or other late-formed tissue), both in the absence and in the presence of water stress (Condon *et al.,* 1987; Craufurd *et al.,* 1991; Ehdaie *et al.,* 1991; Hall *et al.,* 1994; Sayre *et al.,* 1995; Ngugi *et al.,* 1996). Nevertheless, under water limiting conditions negative correlations between Δ and yield is not uncommon (Hall *et al.,* 1994; Brugnoli & Farquhar, 2000). One probable reason for the positive correlation, in the absence of stress, is that variation in Δ is associated with genotypic variation in stomatal conductance which, in turn, is associated with high rates of photosynthesis and consequently higher yield. Under well water and medium water conditions, our genotypes exhibited a positive but non significant association between yield and grain Δ. However leaf and straw Δ exhibited a positive and significant correlation with yield. A detailed account of this correlation has been published earlier (Akhtar *et al.,* 2010). Water use efficiency is another parameter being used as a part of water saving strategies in many breeding programs around the globe. Among our tested genotypes, the highest water use efficiency was exhibited by the genotypes RSP-1(9.48) and RSP-2 (9.2) followed by RSP-3 (7.21), RSP-5 (7.34) and RSP-4 (7.90). Under water limited conditions water use efficiency was high as compared to the well watered conditions (Table 1). The increase in grain water use efficiency (WUE) with water stress may be because of higher rate of reduction in transpiration than photosynthetic assimilation of carbohydrates. Prolonged drought stress can increase WUE significantly (Li, 1999; Saranga *et al.,* 1999; Sun *et al.,* 1996; Akhter *et al.,* 2003), although that effect may not appear regularly (Ehdaie, 1995; Walley *et al.,* 1999). Dingkuhn *et al.,* (1991) showed that under mild water stress leaf Δ was negatively correlated with leaf level water-use efficiency. Coarse rice, in Pakistan, is sown between 20th May to 7th June and 35 days old seedlings are transplanted which normally flower during August to September depending upon photo period sensitivity. Flower development stage in rice is very critical and severe yield losses occur if water stress prevails at this stage (Hasio, 1982; Pinheiro *et al*., 1985). During our preliminary yield trial, under limited water conditions, a moderate level of drought stress was applied at flowering stage to enhance the efficiency of selection by Δ technique. Reduction trend in Δ values, under limiting water conditions, was also reported by Pinheiro *et al.,* (2000). They reported that drought-induced reduction in Δ was accompanied by reduced spikelet fertility and grain yield. They emphasized to exploit the Δ technique in combination with development of early maturing varieties to tackle the issue of water scarcity. In the low land irrigated ecology of Pakistan, early maturing varieties with better yield have been exploited successfully to tackle the issue of water scarcity and yield sustainability. However the use of Δ technique is very rare which needs exploitation in the breeding programs to enhance the breeding efficiency in the low land ecosystem of rice production which is marked with uncertain supply of irrigation water and low erratic rainfall. Early maturing rice varieties fix very well in the rice wheat rotation followed in this ecology of the country. In low land irrigated ecology of rice production, normal date for wheat sowing is 15-30 November and sowing after this date results in 15kg/acre/day yield reduction. About 90% of the rice grown in Pakistan is of basmati type which matures in 150-160 days and is more exposed to drought stress however coarse rice varieties, maturing in 90-110 days, have an edge over the basmati types in their drought tolerance. Genotypes RSP-1 and RSP-2, exhibiting high yield, high water use efficiency and optimum maturity period, 68 to 87 days for 50% heading, which equals 98 to 117 days for maturity respectively, offered the opportunity to select them for commercial release (Tables 1, 2, 3).

Results of the preliminary yield trial (Tables 2-3), under limited water conditions, also supported the lysimeter data (Table 1). Under limited water supply, of 900 mm including 276mm rain fall and 624mm irrigation water, RSP-1 and RSP-2 produced optimum yields (6.73 and 7.17 tons ha^{-1}). Flowering stage is the critical stage in rice and drought stress at this stage can lower the yield up to 35%. Our test genotypes flowered in August and fields received 17.6mm rain fall during this period (Fig. 1). Other factors like temperature and sunshine hours didn't showed any extreme fluctuation during this period (Fig. 1).

Significant differences among the genotypes and G x E interactions were detected. Significant G x E interactions permitted the partitioning of the analysis of variance (Singh & Chaudhery 1979; Phunden & Narayanan, 2004; Ebberhart & Russel, 1966). Pooled analysis of variance detected significant differences among the means of varieties and Var. x Env. Highly significant differences were recoded for environments, Env. + Var. x Env., Env. (Lin.), pooled deviations and pooled error. Significant differences for Var. x Env., exhibited variation in the yield performance of genotypes across the locations. Matus *et al.,* (1977) suggested that significant G x E interaction may be of cross over or non cross over type. A cross over type interaction exhibits a change in the ranking of genotypes across the environments while a non cross over type interaction does not disturbs the ranking order across the environment rather it is the function of degree of response across the environments. Our genotypes exhibited a cross over type G x E interaction. Similar findings were earlier reported by Atta & Shah, (2009); Baker, (1988); Blum (1983), Matus *et al.,* (1997). Pooled deviation when tested against pooled error exhibited significant differences suggesting considerable variation among the genotypes. Both the linear and non linear components were significant for yield performance. Similar results were reported earlier by kumar *et al.,* (2010) and Kulkarni *et al.,* (2000). Finlay & Wilkinson, (1963) defined a stability model by using the mean performance of the genotype over the environments and regression performance in different environments over the respective environmental mean. This model defined the stability of a genotype in terms of b value i.e., if b=1 (average stability), b<1 (above average stability), b>1 below average stability and b=0 (absolute stability).

Eberhart and Russell made further improvements in the stability parameters and defined a stable variety as one with regression coefficient of unity (b=1) and a minimum deviation from the regression line. Using the definition of Eberhart & Russel (1966), a breeder would establish a variety with high mean seed yield while keeping the promise with above criteria. Genotype RSP-2 exhibited 9.44% and 10.74% higher paddy yield over the mean and check variety KS-282. The regression coefficient was less than unity which indicated above average stability of this genotype. Our stability analysis suggested that KS-282 is better adapted to rich or favorable environments while RSP-2 with high mean yield and b value less than one may be recommended for poor environment. Better adaptation of RSP-2 in poor environment is due to the preliminary selection of this genotype for water limited conditions as exhibited by lysimeter studies and preliminary yield trial under water limited conditions.

Salinity is major constraint to agriculture production in Pakistan. About 6 million hectare of land area in Pakistan is affected by salinity which is causing losses, up to Rs. 20 million, every year. It is desirable to breed the varieties having better tolerance to salt stress. Our salt tolerance studies of RSP-2 were based on our previous work to select the salt tolerant coarse rice lines (Ali *et al.,* 2004). In this study genotype RSP-2, previously designated as DM-38/88, exhibited the highest yield over the eighteen genotypes tested under artificially induced salinity conditions (EC: $8.5dSm^{-1}$). Under this salt stress, RSP-2 also showed better performance for chlorophyll concentrations, fertility percentage, number of productive tillers, panicle length and number of primary braches per panicle (Ali *et al.,* 2004). Adverse effects of salinity in rice crop are growth stage specific (Castro & Sabado, 1977). Rice is tolerant during germination, very sensitive during early seedling stage, tolerant during vegetative stage, again becomes sensitive during pollination and fertilization, and then becomes increasingly tolerant at maturity (Salam *et al.,* 2011). These facts obviate that salinity studies at a particular growth stage may not furnish completely reliable estimates (Tun *et al*., 2003). Therefore, during 2011, a yield trial under highly saline field conditions encompassing all plant growth stages was planned to assess salinity tolerance in terms of paddy yield. Under highly saline conditions (EC: 12.92 dSm^{-1}) our genotype RSP-2 produced 15% higher yield over the previously reported salt tolerant cultivar Niab-Irri-9 (Ali *et al.,* 2004; Salam *et al.,* 2011). These results encouraged the simultaneous selection, of rice genotypes, for water limited conditions and salt tolerance by using Δ technique. However more detailed studies on the correlation between salt tolerance and grain Δ are needed to validate our observation. Simultaneous tolerance to drought and salt stress has been correlated earlier with lowering of osmotic potential, under both types of stresses, by proline and various ions accumulation prevailing in the plant environment (in Ben-Hayyim, 1987). However it has been regarded variously as an adaptive response of the plants under both types of stresses (Cabballero *et al.,* 2005; Mostajeran & Rahimi-Eichi, 2009).

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