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Differences in Water Relations and Physiological Characteristics in Leaves of Wheat Associated with Leaf Position on the Plant

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ABSTRACT

The seasonal change in leaf water potential and its components, stomatal resistance, specific leaf weight, photosynthesis rate, the activities of ribulose-1,5-bisphosphate carboxylase and nitrate reductase, and soluble proteins were measured in flag leaves (ninth from base in position), seventh and fifth leaves of wheat *Triticum aestivum* L. cv Kalyansona. Flag leaves had a lower water and solute potential and lower or equal turgor pressure than seventh and fifth leaves. These differences were found to be independent of environment. The rate of photosynthesis and nitrate reductase activity were always lower in fifth and seventh leaves than in flag leaf. The photosynthetic efficiency in flag leaves appeared to be associated with lower stomatal resistance and higher specific leaf weight. The relations between leaf water potential and relative water content showed a change with leaf position. This change possibly allows flag leaf to maintain its functional efficiency despite its lower water potential.

The physiological activity of a leaf, particularly photosynthesis, depends on leaf age, genotype, and assimilate demand by sinks in addition to effects of environment (11, 23, 32). Leaf water status influences all aspects of growth and development (2, 13, 22). At ψ^1 of −1.0 to −1.5 MPa, most physiological processes such as leaf expansion, stomatal conductance, photosynthesis rate, and nitrogen metabolism are reduced (5, 13).

In a recent study (Aggarwal *et al.*, unpublished), it was observed that the leaf ψ decreased to −2.0 MPa at flowering in irrigated wheat plants when dry matter production was at its peak and the flag leaf was most active (10, 25). A similar change in water relations at flowering has also been observed in sorghum (12, 24) and soybean (33). These studies also indicated that though leaf ψ was reduced at flowering, the physiological activity of the leaf was not impaired, rather it was improved. This observation contrasts with earlier studies showing sensitivity of physiological processes to small decreases in water potential (4, 13). It is, therefore, necessary to study the various physiological activities simultaneously with water relations at different growth stages.

In the present investigation, seasonal changes in ψ, ψ_s, and P have been studied concurrently with the Phs, the activities of RUBISCO and NR, r, and SLW in flag leaf and lower leaves of wheat. Pressure-volume curves were made to determine any change in water relations associated with the change in leaf position. An attempt has also been made to separate ontogenetic response from environmental effects experienced by the leaves after their emergence.

MATERIALS AND METHODS

Plant Material. Seeds of *Triticum aestivum* L. cv Kalyansona were sown in lines 20 cm apart in three field plots of 5 × 5 m on November 15, 1980. Fertilizer at a rate of 75:60:60 kg/ha N:P:K was broadcast in the field prior to sowing. After germination, the distance between plants was maintained at 2.5 to 3.0 cm. In 20-d-old plants, main stems that were to be used subsequently for the measurements, were tagged. The field was irrigated as per agronomic practice at crown-root, tillering, jointing, booting, flowering, and grain-filling stages.

Sowing as above was repeated on December 15, 1980 and January 10, 1981. These sowings along with the earlier one provided, in the middle of February 1981, fully-expanded, non-senescent, unshaded, and physiologically active fifth, seventh, and ninth (flag) leaves, respectively, on the main stems.

Methods. *(a) Leaf Water Potential (ψ), Solute Potential (ψ_s), Turgor Pressure (P), and Relative Water Content (RWC).* Leaf ψ was measured with a pressure chamber (Soil Moisture Equipment Corp.) following Scholander *et al.* (20). Prior to this measurement, the wall of the chamber was lined with moist filter paper and the leaves were enclosed in a moist polythene bag to reduce the evaporative loss (26). Immediately after recording the balancing pressure, leaves were removed from the chamber, placed in a glass tube, and killed by pouring liquid N_2 over them. These samples were then stored at −20°C until further use. For determination of ψ_s, the leaves were thawed and their sap was extracted with a small hand press. The sap was loaded onto a precalibrated osmometer (5130B, Wescor Inc.). The resulting values of ψ_s were corrected for apoplastic dilution, itself estimated from pressure-volume curves (see below). Turgor pressure was calculated as the difference between ψ and the corrected ψ_s.

The relationship between ψ and RWC (pressure-volume curve) was determined by using a pressure chamber (8, 27). The leaves were cut before dawn and saturated with water for 1 h by enclosing them in humid chambers with their bases kept in water. The leaves were then blotted dry, enclosed in a moist polythene bag, and inserted in the pressure chamber. Pressure was raised to the balancing pressure. A preweighed vial containing filter paper was placed on the cut end of the leaf. Pressure was raised by 0.1 MPa and maintained at that level for 2 to 4 min; the vial was then removed and immediately weighed. The entire procedure was repeated 10 times with ever-increasing pressure, and a fresh vial was used every time.

After completing the series, the leaf was weighed (W_f), dried to a constant weight at 80°C and again weighed (W_d). By adding the total weight of sap expressed (W_s) to the W_f, the initial turgid weight (W_t) of the leaf was obtained. The difference between W_t and W_d gave the amount of water in the leaf. Per cent RWC at different stages was calculated as follows:

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[1] Abbreviations: ψ, water potential; ψ_s, solute potential; P, turgor pressure; Phs, rate of photosynthesis; RUBISCO, ribulose-1,5-bisphosphate carboxylase; NR, nitrate reductase; r, stomatal resistance; SLW, specific leaf weight; RWC, relative water content.

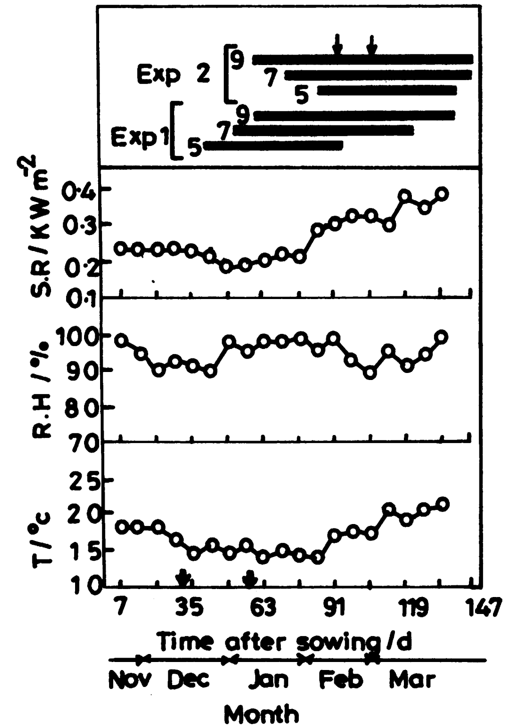

FIG. 1. Mean daily temperature (T), solar radiation (SR), and maximum relative humidity (RH) for entire crop season. Horizontal bars indicate the period from the time of appearance to senescence of different leaf classes in experiments 1 and 2. Arrows on *x* axis indicate the dates of second and third sowings, respectively. Arrows on the bars indicate the days of measurements in experiment 2.

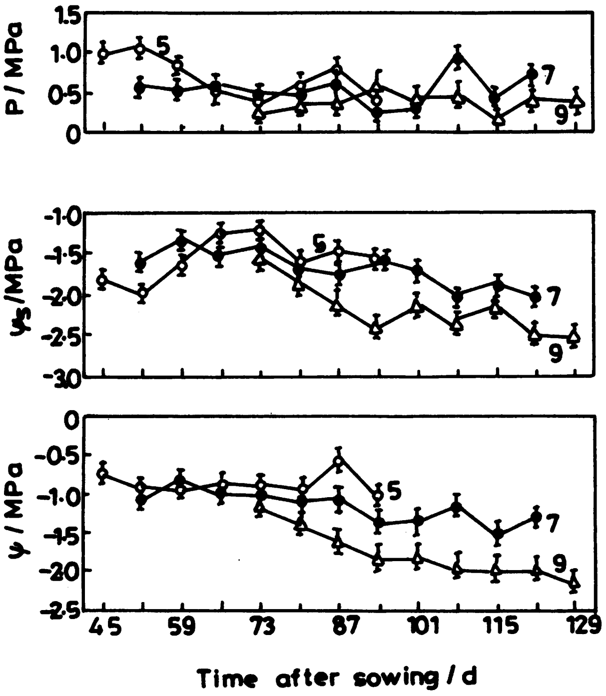

FIG. 2. Leaf water potential (ψ), solute potential (ψ_s), and turgor pressure (P) in the leaves of wheat against time. Values are means ± SE of observations on three replicates (each of two leaves) of each class. The numbers on the lines are leaf numbers. Vertical bars represent SE.

RWC (at i^{th} stage)

$$= \frac{W_t - W_s \text{ expressed by } i^{th} \text{ stage} - W_d}{W_t - W_d} \times 100$$

The results were plotted as ψ^{-1} *versus* % RWC. The initial ψ_s (at full turgor) was obtained by extrapolating the straight line to 100% RWC. The volume of water remaining in the tissue at $\psi^{-1} = 0$ was the volume of apoplastic water. The point at which the straight line joined the curvilinear line was taken as the point of zero turgor.

(b) Specific Leaf Weight (SLW). Fresh weight of the lamina of the different leaves was recorded immediately after removing them from plants in the field. Their area was measured on an automatic area meter (model AAM 7; Hayashi Denkoh Company, Japan). The leaves were then dried at 80°C for 72 h and again weighed. SLW was calculated as the dry weight per unit leaf area.

(c) Stomatal Resistance and Rate of Photosynthesis. Stomatal resistance (r) of both upper and lower surfaces was measured with a diffusive resistance meter (LI 65, LICOR). The rate of photosynthesis (Phs) was determined by feeding the leaves with $^{14}CO_2$ in a closed system (21).

(d) RUBISCO, NO_3^- Reductase, and Soluble Protein. The activities of semipurified RUBISCO (18) and NR were measured by the ^{14}C incorporation (3) and *in vivo* method (15), respectively. The amount of soluble protein was determined by the method of Lowry *et al.* (17).

(e) Weather Parameters. Data on temperature, RH, and solar radiation were obtained from the agro-meteorological laboratory situated adjacent to the experimental plots (Fig. 1).

Experimental Details. *Experiment 1.* In this experiment, measurements of different variables were made at weekly intervals on fifth, seventh, and ninth (flag) leaves from the time of their appearance until senescence. The variables measured were: ψ, ψ_s, P, fresh weight:dry weight ratio, SLW, r, Phs, RUBISCO, NR, and soluble proteins. For each measurement, three replicates (one per plot) with each leaf class were taken. A replicate consisted of two leaves for the measurement of ψ, ψ_s, and P, and of five leaves for rest of the variables. Sampling was always between 10.30 AM and 12 noon.

Experiment 2. This experiment was designed to separate ontogenetic effects from environmental effects. Measurements of the different variables were carried out on February 12 and 26 in fully expanded and nonsenescent fifth, seventh, and ninth leaves obtained from plants sown on different dates. The data reported is the mean of measurements made on both days. The variables measured were: ψ, ψ_s, P, fresh weight:dry weight ratio, r, Phs, RUBISCO, and NR. For each measurement, three replicates with each leaf class were taken, as in experiment 1.

Pressure-volume curves were made for the fifth and flag leaves. The measurements were made between February 10 and 25 on 10 fully expanded and nonsenescent leaves of each class. In 1 d, not more than two leaves of each class were measured.

RESULTS

Experiment 1. Seasonal profiles of ψ showed that the fifth leaf maintained a constant level of −0.8 MPa, whereas ψ of seventh and flag leaves decreased with age from about −1.0 to −1.4 and −2.1 MPa, respectively. ψ_s also varied with time in all the three leaves (Fig. 2). As a result, P also showed fluctuations. The flag leaf had a lower ψ, ψ_s, and P than both fifth and seventh leaves.

With higher leaf position, the fresh weight:dry weight ratio was lower and the specific leaf dry weight was higher (Fig. 3).

Stomatal resistance of both upper and lower epidermis was different in the leaves at different positions. The flag leaf had a relatively lower r than the seventh and fifth leaves (Fig. 3).

Initially both fifth and seventh leaves had higher photosyn-

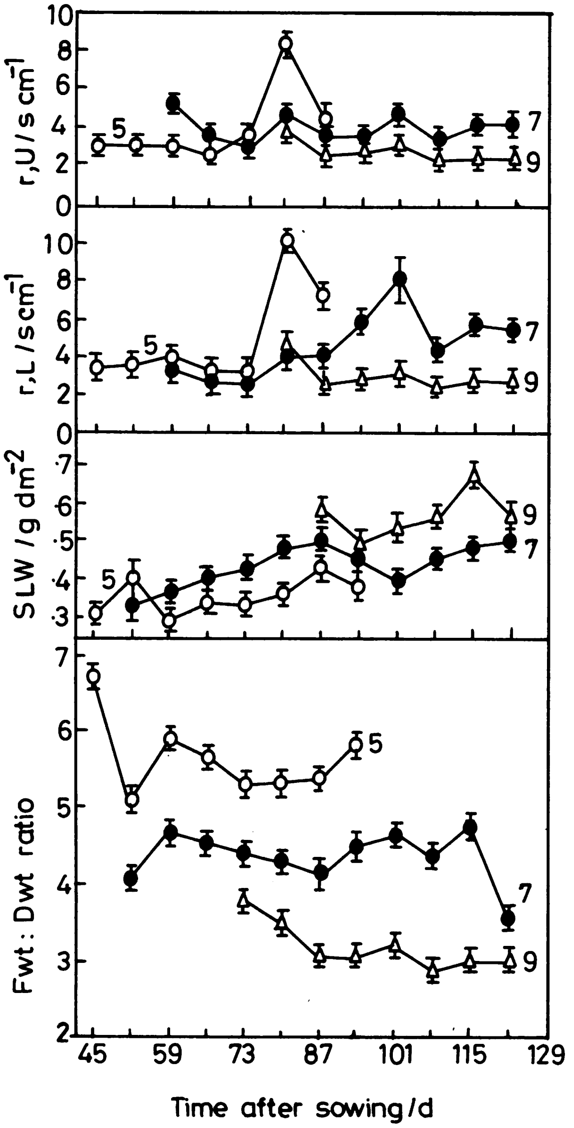

FIG. 3. Fresh weight:dry weight (Fwt:Dwt) ratio, specific leaf dry weight (SLW), and stomatal resistance (r) of upper (U) and lower (L) epidermis in the leaves of wheat against time. Values are means ± SE of observations on three replicates (each of five leaves) of each class. The numbers on the lines are leaf numbers. Vertical bars represent SE.

thetic rates, but subsequently the rate was lower (Fig. 4). By comparison, the flag leaf had a higher rate of photosynthesis throughout. The activity of RUBISCO varied with time, but there were no large differences among the different leaves except from 73 to 87 d after sowing, when the flag leaf had a higher activity. However, the flag leaf maintained a higher activity of NR than the seventh leaf which in turn had higher activity than the fifth leaf (Fig. 4). The amount of soluble protein was not significantly different among the different leaves (Fig. 4).

Experiment 2. The water potentials of different leaves showed the same pattern, as in experiment 1 even when determined on the same day in the leaves of comparable physiological age (Fig. 5). The flag leaf had a lower ψ than the seventh and the fifth leaves. The ψ_s of all leaves were −0.5 MPa lower than their ψ; this resulted in a constant P of 0.5 MPa. Fresh weight:dry weight ratios were 5.0, 4.3, and 3.5 for the fifth, seventh, and ninth leaves, respectively. Stomatal resistance of the upper surface in the flag leaf was lower than in the leaves of lower insertion (Fig. 5).

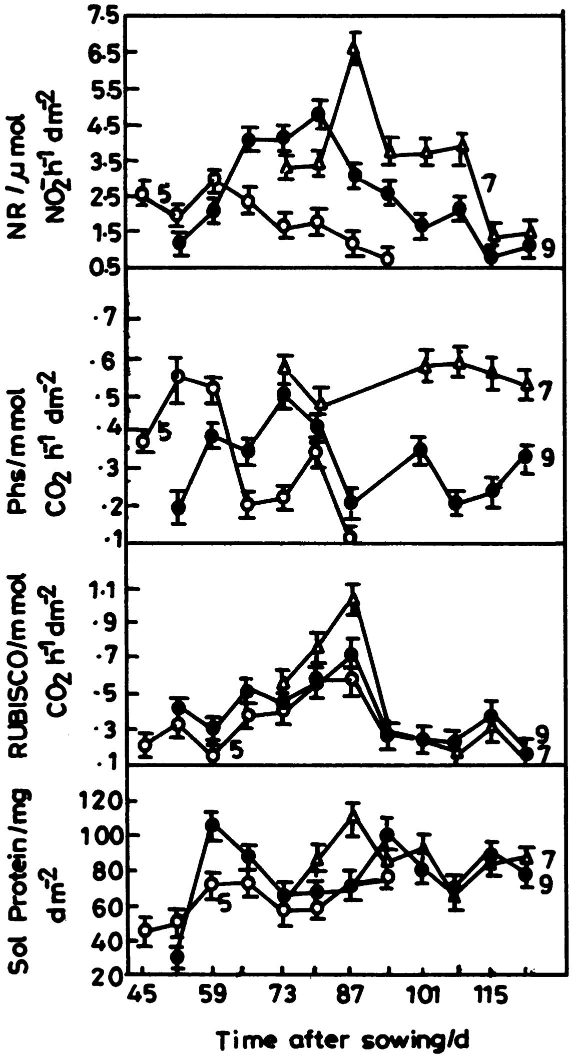

FIG. 4. Activities of RUBISCO and NR, Phs, and the amount of soluble protein in the leaves of wheat against time. Values are means ± SE of observations of three replicates (each of five leaves) of each class. The numbers on the lines are leaf numbers. Vertical bars represent SE.

The effect of leaf position was evident in the Phs and NR activity as well; both these being higher in the flag leaf than in the other leaves (Fig. 5). However, the RUBISCO activity was not significantly different in the three leaves.

Pressure volume curve: ψ of flag leaf decreased rapidly for a small change in water content as compared with the fifth leaf where the decrease was slower (Fig. 6). The fifth leaf had more symplastic water and attained zero turgor at a relatively lower water content than the flag leaf (Table I). However, ψ_s at zero and full turgor were higher in fifth leaf than in the flag leaf (Table I). The apoplastic water in the seventh leaf was not measured but was assumed to be 30.7% (mean of fifth and flag leaf).

DISCUSSION

The experiments carried out in this study indicate that the flag leaf is different from the lower leaves, both in water relations and physiological characteristics. Since observations made in uniform environment also indicated the same pattern of results (Fig. 5), it can be concluded that the responses were not due to environment at the time of measurements (Fig. 1). However, the climate which different leaves had already experienced from the time of leaf primordia initiation to leaf emergence could possibly

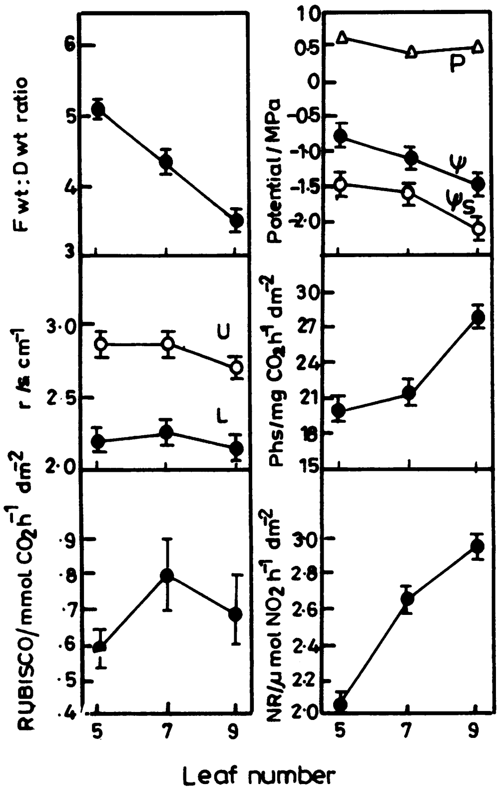

FIG. 5. Water relations, stomatal resistance, and physiological activities in the leaves of wheat. All determinations were made on the same day using fully expanded and nonsenescent leaves. Values are means ± SE of observations of three replicates (each of five leaves) of each class. The numbers on the lines are leaf numbers. Vertical bars represent SE.

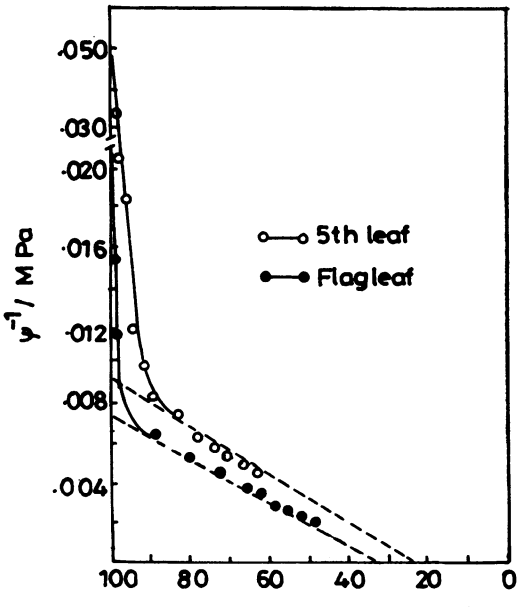

FIG. 6. Relationship between water potential (ψ) and RWC in the fifth and flag leaves of wheat. Values are representative of one determination; 10 determinations were made for each leaf class.

Table I. *Water-Relations Parameters Obtained from the Pressure-Volume Curves of the Fifth and the Flag Leaf of Wheat*

Values are means ± 1 SE of 10 observations.

Parameters	Fifth Leaf	Flag Leaf
Solute potential (full turgor), MPa	−1.00 ± 0.03	−1.22 ± 0.03
Water and solute potential (zero turgor), MPa	−1.31 ± 0.06	−1.39 ± 0.03
Relative water content (zero turgor), %	83.2 ± 1.5	91.2 ± 1.7
Symplastic water, %	72.9 ± 1.9	65.6 ± 1.6
Apoplastic water, %	27.1 ± 1.9	34.4 ± 1.6

have an effect on the behavior of different leaves.

It was observed that the flag leaf consistently maintained a higher Phs and nitrogen assimilation (assessed as NR activity) despite having a lower ψ than the fifth or seventh (lower in position) leaves (Figs. 2 and 4). The higher Phs could not be ascribed either to RUBISCO activity or the amount of soluble protein, which did not differ significantly among these leaves. However, it is important to note that the flag leaf had a higher SLW (dry matter per unit area) and a lower stomatal resistance (Figs. 3 and 5). Both these characters have often been shown to be important in regulating the Phs (29, 31).

In fact, a higher specific leaf weight is apparently associated with the smaller mesophyll cell size but greater cell number cm^{-2} and greater cell wall mass than the leaves of lower positions (7). The smaller cell size in turn is strongly correlated with higher rates of photosynthesis (14, 29). Similar results were reported by Wilson (30) with a *Panicum* species. A greater number of smaller cells have an increased surface:volume ratio (30) and are considered photosynthetically more efficient (9). Our studies in wheat, therefore, suggest that ontogenetic changes in leaf structure are associated with their functional efficiency. Indeed, the relationship between Phs and vein density and other structural features has been demonstrated in studies where flag leaves of several species were used (1, 19).

A pressure-volume curve was derived to assess whether there was any change in water relations of the leaves at different positions (Fig. 6). It was observed that at any given water content, the flag leaf had a lower ψ than the lower leaves. This property of flag leaves can also be ascribed to the different cellular structure of the leaves since the cells with thicker cell walls, as postulated for the flag leaf, are expected to show a sharper decline in their ψ in response to a given change in water content than the cells with less rigid cell walls, as in the other leaves (6, 16, 28). Therefore, a flag leaf will reach lower values of water potential faster than the other leaves as the sun rises, and it would maintain this for the whole day although the water content would not decrease much. Since the physiological efficiency, as exhibited by Phs or NR activity is maintained high, this could be an adaptive mechanism of the flag leaf. Simultaneously, the change in water relations associated with flowering in leaves should provide an increased soil-plant water potential gradient, to ensure extraction of the remaining water in the soil profile (33).

It is, therefore, apparent that the flag leaf of wheat has distinctive properties when compared to the other leaves. Its physiological efficiency despite a lower water potential seems to be due to its structural rather than enzymic characteristics.

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