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Full Length Article

Nitrogen Affects Leaf Expansion and Elongation Rates During Early Growth Stages of Wheat

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Abstract

Nitrogen fertilization strongly influences the wheat growth particularly in N deficient soil. This study quantified the effect of N sources on emergence, growth rate, chlorophyll content, phyllochron and dry matter of wheat. Wheat (AC Barrie variety) was grown in glasshouse at the University of Bangor, Wales, UK in pots comprising five N treatments *i.e*., ordinary soil (0 kg N ha^{-1}), 100 kg N ha^{-1} as nitram, compost, farmyard manure (FYM) @ 12.5 and 25 t ha^{-1}. Nitram N delayed seedling emergence, improved chlorophyll contents, leaf length, and plants height. Linear elongation and leaf appearance rates were greater for manure over control. Linear elongation rate decreased with appearance of leaves. Phyllochron values decreased as the number of leaves on primary tiller increased. In conclusion, the inorganic N reduced leaf numbers but increased leaf elongation rate, whereas organic source of N increased roots biomass and appearance of leaves. © 2019 Friends Science Publishers

Key words: Wheat growth; Leaf elongation rate; Leaf appearance rate and phyllochron
Abbreviations: FYM (farmyard manure), LER (leaf expansion rate) DUR (duration of extension)**,** GDD (growing degree days), LAR (leaf appearance rate), SPAD (soil plant analyses development)

Introduction

Enhanced early vigour, linear leaf elongation rate (LER) and stand establishment are well documented physiological traits that potentially improve wheat productivity (Botwright *et al.*, 2002). Plant dry matter production depends on a number of factors including leaf emergence and elongation rates, leaf area and tillering (Borràs-Gelonch *et al.*, 2012). Leaf appearance rate (LAR) has been characterised by the value of the phyllochron -a termed used for the period between the appearances of successive leaves on the culm (Eggers *et al.*, 2004). The plant phyllochron between leaf number n and n +1 have been reported to be affected by combination of genetic and environmental factors (Toyota *et al.*, 2014). Phyllochron is highly dependent on air temperature (Porter and Gawith, 1999). Severe N stress decrease LAR (Laghari *et al.*, 2013). Phyllochron values can be extended with increasing population and increasing N fertilizers (Martinez-Eixarch *et al.*, 2013). However, Hall *et al.* (2014) reported that the currently available evidence does not support the plastic response of wheat flowering timing to nitrogen. The value of phyllochron can be calculated in different ways: (i) Haun (1973) scale who reported it as the "inverse of the rate of leaf emergence" in units of degree-days per leaf; or (ii) from the thermal time between the emergence of two consecutive leaf tips (Wilhelm and McMaster, 1995).

Temperature, light, water supply (Sánchez *et al.*, 2014) and nutrition (Martinez-Eixarch *et al.*, 2013) are the major factors controlling photosynthetic efficiency of most crops species. In the humid temperate conditions, the photosynthetic efficiency of plants depends primarily on the amount of light intercepted (Villegas *et al.*, 2016), which is more likely to be affected by N (Martinez-Eixarch *et al.*, 2013). Some researchers for example, Alzueta *et al.* (2012) reported negligible differences for early growth pattern in N fertilized and control plots, whereas other found significant differences (Martinez-Eixarch *et al.*, 2013; Akhtar *et al.*, 2018a, b; Khan *et al.*, 2018). The seedling emergence, leaves and stem's growth rates are accounted for early growth development (Richards and Lukacs, 2002). Therefore, the need for exploring variation in each of these characteristics for improving early growth pattern of wheat in relation to N provides research opportunities. This study hypothesised that N sources will have variable effects on growth, emergence, and elongation rates of these components. The quantification of these plants component's growth and rates in temperate humid climatic conditions will provides information on biologically optimal crop N amount and sources for designing crop N management schemes.

The development of plants organism is based on

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thermal degree-days – a term used for the amount of heat absorb by the plants during a specific time (Leblanc *et al.*, 2003). The air temperature below 6.9°C before anthesis reduced wheat production (Villegas *et al.*, 2016) and is considered base temperature (Tbase), which is used for calculation of thermal degree-days by mean-minus-base (Leblanc *et al.*, 2003) or sine-wave method (Allen, 1976) which is needed for estimating phyllochron values. Hence, 2nd hypothesis was that extending the cumulative thermal days had more strong effects in organic sources of N on leaf and tiller appearance than inorganic N source.

Previous literatures studies yielded no significant contribution toward the studies of LAR and phyllochron and its impact on the early growth pattern in wheat, therefore we conducted this experiment with objectives to 1) investigate the influence of N sources on leaves and tillers elongation, phyllochron values and its impact on early growth patterns in wheat, 2) to quantify the relative increase in length of leaves or stem to the various forms of nitrogen application.

Materials and Methods

Site Description

The study was conducted at the Pen-y-Ffrid research station in glasshouse, the University Bangor, Wales (53° 12' N, 4° 09' W, with 292 feet elevation). The glasshouse was kept as a controlled environment (temperature = 20°C in day, 18°C in night; photoperiod = 16 h and relative humidity of 65–70%) for the research period.

Materials and Treatments

Five N treatments including 1) ordinary soil, 100 kg N ha^{-1}, 3) compost (10 tons ha^{-1}), 4) farmyard manure (FYM) at the rate of 12.5 tons ha^{-1} and 5) 25 tons FYM ha^{-1} were used. The soil, compost and FYM all were obtained from Humax, West land Garden (www.humax.co.uk). Nitram (34%) was used as inorganic source of N. The soil was loam top. The pots were filled with ordinary soil leaving a space of only 3 cm from top in case of ordinary soil, nitram or compost treatments, but 10 cm in case of FYM treatments. Out of 10 cm space, 25 tons FYM ha^{-1} occupied 7 cm, whereas 12.5 tons FYM ha^{-1} occupied 3 cm space, thus, an additional 3 cm layer of ordinary soil was added to 12.5 tons FYM ha^{-1} treatment to make the top distance uniform for all the pots. After adding the FYM or nitram, the soil was mixed very well to get a uniform well-mixed top layer and FYM and/or nitram. Six seed of wheat (variety AC Barrie; hard red wheat for bread) was sown in pots (490.62 cm^2 area and 30 cm height) in six replicates and thinned to three seedlings after complete emergence (7 days after sowing). Initially, the gravitational water contents were monitored (0.25–0.30 m^3 $m^{-3)}$ daily for 15 days, and thereafter at two days interval using moisture tester (Delta probe), water was added accordingly as spraying irrigation.

Observations and Measurements

Emergence was recorded as the day's difference between sowing and complete seedling emergence. Plant height was recorded continuously since emergence till harvesting daily. Weeds per pot were recorded at the end of the experiment. Nitrogen deficient leaves were recorded by visual observations for nitrogen deficient symptoms. The leaf length and width of 5th leaf on primary tiller at growth stage 16 (Zadoks *et al.*, 1974) were recorded to calculate the leaf area as the product of leaf length, width and factor 0.8 (Rebetzke and Richards, 1999). Seedlings were uprooted from each pot after seven days of sowing at growth stage 11 (Zadoks *et al.*, 1974). The uprooted seedlings were separated into components (shoots and roots) for dry weights. These components were kept in oven at 50°C temperature until constant weight for 30 h to record dry weights. At growth stage 31 (Zadoks *et al.*, 1974) all the three plants in each pot were uprooted for recording dry weight of individual plants traits including tillers, leaves and roots. The dry weights of the components were measured after drying at temperature 50°C for 48 h.

SPAD value were recorded at growth stages of 12, 14, 15, 16 and 17 (Zadoks *et al.*, 1974) using SPAD meter 502. Initially daily observations were made to record total number of leaves and tillers, but when the plants reached growth stage 26 (Zadoks *et al.*, 1974), then observation were prolonged to be taken after each two days or even more. These SPAD values were converted into chlorophyll content of leaves (Ling *et al.*, 2011).

Leaf length measurements were taken for the first five leaves on the primary tiller, whether fully emerged or emerging daily until the leaf's length become constant and averaged. The linear leaf elongation rate (LER, mm °C per days) were calculated as proposed by Rodríguez *et al.* (1998), using an effective model best fitted to the experimental data, mathematically:

$$y = a + b.x \; if \; x \leq c \;\&\; y = a + b.x \; if \; x > c ------(eq.1)$$

Where, y is total leaf length (mm), a is the intercept (mm), b is the slope (value of LER in mm °C day^{-1}), x is the thermal time since leaf emergence (°C days), and c the time when leaf elongation stopped. Thermal times in term of thermal degree-days were calculated using a common method mean minus-base (Leblanc *et al.*, 2003) using equation;

$$CTU = \sum_{i}^{n} \left(\frac{T_{max} - T_{min}}{2} \right) - T_{base} -----(eq.2)$$

Where, CTU represents the cumulative thermal units in degree-days, i is the starting date to cumulate degree days, n is the time required to complete development, $Tmax$ and $Tmin$ are the daily maximum and minimum temperatures, and $Tbase$ =6.9 is the base temperature for wheat. The leaf appearance rate (LAR) was calculated by regressing the appeared leaves against the thermal units (as per eq-2) from emergence till harvesting using linear regression equation:

Table 1: Days to emergence, leaf area (cm^2), weeds pot^{-1}, N deficient leaves, and plant height (cm) as affected by different levels and form of N

Treatments	Days to emergence	Leaf area (cm^2)	Weeds per pot[I]	N deficient leaves[I]	Plant height (cm)
Ordinary soil	4.5	25.2	2.8	2.7	47.9
100 kg N ha^{-1}	5.3	26.4	1.8	2.3	53.4
Compost	4.0	25.7	2.2	1.2	50.0
FYM 12.5 t ha^{-1}	4.5	24.4	3.0	1.0	49.9
FYM 25 t ha^{-1}	4.5	26.7	1.7	1.5	50.3
s.e.d. (*df* 20)	0.318	1.677	0.214	0.173	1.741
Significance	**	NS	NS	**	**

Analyses were made on square root transformed data

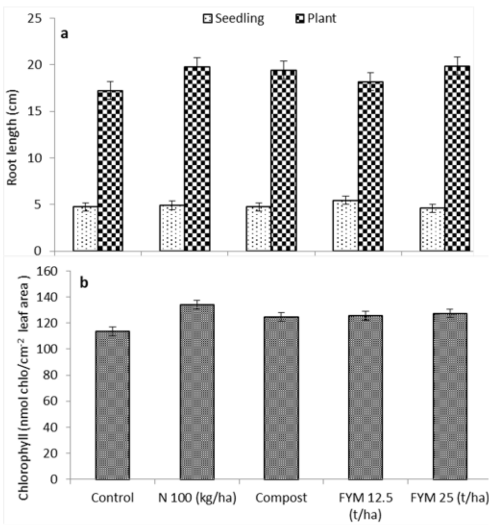

Fig. 1: The effect of N fertilization on (**a**) root length (cm) and (**b**) chlorophyll content (nmol chlorophyll /cm^{-2} leaf area) of wheat. Vertical bars are standard error of mean (n=6)

$$Y = a + bx - - - - - (eq. 3)$$

Where Y is the number of leaves per plant, a is the intercept (leaves $plant^{-1}$), b is the leaf appearance rate (leaf °C d^{-1}, LAR) and x is the accumulated thermal time. Length of phyllochron were calculated according to huan stage (Wilhelm and McMaster, 1995) for leaves emergence in thermal days (°C days) as;

$$Huan\ stage = \left[\frac{L_n}{L_{n-1}}\right] + (n-1) - - - - - (eq. 4)$$

Where L_n is the length of the youngest leaf blade above the collar of the subtending leaf, $L_{(n-1)}$ is the length of the blade of the subtending leaf, and *n* is the total number of leaves that are visible on the tiller. Phyllochron values (°C day) were ascertained by dividing the huan stage with thermal days (°C days, as per eq. 2).

Statistical Analysis

Five treatments repeated six times were evaluated in randomize complete block design. Analysis of variance (ANOVA) was used to detect the significance of treatments effects on the different variables measured. In case of significant differences, standard errors (Gomez and Gomez, 1984) were used to separate the means. Regression analyses were carried out for calculating various physiological observations. The statistical software (GenStat, 2005) was used for analysis of all data.

Results

Seedling Emergence and Early Growth

Seed emerged earlier in compost treatment than control, while inorganic N delayed emergence but increased plant height by 12% with respect to ordinary soil (Table 1). In other fertility treatments, no obvious variations in plants heights were noted. Nitram application resulted in taller plants compared to control (Table 1). Plants in ordinary soil have more N deficiency symptoms than fertilized pots (Table 1). Leaf area and weeds per pot were not affected by N treatments (Table 1). Both seedling components (shoots and root) after 7 days of sowing and 31 days older plant's components (shoot, leaves and roots) dry weights were not statistically different among the treatments (Table 2). However, N applied @ 100 kg ha^{-1} produced 25%, compost 33%, 12.5 tons FYM ha^{-1} 8% and 25 tons FYM ha^{-1} 8% higher root's dry weight over control soil (Table 2). These results showed that the compost treatment was more efficient in root development than other treatments.

Root Lengths and Leaf Chlorophyll Contents

No differences in root lengths for wheat seedling (7 days older) were noted in N treatments (Fig. 1a), however, significant differences were observed for 31 days older wheat plant (Table 3). The 31 days older plant's roots were greater with nitram, compost and 25 t FYM ha^{-1} compared to control (Fig. 1a). Chlorophyll contents were higher for nitram than organic sources of N or control soil (Table 3, Fig. 1b). However, among the organic sources of N, no significant ($p < 0.05$) differences were noted for leaf chlorophyll content.

Leaf Length and Elongation Rates

Significant differences were observed in leaf length among the treatments (Table 3, Fig. 2a), that was higher for 100 kg N ha^{-1} than other treatments. Similarly, the organic sources of N had more leaf length than the control pot (Fig. 2a). Linear leaf elongation rate (LER, mm °C per days) was higher for earlier emerged leaf over subsequent leaves (Fig. 2b). Among the N treatments, no significant differences in the LER of 1^{st} leaves were noted, however for 3^{rd} and 5^{th} leaf, the LER was significant (Table 3, Fig. 2b). The LER was greater for nitram treatments for 2^{nd} leaf, however for 5^{th} leaf, the LER was greater with organic sources of N. These results indicate that later emerged leaves were strongly affected and elongated with organic N sources,

Table 2: Dry weights of wheat seedlings (7 days older) and 31 days older plants as affected by levels and form of N

Parameters	Treatments					s.e.d. (20)
	Ordinary soil	100 kg N ha^{-1}	Compost	FYM 12.5 t ha^{-1}	FYM 25 t ha^{-1}	Sign.
Seedling (7 days after sowing dry weight per plant (mg))						
Roots	10.72	12.16	12.60	12.22	11.82	0.855^{NS}
Shoots	23.39	20.77	21.36	22.02	22.29	1.621^{NS}
31 days older plants dry weight per plant (g)						
Roots	0.12	0.15	0.16	0.13	0.13	0.012^{**}
Shoots	0.71	0.86	0.85	0.78	0.81	0.097^{NS}
Leaves	1.19	1.31	0.97	1.05	1.07	0.139^{NS}

s.e.d=Standard errors of differences of means, **=Significant at $p \leq 0.01$, NS=Non significant

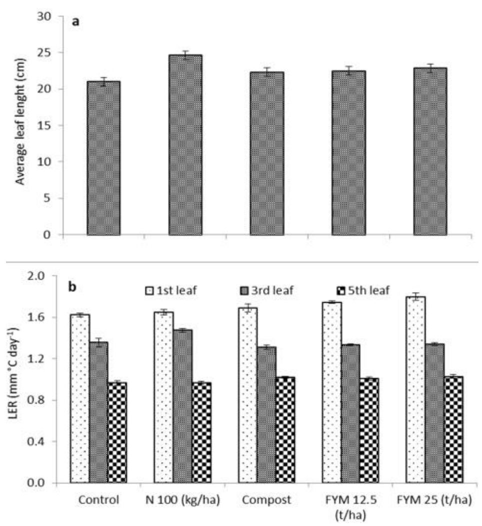

Fig. 2: The effect of N fertilization on (**a**) average leaf length (cm) and (**b**) linear elongation rate (LER, mm °C day^{-1}) of wheat leaves. Vertical bars are standard error of mean (n=6)

whereas the earlier emerged leaves i.e. 3rd elongation was more apparently affected by inorganic N sources. This mean that for 1st leaf the soil available N is sufficient for optimum LER, the nitram is only available for 3rd leaf, whereas the organic source of N provided the N upon mineralization to the subsequent leaves.

Leaf Appearance and Phyllochron

Leaf appearance rate (LAR) was significantly greater in organic fertilized plots over control (Table 3), and maximum LAR was observed in 25 tons FYM ha^{-1} treatment (Fig. 3a). However, no difference for LAR was noted between 12.5 or 25 tons FYM ha^{-1}. Similarly, nitram had lower LAR than organic N but more than control. Leaf emergence on the primary tiller was faster (smaller phyllochron) for compost treatment, followed by 12.5 tons FYM ha^{-1}, but delayed (more phyllochron values) with nitram and 25 tons FYM ha^{-1} (Fig. 3b). This indicated that the availability of N for longer duration has increased the growth of plants leaves and thus had delayed the extrusion of following leaves.

Table 3: ANOVA table (error df = 20) for various studied parameters of wheat as affected by nitrogen fertilization under controlled condition

Parameters studied	MSE	F-ratio	Probability	CV (%)
Average leaf length (cm)	0.52193	4.30	0.011^{*}	3.09
Root length of 7 days older seedling (cm)	2.74797	0.24	0.912^{NS}	1.53
Root length of 31 days older plant (cm)	0.68317	3.01	0.043^{*}	16.85
Chlorophyll contents (nmol /cm^{-2} leaf area)	6.08400	21.31	0.000^{**}	1.69
1st leaf linear elongation rate (mm °C day^{-1})	0.01034	0.11	0.978^{NS}	7.57
3rd leaf linear elongation rate (mm °C day^{-1})	0.00743	3.22	0.034^{*}	6.33
5th leaf linear elongation rate (mm °C day^{-1})	0.00349	5.32	0.004^{**}	6.26
leaf appearance rate (leaf per °C day)	0.00750	4.30	0.011^{*}	5.07
Phyllochron (per °C day)	2.99301	3.62	0.022^{*}	3.70

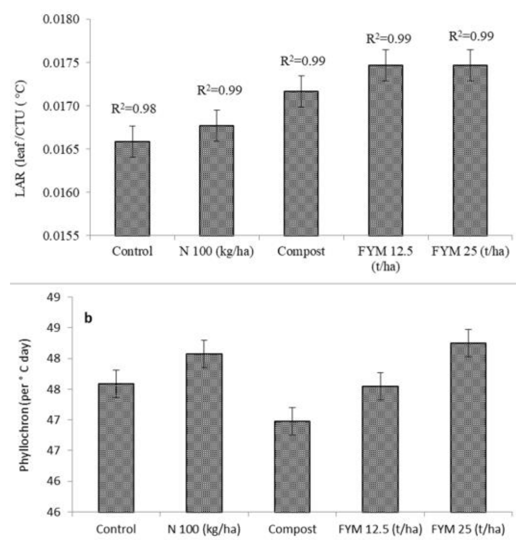

Fig. 3: The effect of N fertilization on (**a**) leaf appearance rate (LAR, leaf per °C day) and (**b**) phyllochron (per °C day) of wheat. Vertical bars are standard error of mean (n=6). R^2 represent the coefficient of determination for each treatment in response to thermal degree days

Discussion

Nitrogen availability strongly affects the growth and development of cereal crops (Ibrahim and Khan, 2017; Khan *et al.*, 2017, 2018). Thus, the N is essentially needed to be supplied from external sources as commercial fertilizer (Romero *et al.*, 2013) or organic source (Akhtar *et al.*, 2018a, b). The N availability affect the plant growth through two major mechanisms *i.e.*, acts as integral component of the chlorophyll and thus affect the photosynthetic efficiency of plants (Errecart *et al.*, 2012) or improve the vegetative growth of the plants and thus increase the leaf areas and chlorophyll for improving the plant photosynthetic efficiencies (Khan *et al.*, 2008; Akhtar *et al.*, 2018b).

In present study, organic and inorganic N sources were optimized for their effects on leaf emergence, tillering, early plant growth pattern and phyllochron values in spring wheat. Inorganic N resulted in more leaf lengths, higher rate of leaf elongation, and chlorophyll content than control. Leaf emergence rate was not necessarily linear (Longnecker and Robson, 1994) across the developmental stages, however, has higher rate of expansion in latter stage of the development then early stage of the development. The inorganic N had less effect on the leaf appearance rate but increased the elongation rate and subsequently the chlorophyll content. Nitrogen deficiency resulted in mark reductions in leaf size in present investigation. Tolley-Henry and Raper (1986) also observed that the leaf size and elongation of leaves depends on the N availability. Leaf emergence and early expansion rate is also affected by the stored N reserve material in seed irrespective of the seed sizes (Khan *et al.*, 2005). The N is readily available when inorganic N placed in the pots, when compared to the organic N. The higher growth rate of leave increased the green colour of the leaves in pots having inorganic N (Tamaki *et al.*, 1999), and thus more chlorophyll could be attributed to more N availability. Likewise, decomposition of 25 tons FYM ha^{-1} have released more mineral N than lower rate of FYM, and thus increased the leaf elongation rate, area and subsequently the chlorophyll contents.

Phyllochron is the time duration in term of thermal units between the appearances of two consecutive leaves. The organic N sources resulted in apparently non-availability of N for initial leaves emergence (Malhi *et al.*, 2001) and growth due to initial immobilization of N, but the latter decomposition resulted in more N in organic (Khan *et al.*, 2015; Muhammad *et al.*, 2018) or greater moisture conservation (Ali *et al.*, 2018) had positive effects on leaves growth. The phyllochron values were greater for inorganic N and 25 tons FYM ha^{-1} than compost or 12.5 tons FYM ha^{-1}. Duru and Ducrocq (2000) reported that when N is supplied the leaf growth rate and duration of expansion is increased. During early growing period, the leaves and tillers per plant steadily increased but the abrupt increase observed during later stage (around 270°C days), was due to greater N availability, mineralization and N uptake efficiency of the plants. The available N was greater in nitram or due to greater mineral availably as a result of large amount of FYM decomposition. Leaves and tiller per plant changed in response to the amount of N in the growth medium and in tissues (Duru and Ducrocq, 2000). Phyllochron values (°C days) decreased as the number of leaves on primary tiller increased, and the rate of expansion increased. Skinner and Nelson (1995) reported that a decrease in the leaf growth rate or increase in the plastochron would decrease the phyllochron values, and hence more leaves per plant (Rodríguez *et al.*, 1998), caused greater LAR due to increased decomposition of the FYM. The LAR in our results decreases with increase in leaf number on the primary tiller is supported by the Skinner and Nelson (1994).

Root dry matter was less in control plots than fertilized, that might be due to the availability of nitrogen in fertilized plots, increasing the dry matter production. Bolovic *et al.* (2005) reported greater dry weight, chlorophyll and carotenoids contents in plants grown in fertilized soil compared to unfertilized. Nitrogen is also associated with the green surfaces of the crop canopy and with the dry matter component, and hence the more N available to the crop might have increased the dry matter (Ercoli *et al.*, 2008). The improvement in leaf length, numbers and rate of growth all are accountable for more dry matter production in wheat. Hati *et al.* (2006) reported more root density and length in manure treated plots than in inorganic N. These greater length and density may lead to the higher dry matter production in the organic N treatments. These results indicated that any attempt to simulate early growth and phyllochron values in spring wheat under various form of N, the deficiency on the leaves should be taken into account, which were essentially higher in control soil than plots having organic sources of N. We believe that studying the effects of N form and levels on cell division and leaf primordia will aid in the better understanding of leaf growth rate, expansion and phyllochron.

Conclusion

Plants root dry weight was greater for compost than control or FYM treatments. The phyllochron values were higher for organic N compared to inorganic N, with greater leaf appearance rate. The average leaf length was higher with inorganic N, whereas LER was maximum for first leaf and decreased with subsequent leaves. However, the organic sources of N had increased the LER of latter emerged leaves. The chlorophyll contents were higher for 100 kg N ha^{-1} than organic N and/or ordinary soil.

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