

## The Effect of Differential Irrigation, Time and Frequency of Nitrogen Application on Proline Accumulation in Wheat Grown under Qatari Conditions

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**ABSTRACT** Wheat (*Triticum aestivum* cv. Condore) was given three irrigation treatments and a split-dose of urea during the winter of 1980-1981 to examine some of the yield components and to estimate proline accumulation through the effects of water stress and nitrogen status of the tissue.

Height of the plant was measured, tiller number per plant was counted and free-proline was determined during the various stages of the crop growth. Under nitrogen deficiency and water stress, proline accumulation was impaired in wheat leaves. Proline accumulation was rapid under reasonable nitrogen levels and water stress. The results indicate that proline accumulation in wheat is correlated with the nitrogen status of the leaf tissue. This should be taken into account in breeding programs where free-proline accumulation is used to indicate drought resistance.

Proline, which may accumulate faster than any other amino acid in plant tissues, may accumulate under several conditions including water stress (Barnett and Naylor 1966, Hsaio 1973, Singh *et al.* 1972, Stewart and Hanson 1980), salinity (Chu *et al.* 1976) and low temperature (Chu *et al.* 1974). There are instances, however, where proline accumulation is induced neither by water stress (Capdevila and Dure 1977) nor under saline conditions (Stewart *et al.* 1978). Goring and Thien (1979) argued that proline accumulation due to mineral deficiency cannot be explained in terms of water stress and indicated that nitrogen deficiency may cause proline accumulation in maize shoots and roots. Elmore and McMichael (1981) have shown that proline accumulation by plant tissue is not only a result of water stress but also of nitrogen status (low or high) of the tissue.

The present study was aimed at gaining information specifically on the combined role of scheduling irrigation and nitrogen fertilizer on the accumulation of

proline in wheat (variety Condore) leaves grown under Qatari conditions where drought and nitrogen deficient soils are prevailing. The wheat variety Condore under Qatari conditions proved to be superior in performance and agronomic characteristics compared to the other grown varieties.

### Material and Methods

The experiments were carried out at Rodat Al-Faras Farm near Doha. The soil samples were analyzed following the methods described by Richards (1954), Chapman and Pratt (1961); (Table 1). *Triticum aestivum* (cv. Condore) seeds were sown on the first of December 1980 using a hand-drill at the rate of 270 seeds/plot. The plot size was 8.1 m<sup>2</sup> (3.60 × 2.25 m) and each plot was surrounded by a ridge 75 cm wide. A completely randomized block design with 4 replicates was used. Three irrigation treatments were adopted and designated as wet (W = 13 waterings), medium (M = 10 waterings) and dry (D = 7 waterings). The number of irrigations was applied on the basis of the crop growth stages, expected rain (virtually none) and water economy. All treatments were given irrigation (500 litres of m<sup>3</sup> of water/plot) at sowing to initiate germination and to establish a common starting point. The irrigation water used has electrical conductivity of 3000-3600 mmhos/cm at 25°C (Ismail 1983).

Split dose application of the nitrogen fertilizer (urea, 46% N) was adopted. The following nitrogen treatments were compared: No N, 50 kg N/ha (44 g/plot) applied at sowing time and before the first irrigation on the first of December for the W, M and D treatments; second dose 100 kg N/ha (88 g/plot) applied for the (M) and (D) treatments with the second irrigation, for the (W) treatment with the third irrigation on 15<sup>th</sup> December 1980; third dose – 150 kg N/ha (132 g/plot) was applied when the crop was 42 days old on 11<sup>th</sup> January 1981 with the third irrigation for (D) and the fourth irrigations for (M and W). Usually the fertilizer was first broadcast and thereafter water was allowed to enter the plot.

#### *Determination of Water Content and Estimation of Proline*

Leaf 3 (fully expanded) in the first two stages of growth and the flag leaf in the following stages were sampled from the different treated plots. Three replicates (leaves) were used. Watering was always made 5 hr before the start of the experiment at 7 a.m. in the morning and the time of day when water content samples were taken was 11 a.m. The water content was determined following the method of Garg *et al.* (1981). The proline was determined following the method of Bates (1973) at 12 a.m. Approximately 0.5 g of the plant material was used for the estimation of proline. Purified proline was used to construct the standard curve and the proline concentration of the sampled leaves at the different stages of growth determined from the curve (Batanouny and Ebeid 1981).

**Table 1.** Analysis of soil used at Rodat Al-Faras Farm. (Depth of soil 0-25 cm).

Characteristic	Value
1. Clay %*	40
2. Sand %	33
3. Silt %	27
4. Field capacity	25%
5. Wilting point	13%
6. pH	7.8
7. CaCO <sub>3</sub>	17%
8. Organic matter	0.78%

\* Size fractions of (a) Clay = < 0.002 m, (b) silt = 0.05-0.002 m and (c) sand = 0.05-2 mm.

#### *Determination of Plant Height and Tiller Number*

These were determined at the 'milk-dough kernel' stage. The average height and the average number of tillers/plant were determined by taking representative plants from each plot, measuring heights and counting tillers, and calculating means.

### **Results**

#### *Water Content of Leaf*

The leaf water content of the (D) irrigation treatment was uniformly 50-60%; that of the (M) irrigation treatment was between 60-70% and that of the (W) irrigation treatment was between 75-80% of the fresh weight irrespective of +N or -N.

#### *Average Plant Height and Average Number of Tillers/Plant*

The differential irrigation treatment showed a substantial reduction in height from (W) irrigation treatment to (D) irrigation treatment (Table 2). Fertilizer application resulted in significant increases in plant height. There were noticeable differences in the number of tillers with irrigation and the applications of the nitrogenous fertilizer.

#### *Proline Content of Leaves*

At the seedling stage (1-3 leaf), all the plants accumulated very little proline and there were no significant differences between treatments.

**Table 2.** The effect of the irrigation and nitrogen fertilizer treatments on the plant height and number of tillers per plant (kernels in milk-dough stage)  $\pm$  S.E. given.

Irrigation treatment*	Average plant height in cm		No. of tillers/plant	
	+ N	- N	+ N	- N
D	76.0 $\pm$ 2.0	65.0 $\pm$ 1.8	6.5 $\pm$ 0.8	4.9 $\pm$ 1.0
M	83.0 $\pm$ 3.0	78.0 $\pm$ 0.9	10.0 $\pm$ 0.35	8.1 $\pm$ .70
W	95.0 $\pm$ 0.3	84.0 $\pm$ 0.5	14.5 $\pm$ 0.5	11.0 $\pm$ 1.2

\* Irrigation treatments: D = dry (7 waterings), M = medium (10 waterings), W = wet (13 waterings).

At the tillering stage, differential irrigation had no significant effect on the amount of proline accumulated, but the availability of the fertilizer significantly increased proline accumulation.

During the flag-leaf out and head in boot stage, significant higher (3 times) proline concentration was measured in the (D) irrigation treatment with N compared to corresponding (M and W) treatments. At this and later stages, N availability also significantly increased the amount of the proline accumulating in the M and W treatments.

During the last two stages (flowering and kernels in milk-dough stages), the (D) irrigated plants with N accumulated up to 4 times more proline than any other treatment. On the absence of N, differences in waterings did not affect proline accumulation.

The proline content in the (M and W) irrigation treatments with N was significantly higher in the last 3 stages of growth compared to the first two stages of growth (Table 3).

### Discussion

The effects of moisture stress on crop growth are due to the effects of plant water deficits which restricts physiological processes such as photosynthesis, translocation, and respiration (Farah 1981). Correct timing of nitrogen application appears to be particularly important for its efficient utilization as it has been estimated that not more than 20-50% of fertilizer nitrogen is recovered by arable crops (Cooke 1967).

The results in this investigation show that growth in wheat, as manifested by the height of the plant and the number of tillers in wheat (variety Condore), was severely reduced by N deficiency and infrequency of irrigation (Table 2). Because of aridity of the environment in Qatar (evaporation rate exceeds 7 mm per day in

**Table 3.** Proline content of wheat leaves at different stages of growth, differential irrigation and two N regimes.

Stage of growth	No. of waterings	Proline content + N	( $\mu$ moles/g fresh weight) – N
Seedling, 1-3 leaf stage	D (2)*	0.40 d †	0.45 d
	M (2)	0.48 d	0.55 d
	W (3)	0.46 d	0.50 d
Tillering	D (3)	0.93 cd	0.36 d
	M (4)	0.65 d	0.38 d
	W (4)	0.45 d	0.31 d
Flag-leaf out, head in boot	D (4)	5.50 b	0.47 d
	M (6)	1.55 c	0.45 d
	W (8)	1.85 c	0.54 d
Flowering stage, 50% of inflorescence was at anthesis	D (5)	6.20 b	0.35 d
	M (8)	1.75 c	0.56 d
	W (10)	1.90 c	0.54 d
Kernels in milk-dough stage	D (7)	8.20 a	0.67 d
	M (9)	2.00 c	0.53 d
	W (12)	1.95 c	0.54 d

\* Actual waterings applied when proline was estimated.

† Means followed by the same letter are not different according to Duncan's Multiple Range Test at  $P = 0.05$ .

Summer) and the salinity of the irrigation water (3000-4000 micromhos/cm), frequent irrigations were considered necessary to provide adequate moisture and to provide efficient leaching in order to prevent salt accumulation.

The differential irrigation in the present study combined with the increasing dose of nitrogen resulted in the accumulation of proline but to different concentrations at the different stages of growth. The results show that proline accumulated in wheat leaves when water stress was increased, and nitrogen was supplied (D irrigation treatments). Free proline content of the leaves of plants was low at all stages of growth when plants were well watered (W-irrigation treatments) and had not received N-fertilizer. The results depicted here for N deficiency are consistent with those of Thomson *et al.* (1960) who showed that under N deficiency proline was not accumulated in turnip, while it was accumulated with other mineral deficiencies; the results in this investigation are in agreement with those of Elmore

and McMichael (1981) who showed that proline accumulation in cotton was not only induced by water stress but also by the N status of the plant.

A salient feature in this study was that the concentration of proline increased in the last stages of growth of the N-fertilized crop even in the unstressed plants. Whether this was due to the splitting and doubling of the N dose or whether it was due to the interaction of the soil type (calcareous clay loam with greyish clay in the subsoil due to anaerobic conditions, see Table 1) or time of nitrogen application (Clapp 1973) and its subsequent effect on the nutritional status of the crop or was it a drought response, remains to be carefully investigated.

The mechanism of free proline accumulation in plant tissue whether due to water stress or N level or saline conditions is still unresolved and equivocal (Barnett and Naylor 1966, Palfi *et al.* 1974, Stewart and Lee 1974, Shobert 1977, Stewart 1980, Elmore and McMichael 1981). From the present experiments, under Qatari conditions, it can only be concluded that free proline accumulation in droughted wheat requires adequate nitrogen and developmental maturity and that accumulation is only produced by water stress when adequate N is present, so that artificial selection to increase proline accumulation is only possible under water stress combined with adequate soil – N (see Goring and Thien 1979).

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## تأثير المقننات المائيه وميعاد الإضافة وتجزئة السماد النيتروجيني على البرولين في نبات القمح المنزرع في قطر

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زرع طرز القمح كندور في حقل روض الفرس بالقرب من الدوحة في ديسمبر ١٩٨٠ في أحواض مساحة كل منها ٣,٦٠ × ٢,٢٥ م باستخدام - أربعة مكررات لكل معاملة، وصممت التجربة في قطاعات كاملة العشوائية.

في هذا البحث، تمت دراسة تأثير عدد مرات الري وميعاد إضافة وتجزئة سماد اليوريا على تجمع وتكدس الحمض الأميني الحر - برولين - في أوراق نبات القمح على طول موسم النمو.

اتضح من هذا البحث أن كمية البرولين الحر المتجمعة مرتبطة ارتباطاً وثيقاً بكمية النيتروجين المضاف لنبات القمح، وأن تجمع البرولين ليس نتيجة الجهد المائي فقط.

نستنتج من هذا البحث أن كمية البرولين المتجمع يمكن استعماله كمؤشر مقاومة جفاف في إمداد البرنامج المعد من قبل وزارة الزراعة لانتخاب أصناف قمح صالحة للنمو في البيئة القطرية.