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Research Paper

CERTAIN GROWTH ATTRIBUTES OF ONION (*Alliums cepa* L.) AT SEED GERMINATION STAGE UNDER ASEPTIC SODIUM CHLORIDE STRESSED CONDITIONS

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Abstract

In this experiment, NaCl stress was assessed on local Nasar-puri cultivar of onion (*Allium cepa* L.) under *in-vitro* conditions. The 4-weeks cultures with three levels of NaCl stress were maintained in MS plant nutrition medium i.e. MS_0 (control), MS_1 (MS_0 + 100 mol m^{-3} NaCl) and MS_2 (MS_0 + 150mol m^{-3} NaCl). Seed germination rate was decreased significantly from MS_0 to MS_1 (35 %) and MS_0 to MS_2 (65%). NaCl stresses cause a number of growth retarding effects on different morpho-biochemical parameters. Plant growth attributes such as plant biomass, total chlorophyll contents were decreased while chlorophyll b and total carotenoids were increased ($p<0.05$). Nitrate contents and total sugars were decreased with the increase in NaCl stress, while reducing sugar and proline contents of plant were increased. In conclusion, salinity has shown different detrimental plant growth retarding effects on Nasar-puri cultivar of onion.

INTRODUCTION

Onion (*Allium cepa* L.) is the second most valuable vegetable crop, grown throughout the world [1]. It is cultivated in at least 170 countries of the world[2]for being essential in food and medicine [3,4].Plant grows best in a sunny sheltered position in a rich light with well-drained soil [5], whilecool weather is required for seedling stage and hot dry summerfor its bulb's ripening and long days favor bulb growth [6]. Salinity remains an important abiotic factor in Pakistan with 6.30 million hectares of land is salt affected. Onion crop mostly cultivated in the regions irrigated with canal water. Such irrigation system encourages salts accumulation in agriculture lands and converts into unproductive land with the passage of time [7]. Salinity is a mixture of chlorides, sulfates, carbonates and bicarbonates of calcium, magnesium, sodium, and potassium [8,9]. Salinity has 2-folds effect on plant growth i.e. prevent water availability to roots (osmotic stress) and uptake of ions of salts increases toxic levels in certain tissues [10].

Salinity is a significant but harmful growth limiting factor in agriculture [11]. Under saline conditions, reduction in plant growth is a result of consequence alteration in the physiological processes like as free metallic ions concentration, availability of water and purity of mineral nutrition. On these factors, carbon catabolism through photosynthesis and its utilization depends [12]. Reduction in photosynthesis rate under salinity stressed conditions is related stomatal conductance that limited the carbon fixation rate in plants [13]. While under aseptic conditions, plant does need to fix carbon but uptake of carbon source and mineral ions remains

difficult under salt stressed cultures. *In-vitro* cultures are precise in volume and salt quantity which can gives us exact reflection of specific stress on plants growth[14]. Under *ex-vitro* conditions, plant has to face a number of environmental factors like as light, water, temperature and imbalanced salts in soil.

By keeping in view the above reports, aim of present work was to measure the toxic effects of salinity on seed germination and seedling stage of onion (*Allium cepa* L.) local cultivar Nasar-puri.Onion like cold season for germination while long day with high shining sunlight for its maturity or yield production. Such area is irrigated with canal water and could be affected with drought as well as salinity. Both of these abiotic stresses causes growth limitations in all living cells even whole organisms including onion among other plants. The designed work could be useful to determine the basic plant health changes at the early growth stage or even its germination. Since water deficit or saline stresses remains toxic for plant growth at global level threat.

MATERIALS AND METHODS

For present experiment, a local onion (*Allium cepa* L.) cv., Nasar puri was selected. Healthy seeds were screened and sterilized in Laminar Air Flow Cabinetby washing with70% ethanol for I min than stirred in 20% commercially available Robin® bleach [5% sodium hypo-chloride (NaOCl)] for 15 min on magnetic stirrer. Seeds were washed 3 times (3x5 min) in distilled H_2O. Two seeds were cultured in test tubes on MS (Murashige and Skoog,[15] basal plant nutrient salts with B_5 vitamins [16]) medium. Three NaCl levels were raised in MS medium like as MS_0 (control), MS_1 (MS_0 + 100 mol m^{-3} NaCl) and MS_2 (MS_0 + 150 mol m^{-3} NaCl) with 5 replicates. Cultured test tubes were incubated under dark conditions in growth chamber for 2-days. On initiation of seed germination, they were shifted to light (32 to 36 µmol $m^{-2}s^{-1}$) conditions in the growth chamber at 25 ± 2ºC for two weeks. Seed germination rate was recorded. Similarly, germinated seeds from MS_0 were also sub-cultured on MS_0, MS_1 and MS_2 and incubated in light conditions for 4-weeks.

After 2-weeks of culture, plants were removed from each culture and washed with tap water. They were dried on filter-paperand plant biomass parameters were measured and relative water contents (RWC) were expressed [17,18] by *RWC (%) = [(FM - DM)/TM] * 100*.

Proline and chlorophyll contents were analyzed in fresh tissue as by Bates et al., [19] and Arnon[20], Porra, [21] respectively. Plantlets of each treatment were dried in electric oven for 3 days at 105°C than subjected for other bio-chemical contents like as total protein as described by Lowery etal.,[22]. Total sugar contents were determined according to the method of Montgomery,[23] and reducing sugarsas by Miller [24]. Morris and Riley, [25] method was applied for nitrate contents analysis.

Almost 5-plants or replicates per treatment were arranged in one way completely randomized design. The results of each treatment were compared through (ANOVA) for data significance at 0.5% by using COSTAT Computer Package (*CoHort Software, Berkeley,* USA).

RESULTS AND DISCUSSION

In 2-weeks seed germination culture, saline sensitivity of onion (*Allium cepa* L.)was assessed when seeds were exposed to different levels of sodium chloride (NaCl) in MS basal plant nutrients under aseptic conditions. Germination ability in seeds of onion cv., Nasar Puri was subjected to NaCl stress. It was reduced with increase in NaCl stress (MS_1 and MS_2) level significantly ($p<0.05$). Maximum seed germination was observed in MS_0 (control) culture and almost no seed germination in 100mol m^{-3} NaCl stressed (MS_2) cultures (Table 1). Such decrease in seed germination also reported in different crop plants like cucumber (*Cucumis sativus* L.) by Carvalho and Kazama, [26] and gliricidia (*Gliricidia sepium*) by Farias *et al*., [27]. It is same to that an increase in salts quantity in agriculture lands, which promotes hyper-osmolarityof soil lead to restrict absorption of water through seed integument. Un-availability of water to embryo within the integument causes inhibition of seed germination [28].Uptakes of water by seeds trigger activation of antioxidant (*superoxide dismutase, catalase* and *ascorbate peroxidase*) enzymes[29,30].First seed germination was observed in control (MS_0) cultures than

in MS_1 and MS_2. This delay reflects in correspond to reduction in seed viability through slow down degradation of seed reserves at its germination stage like as in barley (*Hordeumvulgare* L.) and bean (*Phaseolus vulgaris* L.) seeds [31,32].

Similar cultures were maintained for 4-weeks, when seeds were germinated on MS_0 medium and sub-culture on MS_0, MS_1 and MS_2 medium. In these 3-cultures, different growth attributes like as shoot height and root length of seedlings were observed. Very similar growth response was observed like as in seed germination. A significant decrease in lengths of root and shoot with increase in NaCl level was measured (Table 1). It indicates that NaCl stress limit the growth of shoots and roots including their relative water contents (RWC) in parallel to increase in its concentration under aseptic and surely could be similar to by irrigation water under *in-vivo* conditions [27]. Low seedling growth rate or low accumulation of plant dry mass under high salts stressed levels explain the fact that when seeds absorb limited salty water becomes toxic for its tissues and consequently disturbs the physiological processes [26,33,34].

Application of NaCl in plant growth medium under aseptic conditions couldaffect differently at seed germination stage and after its germination [35]. After shoot emergence from the seed root is a first plant organ that directly affected by salt stress and it has been considered most salt sensitive ones[36]. Neumann,[37] has indicated that salinity inhibit root growth rapidly as well as in alteration water uptake capacity also losses. Shoot cannot receive correct amount of essential minerals nutrients from soil. Up-take of minerals from soil contains imbalance mineral nutrients cause abnormalities in the physiological processes[38], while morphological characters are always being the expression of inside ongoing physiological processes [39]. Like as total protein contents were decreased significantly under 100 and 150mM NaCl stressed cultures, while proline contents were increased (Table 1). With the increase in proline contents reducing sugars also showed same increasing pattern with increase in NaCl stresses ($p<0.05$).

Salinity also interferes in biosynthesis of plant carbon sources like as total carbohydrates were decreased significantly and nitrate metabolism also decreased significantly. Both carbohydrates and nitrates are directly or indirectly associated with plant pigmentation as well as rate of anabolism in cell chloroplast. Under saline stress, chlorophyll contents were decreased like as its reduction has been reported in some salt sensitive plant species under elevated salinity concentrations [40,41,42,43].Decrease in chlorophyll content depends on level of salinity stress, time of exposure and plant species. In contrast, non-significant alterations in chlorophyll contentsalso reported in salt tolerant species plants [44,45]. It means that chlorophyll contents could be used as sensitive indicator or marker of cellular metabolic state [46]. Meanwhile, chlorophyll b contents and total carotenoids were increased with increase in NaCl supply (Table 1).

Fluctuations in chlorophyll contents under salt stressed plants are best indication for abiotic environmental stressed plants. According to data on free amino acids under salt stressed cultures, proline and Glycinebetaine contents were increased. Accumulation of proline and other amino acids including reducing sugars as well as carotenoids may be acting as organic osmolytes. These osmolytesare the bestosmoregulators or osmomodulators being responsible in protection of plant tissue from stress injury. Low to higher proline contents in control to salt stressed cultures indicates the proline may be helping plants to tolerate against saline stresses[47,48,49].

Onion is one of the most important nutritional as well as medicinal food crops. Its yield is decreasing with the passage of time because of increasing level of salinity. This crop is irrigated with canal running water, which brings a mixture of salts from hills to lower leveled agriculture land. Yield of local onion cultivar Nasar Puriis also decreasing time to time like other crops. Deposition of high NaCl concentrations in soil is losing productivity of land. It exerts negative effect on biological aspects of the cells or tissue of onion seeds and its developing shoots. Meanwhile, some free organic amino acids and a specific group of reducing sugars and carotenoids are synthesized on the basis of osmotic signal activation. These organic molecules can develop salt tolerance or resistance at specific level or time but not as a whole life cycle of plants. On the basis of these biomarkers researchers need to select salt tolerant onion cultivars and to inform the farmers which cultivar should use for cultivation in salty agriculture land.

Table 1. Certain growth attributes of onion (*Alliums cepa* L.) local cultivar Nasar Puri at seed germination stage under aseptic sodium chloride stressed conditions (4-weeks culture)

#s	Characters	MS_0	MS_1	MS_2	Significance
A. *Plant Biomass Attributes*					
a.	Seed germination rate(%)	^{a}95.00±5.000	^{b}65.00±6.124	^{c}35.00±6.124	***
b.	Plant height (cm)	^{a}5.50±0.354	^{b}2.90±0.292	^{c}1.92±0.177	***
c.	Root length (cm)	^{a}4.38±0.156	^{b}3.18±0.107	^{c}1.68±0.086	***
d.	Fresh mass (g)	^{a}0.661±0.020	^{b}0.321±0.007	^{c}0.141±0.003	***
e.	Dry mass (g)	^{a}0.056±0.001	^{b}0.027±0.000	^{c}0.012±0.000	***
f.	RWC (%)	^{a}57.515±2.225	^{b}23.556±0.885	^{c}5.692±0.471	***
B. *Plant Bio-chemical Attributes*					
a.	Chl ab (mg g^{-1})	^{a}0.440±0.005	^{b}0.342±0.010	^{c}0.260±0.006	***
b.	Chl b (mg g^{-1})	^{c}0.119±0.004	^{b}0.141±0.001	^{a}0.169±0.001	***
c.	Total carotenoids (mg g^{-1})	^{c}0.101±0.003	^{b}0.203±0.003	^{a}0.365±0.003	***
d.	Nitrate contents (mg g^{-1})	^{a}0.163±0.002	^{b}0.115±0.002	^{c}0.048±0.002	***
e.	Total proteins (mg g^{-1})	^{a}0.259±0.003	^{b}0.170±0.002	^{c}0.152±0.002	***
f.	Proline contents (mg g^{-1})	^{c}0.269±0.003	^{b}0.419±0.002	^{a}0.562±0.002	***
g.	Total sugars (mg g^{-1})	^{a}0.163±0.002	^{b}0.127±0.002	^{c}0.087±0.005	***
h.	Reducing sugars (mg g^{-1})	^{c}0.014±0.001	^{b}0.049±0.003	^{a}0.073±0.003	***
i.	Glycinebetaine contents (mg g^{-1})	^{c}0.344±0.003	^{b}0.391±0.003	^{a}0.448±0.005	***

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