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Effect of irrigation scheduling on physiological characters and yield of different cultivars of wheat under varying sowing dates

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Abstract
A field experiment was conducted under loamy sand soil during two consecutive *Rabi* seasons of 2016-17 and 2017-18 at Research Farm, Rajasthan Agricultural Research Institute, Sri Karan Narendra Agriculture University, Durgapura, Jobner. The experiment comprises four irrigation scheduling treatments (Irrigation at 0.6 ETc, 0.8 ETc, 1.0 ETc and 1.2 ETc), three cultivars (Raj 4120, Raj 4079 and Raj 4238) and three dates of sowing (15^{th} November, 30^{th} November and 15^{th} December) assigned, respectively to main plot, sub plot and sub-sub plots were replicated three times in split plot design. Results revealed that irrigation scheduling at 1.2 Etc significantly influenced the RWC, MSI, Chlorophyll, Protein content and yield of different cultivars of wheat under varying sowing dates according to pooled analysis.

Keywords: Wheat, chlorophyll content, membrane stability index, relative water content

1. Introduction

Wheat is the most important cereal crop, it's stable diet for more than one third of the world population and contributes more calories and protein to the world diet than any other cereal crop (Abd-El-Haleem *et al*., 2009) [1]. Water is the most severe stress and the main cause of significant losses in growth and productivity of crop plants (Ludlow and Muchow, 1990) [12]. Water stress induces significant alterations in plant physiology and biochemistry. Some plants have a set of physiological adaptations that allow them to tolerate water stress conditions. The degree of adaptations to the decrease of water potential caused by drought may vary considerably among species (Save *et al*., 1995). Plant response to water stress include morphological and biochemical changes and later as water stress become more sever to functional damage and loss of plant parts (Sangtarash, 2010) [25]. Researchers linked various physiological responses of plant to drought with their tolerance mechanisms, such as: pigment content and stability and high relative water content (Clarke and McCaig, 1982) [5]. Drought tolerant wheat species can be characterized by growth response, changes in water relations of tissues exposed to low water potential, stomatal conductance, ion accumulation and changes in the fluorescence induction parameters under water stress (Blum, 1988) [4]. In recent years, the screening of plant fluorescence signatures is developing as a specific tool which could be applied to detect the functioning and health status of plants (Lichtenthaler *et al*., 1999; Samson *et al*., 2000) [11, 24]. The ability of plants to maintain membrane integrity under drought is what determines tolerance towards drought stress (Vieira Da Silva *et al*., 1974). Membrane stability is a widely used criterion to assess crop drought tolerance (Premachandra and Shimada, 1988) [19]. Understanding of physiological mechanisms that enable plants to adapt to water deficit and maintain growth and productivity during stress period could help in screening and selection of tolerant genotypes and using these traits in breeding programs (Zaharieva *et al*., 2001). The main objective of this study was to determine the effect of water stress- imposed by planting different wheat cultivars in different dates of sowing on various physiological parameters and yield to find out the best and most simple tool which could be used for screening wheat varieties for drought tolerance.

2. Materials and Methods

The field experiment was conducted during *Rabi* season 2016 and 2017 at Research farm, Rajasthan Agricultural Research Institute, Sri Karan Narendra Agriculture University, Durgapura, Jobner, Rajasthan (75° 47' East longitudes, 26° 51' North latitude and at altitude of 390 m above mean sea level). The soil of experimental field was loamy sand in texture, slightly alkaline in reaction containing 0.25% organic C, with *p*H 8.2, EC 0.15ds m^{-1}, available nitrogen 136.5 kg ha^{-1}, phosphorous 33.30 kg ha^{-1} and potassium 195.45 kg ha^{-1}. The meteorological data was recorded daily from sowing to harvest from meteorological observatory situated near the experimental farm (Table 1.). The experimental site characterized by aridity of the atmosphere and extremity of temperature both in summer (45.5ºC) and winter (4ºC). Under semi-arid climatic conditions, the area receives 500-700 mm per annum rainfall which is mostly occurring during July to September. Rainfall received during the wheat growing season (Nov. to April) was 22.9 mm. The mean monthly maximum and minimum temperatures during the wheat growing season (Nov. to April) varied from 21.55 to 38.32 and 6.05 to 23.25ºC, respectively. The cumulative bright sunshine hours during the growing season varied between 6.70 to 10.05 hrs. The experiment was laid out in Split plot design with three replications. Thirty six treatment combinations were investigated. Treatments comprises four irrigation levels: I_1 (0.6 ETc), I_2 (0.8 ETc), I_3 (1.0 ETc) and I_4 (1.2 ETc), three cultivars: C_1 (Raj-4120), C_2 (Raj-4079) and C_3 (Raj-4238) and three dates of sowing: D_1 (15th Nov.), D_2 (30th Nov.) and D_3 (15th Dec.). In the recommended irrigation treatments applied at different irrigation intervals according to ET_C level with the help of water meter. Standard crop production practice and methods were followed for weeding, fertilizer application and crop protection management to grow the crop. Crop was harvested manually in the end week of March and First week of April when 80% of the grains turned to golden colour. Grain and biological yield were recorded at the harvest. Least significant difference at 0.05% level of probability was used to test the significance of differences among treatment means. Physiological parameters calculated with the following formulas:

2.1 Relative water content (%)
Relative water content (RWC) of fresh flag leaves were measured by the method given by Barrs and Weatherly, 1962. Leaf segments (1 cm^2) were initially weighed and floated over the distilled water for 4 hours and turgid weight was recorded. Dry weight was obtained after drying the leaf segments at 80 ºC for 48 hours. The RWC was calculated as

$$\text{RWC (\%)} = \frac{\text{(Fresh weight - Dry weight)}}{\text{(Turgid weight – Dry weight)}} \times 100$$

2.2 Membrane stability index
The membrane stability index (MSI) was determined as per method prescribed by Sairam, (1994). Leaf samples of 200 mg were placed in test tube filled with 25 ml double distilled water and kept in water bath at 45ºC for 30 minutes. Then cooled at room temperature and electrical conductivity was recorded by conductivity meter (C_1). Subsequently the another plant samples of 200 mg with 25 ml distilled water was placed on boiling water bath at 100ºC for 10 minutes and after cooling, electrical conductivity was recorded (C_2). The MSI was calculated as

$$\text{MSI} = \left(1 - \frac{C_1}{C_2}\right) \times 100$$

2.3 Chlorophyll content
Chlorophyll content was worked out at 40 and 80 DAS. Hiscox and Israelstem (1979) demonstrated that the absorption spectrum (600-680nm) for chlorophyll extracted in DMSO was virtually identical to that for extracted in 90 per cent acetone. Accordingly chlorophyll was extracted in DMSO and transmittance was recorded with spectro-photometer at 645 and 663 nm. Arnon's equation (1949) [2] was used to work out chlorophyll content as here under

$$\text{Chlorophyll "a" (mg g}^{-1}\text{ fresh weight of leaves)} = \frac{(12.7 \times A_{663}) - (2.69 \times A_{645})}{1000} \times \frac{\text{Volume of DMSO}}{\text{Weight of leaf sample}}$$

$$\text{Chlorophyll "b" (mg g}^{-1}\text{ fresh weight of leaves)} = \frac{(22.9 \times A_{645}) - (4.65 \times A_{663})}{1000} \times \frac{\text{Volume of DMSO}}{\text{Weight of leaf sample}}$$

Total chlorophyll content was worked out by adding chlorophyll "a" and chlorophyll "b" as under:

$$\text{Total Chlorophyll (mg g}^{-1}\text{ fresh weight of leaves)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

3. Results and Discussion
Statistical analysis of data for relative water content showed that different levels of irrigation significantly influenced. The treatment I_4 (Irrigation at 1.2 ETc) proved superior over rest of the treatments in relation to RWC with the respect values (75.01 and 66.54%) at anthesis and 15 days after anthesis, respectively which remained at par with I_3 (Irrigation at 1.0 ETc) according to pooled analysis. With irrigation scheduling I_1 (Irrigation at 0.6 ETc) relative water content significantly decreased. According to (Rahman *et al*., 2007) [21], plants grown under water stress conditions decrease the intracellular water by increasing of osmotic compounds to absorb water from the soil powerfully. It seems there is a direct relationship between the soil moisture content and relative water content of leaf so that reduction in soil moisture and increasing water stress reduces relative water content of leaf. The results revealed that different levels of irrigation showed significant variation in the membrane stability index of wheat. The treatment I_4 (Irrigation at 1.2 ETc) proved superior over rest of the treatments and remained at par with I_3 (Irrigation at 1.0 ETc). The most observable indirect effect of drought on plant performance reduces osmotic potential which resulted in reduced water availability of plants. These results are in agreement with Jaleel *et al*., (2007) [7] in *Catharanthus roseus* and in *Brassica juncea*. Also, Rao *et al*., (2012) [20] reported that, the membrane stability index of maize plants was significantly increased in response to the treatment with drought stress. Data (Table 2.) revealed that significantly higher value (2.76 mg g^{-1}) of chlorophyll content was recorded under the treatment I_4 (Irrigation at 1.2 ETc) which proved superior over rest of the treatments and remained at par with I_3 (Irrigation at 1.0 ETc) with respective value (2.73 mg g^{-1}) on pooled data basis. This may be attributed to better root growth, resulting in higher water and nutrient uptake which resulted in increased chlorophyll content in leaves. Higher root density had a large influence on plant water status through its effect on water uptake from soil (Patidar and Mali, 2004) [18]. Highest chlorophyll content was observed in Raj 4079 (2.71%) and lowest in Raj 4120 (2.40%) at flag leaf stage. The leaf chlorophyll content decreased in Raj 4238 and Raj 4120 by 2.65 and 12.91% at flag leaf stage, respectively, as compared to Raj 4079, when averaged across all the genotypes taken for study this differential magnitude of decrease in chlorophyll content shows the presence of genetic variability for chlorophyll retention in these wheat genotypes. Ristic *et al*., (2007) [22] also observed the genetic variability in chlorophyll content in wheat lines exposed to high temperature. Thus, the amount of chlorophyll in leaves might be crucial in realizing higher yield under high temperature

stress. Membrane stability index declined significantly under high temperature stress in all the genotypes. The percent decline was least in Raj 4079 at (8.11%) at 15 DAA from anthesis, while Raj 4120 (16.57%) exhibited highest decline in MSI at 15 DAA from anthesis in pooled data analysis (Table 1.). It has been reported that stable cell membrane that remains functional during stress appears to control adaptation to high temperature (Gupta *et al*., 2000) [6]. Sairam *et al*., (2000) [23] reported that membrane disruption may alter water, ion and organic solute movement, as well as photosynthesis and respiration. In present investigation also, close link between membrane stability was observed in heat tolerant Raj 4079 and Raj 4238 cultivars. Leaf RWC is proposed as more important indicator of water status than other water potential parameters under drought stress conditions. During plant development, drought stress significantly reduced RWC values (Siddique and Islam, 2000). Significant difference in RWC was observed between cultivar at various stages and our results showed reduction in RWC in all the three cultivars at all stages of growth and more reduction were recorded in drought susceptible cultivar Raj 4120 (Table 1.). This deviation in RWC may be attributed to differences in the ability of the cultivars to absorb more water from the soil and the ability to control water loss through the stomata. It may also be due to differences in the ability of the tested cultivars to accumulate and adjust osmotically to maintain tissue turgor and hence physiological activities. Highest RWC was recorded at anthesis stage and decreased gradually to 15 DAA and the highest RWC value observed in drought tolerant cultivars Raj 4079 (Table 1.). These finding are in agreement with (Mamnouie *et. al*., 2006) [13]. Chlorophyll, relative water content and membrane stability index showed wide differences among crop sown on various dates (Table 2.). While comparing the planting windows, the suitable time of seed planting was 15th November with respect to chlorophyll content, membrane stability index and relative water contents. The relative water content controls the leaf tissue turgor pressure which ultimately maintains the activities of leaf resulting to high rate of photosynthesis. Similarly, high chlorophyll contents might also contribute to higher photosynthetic rate and significant positive correlation between chlorophyll content and photosynthesis rate was reported in earlier findings (Thomas *et al*., 2005). Pande and Verma, (2011) [16] have also documented the adverse effects of delayed sowing and wide variations among sowing dates for chlorophyll content, relative water content and membrane stability index.

Data on grain yield (Table 2) indicated that it was markedly influenced due to different irrigation schedules. The treatment I_4 (Irrigations at 1.2 ETc) recorded significantly higher grain and biological yield of wheat (5136 and 1250 kg ha^{-1}) in pooled analysis, respectively over I_1 and I_2 and at par with I_3 (Irrigations at 1.0 ETc). While significantly lowest grain and biological yield (3442 and 7445 kg ha^{-1}) in pooled analysis was recorded under the treatment I_1 (Irrigations at 0.6 ETc). Higher grain and biological yield under the treatment I_4 and I_3 might be the result of cumulative effect of improvement in growth and yield attributes such as effective tillers, number of grains $spike^{-1}$ test weight, spike length, number of spikes per unit area, number of spikelets $spike^{-1}$. It was also found that with sufficient moisture in the soil profile under I_4 irrigation schedule, plant nutrients particularly nitrogen, phosphorus and potassium were more available and might have translocated to produce more dry matter. Secondly, higher yield with higher levels of irrigation might be due to its key role in root development by reducing mechanical resistance of soil, higher transpiration, greater nutrient uptake and more photosynthesis due to metabolic activities in plant (Bhunia *et al*., 2006) [3]. The other reason of yield increase might be that irrigation scheduling at 1.2 ETc and 1.0 ETc throughout growth and reproductive phase created longer reproductive period with larger photosynthetic surface and reproductive storage capacity to attain higher allocation of net photosynthates to yield. The results obtained by (Sharma and Pannu, 2008, Sarwar *et al*., 2010, Kumar *et al*., 2015 and Mishra and Kushwaha, 2016) [10, 14] also confirm the findings of present investigation.

Since, wheat yield is a complex process and governed by interaction between source (photosynthesis and availability of assimilates) and sink component (storage organs). Thus, as a consequence of marked improvement in both these regulative process as evidenced from higher accumulation of biomass and nutrients as well as yield components under cultivar Raj 4079 led to significant increase in grain and biological yield. Further, the yield of wheat is dependent on two most important components namely spikes per unit area and weight of grains (test weight). Thus, due to more number of grains by virtue of increased number of spikes and more test weight under Raj 4079, increased the grain yield over Raj 4238 and Raj 4120, and remained at par with cultivar Raj 4238. Since, biological yield is a sum of grain and straw yield produced by the crop, the increased grain yield under Raj 4079 might have resulted in higher biological yield in this cultivar. The marked variation in various yield components and yield between cultivars was observed by (Pandey *et al*., 1999, Nainwal and Singh, 2000, Sardana, 2001 and Singh *et al*., 2007) [17, 15, 26].

Grain, biological yield of wheat increased significantly when sowing of wheat on 15th November. Grain and biological yield decreased significantly as sowing was delayed from 15th November. This might be due to cumulative effect of poor expression of vegetative growth and yield contributing characters *i.e*., number of spikes, ear length, grains $spike^{-1}$ and test weight under late sown conditions accompanied with high temperature and hot winds which leads toward forced maturity of the crop and ultimately resulted in lower grain and biological yield. The early sown crop, on the other hand, having favorable cool weather conditions for longer duration recorded better growth and yield attributes resulted in greater productivity (Kulhari *et al*., 2003) [9].

Table 1: Effect of irrigation scheduling, cultivars and varying sowing dates on relative water content (%) and membrane stability index (%) of wheat

Treatment	RWC at anthesis stage	RWC at 15 DAA	MSI at anthesis stage	MSI at 15 DAA
Irrigation scheduling				
I_1 (Etc 0.6)	63.52	55.87	52.04	46.40
I_2 (Etc 0.8)	69.90	61.68	58.08	50.02
I_3 (Etc 1.0)	74.52	66.11	61.64	52.75
I_4 (Etc 1.2)	75.01	66.54	62.49	53.01
SEm±	0.60	0.49	0.38	0.41
CD(P= 0.05)	1.86	1.51	1.16	1.26
Cultivars				
V_1 (Raj 4120)	68.45	60.72	56.50	46.67
V_2 (Raj 4079)	72.23	63.81	59.91	52.76
V_3 (Raj 4238)	71.53	63.12	59.28	52.21
SEm±	0.28	0.30	0.24	0.35
CD(P= 0.05)	0.81	0.85	0.69	1.00
Date of sowing				
D_1 (15th NOV.)	73.97	67.31	62.02	53.48
D_2 (30th NOV.)	71.69	64.34	60.08	51.33
D_3 (15th DEC.)	66.56	56.00	53.59	46.83
SEm±	0.28	0.26	0.27	0.27
CD(P= 0.05)	0.79	0.73	0.75	0.75

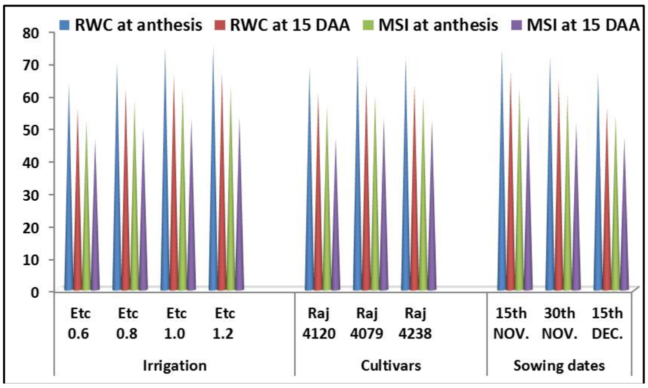

Fig 1: Effect of irrigation, cultivars and sowing dates on relative water content and membrane stability index of wheat

Table 2: Effect of irrigation scheduling, cultivars and varying sowing dates on chlorophyll content (mg g^{-1} fresh weight) and heat use efficiency of wheat

Treatment Irrigation scheduling	Chlorophyll content at flag leaf stage	Protien content	Grain yield	Biological yield
I_1 (Etc 0.6)	2.33	11.02	3442	7445
I_2 (Etc 0.8)	2.54	11.22	4600	10130
I_3 (Etc 1.0)	2.71	11.30	4986	10967
I_4 (Etc 1.2)	2.76	11.34	5136	11250
SEm±	0.03	0.10	49	102
CD(P= 0.05)	0.10	NS	151	315
Cultivars				
V_1 (Raj 4120)	2.40	11.13	3788	7957
V_2 (Raj 4079)	2.71	11.25	4974	11051
V_3 (Raj 4238)	2.64	11.28	4861	10835
SEm±	0.02	0.06	39	80
CD(P= 0.05)	0.08	NS	113	229
Date of sowing				
D_1 (15^{th} NOV.)	2.73	11.25	5201	11411
D_2 (30^{th} NOV.)	2.62	11.22	4780	10520
D_3 (15^{th} DEC.)	2.40	11.18	3641	7912
SEm±	0.02	0.05	31	58
CD(P= 0.05)	0.06	NS	86	164

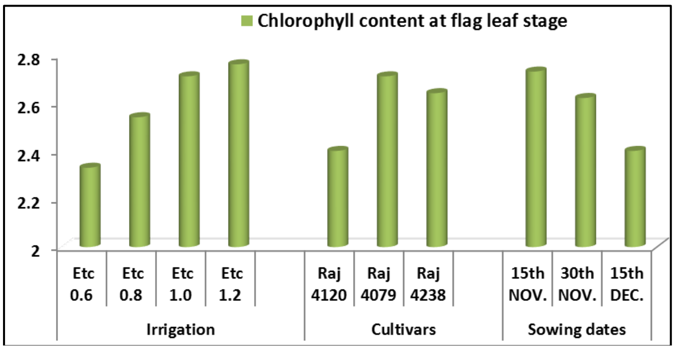

Fig 2: Effect of irrigation, cultivars and sowing dates on chlorophyll content at flag leaf stage of wheat)

4. Conclusions

Growth and photosynthesis are two of the most important processes abolished, partially or completely, by water stress (Kramer and Boyer, 1995) [8], and both of them are major cause of decreased crop yield. The best option for crop production, yield improvement, and yield stability under soil moisture deficient conditions is to develop drought tolerant crop varieties. A physiological approach would be the most attractive way to develop new varieties rapidly (Turner and Nicolas, 1987). Looking overall results, it is clear that these parameters could explain some of the mechanisms which indicate tolerance to drought; however, their relevance in describing the varietals variability is significant.

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