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Effect of nitrogen fertilizer on growth attributes of NaCl stressed barley (*Hordeum vulgare* L.) B90068 genotype

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Abstract

Salinity is the big threat of drought as well as flooded areas for crop production. In this experiment, effects of urea fertilizer (50kg N ha^{-1}) application on rate of vegetative growth and yield production of NaCl (0, 100 and 200mM) stressed barley (*Hordeum vulgare* L.) genotype B90068 was assessed. High NaCl level decreased plant biomass, net CO_2 assimilation (*A*) rate, K^+ concentration and grain yields but Na^+ and Cl^{-1} contents increased significantly especially in salt sensitive cultivar. With urea application, plant growth, photo-assimilation rates and plant yield was maximum in control plants as well as each increased than NaCl stressed plants ($p \leq 0.05$). It indicates that influence of urea fertilizer, which is playing a significant role in barley production even when grown in saline conditions. Among NaCl stressed plants, carotenoids were increased while chlorophyll contents were decrease significantly. A non-significant alteration in in Na^+/K^+ and K^+/Cl^-, which were decreased with NaCl application while reversed under the influence of urea fertilizer. Transpiration (*E*) rate and sub-stomatal CO_2 concentrations observed upward under NaCl stress than control plants, while down towards normal form with application of urea but gs (stomatal conductance) noted as reversed than *E* and *Ci*. Application of NaCl in root medium is decreasing plant growth and its final yield while influence of urea fertilizer reduces ($p \leq 0.05$) the deleterious effects of NaCl stress.

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Introduction

Barley is one among the major cereal crops after wheat, rice and maize with 148.78 million tons annual production (Kathy *et al.,* 2016). It is cultivated and consumed as feed from more than 10,000 years ago (Gujral and Gaur, 2005; Salamini *et al.*, 2002) with wide utilizations for animal feed (60%), malt production (30%), seed production (7%) and human food (3%) (Baik and Ullrich, 2008; Dawson *et al.*, 2015). Increasing world's population and drought in crop cultivation area are demanding good quantity of water. At present, it is fulfilled with recycling of drainage water especially for crop production (Yordanov *et al.*, 2003), while it is inducing salinity and reducing plant growth and its production significantly (Tabatabaei and Ehsanzadeh, 2016). Barley species has been considered as most salt-tolerant then other cereal crops.

Survival of plant genotypes on saline area have to adopt a number of physio-chemical mechanisms. As salinity imbalance the concentration of certain ions, which increases osmotic potential of soil solution and causes deteriorations of soil structure (Munns, 2002; Saeed *et al.*, 2009). High osmotic potential of soil solution inhibits uptake of water, which causes inhibitions of cell enlargement, biosynthesis of cell wall, gas exchange attributes and development of buds (Munns and Tester, 2008). Systematic transportation of salts from roots to shoots apexes induces ion-specific stress within plant organs including leaves. Leaf mortality increases due to their chlorosis and necrosis, which decrease an active area for cellular metabolic activity including photosynthesis (Munns, 2005; Witzel *et al.*, 2014; Yeo and Flowers, 1986).

Traditionally, cultivation of plant genotypes on saline area are selected on the basis of their grain yields (Kiani-Pouya and Rasouli, 2014) but from last decades some specific physiological parameters (Ashrafi *et al.*, 2014; Widodo *et al.* 2009) are included like as rate of photo-assimilations, ability to retain K^+ (Chen *et al.*, 2005; Ligaba and Katsuhara, 2010; Witzel *et al.*, 2014) and avoidance of Na^+ accumulation (Wu *et al.*, 2013).

A continuous mono-cropping system on a land area has also been decreasing soil fertility and its pH, which is increasing soil erosion (Desta, 1987). Application of nitrogen fertilizers is increasing growth and yield attributes of barley on soil erosion area (Agegnehu *et al.*, 2006; Blanchard, 1986; Desalegn *et al.*, 2016; Ozturk *et al.*, 2003). Meanwhile, inorganic soil nitrogen and aerobic nitrogen released from organic matter's decomposition (NO_2^-, NO_3^- and NH_4^+) is directly available to uptake as plant nutrition. Crop plants prefer to uptake NH_4^+ for its usage in protein synthesis directly, while other first have to reduce to NH_4^+ form (Baethgen *et al.*, 1995; Kolovos, 2010). Application of NH_4^+ increases 7 to 47% yield and NO_3^- enhances plant tillering (Prystupa *et al.*, 2003).

Major constraint for cereal's production is low availability of nitrogen, as barley responded significantly better under balanced N fertilizer application (Cheng *et al.*, 2002; Chu *et al.*, 2007; Foster *et al.*, 2004). In this study, selected barley genotype may possess diverse physiological traits including nitrogen use efficiency under saline stressed root medium. To unravel the beneficial traits in response to salt tolerance and ranked for its contribution in increasing the grain yield under influence of nitrogen. Effects of salt stresses in presence or absence urea fertilizer on net photo-assimilation rate, chlorophyll contents, Na^+, K^+ contents, nitrogen and grain yields of barley genotype is assessed in earthen pots filled with soil. To find out the possible elevated traits with urea fertilizer application to endeavor or maintain good grain yield character in saline stressed plants.

Materials and methods

Plant materials and growth conditions

For this experiment, a local barley genotype B90068 with specific adopted characters was selected. Experiment was conducted under green-house conditions. Healthy seeds were collected and washed with 10% sodium hypochlorite for 5min for surface sterilization. Seeds were rinsed thoroughly with distilled water. Ten to twelve seeds of each cultivar were sowed in earthen pots, which filled with pure-sand (20kg each pot) as thoroughly washed with tap-water.

Five pots (replicates) per treatment were arranged in a randomized complete block design. After two weeks of seed sowing, 100ml plant Hoagland nutrient solution was supplied to each pot daily (Hoagland and Arnon, 1950).

Salinity (NaCl) and urea fertilizer applications

First number of germinated plants per pot were reduced to 4 plants. Three levels of NaCl treatment were maintained when 3rd leaf of plants expanded completely. In this experiment, 2 levels of salt gradients were raised to 100 and 200mM NaCl against control pots contains normal sand (considered 0 mM NaCl). The salt levels were raised gradually with an increments of 25mM NaCl per day. These 3-levels of NaCl stresses were maintained with one level of urea fertilizer at the rate of 50kg N ha^{-1}. After both salt and urea fertilizer treatments, plants were irrigated with tap-water for 2-weeks. For morph-physiological data collection plants harvested on 45th day of sowing.

Measurements of morphological attributes

Five individual plants from each pot per treatment and control were harvested for comparative estimation of morphological traits. Plants were cutted into roots and shoots and weighed for their fresh weights (F.Wt). Shoot lengths (SL) and root length (RL) of each plant from crown to leaf tip than to root tip were measured respectively. Number of tillers (NT) and leaves (NL) per plant were counted. Shoots and roots were dried at 72°C in electric oven for 5-days and root dry weight (RDW) and shoots dry weight (SDW) were measured.

Flag leaf relative water content (%)

For relative water content (RWC) determination, flag leaves were collected at heading plant growth stage (Pask *et al.*, 2012). Five mid-sections ($5cm^2$) from each plant per treatment were excised and weighed in a glass-tube for fresh weight (FW) than tubes filled with distilled water and incubated in refrigerator (4°C, dark). After 24 hrs, tubes were dried with paper towels then weighted for turgid mass (TM).

Leave sections of each treatment were dried at 70°C for 72h separately then dry mass was obtained on balance with 0,001g precision. The RWC (%) was calculated by applying this above equation:

$$RWC\ [\%] = \frac{FM - DM}{TM - DM}\ x\ 100$$

Flag leaf chlorophyll contents

Leaf chlorophyll contents were also estimated at plant heading stage with *SPAD-502* (*Konica-Minolta*, Japan) between 10:00 to 13:00 hrs. Average Chl contents were calculated from optical density (OD) of average five flag leaves of each treated plants. Fresh flag leave were fine-chapped than agitated in 90% acetone for chlorophyll extraction. Samples were stand under dark for 30 minutes before OD measurements (Arnon, 1949; Lichtenthaler and Wellburn, 1983).

Gas-exchange characteristics

Plant gas exchange attributes like as net photosynthesis (*A*), transpiration rate (*E*), sub-stomatal CO_2 (*Ci*) and stomatal conductance (gs) of flag leaves measured on at grain-filling stage (Azizian and Sepaskhah, 2013) with infrared gas analyzer (LCA-4 ACD, UK). Abaxial surface of fully expanded mid-lamina portion of flag leaf was targeted for these measurements from 11:00am to 12:30pm. At data recoding time, some system specifications were observed like as 251 µmol s^{-1} gas flow rate (U), $11.25cm^2$ area of leaf surface in leaf chamber; 99.8kPa ambient pressure; H_2O vapor pressure 7.0-8.9 mbar, 351µmol mol^{-1} ambient CO_2, 34.2°C-39.3°C temperature of leaf chamber; 403.4mol m^{-2} s^{-1} air flow rate (Us), 41.2% relative humidity and 1099µmol m^{-2} s^{-1} *PAR*.

Free ionic concentrations

Nutrient concentration like as Na^+ and K^+ measured in flag leaves and roots. Dried plant material was powdered and digested as by Parida and Das, (2005). Concentrations of Na^+ and K^+ were determined with flame photometer (Model 420, Cambridge, UK) by following method by Loutfy *et al.*, (2008). Chlorides calculated by following formula reported by Chen *et al.*, (2001) as below:

$$\boldsymbol{Cl\ ions}\ (ppm) = \frac{(Volume\ x\ Normality\ x\ Extract\ volume\ x\ 35.5\ x\ 10^3)}{Volume\ of\ solution\ used\ in\ determination}$$

Measurement of nitrogen contents

Total nitrogen (N) contents were estimated with macro-Kjeldahl apparatus. Copper sulphate was used as a catalyst (AOAC, 1995). Briefly, fresh samples of flag leaf were weighed and dried in tin foil for 12h at 70°C for removal of error due sample moisture. Sampled cooled down at room temperature than its 100g was subjected for N analysis with N-Kjeldahl (FP-428, Leco Corp, USA) apparatus. Concentration of N was denoted g N per 100g (%N) of used sample (Simonne *et al*., 1997).

Yield and yields collection

For yield data, plants were harvested at maturity stage. Grains of 4 plants per pot per treatment were separated from straw and dried for 2 hours (12:00 to 14:00h) under sunlight. Biomass yields were measured by counting the average number of grains per spike and by weighing average weight of 100 grains per treatment.

Data significance analysis

Collected data of experiment was analyzed for treatment variance with ANOVA on CoStat (3.03) *CoHort* software, Berkeley. Significant means differences among the treatments were subjected for further assessment by Duncan Multiple Range (DMR) test at 5% (Behrens, 1997; Henley, 1983).

Results and discussion

Plants are major food source for all living organisms, however their growth reduction could be caused often under variable environments including cold to heat, drought to flood and soil salts to air-pollutants (Mahajan *et al*., 2005). These abiotic environmental factors induces changes in growth attributes subsequently (Atkinson and Urwin, 2012; Lipiec *et al*., 2013). Uptake of water is being crucial for plant growth limitation, which reflects its properties in the form of altered attributes of plant physiology and then its morphology (Chaves *et al*., 2002; Chaves *et al*., 2009).

One of most common salts of irrigation water is sodium chloride (NaCl) is losing intracellular water contents. It interacts with nitrogen metabolism as well as assimilation at several steps including several other secondary metabolisms (Debouba *et al*., 2006, 2007; Helal and Mengel, 1979; Parida and Das, 2004). Influence of nitrogen fertilizer could be involved to combat adverse impacts of salinity on plants (Murtaza *et al*., 2014).

Since it depends on optimal fertilizer application to saline soils (Sultana *et al*., 2001), which increases net photo-assimilation characters as well as plant growth and their yields (Abdelgadir *et al*., 2010; Esmaili *et al*., 2008).

In this experiment from control to salt stressed conditions, vegetative growth of plants reduced (0, 100, 200mM NaCl) considerably. Very similar results for rice (Shahbaz and Zia, 2011), wheat (Ashraf and Ashraf, 2012), sunflower (Shahbaz *et al*., 2011) and also for eggplant (Abbas *et al*., 2010) has been reported. Variation in growth rate of barley B90068 genotype plants under NaCl stressed conditions are observed that might be governed by variation in cellular biocontents and other physiological characters including photosynthetic rate, chlorophyll contents or water use efficiency etc.

Among these plants differential growth responses may be due to change in capability of plants for water absorption by roots due to alteration in biochemical mechanisms under salt stressed conditions (Ziaf *et al*., 2009). Application of urea fertilizer from control to salt stressed plants increase in plant growth to its yield and yield attributes was observed (Table 1).

Same in agreement with Maas and Hoffman (1977) in plants like as chickpea (Garg and Chandel, 2011), corn (Absalan *et al*., 2011), tomato (Maggio *et al*., 2007) and wheat (Murtaza *et al*., 2014). Meanwhile, total N uptake decreases among salt stressed plants and it increases with application of urea fertilizer but N concentration remain constant or unchanged when soil is optimal with N level (Hu *et al*., 2006; Hu and Schmidhalter, 2005).

Table 1. Effect of nitrogen fertilizer on growth attributes of NaCl stressed barley (*Hordeum vulgare* L.) B90068 genotype.

#s	Characteristics	T_0N_0	T_0N_1	T_1N_0	T_1N_1	T_2N_0	T_2N_1	*p-sig.*
A. Morphological attributes								
a.	Stem length (cm)	^{b}25.80±1.126	^{a}31.46±0.549	^{d}22.59±0.527	^{bc}24.76±0.423	^{e}18.76±0.410	^{cd}23.29±0.344	2.084***
b.	Root length (cm)	^{c}12.52±0.072	^{a}19.46±0.163	^{d}10.95±0.150	^{b}14.87±0.077	^{f}7.683±0.088	^{e}9.483±0.084	1.637***
c.	No of tillers/plant	^{b}4.803±0.113	^{a}7.073±0.096	^{d}3.801±0.020	^{c}4.583±0.071	^{e}3.233±0.032	^{e}3.287±0.020	4.531***
d.	Stem F.Wt (g)	^{b}5.973±0.144	^{a}7.667±0.090	^{d}4.303±0.056	^{c}5.013±0.088	^{e}3.157±0.097	^{e}3.437±0.055	2.076***
e.	Root F.Wt (g)	^{b}4.580±0.058	^{a}6.053±0.072	^{c}3.871±0.061	^{b}4.623±0.049	^{e}3.217±0.055	^{d}3.467±0.050	2.700***
f.	Flag leaf F.Wt (g)	^{b}0.491±0.005	^{a}0.696±0.004	^{d}0.343±0.003	^{c}0.393±0.003	^{f}0.272±0.003	^{e}0.304±0.001	4.080***
g.	Flag LA (cm^{-2})	^{b}5.643±0.127	^{a}7.263±0.094	^{d}4.373±0.049	^{c}5.267±0.055	^{e}3.997±0.041	^{d}4.353±0.046	1.023***
h.	Stem D.Wt (g)	^{c}0.364±0.008	^{a}0.504±0.004	^{d}0.307±0.010	^{b}0.407±0.009	^{d}0.378±0.006	^{bc}0.332±0.016	2.182***
i.	Root D.Wt (g)	^{ab}1.392±0.116	^{a}1.541±0.149	^{bc}1.165±0.037	^{bc}1.644±0.228	^{d}1.501±0.113	^{c}1.322±0.059	0.211***
j.	Flag leaf D.Wt (g)	^{bc}0.032±0.001	^{a}0.044±0.000	^{b}0.034±0.002	^{a}0.042±0.000	^{c}0.029±0.000	^{bc}0.032±0.000	1.926***
B. Flag leaf physiological attributes								
a.	RWC (%)	^{c}80.85±1.689	^{a}87.71±0.331	^{b}83.97±1.114	^{a}88.15±0.157	^{c}78.13±0.292	^{c}80.55±0.199	9.070***
b.	Chl a/Chl b	^{c}1.871±0.062	^{d}1.669±0.017	^{b}2.085±0.013	^{d}1.751±0.029	^{a}2.771±0.024	^{b}2.008±0.030	5.056***
c.	Chl ab/Car.	^{c}0.606±0.003	^{a}0.615±0.006	^{d}0.597±0.001	^{b}0.611±0.094	^{d}0.576±0.001	^{e}0.600±0.001	5.697***
d.	Na^+/K^+	^{a}0.962±0.030	^{a}0.764±0.071	^{a}1.280±0.002	^{a}0.784±0.044	^{a}2.036±0.018	^{a}1.672±0.026	7.569^{ns}
e.	K^+/Cl^-	^{a}0.418±0.006	^{ab}0.466±0.019	^{ab}0.325±0.001	^{ab}0.460±0.011	^{b}0.232±0.001	^{b}0.307±0.003	2.657^{ns}
f.	*A*, µmol m^{-2} s^{-1}	^{b}7.047±0.062	^{a}8.037±0.064	^{d}6.601±0.044	^{c}6.863±0.055	^{f}4.473±0.038	^{e}5.121±0.044	3.800***
g.	*E*, mol m^{-2} s^{-1}	^{b}2.477±0.027	^{a}3.256±0.032	^{c}2.281±0.040	^{c}2.361±0.040	^{c}2.327±0.046	^{c}2.303±0.035	2.809***
h.	*gs*, mol m^2 s^{-1}	^{a}0.062±0.006	^{bc}0.047±0.003	^{a}0.059±0.001	^{ab}0.053±0.003	^{ab}0.055±0.002	^{c}0.041±0.002	0.005**
i.	*Ci*, µmol^{-1}	^{c}87.33±2.751	^{d}77.57±2.888	^{ab}99.13±1.211	^{d}77.63±3.324	^{a}101.9±2.976	^{ab}92.35±2.112	6.778***
j.	Nitrogen (%)	^{b}4.241±0.069	^{a}5.590±0.356	^{cd}3.080±0.081	^{b}4.063±0.082	^{d}2.983±0.086	^{c}3.543±0.073	9.102***
C. Yield and yield attributes								
a.	No of grain spike^{-1}	^{ab}36.63±1.068	^{a}38.24±1.081	^{c}32.12±1.066	^{bc}33.59±1.274	^{c}31.19±0.642	^{c}32.64±0.980	0.003**
b.	100 grains Wt (g)	^{b}4.971±0.075	^{a}5.301±0.079	^{d}3.943±0.033	^{c}4.351±0.035	^{e}3.311±0.023	^{e}3.461±0.029	1.134***
c.	Spike length (cm)	^{b}15.83±0.504	^{a}17.45±0.078	^{b}15.21±0.136	^{b}15.47±0.104	^{c}13.77±0.406	^{c}14.26±0.038	1.146***

LA – leaf area; Car – carotenoids; Wt – weight; F – fresh; D – dry; N – nitrogen; Sig – significance; A - assimilation rate; E - transpiration rate; Ci - sub-stmatal CO_2; gs - stomatal conductance.

Generally, +ve correlation has been studied between growth and photosynthesis and reduction in growth due to decline in photosynthesis under saline conditions (Sabir *et al.*, 2009; Shahbaz *et al.*, 2011). However, a significant reduction with salt stress in photosynthesis noted in this current study. It could be due to deficiency in rate of necessary photo-assimilates and imbalance water uptake efficiency (Kanwal *et al.*, 2011). Significant effect of salinity was found on rates of CO_2 assimilation (*A*), stomatal conductance (*gs*), transpiration rate (*E*) and sub-stomatal CO_2 (*Ci*), as shown in Table 1. Decrease in A and gs, while increase in E and Ci was noted in plants under saline stress (Shabala and Munns, 2012; Sudhir and Murthy, 2004; Zhang and Shi, 2013).

Gas exchange (CO_2) parameters are best supportive indicator and have direct link with net plant production (Piao *et al.*, 2008). High *Ci* rate decrease *gs* efficiency (CO_2 uptake), while increases water loss under salt stress (Table 1). Overall reduction in gases exchange rates causes closer of leaf stomata due to accumulation of Na^+ and Cl^- with efflux of K^+ causes to decrease in photosynthetic electron transport and gs rate (Ghosh and Singh, 2005; Hussain *et al.*, 2012; James *et al.*, 2006; Jarolimek *et al.*, 1999; Tavakkoli *et al.*, 2011).

Even salt stress reduces N contents due to increase in Na^+ content uptake in comparison to others, which causes imbalanced cellular nutrient elements for plant growth.

Likely to antagonistic relations between K^+ and Na^+ under salinity stress (Eisechie and Rodriguez, 1999; Geissler *et al.,* 2009; Hariadi *et al*., 2011; Maggio *et al*., 2001; Nandy *et al*., 2007; Parida *et al*., 2002). Low ratios of Na^+ and K^+ disturb the rate of plant metabolism and ultimately reduction in plant growth rate (James *et al*., 2008; Morshedi and Farahbakhsh, 2012; Munns and Tester, 2008; Rahnama *et al*., 2010). An increase in N contents and K^+ and Na^+ with application of urea fertilizer from control to saline stressed plants (Table 1). Salt stressed plants presented low ratios of chlorophyll and carotenoids while reversed with the application of urea. Plants with higher chlorophyll contents produces higher grain yield than with low chlorophyll contents (Akbari *et al.,* 2012; Khosravifar *et al*., 2008). It is being a useful criteria either to determine plants are growing under stressed environment or under observation genotype is salt tolerant of salt sensitive (Pak *et al.,* 2009; Tiwari *et al*., 2010; Zhao *et al*., 2007).

A large number of factors including genotype, plant growth stage, epidemic diseases, soil nutrient contents and other abiotic stresses are affecting chlorophyll contents. It is most important component that provides a suitable site for photo-assimilation generation of reducing powers from sun-light. However, chlorophyll contents are susceptible to saline stresses, which have significant effects on plant yield and its quality (Ali *et al*., 2004; Giunta *et al*., 2002; Gomez-Becerra *et al*., 2010; Kiani-Pouya and Rasouli, 2014). Relative water contents (RWC) also observed dynamic under salt stress to nitrogen applications (Table). It decreases with increase in salinity level but reversed under the influence of urea fertilizer (Belanger and Richards, 2000; Ebrahimian and Bybordi, 2011a, 2011b; Glibert *et al*., 2006; Munns and Tester, 2008).

Conclusions

Salinity is top-most important destructive abiotic plant stress. It imposes ionic imbalance and osmotic stresses, which causes ion toxicities and reduces barley plant growth rate markedly. Relatively better growth performance due to urea fertilizer application is correlated to net photo-assimilation rate positively. Saline level of soil nutrient regimes is deceitful for specific nutrient uptake by root and then translocation as well as disturbance of mineral balance also decreases the availability of nutrient in substrate to plants. Application of nitrogen in the NO_3^- form to soil may decreases the uptake rate of Cl^-. Better vegetative and reproductive growths under NaCl stresses are measured with urea fertilizer application at 100mM NaCl stress than 200mM NaCl stress. Improvement in plant biomass by urea application is due to increase in photosynthesis rate under NaCl stresses. Both attributes could be used as morphological marker for possible selection of a genotype for a selected agriculture land cultivation in upcoming crop season.

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