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Physiological response, molecular analysis and water use efficiency of maize (*Zea mays* L.) hybrids grown under various irrigation regimes

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With a view to study the effects of irrigation scheduling on the water use efficiency and physiological response and molecular basis of maize hybrids of different maturity groups, a field experiment was conducted at Water Management Research Center (WMRC), Belvatagi, University of Agricultural Sciences, Dharwad, India during 2010-2011 *rabi* season in Malaprabha Command Area'. The experiment was laid out in split plot design with three replications. The main plot comprised four irrigation levels (0.4. 0.6 and 0.8 irrigation water/cumulative pan evaporation (IW/CPE) ratio and irrigation at critical growth stages of maize) and subplots were three maize hybrids [PEEHM-5 (extra early), PEHM-2 (early) and 900 M gold (full season)] were tested. The results reveal that significantly higher grain yield (P<0.05) was at 0.8 IW/CPE ratio followed by irrigation at critical growth sages of maize. Among the maize genotypes tested, full season 900 M Gold recorded significantly greater grain yield (84.61 q ha^{-1}) over PEHM-2 (early) and PEEHM-5 (extra-early). The moisture stress due to 0.4 IW/CPE ratio prolonged the days which reached 50% anthesis and 50% silking thus widening the anthesis-silking interval. Significant positive correlations (P<0.05) of maize grain yield with 100 seed weight (r=0.81), cob length (r=0.83), harvest index (r=0.82) and water-use efficiency (WUE; r=0.61) were found. The RWC decreased significantly (P<0.05) from 82.53 to 75.24% with increasing moisture stress on account of variations in the availability of soil moisture in the crop root zone. WUE was significantly low (P<0.05) in 0.8 IW/CPE ratio, despite providing more amount of water which could be attributed to a greater use of water with relatively lesser increase in yield. The present investigation shows that providing four irrigations at critical growth stages of maize hybrids followed by either three or five irrigations seem to have higher WUE. This approach could save water up to 29% with slight reduction of grain yield by 12% over providing full irrigation. Molecular analysis of three hybrids revealed the possibility of introgressing the yield enhancing traits from full season hybrid into early and extra-early hybrids, the latter clustered distinctly with each other. This strategy besides saving water helps tail-end farmers in choosing additional crop for double cropping in the command areas.

Key words: Maize hybrids, IW/CPE ratio, water use efficiency, grain yield.

INTRODUCTION

Maize (*Zea mays* L.) a miracle crop, is grown over a wide range of climatic conditions in semi arid and sub-tropics of Indian continent. Besides, it is a water demanding crop; higher grain yields can be achieved when water and nutrients are not limiting. Occurrence of drought is unpredictable as it can occur at any stage of the crop. However, maize is very sensitive to water and other environmental stresses in the period one week before flowering to two weeks after flowering (Grant et al., 1989; Pandey et al., 2000; Cakir, 2004). Drought during this period result in easily measured increase in the anthesis-silking interval (ASI) as the silk emergence is delayed (Zaidi et al., 2007). Further, the water stress occurring at different crop developmental stages could potentially limit biomass accumulation and consequently reduce grain yield of the maize crop.

Throughout the tropics, periodic drought caused by uncertain and ill distributed rainfall and soils with low water holding capacities cause sizeable reduction in maize yield. In India, majority of maize is grown under irrigated conditions and most farmers in south India cultivate maize under rainfed condition also. Significant yield losses in maize from drought are expected to increase with global climate change as temperature rise and rainfall distribution changes in key traditional areas. There is a need to identify suitable management techniques in maize which can withstand water stress situations. Most of the maize grown in the irrigated areas of the Navalgund and Nargund taluks of Dharwad district, Karnataka, India suffers from such water shortages at key developmental stages.

The hypothesis of the study was that under water limited conditions, an early maturing maize hybrid would be a better alternative crop in the area of study. In this context, a field experiment was performed to compare response of maize hybrids of different maturity to varying irrigation schedules in the same location and under the same crop management. Crop development, soil water extraction pattern, biomass and grain yield; and molecular diversity were characterized for maize hybrids. The objectives of this study were: i) to compare agronomic and physiological responses of maize hybrids of different maturity groups to irrigation scheduling; and characterize their molecular diversity and (ii) to quantify the relative yield contribution of maize hybrids and the variations in their water use efficiency (WUE).

MATERIALS AND METHODS

Site description

The field experiment was conducted at Water Management Research Center (WMRC), Belvatagi, University of Agricultural Sciences, Dharwad in Malaprabha Command Area, Karnataka, India during winter season 2010. The experimental site is located in the northern agroclimatic zone (zone-3) of Karnataka at latitude of 15°16' N, and longitude of 75°23' E with an altitude of 579 m above sea level. The soil of the experimental site was analyzed for its physico-chemical properties (Table 1).

The meteorological data gathered during the experimental period are presented in Figure 1. The experimental crop received a very less amount of rainfall (101 mm) during the growing period, only in the month of November. Mean maximum temperature ranged from 31.70 (November) to 37.5°C (March) while the mean minimum temperature ranged from 11.1 (January) to 21.87°C (November). There was an uneven seasonal rainfall distribution coupled with 20.81 mm mean growing season evaporation. The percent relative humidity also declined from November (63.18%) to March (48.96%). Thus, due to frequent drying of top soil (six inches), irrigations were provided based on irrigation water/cumulative pan evaporation (IW/CPE) ratio. The higher temperatures during March resulted in higher evaporation of 6.26 mm which exceeded previous three years average by 0.76 mm (data not shown).

Experimental design and treatments

The experiment was laid out in a split plot design with three replicates using a net plot size of 3.0 x 5.6 m for biometric observations. The maize plants were accommodated in 0.6 m inter-row spacing with 0.2 m intra-row spacing between the plants. Irrigation schedules and maize hybrids were randomized in main and subplots, respectively. The treatment combinations comprised four irrigation schedules [I_1 = 0.4 IW/CPE, I_2 = 0.6 IW/CPE, I_3 = 0.8 IW/CPE, and I_4 = irrigations at critical growth stages of maize that is (i) at knee-high stage (V_5 or 35 DAE), (ii) anthesis stage (VT or 65 DAE) and (iii) grain development (R_4 or 90 DAE)]; and three maize hybrids [H_1=PEEHM5 (extra early), H_2 = PEHM2 (early) and H_3 = 900 M Gold (full season)].

Characteristics of maize hybrids used in the study

PEEHM5 and PEHM2 (extra-early and extra maturing hybrids) were released from IARI, India and are recommended for cultivation in Karnataka state. 900 M gold is a full season single cross hybrid of Monsonto Ltd.

Crop husbandry

Maize hybrids were sown on 2nd November 2010 by marking and opening of shallow furrows at 0.6 m apart and seeds were dibbled uniformly at 0.2 m interval in furrows using a seed rate of 25 kg ha^{-1}. Nitrogen, phosphorus and potash were applied at 150, 75 and 37.5 kg ha^{-1}, respectively to all the plots. Entire doses of P_2O_5 and K_2O were applied at planting, while N was applied in three splits that is ⅓ each at the time of sowing, at vegetative stage and before flower initiation stage. The experimental plot was maintained weed free throughout the growth period using pre-emergence

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Abbreviations: WUE, Water-use efficiency; **RWC**, relative water content; **SPAD,** soil plant analysis development system; **IW/CPE**, irrigation water/cumulative pan evaporation ratio; **DAE**, days after emergence; **RAPD**, random amplified polymorphic DNA.

Table 1. Soil physico-chemical properties.

Soil layer (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Bulk density (g cc^{-1})	Field capacity (%)	Wilting point (%)	Soil pH (1:1.25)	EC (dS m^{-1} at 25°C)	Available N (Kg ha^{-1})	Available P_2O_5 (Kg ha^{-1})	Extractable K_2O (Kg ha^{-1})
0-15	13.20	11.30	14.60	60.90	1.36	40.30	20.60	8.50	0.37	210	35	745
15-30	10.40	13.70	15.70	60.20	1.38	41.25	21.10	8.70	0.30	240	38	741
30-45	10.20	12.80	16.70	60.50	1.39	42.50	21.90	8.80	0.25	235	32	743

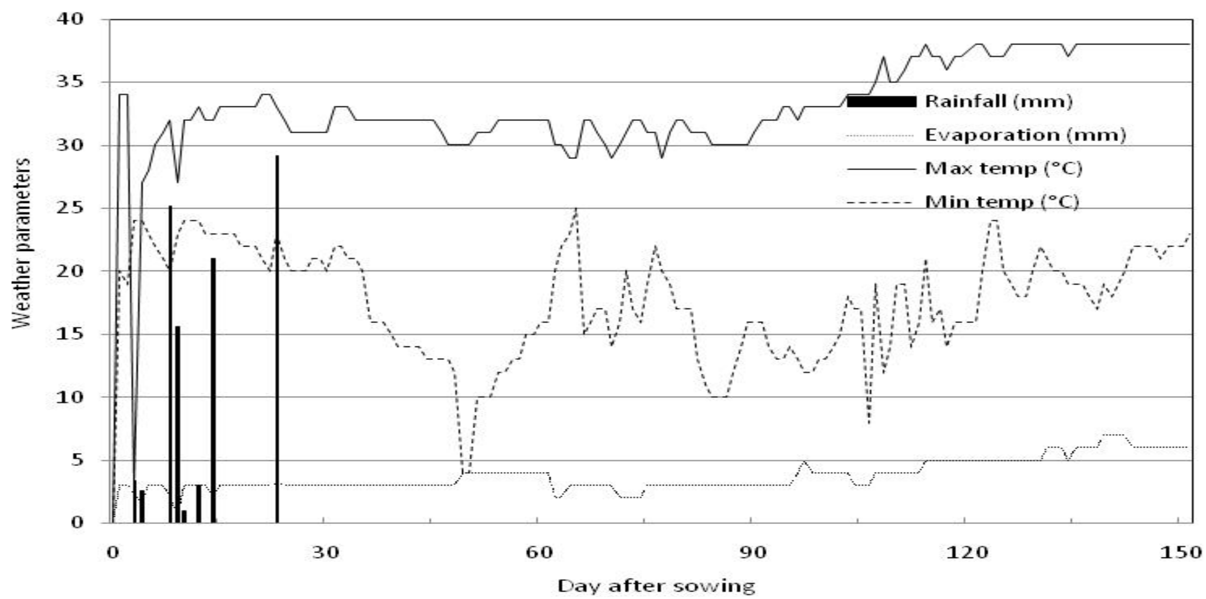


Figure 1. Daily values of rainfall (mm), evaporation (mm), maximum and minimum temperatures (°C) for the period between November, 2010 to March, 2011 (cropping season) at the experimental site.

application of pendimethalin 30 EC at 1.0 kg a.i ha^{-1} followed by manual weeding. Irrigation was applied manually to a depth of 60 mm. The scheduling of irrigation was done based on progressive total of evaporation, after attaining the pre- determined values of cumuli-tive pan evaporation (CPE) (Prihar et al., 1974). Thus, CPE values for different IW:CPE ratios viz., 0.4,0.6 and 0.8 at a constant depth of 60 mm irrigation water (IW) were calculated to be 150, 100 and 75 mm, respectively. The total water use, depth of irrigation water and the number of irrigations provided are presented in Table 2.

Gas exchange measurements and soil plant analysis development system (SPAD) chlorophyll meter values

Leaf gas exchange parameters photosynthesis (P_n), stomatal conductance (g_s), and internal CO_2 concentration (C_i) were measured in the top fully expanded leaf at anthesis stage using a portable infra-red gas analysis system (LI-6400 LICOR, Nebraska, Lincoln, USA) under uniform light conditions. The readings were taken after at an ambient CO_2 concentration of 380 ppm. Three measurements per leaf were taken for each genotype x irrigation combination in each replicate; a total of 48 readings were taken at each time. Gas exchange measurement was taken on a day with sufficient sunlight

Table 2. Total water used (TWU), depth of irrigation water (DIW) and WUE under different irrigation levels.

Treatment	TWU (mm)	DIW (mm)	Number of irrigations
I_1 (0.4 IW/CPE)	240	180	3
I_2 (0.6 IW/CPE)	360	300	5
I_3 (0.8 IW/CPE)	420	360	6
I_4 (critical stages)	300	240	4

and no artificial light source was used for illumination.

Chlorophyll content was determined non-destructively using a SPAD-502 meter (Minolta, Japan), on third fully expanded leaf from the top at 60 DAE (V_{12}) and 90 DAE (R_4) by clamping the SPAD sensor over the leaf lamina. In each plant, five readings were recorded from single leaf.

Soil moisture measurements

Soil moisture was measured gravimetrically before and after irrigation at grand growth, anthesis and at physiological maturity in soil layers: 0 to 15, 15 to 30 and 30 to 45 cm. Soil samples were taken from each plot at about 15 cm away from the crop line. The soil moisture measurements were used to calculate consumptive use and moisture extraction pattern.

Molecular analysis and genomic DNA extraction

Genomic DNA was extracted by cetyltrimethyl ammonium bromide (CTAB) extraction procedure (Doyle and Doyle, 1987). Fresh leaf samples of 1 g were ground to powder in liquid nitrogen and transferred to a 1.5 ml centrifuge tube to which 1 ml of pre-heated (60°C) extraction buffer was added. The extraction buffer consisted of 2% CTAB (w/v), NaCl (4 M), Tris HCl (pH 8.0 1 M) and PVP (0.1%), mercapto ethanol 1% (v/v), RNAse A (2 mg/ml), chloroform: iso-amyl alcohol (24:1) (v/v), ethanol (70%) and TE buffer (Tris HCl, 10 mM (pH 8.0), and 1 mM EDTA (pH 8.0) were the additional solutions required. The samples containing tubes were incubated at 65°C in circulating water bath for 15-20 min. An equal volume of chloroform: iso-amyl alcohol (24:1) was added and mixed for about 5 min. Samples were centrifuged at 12000 rpm for 15 min and the supernatant was decanted and transferred to a fresh tube.

Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) amplification

Amplifications were carried out using a DNA thermal cycler (Mastercycler gradient, Eppendorf). Each 20 µl reaction volume contained about 50 ng of template DNA, 10X PCR Buffer (Tris with 15 mM $MgCl_2$) [Bangalore Genei, India], 2.5 mM dNTP Mix (Bangalore Genei, India), 10 pmols of single decamer primer (Sigma Genosys, India and Quiazen Operon Technologies, Almeda, USA), 3U/µl of *Taq* DNA polymerase (Bangalore Genei, India).

The PCR programme included an initial denaturation step at 95°C for 5 min followed by 39 cycles with 94°C for 1 min for DNA denaturation, annealing at 31.6°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 8 min were carried out. The amplified DNA fragments were electrophoretically separated on 1.4% agarose gel in 1X Tris-acetate-EDTA (TAE) buffer (for each liters of stock contains 4.84 g of Tris base, 1.14 ml of glacial acetic acid and 2 ml of 0.5 M EDTA) and stained with ethidium bromide (10 mg/ml). Thirty five 10-mer primers randomly selected were used in RAPD analysis. A 250 bp DNA ladder (Bangalore Genie) was used as a marker with molecular size of 5000, 4500, 4000, 3500, 3250, 3000, 2750, 2500, 2250, 2000, 1750, 1500, 1250, 1000, 750, 500 and 250 bp. 20 µl of sample was loaded onto each well and amplified DNA was separated with 70 V constant current for 3 h. The amplified pattern was visualized on a UV trans illuminator and photographed.

Data collection and analysis

Observations on number of days to 50% anthesis, days to 50% silking, anthesis -silking interval, cob length, number of grains per row, above ground biomass, 1000-seed weight, grain yield, harvest index and soil moisture extraction pattern were recorded. Above ground biomass was determined using five plants per plot and the samples were oven dried to a constant weight at 80°C. Antheis silking interval (ASI) was computed as the difference between silking and anthesis dates (Kuchanur et al., 2013). Relative water content (RWC) was measured to determine the plant water status of leaf discs sampled from the third leaf from the top adopting the procedure given by Barrs and Weatherly (1962) as:

RWC (%) = (Fresh weight- oven dry weight) / (Turgid weight-oven dry weight) x 100

The water-use efficiency (kg ha^{-1} mm^{-1}) was estimated in terms of grain yield as the ratio between grain yield (kg ha^{-1}) and total consumptive use of water (mm).

The data collected were analysed using analysis of variance (ANOVA) and Fisher's LSD test to determine the significant differences at $P<0.05$ levels between treatment means. All statistical analyses, except for the molecular analyses were performed with MSTATc (Russel, 1986).

RAPD data analysis and scoring

For RAPD data analysis, the bands with same molecular weight and mobility were treated as identical fragments. RAPD products were scored for presence or absence of each amplicon evaluated. Only those bands that could be unequally scored across all the samples were included in the analysis. Pair wise similarity matrices were generated using Jaccard's coefficient of similarity. Data matrices were prepared in which the presence of a band was coded as 1, whereas the absence as 0. The data matrices were analyzed by the SIMQUAL program of NTSYSpc© (version 2.02j) (Rolf, 1998).

Dendrogram of the similarity coefficients was performed using unweighted pair group method of arithmetic means (UPGMA) through the programme, Popgene Version 1.31 (Microsoft windows based Freeware for population genetic analysis).

RESULTS

Physiological responses of maize hybrids to irrigation scheduling

The comparisons of means of irrigation levels, maize hybrids and their interactions are shown in Tables 3 and 4. The above ground biomass (AGB) at harvest was significantly highest in I_3 (259.56 g plant^{-1}) compared to I_1 (164.96 g plant^{-1}). Averaging across irrigation levels, H_3 produced significantly higher AGB (247.64; P<0.05) over other hybrids. Among interaction effects, H_3 produced more AGB in I_3 compared to other treatments (Table 3). Effect of different irrigation regimes and maize hybrids as well as their combined effect on days to reach 50% anthesis was significant (Table 3). Water stressed regimes I_1 and I_2 resulted in more number of days to reach 50% anthesis. Among hybrids, H_3 took 66.75 days to reach 50% anthesis (P<0.05) over other hybrids. The combined effect also showed a similar trend. A same trend was found for number of days to reach 50% silking. Averaging across all hybrids, the interval between anthesis and silking (ASI) was significantly influenced by irrigation schedules. I_1 extended the ASI (8.4 days) while the least was recorded in I_3 (4.11 days; P<0.05). The effects of hybrids and their combined effects was not significant (Table 3). The cob length, number of grains, 100 seed weight and HI were significantly more in I_1 (14.97 cm, 22.40 g, 28.2 g and 46.22%, respectively) (P<0.05) compared to other irrigation schedules. A similar trend was noticed for these yield components in H_3 (14.44 cm, 34.14 g, 27.5 g and 51.71%, respectively) (P<0.05; Table 3).

Grain yield

Averaging across all the maize hybrids, the yield of I_3 was significantly higher by about 3019 kg ha^{-1} than that of I_1 (Table 3). The yield differences were significant (P<0.05) at all the irrigation levels. The hybrid 900 M Gold (H_3) produced higher grain yields in the range 2143 to 2733 kg ha^{-1} than other hybrids. Among interaction effects, H_3 produced higher grain yields in all the irrigation levels and yield increase ranged from 1077 to 2774 kg ha^{-1}, while for H_1, it was in the range of 810 to 3423 kg ha^{-1}; and for H_2 in the range of 446 to 2860 kg ha $^{-1}$.

Gas exchange measurements

Irrigation levels did not show significant differences for P_n, g_s, and C_i (P>0.05). Among hybrids, H_1 had the highest C_i (P<0.05) compared to other hybrids. There were significant interaction effects between irrigation levels and maize hybrids for P_n being significantly highest for H_1 at I_4 (P<0.05) (Table 4).

SPAD chlorophyll meter readings

Averaging across hybrids, I_3 recorded maximum SPAD value both at anthesis and grain filling stages (P<0.05). Among hybrids, H_3 recorded the maximum values (P>0.05). There was a trend in response of hybrids to irrigation levels with respect to SPAD values being higher in H_3 at all the irrigation levels (P>0.05) (Table 4).

Relative water content (RWC)

Significantly higher RWC was recorded at anthesis stage in I_3 which was 8.8% higher than I_1 (P<0.05).

Water use efficiency (WUE)

I_4 had significantly the highest value of 23.80 kg ha^{-1} mm (P<0.05). Providing water at higher frequency, I_3 resulted in decrease in WUE by 4.5 kg ha^{-1} mm. Over all, the WUE for maize hybrids was in the range 17.25-26.16 kg ha^{-1} mm. There were significant interactions between irrigation levels and maize hybrids being highest with H_3 at I_1 (30.12 kg ha^{-1} mm) but was at par with H_3 at I_4 (29.75 kg ha^{-1} mm) (Table 3).

Soil moisture extraction pattern

At sowing, the soil profile was close to field capacity in all the plots. The depletion of soil moisture was higher in the top soil layer due to delayed irrigation (I_1) both at vegetative stage and anthesis stage. On the contrary, lowest depletion was found at all the soil depths in I_3. The interaction effects were not significant (P>0.05) (Data not shown).

RAPD analysis

Random amplified polymorphic DNA analysis of three maize hybrids on 34 primers produced a total of 351 amplified fragments, 202 of which were polymorphic, and the percentage of polymorphism was 57.55. These amplified fragments ranged in size from 250 to 5000 bp (Figure 2). On average, 10.32 bands were amplified per primer and 5.94 were polymorphic (Table 6). Jaccard's coefficient of similarity ranged from 0.70 to 0.74. A highest genetic diversity was observed between H_1 and H_3 (0.73). The dendrogram revealed two distinct clusters; H_1 and H_2 clustered distinctly away from H_3.

DISCUSSION

Effect of irrigation scheduling on physiological responses of maize hybrids

Results of this study show higher grain yield with 0.8 IW/CPE (I_3) on account of higher cob length, cob girth, number of grains per row, and 1000- seed weight. The

Table 3. Yield and yield components of maize (*Zea mays* L) under different irrigation levels.

Treatment	Above ground biomass (g plant^{-1})	Days to 50% anthesis	Days to 50% silking	Anthesis-silking interval	Cob Length (cm)	No. of grains row^{-1}	100 seed weight (g)	Grain yield (kg ha^{-1})	Harvest index (%)	Water use efficiency (Kg/ha-mm)
Irrigation levels										
I_1 : 0.4 IW/CPE	164.96	61.40	69.30	8.44	10.80	20.28	23.33	5124	39.22	21.25
I_2 : 0.6 IW/CPE	230.25	61.30	66.60	5.11	14.07	21.79	25.36	6909	46.10	19.13
I_3 : 0.8 IW/CPE	259.56	60.30	64.60	4.11	14.97	22.40	28.20	8143	46.22	19.29
I_4 : Critical Stage Irrigation	240.27	60.90	66.31	5.22	14.22	21.04	25.23	7168	43.03	23.80
LSD (*P=0.05*)	8.00	0.20	0.32	0.22	0.82	0.29	0.47	1260	0.83	0.42
Maize hybrids										
H_1: PEEHM-5	206.14	57.75	63.92	6.08	12.23	14.55	24.23	5728	36.00	17.25
H_2: PEHM-2	217.50	58.50	63.92	5.58	13.88	15.44	24.80	6318	43.22	19.19
H_3 : 900 M Gold	247.64	66.75	72.25	5.50	14.44	34.14	27.57	8461	51.71	26.16
LSD (*P=0.05*)	2.61	0.18	0.22	ns	0.52	0.19	0.42	720	0.42	0.23
Interaction (I x H)										
I_1 x H_1	148.45	59.00	67.67	9.33	7.90	14.33	22.90	3527	27.85	14.63
I_1 x H_2	158.68	58.00	65.00	8.00	12.07	15.63	23.07	4582	36.98	19.00
I_1 x H_3	187.74	67.33	75.33	8.00	12.43	30.87	24.03	7262	52.84	30.12
I_2 x H_1	196.48	57.33	63.67	5.67	13.10	14.67	23.10	6143	39.46	17.01
I_2 x H_2	226.53	60.00	64.33	4.67	14.33	15.53	24.73	6996	46.39	19.37
I_2 x H_3	267.74	66.67	71.67	5.00	14.77	35.17	28.23	7587	52.46	21.01
I_3 x H_1	247.97	56.67	60.67	4.00	13.60	14.80	27.27	6950	39.43	16.46
I_3 x H_2	243.22	58.00	62.67	4.33	14.80	15.30	25.83	7442	46.85	17.63
I_3 x H_3	287.49	66.33	70.33	4.00	16.50	37.10	31.50	10036	52.39	23.77
I_4 x H_1	231.65	58.00	63.67	5.33	14.30	14.40	23.63	6292	37.26	20.90
I_4 x H_2	241.58	58.00	63.67	5.33	14.30	15.29	25.57	6251	42.68	20.76
I_4 x H_3	247.59	66.67	71.07	5.00	14.07	33.43	26.50	8959	49.16	29.75
LSD (*P=0.05*)	10.42	0.72	0.89	ns	2.06	0.75	ns	2890	1.69	0.92

ns = Not significant

significant increases in these yield components were due to beneficial effect of sufficient moisture available in the soil. This result is in conformity with the findings of Farshad et al. (2008) who showed that missing single irrigation at any of the growth stages in maize significantly decreases grain yield. Scheduling irrigation at critical stages of growth also significantly improved grain yield than providing more irrigation in I_3. The moisture stress encountered in I_1 resulted in more number of days to reach 50% anthesis, days to reach 50% silking and thus widening the interval between anthesis and silking. Continuous stress due to low frequency of irrigation in I_1 also prolonged the days to reach 50% silking by about eight days. In

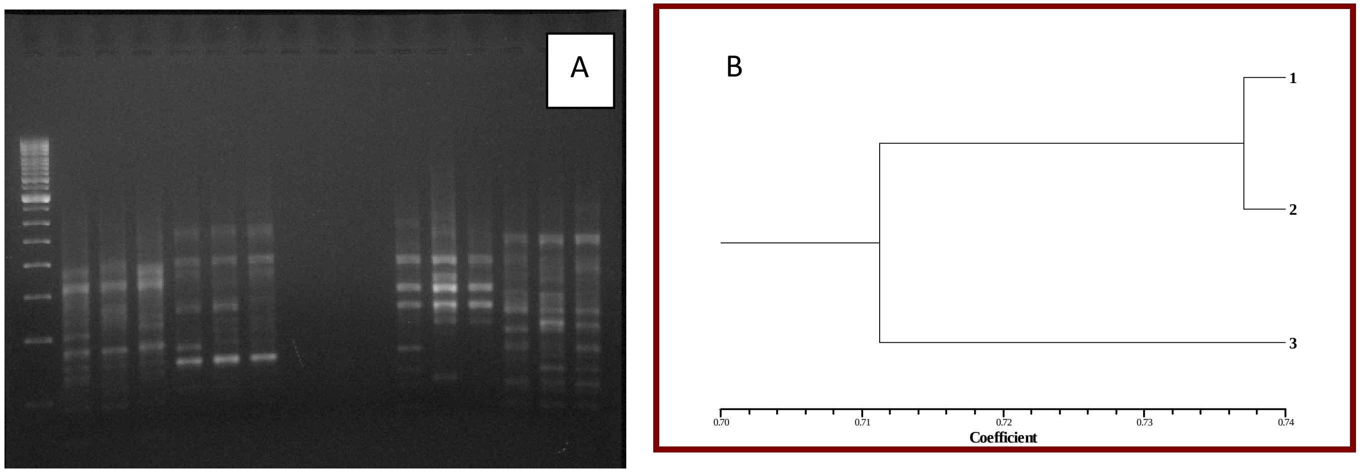
Table 4. Relative water content (RWC), SPAD values and stomatal aperture traits of maize (*Zea mays* L) under different irrigation levels.

Treatment	RWC at anthesis (%)	SPAD values	Rate of photosynthesis (μ mole m^{-2} s^{-1})	stomatal conductance (m mole cm^{-2} s^{-1})	internal CO_2 concentration (ppm)
Irrigation levels					
I_1 : 0.4 IW/CPE	75.24	29.77	6.48	26.50	148.93
I_2 : 0.6 IW/CPE	76.68	36.87	9.43	28.67	134.50
I_3 : 0.8 IW/CPE	82.53	41.29	7.19	25.17	240.46
I_4 : Critical Stage Irrigation	79.52	39.63	12.69	49.67	155.74
LSD (*P=0.05*)	1.34	1.15	ns	ns	Ns
Maize hybrids					
H_1: PEEHM-5	77.74	35.40	9.08	32.75	198.09
H_2: PEHM-2	78.74	36.10	8.75	35.25	168.01
H_3 : 900 M Gold	79.00	39.17	9.01	29.50	143.62
LSD (*P=0.05*)	ns	0.55	ns	ns	9.23
Interaction (I x H)					
I_1 x H_1	73.44	29.05	5.30	21.50	143.77
I_1 x H_2	74.30	28.20	8.54	37.00	168.51
I_1 x H_3	77.99	32.05	5.60	21.00	134.51
I_2 x H_1	72.71	35.29	8.20	27.50	155.24
I_2 x H_2	79.02	35.93	7.90	29.00	133.40
I_2 x H_3	78.33	39.37	12.21	29.50	114.85
I_3 x H_1	84.14	39.31	6.79	29.00	258.56
I_3 x H_2	80.61	41.20	6.56	26.50	234.61
I_3 x H_3	82.84	43.37	8.22	20.00	228.21
I_4 x H_1	80.67	37.94	16.04	53.00	234.78
I_4 x H_2	81.04	39.07	12.03	48.50	135.54
I_4 x H_3	76.85	41.88	10.02	47.50	96.91
LSD (*P=0.05*)	NS	ns	ns	ns	ns

ns = not significant

maize, a wider ASI causes poor synchronization of flowers leading to decline in grain yield. Kuchanur et al. (2013) reported that in maize, moisture stress increased significantly the days required for 50% anthesis, 50% silking and ASI. In this study, the association of ASI with grain yield was significantly negative (r= -0.73; P<0.05) (Table 5). Monneveux et al. (2006) reported in two drought tolerant populations viz. DTP1 and DTP2 that significant yield gains in the populations were associated with a significant increase in number of cobs per plant and grains per ear and significant reductions in ASI. Further, it is evident in the literature that the shortening of ASI is associated with high grain yield under drought (Edmeades et al., 2000; Moser et al., 2006). The increase in grain yield of H_3 was about 32.30% over H_1 and 26.23% over H_2. This might be due to genetic and morphological characteristics of maize hybrids exploiting climatic maxima at important growth stages. In the present study, all the yield traits have contributed for yield increment with significant positive correlations for 1000-seed weight (r= 0.81); cob length (r= 0.83); harvest index (r=0.82); SPAD values (r=0.79) and WUE (r=0.61) (P<0.05) (Table 5). H_3 exhibited a higher HI which might be due to a genetically strong source-sink relation resulting in higher yields. A higher total biomass production in well watered situations has been reported by Moser et al. (2006). Among interaction effects, H_3 performed equally well in all the irrigation levels. The grain yield ranged from 4582 to 7442 kg ha $^{-1}$ in H_2 and from 3527 to 6950 kg ha $^{-1}$. In this study, lesser number of irrigations resulted in reduction in grain number and 100 grain weight. This is in agreement with the findings of Moser et al. (2006) which showed a decrease in kernel number and 1000-kernel weight resulted in lower grain yields due to water shortage in maize.

The RWC decreased significantly (P<0.05) with increasing moisture stress. I_3 had higher RWC by about 82.53% compared to I_1 (75.24 %). This may be attributed to better availability of soil moisture in the crop root zone. The earlier findings in maize revealed that, water potential and RWC (Chen et al., 1990) and relative water content (Schlemmer et al., 2005; Kuchanur et al., 2013)



C	*Maize hybrids*	PEEHM5	PEHM2	900 M Gold
PEEHM5		**1.00**		
PEHM2		**0.74**	**1.00**	
900 M Gold		**0.70**	**0.73**	**1.00**

Figure 2. (A) Amplification of RAPD products from three different maize hybrids (PEEHM5; PEHM2; 900 M Gold) with 4 primers OPP 2, OPP 3, OPP 5, OPP 11. Lane M = 250 bp DNA Marker. (**B)** Dendrogram of the three different maize hybrids (1=PEEHM5; 2= PEHM2; 3=900 M Gold) obtained by RAPD using UPGMA method. **(C)** Similarity matrix computed with Jaccard's coefficient of three maize hybrids obtained from RAPD markers.

Table 5. Associations of growth and yield traits of maize (*Zea mays* L) with grain yield under different irrigation levels.

Correlation	Above ground biomass (g plant^{-1})	Days to 50% anthesis	Days to 50% silking	Anthesis silking interval	Cob length (cm)	Number of grains row^{-1}	100 seed weight (g)	Harvest index (%)	SPAD values	WUE Kg/ha-mm
Grain yield (kg ha^{-1})	0.85**	0.64*	0.37^{ns}	-0.73**	0.83**	0.73**	0.81**	0.82**	0.79**	0.61*

**Indicates significance level P<0.01 ns = not significant

declined under low water conditions. The chlorophyll concentration is a measure of functional stay green (Barker et al., 2005). The chlorophyll content as measured by SPAD values decreased under water stress but it was more drastic under I_1. A higher photosynthetic rate was found in I_4 being highest with H_1 (16.04 µ mole m^{-2} s^{-1}). The other parameters of stomatal aperture traits were not significant. Moderate stress did not significantly change the relative water content (RWC). Severe stress at silking stage did significantly decrease the leaf RWC and increase leaf

Table 6. Total number of amplicons, number of polymorphic bands and per cent polymorphism of maize hybrids.

Primer	Sequence (5' → 3')	Total number of bands (a)	Number of polymorphic bands (b)	Percent polymorphism (b/a*100)
OPP-02	TCGGCACGCA	13	11	84.62
OPP-03	CTGATACGCC	10	08	80.00
OPP-05	CCCCGGTAAC	12	09	75.00
OPP-06	GTGGGCTGAC	13	06	46.15
OPP-07	GTCCATGCCA	10	04	40.00
OPP-08	ACATCGCCCA	14	09	64.29
OPP-09	GTGGTCCGCA	16	12	75.00
OPP-10	TCCCGCCTAC	18	11	61.11
OPP-11	AACGCGTCGG	13	06	46.15
OPP-12	AAGGGCGAGT	09	02	22.22
OPP-13	GGAGTGCCTC	08	04	50.00
OPP-14	CCAGCCGAAC	08	03	37.50
OPP-15	GGAAGCCAAC	05	00	0.00
OPP-16	CCAAGCTGCC	07	02	28.57
OPP-17	TGACCCGCCT	10	06	60.00
OPP-19	GGGAAGGACA	04	01	25.00
RKAZ-1	TCGGATCCGT	10	06	60.00
RKAZ-3	GGCTGTGTGG	12	08	66.67
RKAZ-4	GGCTGTGTGG	11	07	63.64
RKAZ-5	GGCTGTGTGG	06	02	33.33
RKAZ-8	TCGCTCGTAGS	05	04	80.00
RKAZ-9	CGCTCGCGCT	08	03	37.50
K-01	CATTCGAGCC	9	06	66.67
K-06	CACCTTTCCC	16	08	50.00
K-07	AGCGAGCAAG	06	01	16.67
K-08	GAACACTGGG	11	06	54.55
K-09	CCCTACCGAC	12	07	58.33
K-10	GTGCAACGTG	07	05	71.43
K-11	AATGCCCCAG	11	07	63.64
K-12	TGGCCCTCAC	11	07	63.64
K-13	GGTTGTACCC	10	08	80.00
K-14	CCCGCTACAC	19	15	78.95
K-17	CCCAGCTGTG	08	02	25.00
K-18	CCTAGTCGAG	09	06	66.67
	TOTAL	351	202	1832.28
	Average	10.32	5.94	57.55

relative conductivity (Li-Ping et al., 2006).

A highest WUE was observed at I_4 (23.80 kg ha^{-1} mm) than I_1 (21.25 kg ha^{-1} mm). The increased water application resulted in increase in crop water use without a corresponding increase of yield which was reported by Kar and Verma (2005). Providing irrigation at critical stages that is I_4 resulted in better grain yield and WUE over I_2 on account of optimum number of irrigations. This is in agreement with the findings of Jiotode et al. (2002) which revealed better WUE with irrigation at critical growth stages of maize. While Maqsood et al. (2012) reported that providing six irrigations at different growth stages of maize along with higher N rates up to 200 kg ha^{-1} has increased maize grain yield. WUE values for rainfed maize have been reported in the literature in the ranges 11.4 to 14.4 kg ha^{-1}mm^{-1} (Meena et al., 2009); 9.3 to 13.8 kg ha^{-1}mm^{-1} (El-Tantawy et al., 2007); and 11.0 to 18.0 kg ha^{-1}mm^{-1} (Tijani et al., 2008).

Despite providing a highest amount of water (420 mm) in I_3, the WUE was significantly low ($P<0.05$) which could be attributed to a greater use of water with relatively lesser increase in yield. Trooijen et al. (1999) found greater WUE of maize with limited irrigation, but full irrigation of maize was more profitable than limited irrigation. During vegetative growth stages, soil moisture depletion from different soil layers varied with irrigation levels. Moisture extraction was more from the top layer (0-15 cm) irrespective of irrigation levels. However, it was more at I_1 coupled with moisture loss through evaporation from soil. Similar trends were found during anthesis stage except in I_3 wherein depletion of moisture from 15 to 30 cm depth was seen. Maximum moisture extraction from deeper layers may be due to stress as a result of less number of irrigation which encourages more rootgrowth into deeper profiles. It is also reported in other study that drought sensitive inbred maize lines despite having deeper rooting markedly reduced WUE on account of inefficient photosynthesis (Hund et al., 2008).

Based on the water extraction patterns, the lack of significant differences in water extraction at depth among maize hybrids and their interaction with water levels was mainly due to a well developed root system by maize. The relationship between yield and irrigation is affected by factors such as climate, soil properties and irrigation practices (Tolk and Howell, 2003) and determining the level of irrigation needed to optimize profits can be complex and depends on both biophysical and economic factors (English et al., 2002; Payero et al., 2008).

Conclusion

The study focuses to bring interrelations between different maturity group hybrids and their WUE through several physiological, molecular and agronomic analyses. Our findings show that providing four irrigations at critical growth stages followed by either three or five irrigations seem to have higher WUE. This approach could save water up to 29% with slight reduction of grain yield by 12% over providing full irrigation. Further, WUE was increased to a maximum of 23.80 kg ha^{-1} mm with decreased frequency of irrigation at critical growth stages. The RAPD analysis revealed that extra-early and early maturity hybrids clustered significantly differently from full season hybrid. This diversity may be of use in crop improvement to introgress certain traits from full season hybrid into early maturity groups for enhanced productivity while emphasizing on the reduction in number of days to reach physiological maturity. To integrate differential responses during phenological stages, it is suggested that field research be combined with thoroughly calibrated and validated crop-water productivity models to further improve strategies obtained from field experiments. In areas where water supply is going to be a constraint in future, farmers must choose varieties and irrigation strategies to ensure sustainable production. Under these situations per day productivity of crop both based on crop duration and on water use need to be given due consideration with a view to save water resources as well as to have temporal benefit so that farmers will have an option to choose additional crop for inclusion in the double cropping system. This perhaps is the only alternative that net returns can be maximized, especially for the farmers in tail-end regions of canal areas.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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