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Accumulation of Protein, Chlorophyll and Relative Leaf Water Content in Barley (*Hordeum Vulgare* L.) In Relation to Sowing Time and Nitrogen Fertilizer

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Abstract: Field experiments were carried out to study the effects of sowing times and nitrogen fertilizers on accumulation of protein, chlorophyll and relative water content in barley cultivars (*Hordeum vulgare* L.). Results indicated that sowing in the 1st and 2nd week of November resulted higher chlorophyll and RLWC compared to other times. But protein content was higher in 11th December sowing. Increased doses of nitrogen fertilizer increased protein, chlorophyll and RLWC as well.

Key words: Protein, Chlorophyll, RLWC, Barley, Sowing time, Nitrogen fertilizer

INTRODUCTION

The most important use of barley throughout the world is as malt for manufacturing beverages or malt enriched food products. It is also used as fodder crop for domestic animals, poultry and human food in the form of preached grain. In barley, grain protein concentration (GPC) is an important factor determining malting quality, is sensitive to high rates of nitrogen fertilizer[10]. High nitrogen fertilizer rates, particularly when applied in adverse growing conditions (drought), result in excessive GPC[19]. High GPC is undesirable because malt extract is inversely related to GPC[16]. Role of nitrogen is important because it is the building and information substances from which the living material or protoplasm of every cell is made. In addition, it is also found in chlorophyll, the green coloring matter of plants. Chlorophyll enables the plant to transfer of energy from sunlight by photosynthesis. Therefore, nitrogen supply to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Relative leaf water content (RLWC) is another important character, which is directly related to soil water content and also indicate soil water status[26].

As a rainfed crop, barley is generally sown in early November to late December in Bangladesh, which is cool and dry. Farmers of Bangladesh cultivate barley plants without considering proper sowing time and nitrogen fertilizer doses. Which resulted early or delayed exposure to the environment and under or over application of nitrogen fertilizer.

Though few work have been done on growth and grain yield of barley in relation to sowing time and nitrogen fertilizer, accumulation of protein, chlorophyll and relative water content in barley in relation to sowing time and nitrogen fertilizer has not yet studied so far in Bangladesh. So, the present experiment was carried out to find out the suitable sowing time and nitrogen fertilizer doses on accumulation of protein, chlorophyll and relative water content in barley.

MATERIALS AND METHODS

The experiment was conducted in the research field of the Department of Botany, University of Rajshahi with four barley cultivars viz. BB 1, Karan 19, Karan 163 and Karan 351. The soil of the experimental field was silty loam with pH 6.6. The total nitrogen was 0.06%, organic matter 1.04%, phosphorus 13.9 ppm, potassium 0.12 milliequivalent/100g soil, zinc 0.40 ppm and sulphur 8.1 ppm. The experiment was conducted in a split plot design with four sowing times (S_1, S_2, S_3 and S_4 which indicate 5th, 17th and 29th November and 11th December respectively) five N treatments (N_0, N_1, N_2, N_3 and N_4 indicate 0, 30, 60, 90 and 120 kg/h N respectively) in each sowing and cultivars with three replications. Row to row distance was 20 cm and plant-to-plant distance was 5 cm in all the plots, thus 100 plants were counted per square meter avoiding border row. Grain protein content was determined by the micro-Kjeldahl method Jayaraman[14]. Chlorophyll a and b were determined according to the formula proposed by Mackinney[18] and later used by Arnon[2]. The Relative water content of the flag leaf was determined at 8.00 h, 12.00 h and 16.00 h according to Barrs and Weatherly [5]. Statistical Analysis was carried out according to Gomez and Gomez [11].

RESULTS AND DISCUSSION

Sowing times significantly influenced total protein content and grain protein content (Table 1) in barley, as such the maximum total protein content and grain protein content was obtained in S_4 followed by S_3, S_2

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Table 1: Accumulation of total and grain protein content (%) as affected by different nitrogen levels at various sowing times (Arc Sine transformed values).

	Total protein content (%)					Grain protein content (%)				
Treatment	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
N_0	11.93 ab	12.22 ab	12.77 ab	13.04 ab	12.49	8.47 c	8.73 c	8.91 c	9.11 c	8.80
N_1	11.68 cd	12.00 c	12.55 c	12.83 c	12.26	8.70 c	8.97 c	9.14 c	9.34 c	9.04
N_2	11.55 d	11.82 d	12.38 d	12.64 d	12.1	9.18 b	9.45 b	9.63 b	9.83 b	9.52
N_3	11.81 bc	12.08 bc	12.66 bc	12.96 bc	12.38	9.65 a	9.91 a	10.08 a	10.29 a	9.98
N_4	12.02 a	12.33 a	12.90 a	13.19 a	12.61	9.78 a	10.04 a	10.22 a	10.42 a	10.12
Mean	11.80	12.09	12.65	12.93	12.37	9.16	9.42	9.59	9.80	9.49
LSD at 5%	S=0.54		T=0.08			S=0.30		T=0.16		

In a column, values followed by a common letter do not differ significantly at 5% level.

Table 2: Accumulation of chlorophyll a, b, total chlorophyll and chlorophyll a:b of the flag leaf as affected by different nitrogen levels and sowing times.

	Chlorophyll a (mg dm^{-2})					Chlorophyll b (mg dm^{-2})				
Treatment	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
N_0	3.05 d	2.86 d	2.74 c	2.60 c	2.82	1.05 c	0.93 c	0.92 c	0.91 c	0.95
N_1	3.12 d	2.92 d	2.77 c	2.65 c	2.87	1.09 b	0.97 b	0.97 b	0.95 b	1.00
N_2	3.24 c	3.03 c	2.93 b	2.75 b	2.99	1.05 c	0.93 c	0.91 c	0.92 c	0.95
N_3	3.35 b	3.12 b	2.95 b	2.82 b	3.06	1.14 a	1.02 a	1.03 a	1.01 a	1.05
N_4	3.49 a	3.24 a	3.13 a	2.93 a	3.20	1.09 b	0.98 b	0.96 b	0.97 b	1.00
Mean	3.25	3.04	2.91	2.75	2.99	1.08	0.97	0.96	0.95	0.99
LSD at 5%	S=0.22		T=0.04			S=0.09		T=0.02		
	Total chlorophyll (mg dm^{-2})					Chlorophyll a:b (mg dm^{-2})				
Treatment	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
N_0	4.10 e	3.80 e	3.66 e	3.51 e	3.77	2.95 c	3.12 bc	3.70 b	2.93 ab	3.02
N_1	4.21 d	3.90 d	3.74 d	3.60 d	3.86	2.91 c	3.05 c	2.93 b	2.85 b	2.94
N_2	4.29c	3.96c	3.84 c	3.66 c	3.94	3.14 ab	3.29 ab	3.30 a	3.05 a	3.20
N_3	4.49 b	4.15 b	3.98 b	3.83 b	4.11	2.98 bc	3.07 c	2.94 b	2.84 b	2.96
N_4	4.58 a	4.23 a	4.09 a	3.90 a	4.20	3.24 a	3.33 a	3.32 a	3.05 a	3.24
Mean	4.34	4.01	3.86	3.70	3.98	3.04	3.17	3.11	2.95	3.07
LSD at 5%	S=0.14		T=0.03			S=0.47		T=0.09		

In a column, values followed by a common letter do not differ significantly at 5% level.

and S_1. Generally delayed sowing resulted increase in protein content, which is an agreement with the findings of Weston *et al.*[30]. But higher doses of nitrogen fertilizer increased total protein content and grain protein content in the present study. Similar results were reported by Boonchoo *et al.* [7].

Chlorophyll a and b are the most important pigments active in the photosynthetic process. In the present experiment, chlorophyll a, chlorophyll b and total chlorophyll of the flag leaves were comparatively higher for S_1. But chlorophyll a:b ratio was higher for S_2 (Table 2). The S_1 plants contained higher chlorophyll was due to higher heliothermal unit obtained by the plants of that sowing which enhanced more chlorophyll formation and increased the rate of photosynthesis more efficiently in leaves. Chlorophyll formation decreased with delayed sowing in the present study as because of low temperature and scarce soil moisture at the vegetative stages. Reduction of chlorophyll formation due to scarce soil moisture was reported by Ashraf *et al.*[3], Sarker *et al.*[26] Chandrasekar *et al.*[8], Nyachiro *et al.*[22] and Paul *et al.*[24]. It was also found that increased level of nitrogen increased chlorophyll a, chlorophyll b and total chlorophyll in the present investigation. Similar results were reported by Islam *et al.*[13], Shim *et al.*[27] and

Table 3: Accumulation of relative leaf water content (%) of the flag leaf at different times of a day as affected by different nitrogen levels and sowing times (Arc Sine transformed values).

	8.00 h					12.00 h					16.00 h				
Treatment	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
N_0	91.8 e	88.6 d	83.0 c	72.5 c	84.0	74.9 c	72.3 c	67.8 b	59.1 b	68.5	80.4d	77.6c	72.5b	63.6b	73.5
N_1	92.2 d	88.8 d	82.8 c	72.5 c	84.1	72.8 e	70.3 e	65.3 d	57.3 d	66.4	78.7e	75.9d	70.8d	62.0e	71.9
N_2	92.4 c	89.0 c	82.8 c	72.4 c	84.2	75.2 b	72.7 b	67.6 b	59.1 b	68.6	80.9b	78.0b	72.4b	63.4c	73.7
N_3	94.2 b	90.7 b	84.3 b	73.8 b	85.7	74.3 d	71.8 d	66.6 c	58.3 c	67.7	80.7c	77.7c	71.9c	63.1d	73.3
N_4	96.7 a	94.4 a	87.3 a	76.6 a	88.8	78.7 a	76.8 a	70.9 a	62.2 a	72.1	85.2a	83.2a	77.0a	67.3a	78.2
Mean	93.4	90.3	84.1	73.6	85.3	75.2	72.8	67.6	59.2	68.7	81.2	78.5	72.9	63.9	74.1
LSD at 5%	S=0.15		T=0.12			S=0.16		T=0.12			S=0.17		T=0.11		

In a column, values followed by a common letter do not differ significantly at 5% level.

Paul [23]. In the present study, chlorophyll a, b and total chlorophyll were found to be affected by sowing time and nitrogen levels but chlorophyll a:b ratio was less affected.

Relative leaf water content (RLWC) is the relation between the actual and fully turgid water contents of leaf tissue[4] and it represents the severity of dehydration of a leaf when it experiences water stress and is closely associated with the developmental and physiological activities[20]. In the present investigation, early sown plants had the highest RLWC than that of the delayed sown plants for the flag leaves (Table 3). It might be due to scarcity of soil moisture at delayed sowing times. Chandrasekar *et al.*[8] reported that water stress caused a decline in relative water content in both the tetraploid and the hexaploid wheats. Chaves[9] suggested RLWC as an appropriate indicator of plant water status and drought tolerant cultivars can be identified with greater certainty by a high RLWC following a period of drought. Kramer[15] opined that higher RLWC was associated with higher dry matter production rates of the well watered plants because cell turgidity is important in relation to the opening and closing of stomata, expansion of leaves and flowers and movement of water and nutrients to various parts of the plants. Agenbag[1] noticed that water stress decreased RLWC as well as increased leaf diffusive resistance. Wein *et al.*[29] suggested that an increase in the plants' resistance to water flow as soil moisture stress increased can help to maintain suitable plant water status. As plant resistance to water flow definitely increased with soil moisture stress, Hall and Schulze[12] suggested that the constant negative water content is the result of stomatal regulation and of carbon partitioning. However, Steven *et al.*[28] stated that the behaviour of water was accurately portrayed by water potential, turgour potential and osmotic potential and Ma *et al.*[17] noticed a close correlation between leaf water potential and yield. In the present investigation, highest RLWC was noticed at 8:00 am and it decreased at mid-day (12:00 noon). Again, a gradual increase in RLWC was noticed at the later part of the day (4:00 pm) in most cases.. This result was in agreement with Nazrul-Islam[21], Begum and Paul[6], Rahman[25], Sarker *et al.*[26] and Paul *et al.*[24]. The steady decline of RLWC at the mid-day might be due to higher evapotranspiration owing to increased temperature and light intensity. Significant fertilizer differences were also found for RLWC in the present study. Higher RLWC was observed in N_4. It might be due to its greater leaf area index.

Conclusion: The overall results indicated that delayed sowing decreased accumulation of chlorophyll and RLWC but protein content increased with delayed sowing. On the other hand accumulation of protein, chlorophyll and RLWC increased with higher doses of nitrogen fertilizer.

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