

## Spring barley (*Hordeum vulgare* L.) Responses to Soil Injected Liquid Ammonium Nutrition under Different Growth Temperatures

Charles M. Matoka<sup>1,2,S</sup>, Siegfried Schittenhelm<sup>1</sup>, Joerg M. Greef<sup>1</sup> and Stephen G. Agong<sup>3</sup>

<sup>1</sup> Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Braunschweig, Germany

<sup>2</sup> Directorate of Academic Quality Assurance (DAQA), Technical University of Mombasa (TUM), Tom Mboya Avenue, P. O. Box 90420 – 80100, Mombasa, Kenya

<sup>3</sup> Jaramogi Oginga Odinga University of Science and Technology (JOUST), P. O. Box 210 - 40601, Bondo, Kenya

---

**Abstract:** Controlled chamber experiments were performed at Braunschweig, Germany to examine the potential effects of global warming on spring barley (*Hordeum vulgare* L.) shoot and root biomass accumulation, yield forming structures (tillers) and attainable grain yield through the adoption of a novel fertilization technique that injects liquid ammonium nutrition into soil for crop uptake. Three growth temperature regimes; low (Temperate), medium (Intermediate) and high (Tropical) were set to mimic different climatic conditions experienced globally. Each temperature regime comprised of three nitrogen fertilization treatments of which conventional nitrate application and non-fertilized control were compared to soil injected liquid ammonium nutrition herein referred to as Controlled Uptake Long Term Ammonium Nutrition (CULTAN). Each of the three nitrogen treatments was replicated twice in each of the three temperature regimes. The experiment was repeated in two seasons during which chamber randomization was performed eliminate chamber effects.

Nitrogen fertilized barley attained superior grain yields under low temperature regime (LTR) as compared to high temperature regimes (HTR). Similarly, low temperature regimes supported higher shoot and root biomasses, more tillers and ear bearing tillers than the other two temperature regimes. Grain yields were correlated to ear bearing tillers and grain numbers. Low temperature regime supported more grains that weighed less per grain in comparison to warmer temperatures which compensated for less grain numbers by heavy grains. This is the first time interaction effects between soil injected liquid ammonium nutrition (CULTAN fertilization) and temperature regimes have been reported in a cereal crop production model. The results demonstrate that growth temperatures and nitrogen nutrition forms jointly can cause a suite of responses on barley growth and yield formation with potential for exploitation in enhancement of food security.

**Key words:** Barley Grain yield, Cereal production, Crop nutrition, Climate change, CULTAN fertilization technique, Growth temperature and Global food security

---

### I. Introduction

The global food demand has been increasing with serious gaps reported in Asia and sub Saharan Africa [1]. However, attainment of food demands without undermining integrity of the earth's environmental systems one of the greatest current global challenges for humanity [2]. To date, the commonly adopted agricultural systems are among the major forces contributing to environmental degradation [1]. It is projected that human population growth will increase from the current 6.8 to 9.1 billion by 2050, thus doubling consumption for calorie-intensive diets [2]. The scenario will be a repeat of the happenings of between 1961 and 2007 when the world's population more than doubled while agricultural output lagged behind [3]. Current trends suggest that the pace of food production has been lagging and it will continue at the same rate unless innovative technologies are adopted [3]. The global crop yield variability is heavily influenced by fertilizer use, irrigation and climatic factors [4]. Admittedly, climate change and nutrition contribute significantly to the uncertainty bedeviling crop production systems [5, 6]. Adoption of new technologies like irrigation and fertilizer application provide avenues for creating large crop production increases with the potential of closing attainable yield gaps [7]. Recent emphasis on sustainable intensification to ensure crop yield increase on underperforming landscapes while simultaneously decreasing environmental impact have been undertaken [8]. The achievement of optimal agricultural production will remain a mirage because of myriads of constraints [1].

In light of this, a paradigm shift focused on increasing crop production alongside environmental protection needs to be adopted. The initiative has led to development of a phenomenon referred to as 'sustainable intensification of global agriculture' aimed at the promotion of high yielding crop varieties whose

water and fertilizer requirements are minimal [9]. Currently, fertilizer usage significantly contributes towards achievement of increased global food output. More particularly, fertilizer use efficiency is critical in both rain fed and irrigated agriculture [10]. Adequate plant nutrition involving applications of fertilizers play a critical role in ensuring food security [11]. Nitrogen in agricultural soils exist as organic and inorganic forms, with 95% or more being organic [12]. The main inorganic nitrogen forms taken up by plants include nitrate and ammonium [13]. Ammonium usually undergoes nitrification whose end product is nitrate via nitrite. Ammonia oxidation, the first step in nitrification and a key process in global nitrogen cycle is normally facilitated by microbes [11]. The increase in nitrate availability is important for plant nutrition, but also has considerable adverse effects on ground water due to pollution caused by leaching and eutrophication of above ground waters [14]. The apparent inadequacy of nutrients in agricultural fields necessitates application of inorganic fertilizers, especially nitrogen to support satisfactory crop yields.

Since nutrient losses reduce crop productivity besides polluting environment, the adverse effects can be mitigated through a novel fertilizer application method where liquid ammonium nutrition referred to as Controlled Uptake Long Term Ammonium Nutrition (CULTAN) is adopted. The agricultural practice is based upon injection of concentrated liquid ammonium nutrition to support crop growth. The fertilization method mitigates against nitrate related nutritional losses through the exploitation of ammonium ( $\text{NH}_4^+$ ) adsorption capacity onto clay matrix to form deposition-zones of sorption complexes [15, 16, 17]. The fertilization technique presents a great economic potential because of reduced number of farm operations. CULTAN has the potential of promoting benign environmental effects with an increased nutrient availability for cereal and horticultural crop uptake [18, 16]. Alongside nutrition, cereal crop yields are greatly influenced by growth temperatures [19], carbon dioxide concentration [20] and greenhouse gases [21] among other factors. Increased atmospheric temperatures and decreased soil moisture reduce yields substantially as noted in several climate change reports [22]. Very few climate change studies report impact on crop growth and yield output [19]. Global temperatures will continue to rise in the near future hence need to find reliable indicators for identification of plant populations with ability to cope with increasing temperatures. Recent findings by Springate and Kover [23] suggest that plant species with ability to respond to temperature increases by accelerating flowering are more likely to adapt to global warming. The newly developed agricultural technologies like CULTAN fertilizer application can be adopted to surmount crop nutritional challenges while ensuring a benign environment [8, 4]. It is against this background that the study sought to evaluate growth and yield formation responses of spring barley (*Hordeum vulgare L.*) to soil injected liquid ammonium nutrition (CULTAN) alongside conventional nitrate fertilization and non-fertilized barley crop under different growth temperature regimes.

## **II. Materials And Methods**

The experiment was performed in three separate growth chambers at Federal Agricultural Research Centre (FAL), Braunschweig, Germany. Spring barley, *Hordeum vulgare L.* cultivar Maresi was used as a model cereal crop in the study. The seeds were sown singly within an inter- and intra- row spacing of 5 cm at a depth of 2.5 cm in 90 L soil filled container whose planting surface area measured 0.2288 m<sup>2</sup>. The soil was a mixture of top-, sub- and sandy-soil proportions in the ratio of 1:1:1, respectively. It was free draining sandy loam comprising 14.6% clay, 39.6% silt, 45.8% sand and 1.6% humus with a near neutral pH of 7.4. Growth chambers were cubes measuring 3 m (27 m<sup>3</sup>) fitted with 16 lamps on two horizontally adjustable metallic frames. Half of the lamps comprised sodium bulbs while the other eight were fluorescent potassium tubes. The two lamp sets provided 600  $\mu\text{mol m}^{-2}\text{s}^{-1}$  photosynthetic active radiations (PAR) during the 14/10 hrs day/night photoperiod. Chamber relative humidity ranged between 55 – 70%. Five 1 cm diameter holes at the bottom of sowing container drained off excess irrigation water to guard against water-logging and the percolate was reutilized.

### **2.1 Experimental Design**

Temperature was a major treatment whereas nitrogen form was minor. Temperature regimes, Low Temperature Regime (LTR; 6-16 °C) and High Temperature Regime (HTR; 14-29 °C) were established with Medium Temperature Regime (MTR; 10-20 °C) as intermediate. Each temperature regime comprised of three nitrogen fertilizer treatments replicated twice. The three nitrogen treatments included ammonium (CULTAN), nitrate (Conventional) and non-fertilized control. The fertilization treatments were arranged in Randomized Complete Block Design (RCBD) within each temperature regime. The experiment was conducted in two seasons under similar conditions, but growth chambers were swapped to balance out possible chamber effects.

### **2.2 Temperature and Nitrogen Treatments**

Three days prior to sowing, growth chambers were acclimatized to similar temperature conditions of 13/9 °C for day/night, respectively. The condition was maintained from sowing up to onset of tillering stage. At

tillering, each chamber was reset to corresponding LTR, MTR and HTR conditions (Table 1). During change-over, chamber temperatures were raised in a stepwise manner to correspond to the seasonal progression that coincided with four distinct barley growth stages (Table 1). Temperatures were monitored using soil thermometers inserted at 7 cm depth. The crops were considered to have attained subsequent growth stages when at least 50% exhibited descriptions of Zadoks' growth scale [24]. Each temperature regime had three nitrogen treatments (Table 1). Nitrate was provided as  $\text{Ca}(\text{NO}_3)_2$  while ammonium was supplied as diammonium phosphate (DAP) salt dissolved in water. Each treatment received 4 g of nitrogen either as  $\text{NO}_3^-$  or  $\text{NH}_4^+$ . Nitrate was top-dressed whereas ammonium was injected into five different points per treatment. The injection was performed at a depth of 7 cm using 1 cm diameter aluminium rod. The holes were fitted with 20 ml Eppendorf tubes to simulate spoke wheeled injectors used for CULTAN injection under field conditions. After injection, the holes were refilled with soil and marked with thin plastic pegs. The extra phosphorus from DAP in  $\text{NH}_4^+$  treatment was balanced out by supplying 18 g P in the form of  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  to nitrate and non-fertilized treatments. The other essential macro- and micronutrients whose effects were not being assessed were adequately supplied to ensure balanced mineral nutrition.

### 2.3 Carbon Exchange Rates (CERs) and SPAD

Gas exchange measurements were performed on the leaf immediately below flag-leaf (F-1) on the main tillers at onset of grain filling period. Four barley crops in each replicate nitrogen treatment were used. Each leaf was clamped onto 2.5 cm<sup>2</sup> cuvet for CER measurements. SPAD – 502 (Minolta Ltd., Japan), a handheld chlorophyll meter was employed to estimate leaf nitrogen status. CER measurements were performed using HCM-1000 differential infrared gas analyzer (IRGA) (Heinz Walz GmbH, 91090 Effeltrich, Germany) fitted with a leaf cuvet. During measurements, carbon dioxide concentration was regulated at 350  $\mu\text{L L}^{-1}$  while leaf cuvet air temperature was set at 25 °C. The saturating photosynthetic photon flux density (PPFD) was supplied by Walz lighting unit at 1800  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . Diurnal effects were accounted for by switching among treatments and replicates. Maximum CER were calculated using HCM-1000's operation software based on methods of von Caemmerer and Fahquer [25].

### 2.4 Shoot and Root Biomass Estimates

The crops were harvested at physiological maturity and grains processed. Crop growth duration, stem length and total tiller numbers were recorded. Dry shoot and root weights were recorded while grain bearing ears were cleaved off and counted. Tillers per plant alongside average grains per head as well as thousand (1000) grain weights were calculated. Roots were harvested as described by Bloom et al. [26] with slight modifications. Detachable sieves of varying mesh sizes, 2.5, 2 and 1 mm were used. Finer roots were recovered using a pair of forceps. Root and shoot samples were oven dried for 48 hrs at 60 °C for chemical analysis. Sub-samples for dry matter (DM) were dried at 105 °C for 48hrs. Agronomic nitrogen use efficiency (NUE) was estimated as described by Maranville et al. [27] and Moll et al. [28]. Root: shoot ratios, harvest index as well as response index were both estimated.

### 2.5 Data Management and Analysis

Statistical analysis was performed using SAS programme for windows version 9.1 employing general linear model (GLM). The presented data were averages of two seasons. One way Analyses of Variance (ANOVA) was performed to compare mean differences among the three nitrogen treatments within each temperature regime. Two way ANOVA was performed to compare mean differences among various parameters of pooled seasonal data sets of nitrogen treatments in each temperature regime besides individual seasonal comparisons. Similarly, average photosynthetic rates and chlorophyll meter (SPAD) readings were compared within and among temperature regimes. Treatment means were considered significant when  $P \leq 0.05$  and post ANOVA was performed using Tukey test. Tiller numbers were correlated to shoot biomass and grain yield.

## III. Results

The findings of the study were as described herein;

### 3.1 Barley Growth Duration and Stem Lengths

The impact of temperature regimes on barley growth duration and stem elongation was recorded at physiological maturity. Barley took varied durations to attain physiological maturity. Crops grown under LTR required six months (183 days) to attain maturity while those in HTR took four months (122 days) (Fig. 1a). MTR crops required five (5) months (162 days), which was an intermediate duration between LTR and HTR. There were no significant differences among stem lengths of non-fertilized crops in the three temperature regimes. Stem lengths of fertilized crops were not significantly different from those within the same temperature regime. However, stem lengths of fertilized crops increased as growth temperatures reduced (Fig. 1b).

Comparison of non-fertilized control in LTR with ammonium and nitrate fertilized crops expressed 39.3% and 36.1% stem-length increases, respectively. Similarly, HTR ammonium and nitrate fertilized crops recorded 16.9% and 18.6% increases, respectively (Fig. 1b). Comparison of fertilized crops in LTR to their counterparts in HTR recorded that ammonium expressed 23.2% while nitrate registered 18.6% stem length increases (Fig. 1b). MTR stem lengths remained intermediate.

### **3.2 Biomass Accumulation and Partitioning**

To investigate barley root and shoot growth responses to CULTAN fertilization technique, biomass accumulation and partitioning as well as root architecture were assessed. CULTAN fertilized crops developed intensive architectural root network around the injection-points while nitrate fertilized crops did not develop any. The intensive root network proliferated around injection points indicated none or minimal root penetration through the concentrated ammonium injection point. Shoot biomass accumulation in the three nitrogen treatments exhibited increasing trends with decreases in growth temperature (Fig. 2a). Shoot biomasses were significantly different at  $P < 0.001$ . Similarly, root biomass expressed an increasing trend with decreases in growth temperature (Fig. 2b). Non-fertilized crop shoot and root biomasses increased with temperature decrease (Fig. 2a and b). Dry root biomass improvement due to nitrogen fertilizer application in LTR was 191.4% and 154.7% for CULTAN and nitrate, respectively (Fig. 2b). In LTR, CULTAN fertilized crops supported 14.4% higher root biomass over nitrate. The relative biomass increase under HTR demonstrated that nitrate and ammonium gained 127.5 and 86%, respectively (Fig. 2a). Shoot biomass gains in LTR in comparison to HTR were 194, 94, and 32% for ammonium, nitrate and non-fertilized control (Fig. 2a). Similar trends were observed in root biomass increases under low growth temperatures. Non-fertilized crops in LTR recorded the highest root: shoot ratio of 0.18 while nitrate was lowest with 0.08 whereas CULTAN treatment recorded 0.14. Changes in biomass partitioning resulted into a shift in root: shoot ratios under HTR in comparison to those in LTR. The ratios illustrate that non-fertilized and ammonium fertilized crops in LTR partitioned higher biomasses to roots with less apportioned to shoot.

### **3.3 Response of barley yield formation to growth temperatures and ammonium nutrition**

To evaluate the impact of growth temperature and nitrogen form applied, total grain weights and numbers were compared within and among temperature regimes. The results demonstrated that total grain weights and numbers were influenced by growth temperatures and nitrogen form (Fig. 3a and b). Both grain weights and numbers increased with decreasing growth temperatures (Fig. 3a and b). The attained grain yields of non-fertilized crops ranged between 100 and 110 g that translated to about 4 and 5 tonnes  $\text{ha}^{-1}$ . Nitrate fertilized crops yielded 240 g under HTR that translated into 10.5 tonnes  $\text{ha}^{-1}$  while under LTR it supported 310 g which was an equivalent of 13.5 tonnes  $\text{ha}^{-1}$ . Similarly, CULTAN yielded 210 g (9.18 tonnes  $\text{ha}^{-1}$ ) and 335 g (14.64 tonnes  $\text{ha}^{-1}$ ) (Fig. 3a). The yields of fertilized crops were not significantly different from each other within same growth temperature regime; however, they were both significantly different from non-fertilized crops at  $P < 0.001$ . The agronomic nitrogen use efficiency (NUE) attained by ammonium and nitrate fertilized crops in HTR was 105 and 133%, respectively. The agronomic NUE was even higher under LTR where nitrate and ammonium grain weights increased by 214 and 227%, respectively (Fig. 3a).

The percentage increase in grain numbers over non-fertilized control by nitrate and ammonium fertilized crops under HTR was 151 and 145%, whereas LTR attained 171 and 179%, respectively (Fig. 3b). Similarly, the percent gain in grain yield weights attributed to temperature effect was 40, 13 and 7% under LTR for ammonium, nitrate and non-fertilized control in comparison to HTR attained yields. Crops grown in low temperatures attained higher total grain yields supported with lower average weights of 0.043 g per single grain. This was in contrast with the crops under high temperature conditions that attained lower total grain yields despite supporting slightly higher single grain weights of 0.045 g. Thousand (1000) grain weights among fertilized crops ranged between 40 and 45 g. Fertilized treatments in HTR attained harvest index of 0.2. Under LTR, both ammonium and nitrate fertilized crops also had equal harvest indices of 0.1 while non-fertilized crops recorded 0.2.

To determine the effect of growth temperatures and applied nitrogen forms on yield forming structures, fertile tillers and grain bearing ears were counted. The findings demonstrated that tiller numbers influenced the attainable grain yield (Fig. 4a). Fertilized crops supported more tillers and grain bearing ears than non-fertilized crops (Fig. 4b). Generally, tiller numbers increased among fertilized crops as growth temperatures reduced. However, tiller numbers were not influenced by growth temperatures as they remained same in the three growth temperature regimes (Fig. 4a). It emerged that nitrate nutrition was superior to CULTAN under HTR with regard to tiller numbers (Fig. 4a). Average tillers per plant supported by nitrate were five in HTR and six in LTR. Ammonium treatment supported six tillers in HTR and seven in LTR. Tiller bearing ears influenced attained grain yields, whereas both fertile and infertile tillers contributed to the recorded shoot biomasses (Fig. 2a).

The influence of growth temperature and nitrogen forms on physiological performance of barley was assessed. Photosynthetic; carbon exchange rate (CER) measurements were estimated at flowering stage and compared within and among temperature regimes. Fertilizer application significantly influenced photosynthetic rates; however, they were not significantly different among the temperature regimes (Fig. 5a). However, the CER among nitrate fertilized crops consistently decreased as growth temperatures decreased, though the margin was quite low. The intensity of photosynthetic pigments (chlorophyll) was evaluated. Chlorophyll measurements using (SPAD) recorded values ranging between 40 and 45 among the fertilized crops. Non-fertilized crop SPAD values ranged between 20 and 30 across the temperature regimes (Fig. 5b).

#### IV. Discussion

Grain, shoot and root biomass yields supported by CULTAN were superior to nitrate under LTR. Conversely, CULTAN grain and biomass yields in HTR recorded inferior yields in comparison to nitrate treatment. Moreover, grain yields of fertilized crops increased as temperature decreased suggesting temperature as an important factor of cereal grain formation. The accumulated biomass climaxed at physiological maturity after growth duration of 4 months in HTR and 6 months under LTR. The findings are supported by those reported by Trnka et al. [29] who observed that high temperatures supported lower cereal crop yields. Since global temperatures will continue to rise in future because of global warming, there is urgent need to seek reliable indicators of identifying plant populations with capacity to adapt to increasing temperatures. The findings of Springate and Kover [23] reported that plant species with ability to respond to temperature increases by accelerating flowering are more likely to persist in a globally warming environment. These observations are reminiscent with findings of the current study where barley crop under HTR conditions recorded shorter growth durations and equally a reduced grain filling period.

The corollary effect of shorter growth and grain filling periods is lower grain yields with a potential negative effect on food security. Currently, the global food demand has been increasing with serious gaps reported in Asia and sub-Saharan Africa [1]. By 2050, more than 300 million people in sub-Saharan Africa are at risk of starvation if cereal production in the continent is not stepped up. The situation could worsen and lead to further malnutrition of 52 million children in sub-Saharan Africa [1]. Because of such challenges, there is need to spur innovation in response to environmental protection, climate change effects, population growth, crop production and protection challenges as well as new market opportunities [7, 4].

The development and adoption of benign fertilizer application methods such as CULTAN offers a window of opportunity for increased cereal crop production while conserving the environment [16]. Reduction of agricultural activity and related impact on environment through elimination of nutrient overuse in cereal production is an important step towards environmental conservation besides cost reduction [30]. The attainment of food security requires improvement in nutrient and water management for enhanced crop yields [8, 4]. The Consultative Group on International Agricultural Research (CGIAR) reported that the demand for cereals, especially maize in developing countries will double between now and 2050. Since the year 2000, climate change has caused cereal crop consumption to decrease from 117 kg person<sup>-1</sup> year<sup>-1</sup> and is projected to decrease even further to 89 kg person<sup>-1</sup> year<sup>-1</sup> by 2050 [5]. Climate change studies predict considerable warming of sub-Saharan Africa by 2050 with severe outcome on crop yields [10].

The low grain yields under high temperatures were occasioned by rapid crop growth through phenological phases that culminated into shorter growth duration, while low growth temperatures provided adequate growth duration for grain formation and biomass accumulation [29]. The accumulation of photosynthetic products by nitrogen fertilized crops under LTR and HTR was substantial despite non-statistically significant SPAD measurements. Among cereals, high growth temperatures have the potential of driving shorter life cycles that result into less seasonal photosynthesis, shorter reproductive phase and lower grain yields [31]. Cereal vegetative development is accelerated by high temperatures; however, it is the dramatically shorter grain-filling period caused by rising temperatures that portend a major constraint to grain yield attained [32]. The effect of temperature on wheat grain-filling duration resulted into lower yields in Western USA than in northern Europe [30]. When wheat growing areas experienced about 25 °C, grain-filling was significantly reduced, after which additional 1 °C temperature rise shortened reproductive phase by 6% and grain-filling duration by 5% to concomitantly reduce grain yield and harvest index by large margins. Barley grain yield is majorly influenced by grain numbers rather than sizes which remain relatively constant and stable components [33]. Nitrogen nutrition influences barley leaf pigmentation and photosynthetic rates with significant effects on biomass partitioning in the root or shoot [34].

In the study, tillering was highly responsive to temperature and nitrogen form applied. These findings are reported by those of Garcia et al. [35, 36] who reported that cultivar and nitrogen fertilizer forms influenced cereal tillering ability. The effect of tillering ability on grain formation emphasizes its contribution to the attained yield as observed by Yan et al. [37] and Li et al. [38]. Alongside grain yield improvement, partial NO<sub>3</sub><sup>-</sup> occurrence in mixed nitrogen nutrition is thought to play a key role in ammonia toxicity alleviation [39], though

the mechanism remains unknown [40, 41, 42]. Although partial  $\text{NO}_3^-$  is associated with enhancement of plant tissue  $\text{NH}_4^+$  assimilation, the pathway involved is also yet to be unravelled [43]. CULTAN fertilizer application technique though initially had high concentrations of ammonium, it gradually underwent oxidation through microbial activity to subsequently produce nitrate that possibly made available mixed nitrogen nutrition ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) for crop uptake [44, 45]. Before oxidation, uptake of ammonium nutrition generally causes toxicity-symptoms as reported by Schittenhelm and Menge-Hartmann [46]. When oxidized, CULTAN provides mixed nutrition with potential for root growth proliferation around the injection zones so as to support dense architectural network [16, 47, 48, 49].

The possible availability of mixed nitrogen nutrition could have induced enzymes that enhance  $\text{NO}_3^-$  uptake thus facilitating increased  $\text{NH}_4^+$  assimilation among barley crops fertilized through CULTAN method [50]. Elsewhere, Wang and Below [51] reported a strong correlation between cytokinines and tillering under mixed nitrogen nutrition which suggest that hormones could be involved in tiller initiation and development. The argument is supported by ammonia toxicity remediation attempts attributed to changes in plant hormone balance [52]. Root growth and development is a function of ammonium and nitrate within the rhizosphere [26, 53]. This is highly possible since nitrogen form of energy uptake requirements vary greatly with ammonium assimilation consuming much lesser energy than nitrate [54, 55]. The application of similar findings form the basis of rice bioengineering focused on nitrogen uptake and assimilation [41]. It is perhaps because of such factors that superior grain yields in LTR favoured CULTAN fertilization technique over conventional nitrate. The findings clearly demonstrate the potential impact of climate change on cereal crop yield output in different regions. The impact of climatic changes especially rising temperatures may concomitantly reduce attainable cereal crop grain and biomass yields while cooler temperatures would enhance attainment of higher yields.

The interplay between nitrogen nutrition form and temperature seem to have impacted upon yield and yield forming structures. In response to nitrogen form applied, grain numbers per ear tended to compensate for high grain numbers by supporting less heavy grains, thus expressing a negative correlation between grain numbers per ear and average grain weights. This observation is supported by findings of Frank et al. [33] who reported a trade-off between grain numbers and weights. The study clearly demonstrated potential of the upcoming fertilizer application technology focused on driving crop yield enhancement under changing climatic conditions. Since the experimental conditions mimicked an environment that is increasingly becoming warmer both in the tropics and temperate regions, the results are indicative of a globally warmed environment that would present cereal crops with shorter growth and grain filling periods with subsequent lower yield outputs, higher disease and pest infestation intensities with poor marketable grain yields. In addition, the ability of a single CULTAN injection operation to provide adequate nitrogen nutrition necessary for the sustenance of crop growth throughout the entire season emphasizes its comparative advantage in an attempt to minimize the number of farm operations from the three split applications undertaken to administer nitrate fertilizers [56]. The corollary effect of fewer farm operations under CULTAN can lead to reduced costs with increased profit margins for enhanced food security. Since  $\text{NH}_4^+$  nutrition usually adsorbs onto clay soil thus potentially reducing losses through leaching besides suppressing environmental pollution hence improving nitrogen use efficiency and subsequently attainable grain and biomass yield as reported by Raun and Johnson [57].

## V. Conclusions

It can be concluded from this study that growth temperatures interacted with soil injected liquid ammonium nutrition applied through CULTAN technique to cause a suite of growth responses that impacted on cereal crop yield and yield forming factors. Crop growth rate among different phenological stages were greatly influenced by temperature regimes with concomitant effects on attainable yield. Warmer growth chamber conditions (HTR) resulted in faster growth rates with poorly developed grain forming structures that supported reduced grain yields. Cooler growth conditions provided longer tillering and grain filling periods that enhanced attainable grain yields. The oxidation of  $\text{NH}_4^+$  into  $\text{NO}_3^-$  and possible occurrence of mixed nitrogen nutrition for barley crop uptake seemed to have contributed towards the attained grain and biomass yield superiority among CULTAN fertilized crops as compared to sole nitrate fertilized crops. The interaction effects between soil injected liquid ammonium and growth temperatures may potentially be exploited to improve cereal crop production for enhanced global food security.

## Acknowledgements

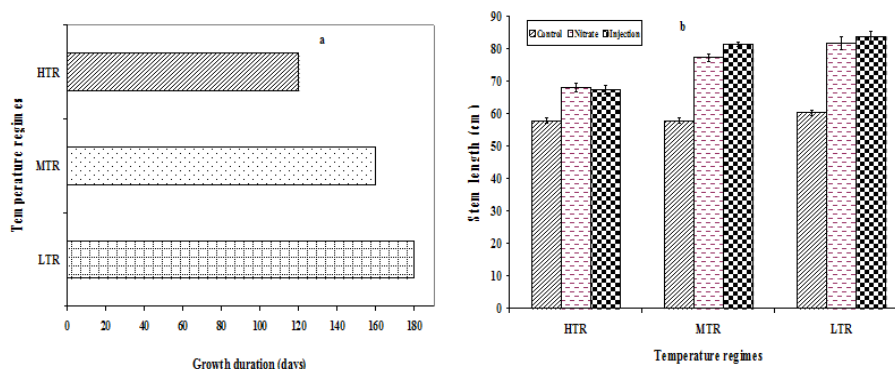
The authors wish to thank B. Arnemann, M. Kuechenthal, Edwin Matoka and S. Peickert for their technical assistance. The financial support provided by the German Academic Exchange Services (DAAD) is sincerely appreciated.

**Table And Figures**

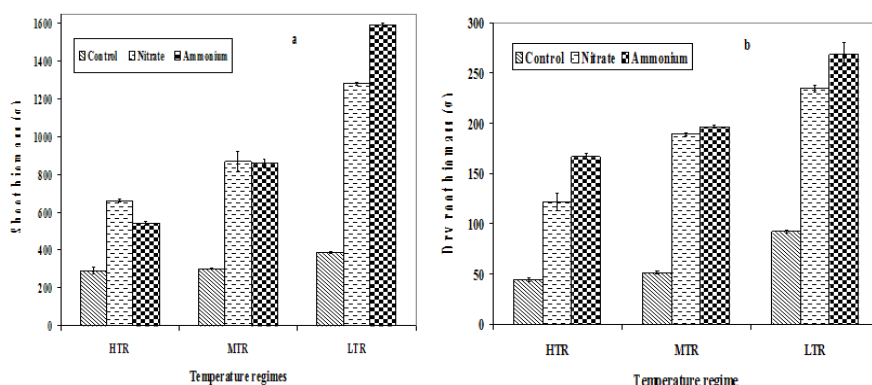
**Table 1: Summary of crop growth stages and nitrogen treatments in the three temperature regimes**

Treatments		Crop Growth Stages			
		Tillering start to end	Tillering end to booting	Flowering Start to end of flowering	Flowering to Physiological Maturity
Temperature	LTR (d/n*) °C	6/2	1/7	4/10	16/12
	MTR (d/n*) °C	10/6	5/11	8/14	20/16
	HTR (d/n*) °C	14/10	9/15	4/20	29/25
Nitrogen Regimes	T <sub>1</sub> - Non-fertilized control (No nitrogen fertilizer application) adopted as a check treatment				
	T <sub>2</sub> - Conventional nitrate fertilizer application of Ca(NO <sub>3</sub> ) <sub>2</sub> to provide 4 g N				
	T <sub>3</sub> - CULTAN technique injected as 4 g N/container as (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>				

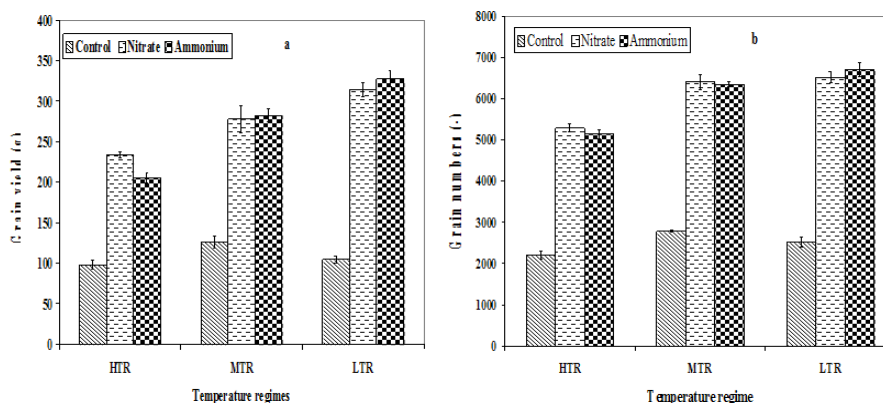
\* day/ night temperatures within each growth temperature regime performed at the four stages



**Fig. 1a-b: Barley growth durations under (a) and stem lengths (b) in response to the interaction between nitrogen treatments and growth temperatures**



**Fig. 2a-b: Shoot (a) and root biomasses (b) of barley crop fertilized by different nitrogen forms and subjected to three different growth temperatures**



**Fig. 3a-b: Total barley grain weights (a) and numbers (b) attained by the three different nitrogen fertilization regimes in the three different growth temperatures**

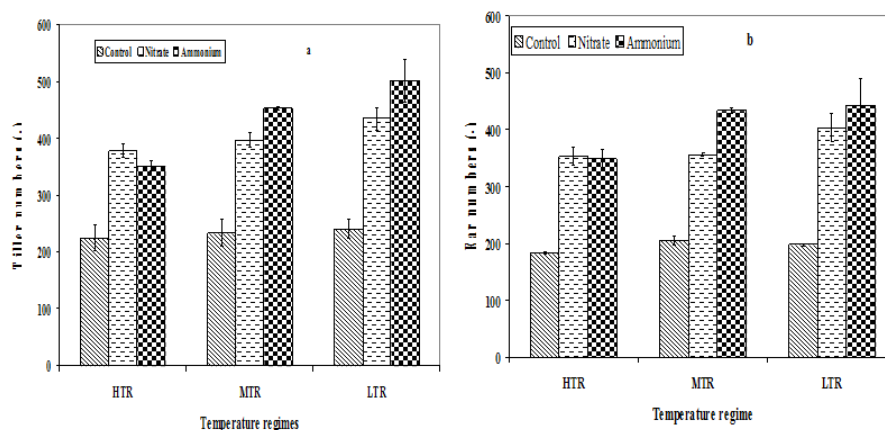


Fig. 4a-b: Total number of barley tillers (a) and ear bearing tillers (b) at crop maturity in the three nitrogen treatments across the three temperature regimes

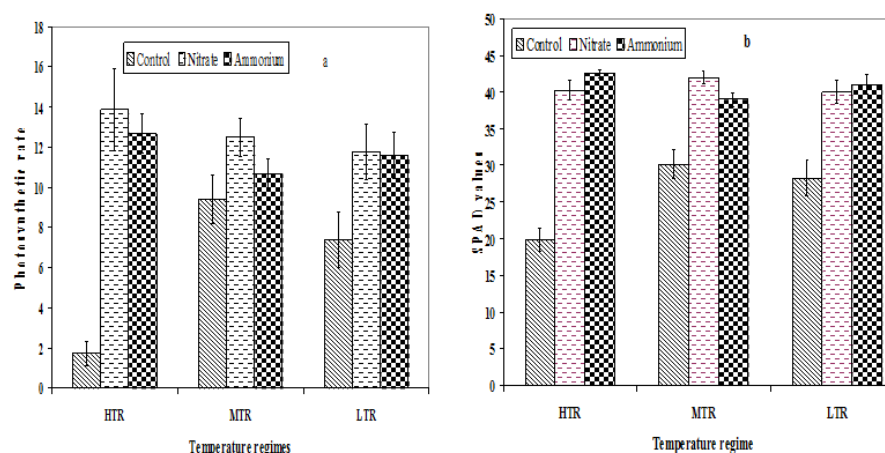


Fig. 5a-b: Average photosynthetic rates (a) and SPAD values of barley at flowering stage in the three nitrogen treatments across the three temperature regimes

## References

- [1]. Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C. Food Security: The Challenge of Feeding 9 Billion People. *Science*, 2010, 327: 812-818.
- [2]. Tilman, D., Balzer, C., Hill, J. and Befort, B.L. Global food demand and the sustainable intensification of agriculture. *Proceedings of National Academy of Sciences*, 2011, 108: 20260-20264.
- [3]. Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockstrom, J., Sheehan, J., Siebert, S., Tilman, D., Zaks, D.P.M. Solutions for a cultivated planet. *Nature*, 2011, 478: 337-342.
- [4]. Mueller, N.D., Gerber, J.S., Johnston, M., Ray, D.K., Ramankutty, N and Foley, J.A. Closing yield gaps through nutrient and water management. *Nature*, 2012, 490: 254-257.
- [5]. Inter-Governmental Panel on Climate Change. Assessment of Global Climate Change. Inter-Governmental Panel on Climate Change, Washington, DC, USA, 2008.
- [6]. Rosenzweig, C. and Parry, M.L. Potential impact of climate change on world food supply. *Nature*, 1994, 367: 133 - 138.
- [7]. Finger, R., Hediger, W., Schmid, S. Irrigation as Adaptation Strategy to Climate Change: A Biophysical and Economic Appraisal for Swiss Maize Production. *Climate Change*, 2011, 105: 509-528.
- [8]. Cassman, K.G., Dobermann, A., Walters, D.T., Yang, H. Meeting cereal demand while protecting natural resources and improving environmental quality. *Annual Review of Environmental Resource*, 2003, 28: 315 - 358.
- [9]. Cassman, K.G. Ecological intensification of cereal production systems: Yield potential, soil quality and precision agriculture. *Proceedings of National Academy of Sciences*, 1999, 96: 5952-5959.
- [10]. Ryan, J., Sommer, R. and Ibrici, H. Fertilizer Best Management Practices: A perspective from the Dryland West Asia-North Africa Region. *Journal of Agronomy Crop Science*, 2012, 198: 57-67.
- [11]. Leininger, S., Ulrich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C. and Schleper, C. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*, 2006, 442: 806-809.
- [12]. Miller, A.J. and Cramer, M.D. Root nitrogen acquisition and assimilation. *Plant Soil*, 2004, 274: 1-36.
- [13]. Tischner, R. Nitrate uptake and reduction in higher and lower plants. *Plant Cell Environment*, 2000, 23: 1005 - 1024.
- [14]. Dang, H., Li, J., Chen, R., Wang, L., Cuo, L., Zhang, Z., Klotz, M.C. Diversity, abundance and spatial distribution of sediment ammonia oxidizing betaproteobacteria in response to environmental gradients and coastal eutrophication in Jiaozhou bay, China. *Applied Environmental Microbiology*, 2010, 76: 4691-4702.
- [15]. Janzen, H.H., Lindwall, C.W. Optimum application parameters for point injection of nitrogen in winter wheat. *Soil Science Society of American Journal*, 1989, 53: 1878-1883.



- [16]. Sommer, K. CULTAN cropping system: Fundamentals, state of development and perspectives. In: Nitrogen in a Sustainable Ecosystem: From the Cell to the Plant. Eds. Martins-Loucaó M.A. and Lips S.H. Backhuys Publishers, Leiden, Netherlands. pp. 361 – 381, 2000.
- [17]. Wakimoto, K. Utilization advantages of controlled release nitrogen fertilizer on paddy rice cultivation. Japan Agricultural Research Quarterly, 2004, 38: 15 – 20.
- [18]. Sommer, K. 1995. Ammonium and phosphate nutrition of plants grown in saturated solutions. Dahlia Greidinger Memorial International Symposium on Fertigation, Technion – Israel Institute of Technology, Haifa, Israel. pp. 155–164.
- [19]. Lobell, D. and Asner, G. Climate and management contributions to recent trends in U.S. agricultural yields. Science, 2003, 300: 1500-1505.
- [20]. Long, SP. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO<sub>2</sub> concentrations: Has its importance been underestimated? Plant Cell Environment, 1991, 14: 729-739.
- [21]. Maggs, R. and Ashmore, MR. Growth and yield responses of Pakistan rice (*Oryza sativa* L.) cultivars to O<sub>3</sub> and NO<sub>2</sub>. Environmental Pollution, 1999, 103: 159-170.
- [22]. Hoogenboom, G., Tsiji, GY., Jones, TW., Singh, U., Godwin, DC., Pickering, NB., Curry, RB. Decision Support System to Study Climate Change Impacts on Crop Production. pp. 51-75, In: Rosenzweig C. et al (ed.) Climate Change and Agriculture: Analysis of Potential International Impacts. ASA Special Publication No. 59, American Society of Agronomy, Madison, WI. 1995.
- [23]. Springate DA and Kover PX. Plant Responses to elevated temperatures: A field study on phenological sensitivity and fitness responses to simulated climate warming. Global Change Biology, 2014, 20: 456-465.
- [24]. Zadoks, JC., Chang, TT. and Konzak, CF. A decimal code for the growth stages of cereals. Weed Research, 1974, 14: 415 – 421.
- [25]. Von Caemmerer, S. and Farquhar, GD. Some relations between the biochemistry of photosynthesis and the gas exchange of leaves. Planta, 1981, 153: 376 - 387.
- [26]. Bloom JA. Interactions between inorganic nitrogen and root development. Journal of Plant Nutrition and Soil Science, 1997, 160: 253–259.
- [27]. Maranville, JW., Clark, RB., Ross, WM. Nitrogen efficiency in grain sorghum. Journal of Plant Nutrition, 1980, 2: 577–589.
- [28]. Moll, RH., Kamprath, EJ. and Jackson, SB. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agronomy Journal, 1982, 74: 562 – 564.
- [29]. Trnka, M., Dubrovský, M., Semerádová, D. and Zalud, Z. Projections of uncertainties in climate change scenarios into expected winter wheat yields. Theoretical and Applied Climate, 2004, 77: 229 - 249.
- [30]. Hatfield, J., Boote, K., Fay, P. Agriculture. In: The Effects of Climate Change on Agriculture, Land Resources, Water Resource and Biodiversity. Washington, DC: U.S. Climate Change Science Program and the Subcommittee on Global Change Res pp. 2008, 21– 74.
- [31]. Ainsworth, EA., Ort, D.R. How do we improve crop production in a warming world? Plant Physiology, 2010, 154: 526–530.
- [32]. Mishra, V. and Cherkauer, KA. Retrospective droughts in the crop growing season: Implications to corn and soybean yield in the mid-western United States, Agriculture. Forest Metrology, 2010, 150: 1030–1045.
- [33]. Frank, AB., Bauer, A., Black, AL. Effects of air temperature and fertilizer nitrogen on spike development of spring barley. Crop Science, 1992, 32:793–797.
- [34]. Zivcak, M., Brestic, M., Hazem, M. and Govindjee, K. Photosynthetic responses of sun- and shade-grown barley leaves to high light: Is the lower PS<sub>II</sub> connectivity in shade leaf associated with protection against excess of light? Photosynthetic Research, 2014, 119: 339–354.
- [35]. Garcia del Moral, LF., Ramos, JM. and Recale, L. 1994. Tillering dynamics in winter barley as influenced by cultivar and nitrogen fertilizer: A field study. Crop Science, 25:179–181.
- [36]. Garcia del Moral, MB. and Garcia del Moral, LF. Tiller production and survival in relation to grain yield in winter and spring barley. Field Crop Research, 1996, 44: 85–93.
- [37]. Yan, J., Zhu, J., He, CX., Benmuosa, M. and Wu, P. Quantitative trait loci analysis for the developmental behaviour of tiller number in rice (*Oryza sativa* L.). Theoretically Applied Genetics, 1998, 97: 267 – 274.
- [38]. Li, X., Qian, Q., Fu, Z., Wang, Y., Xiong, G., Zeng, D., Wang, X., Liu, X., Teng, S., Hiroshi, F., Yuan, M., Luo, D., Han, B. and Li, J. Control of tillering. Nature, 2003, 422: 618–621.
- [39]. Houdusse, F., Zamarrano, AM., Garnica, M., and Garcia-Mina, J. The importance of nitrate in ameliorating the effects of ammonium and urea nutrition on plant development: the relationships with free polyamines and plant praline contents. Functional Plant Biology, 2005, 32: 1057-1067.
- [40]. Britto, DT., Kronzucker, HJ. NH<sub>4</sub><sup>+</sup> toxicity in higher plants: a critical review. Journal of Plant Physiology, 2002, 159: 567–584.
- [41]. Britto, T., Kronzucker, HJ. Bioengineering nitrogen acquisition in rice: can novel initiatives in rice genomics and physiology contribute to global food security? Bioassays, 2004, 26: 683–692.
- [42]. Cruz, C., Castillo, M., Dominguez, CN., Juanarena, N., Aparicio-Teja, P., Lamfus, C., Botella, MA. The importance of nitrate signalling in plant ammonium tolerance: Spinach as a case study. In Actas XV reunion de la Sociedad Espanola de Fisiologia Vegetal y VIII congreso Hispano-Luso. 2003, 297 (Sociedad Espanola de Fisiologia Vegetal: Palma de Mallorca).
- [43]. Redinbaugh, MG. and Campbell, WH. Glutamine synthetase and ferredoxin dependent glutamate synthase expression in maize (*Zea mays*) root primary response to nitrate. Plant physiology, 1993, 101: 1249 - 1255.
- [44]. Matoka, CM., Menge-Hartmann, U., Schittenhelm, S., Schubert, S., Schnell, S., Greef, JM. and Tebbe, CC. Bacterial community response to liquid ammonium nutrition for spring barley (*Hordeum vulgare* L.) production. In: 10<sup>th</sup> International Symposium on Wetland Biogeochemistry: pp. 83. Frontiers in Biogeochemistry, 1<sup>st</sup> – 4<sup>th</sup> April, 2007, Annapolis, Maryland, USA.
- [45]. Matoka, CM. Bacterial community responses to soil-injected liquid ammonium nutrition and effect of temperature on barley (*Hordeum vulgare* L.) grain yield formation, PhD thesis. Cuvillier Verlag Goettingen, Germany, 2008, ISBN 978-3-86727-507-1.
- [46]. Schittenhelm, S. and Menge-Hartmann, U. Yield formation and plant metabolism of spring barley in response to locally injected ammonium. Journal of Agronomy Crop Science, 2006, 192: 434 - 444.
- [47]. Sommer, K. Grundlagen des 'CULTAN'-Verfahrens. In: M. Kücke, ed. Anbauverfahren mit N-Injektion (CULTAN) 'Ergebnisse, Perspektiven, Erfahrungen'. Landbauforschung Voelkenrode Sonderheit, 2003, 245: 1-22.
- [48]. Zhang, XK. and Rengel, Z. Role of soil pH, Ca supply and banded fertilizers in modulating ammonia toxicity to wheat. Australian Journal of Agricultural Research, 2000, 51: 691-699.
- [49]. Zhang, XK. and Rengel, Z. Temporal dynamics of gradients of phosphorus, ammonium, pH and electrical conductivity between diammonium phosphate band and wheat roots. Australian Journal of Agricultural Research, 2002, 53: 985 – 992.
- [50]. Kronzucker, H., Glass, ADM. and Siddiqi, MY. Inhibition of nitrate uptake by ammonium in barley: Analysis of component fluxes. Plant Physiology, 1999, 120:283–291.

- [51]. Wang X. and Below FE. Cytokinins in enhanced growth and tillering of wheat induced by mixed nitrogen source. *Crop Science*, 1996, 36: 121 - 126.
- [52]. Gerendas, J., Zhu, Z., Bendixen, R., Ratcliffe, S., and Sattelmacher, B. Physiological and Biochemical Processes Related to Ammonium Toxicity in Higher plants. *Journal of Plant Nutrition and Soil Science*, 1997, 160: 239–251.
- [53]. Bloom, AJ., Meyerhoff, PA., Taylor, AR., Rost. TL. 2003. Root development and absorption of ammonium and nitrate from rhizosphere. *Journal of Plant Growth Regulators*, 21: 416–431.
- [54]. Bloom, AJ., Sukrapanna, SS., Warner, RL. Root respiration associated with ammonium and nitrate absorption by barley. *Plant Physiology*, 1992, 99: 1294 - 1301.
- [55]. Bloom, AJ., Jackson, LE, Smart, D.R. Root growth as a function of ammonium and nitrate in the root zone. *Plant Cell Environment*, 1993, 16: 199–206.
- [56]. Sowers, KE., Pan, WL. and Smith, JL. Nitrogen use efficiency of split nitrogen applications in soft white wheat. *Agronomy Journal*, 1994, 86: 942 – 948.
- [57]. Raun, WR. and Johnson, GV. Improving Nitrogen Use Efficiency for Cereal production. *Agronomy Journal*, 1999, 99: 357–363.