

## **Effect of Salinity on Growth and Some Photosynthetic Pigments of Improved Population in *Puccinellia ciliata* (Poaceae)**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors IY and AU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author YE managed the analyses of the study. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/CJAST/2020/v39i130481

#### **Editor(s):**

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Complete Peer review History: <http://www.sdiarticle4.com/review-history/54317>

**Original Research Article**

**Received 01 December 2019**

**Accepted 06 February 2020**

**Published 12 February 2020**

### **ABSTRACT**

*Puccinellia* (*Puccinellia ciliata* Bor.) fairly resistant to salinity and used as forage for livestock in China, Australia, and Turkey. In this study, our objective was to determine the effects of salinity on growth and various photosynthetic pigments of an improved population of *Puccinellia* via recurrent selection. To accomplish this, effects of salinity on seedlings growth of homogenous *Puccinellia* was examined, one week after emerging of radicle from seeds. Seeds were germinated on Murashige and Skoog (MS) medium with 6% agar. Seedling growth was studied under different levels of NaCl salinity (0, 10, 20, 30, 40  $\mu$ S/cm). Salinity applications were carried out for 6 weeks. Cultures were maintained in growth chambers at  $24\pm 2^\circ\text{C}$  and 16/8 light/dark conditions. Germination was scored during 2 weeks after culture initiation. The experiment was performed in a completely randomized

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design with three replicates. Plant growth parameters such as the number of radicle and tillers, maximum radicle and shoot length, plants fresh and dry weights were investigated. Photosynthetic pigments such as total chlorophyll, chlorophyll a, b, chlorophyll a/b ratio, total carotenoid,  $\beta$ -carotene, lutein and neoxanthin were examined. The maximum values for tiller number per plant, the maximum length of shoot and chlorophyll b were found in the 20  $\mu\text{S/cm}$ , while the maximum length of the radicle was recorded at 10  $\mu\text{S/cm}$  NaCl treatment. It was concluded that low salinity levels (10-20  $\mu\text{S/cm}$ ) increased seedling growth, while high salinity levels (30 and 40  $\mu\text{S/cm}$ ) inhibited the growth significantly. These results indicate that *P. ciliata* is a promising salt-tolerant and can be grown productively under low to moderate saline conditions between 10-20  $\mu\text{S/cm}$ .

**Keywords:** Germination; growth; halophyte; *Puccinellia*; salinity tolerance.

## 1. INTRODUCTION

Salinity significantly restricts plant growth [1]. Sodium toxicity and osmotic effect are the main factors that reduce the growth of plants under salinity conditions. Due to its high salinity / sodicity, such soils are not suitable for plant growing. Therefore, forage plants can be grown in such areas. [2]. *Puccinellia* (*Puccinellia ciliata* Bor – Poaceae) is a perennial pasture grass located near the Aegean Sea on the west coast of Turkey. It is suitable for forage with the use of salt water in alkaline and calcareous soils [3]. *Puccinellia ciliata* - Poaceae is highly resistant to salinity, and previously it has shown much better performance than most of the grasses on water logged environment [2]. As stated by Reddy and Vora [4], salinity-induced reductions in chlorophyll content, and it may increase from the inhibitory effects of salt on Chl synthesis or the acceleration of Chl degradation. With the increase of NaCl concentration, salinity treatment resulted in significant reduction in both shoot and dry weights of buffalograss cultivars blue grama ecotypes [5] *Puccinellia ciliata* [2] and *Puccinellia distans* [6]. Other researcher reports indicate that higher salinity conditions significantly affect the tiller production of *Chloris gayana* plants [7]. There is "puccinellia" population improved by our breeding studies under the salinity, alkalinity and waterlogged conditions. There is a little information about salt tolerance of the homogeneous/heterozygote population in *Puccinellia ciliata*. Hence, the present study was designed with the aim of assessing the suitability of this population for salinity tolerance.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material and Extracts Preparation

Our working team has conducted the population breeding of *Puccinellia ciliata* via recurrent

selection methods since 2010. Healthy seeds of pre-registration at the end of cycle-2 were used as material. For seed sterilization, seeds were washed under running tap water for an hour. Subsequently, seeds were surface-sterilized in 70% EtOH for 5 min, then in 2.25% NaOCl (including 3-4 drops tween 20) for 8 min. Then, seeds were rinsed three times for 5 min each with sterile distilled water. Surface-sterilized seeds were placed in to different NaCl solutions overnight (0, 10, 20, 30, 40  $\mu\text{S/cm}$  established with NaCl). For germination, seeds were placed on 6% agar. One week after germination, seedlings were replaced on Murashige and Skoog (MS) medium (Murashige and Skoog, [8]) with different concentrations of NaCl (10, 20, 30, 40  $\mu\text{S/cm}$ ). Medium without salt was used as control. Agar-agar was used for a gelling agent (6%). a pH of medium adjusted to 5.8 using 1M NaOH or HCl. 25 seeds per glass were planted. Salinity was applied for six weeks.

### 2.2 Growing Conditions, Measurement of Growth Parameters and Photosynthetic Pigment Analysis

Cultures were inoculated in a growth chamber for 16/8-h (light/dark) photoperiod provided by fluorescent light ( $36 \text{ mmol}^{-1} \text{ m}^{-2}$ ) at  $24 \pm 2^\circ\text{C}$ . All tests were conducted as a completely randomized design with three replicates. The radicle and tiller number, maximum radicle and shoot length (mm), fresh and dry weight (mg) of the plant were evaluated. Plants were treated at  $70^\circ\text{C}$  for 24 hours for the determination of dry weight.

Total chlorophyll, chlorophyll a, b, chlorophyll a/b ratio, total carotenoid were determined according to the method described by Wellburn [9].  $\beta$ -carotene, lutein and neoxanthin contents were recorded as absorbance values at 477.8, 461 and 453.4 nm, respectively. The concentrations of chlorophylls a (Ca) and b (Cb) and total carotenoids (Cc+x) in micrograms per milliliter of

pigment extracts were calculated using equations of Wellburn [9].

$$Ca = 12.47A665.1 - 3.62A649.1$$

$$Cb = 25.06A649.1 - 6.5A665.1$$

$$Cc+x = (1000A480 - 1.29Ca - 53.78Cb)/220$$

### 2.3 Statistical Analysis

All the data were statistically analyzed with analysis of variance (ANOVA) procedures using the SPSS software (SPSS Inc. [10]). The differences between the means were compared by the least significant difference by employing the Duncan Multiple Range Test ( $p \leq 0.05$ ).

The regression graphs and curve fitting were generated by SPSS using replication values for each observed parameters. Here, the most suitable curve function is selected to the highest  $R^2$ .

## 3. RESULTS AND DISCUSSION

### 3.1 Growth Parameters

Duncan's Multiple Range Test ( $P < 0.05$ ) indicated significant differences among means of some growth parameters at different salinity levels (Table 1).

Seeding data indicated that all parameters except maximum radicle length significantly affected by salinity (Table 1), and radicle number, fresh and dry weight decreased accordingly with increasing salinity. The lowest values were statistically observed in high salinity levels (30 and 40  $\mu\text{S}/\text{cm}$ ). Lower salinity levels had stimulating effects on 10  $\mu\text{S}/\text{cm}$  for radicle length, 20  $\mu\text{S}/\text{cm}$  for shoot length and tiller number compared to control. 50% decrease in the dry weight of plants occurred at 30-40  $\mu\text{S}/\text{cm}$ . Results of the present study were in agreement with the finding of Haider et al. [2] who observed 22 mg decreased at 40  $\mu\text{S}/\text{cm}$ . Besides, they

found linear relationships for a fresh and dry weight of shoot and radicle against increasing salinity in the root zone. Jenkins et al. [1] stated that in *Puccinellia*, total radicle dry mass was 80-85% greater under saline waterlogged than saline drained conditions. This is in agreement with the results of Alshammery et al. [11]. Significant reduction in dry matter with increase in salinity was also observed for *Puccinellia distans* [12]. The authors found the maximum length and weight of the radicle and the shoots were seen at 4 dS/m salinity level [13]. Results revealed that under salinity stress, nutrient and water absorption by radicle and shoot were reduced. In line with the results of present study, Jenkins [14] also reported 50% reduction in growth of shoot under 25-28 dS/m levels of salinity. In *P. ciliata*, the 250 mM the salinity concentrations under waterlogged conditions causing a 20% increase in shoot weight. At 2.1  $\text{g}\cdot\text{kg}^{-1}$  NaCl, total radicle weights increased under hypoxia. In a comparison to the performance of two halophytic kinds of grass at salt land sites in Western Australia, Jenkins et al. [1] observed that the salinity level (300 mM NaCl) under drained conditions reduced shoot dry mass by 50%.

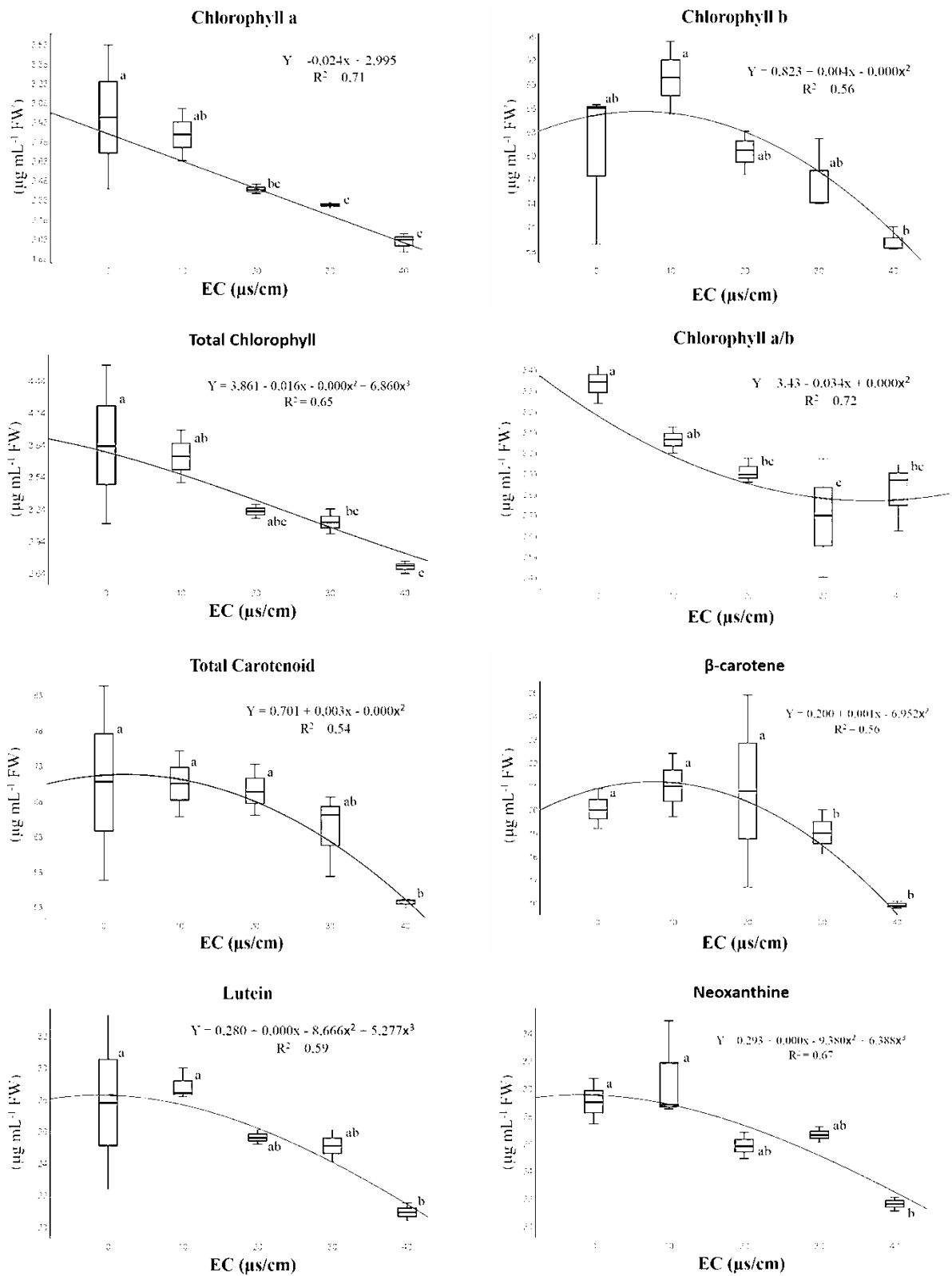
### 3.2 Photosynthetic Pigments

Significant effects of NaCl treatments were observed on chlorophyll a, b, total chlorophyll, chlorophyll a/b, total carotenoid,  $\beta$ - carotene, lutein and neoxanthine contents (Fig. 1). According to  $R^2$  value, much more expressive decrease in Chl a and total Chl were detected in response to salinity, whereas chlorophyll a/b, total carotenoid,  $\beta$ - carotene, lutein and neoxanthin showed quadratic trends. The salinity dose, 10  $\mu\text{S}/\text{cm}$ , triggered the content of chlorophyll b, total carotenoid,  $\beta$ - carotene, lutein and neoxanthine contents. Higher salinity doses, 30 and 40  $\mu\text{S}/\text{cm}$  caused significant decreases in all pigment parameters.

**Table 1. Some growth parameters of *Puccinellia ciliata* subjected to salinity for six weeks**

NaCl ( $\mu\text{S}/\text{cm}$ )	Tiller number per plant	Maximum length of shoot (cm)	Number of radicle	Maximum length of radicle (mm)	Fresh weight of plants (mg)	Dry weight of plants (mg)
0	3.30±0.16 ab	4.33±0.25 c	2.90±0.22 a	5.4±0.89	8.29±0.15 a	0.94±0.22 a
10	3.06±0.22 abc	5.64±0.34 ab	2.80±0.20 a	6.5±1.67	8.13±0.16 a	0.68±0.18 b
20	3.46±0.17 a	6.06±0.48 a	2.20±0.20 b	4.7±0.56	6.32±0.37 ab	0.56±0.40 b
30	2.86±0.17 bc	4.64±0.37 bc	2.06±0.18 b	4.8±0.54	6.26±0.8 ab	0.42±0.16 c
40	2.70±0.11 c	4.39±0.28 c	1.90±0.20 b	4.1±0.50	5.00±0.46 b	0.22±0.32 d

Values are means of three replicates  $\pm$  SE. Means with different superscripts within a row differ significantly according to Duncan's Multiple Range Test ( $P < .05$ )



**Fig. 1. Regression graphs of total chlorophyll, chlorophyll a, b, chlorophyll a/b ratio, total carotenoid, β- carotene, lutein, and neoxanthine contents under different concentrations of NaCl in of Puccinellia ciliate. Horizontal bars represent the standard errors of the mean**

Maximum reduction in chlorophyll a value was observed at 40  $\mu\text{S}/\text{cm}$  level of salinity. Results of our study were consistent with the findings of Kiani-Pouya and Rasouli [15] who found that salt stress significantly reduced chlorophyll a in leaves. The concentration of NaCl had a negative effect on the chlorophyll content of wheat [16]). Results of the present study were in line with the findings of Terletskaia et al. [17] who reported that the chlorophyll content of *T. monococcum*, *T. polonicum*, *T. macha*, *T. aestivum* decreased significantly (by 75-84%) under stress conditions. Salama et al. [18] reported that high salinity level of (200 Mm NaCl) resulted in the swelling of membranes in chloroplasts of sensitive wheat cultivars while it had little effect in the tolerant ones. Total chlorophyll content of wheat genotypes was decreased at 100 mM NaCl as mentioned by Hussain et al. [19]. It has been observed that applying low doses of salt (10  $\mu\text{S}/\text{cm}$  NaCl) to puccinellia plants promotes chlorophyll b content. Different studies over the years also reported the significant decline in chlorophyll contents under saline conditions [19,20,21,22]. The highest chlorophyll a/b values were observed at control conditions. Kiani-Pouya and Rasouli [15] found that chl a/b remained unchanged with increasing salinity at late tillering, but significantly reduced at the anthesis stage. They found that both chl a and b failed to be used as the indicator of salinity. In puccinellia, it was found that the plants were resistant to 30  $\mu\text{S}/\text{cm}$  NaCl in terms of carotenoid and total  $\beta$ -carotene. In another study, chlorophyll and carotenoid of wheat were stimulated by 14  $\text{dS}\cdot\text{m}^{-1}$  NaCl [23]. Oludare Agbolade et al. [24] also reported significant reduction in chlorophyll a, b, total chlorophyll and carotenoids contents under the application of both selenium and salinity stress in wheat plants. On the other hand, Ben-Abdallah [25] observed a decrease in  $\beta$ -carotene and lutein of *Solanum nigrum* at concentrations of 150 mM NaCl. Similarly, Kahrizi and Sedghi [26] reported that  $\beta$ -carotene contents were considerably increased under increasing level of salt stress. Norshazila et al. [27] stated that the absence of light would increase the lutein content in pumpkin plants, but the synthesis of  $\beta$ -carotene could not occur. Abadia et al. [28] reported that salt stress did not induced significant changes in chlorophyll a, b, chlorophyll a/b,  $\beta$ - carotene, lutein and neoxanthine in barley.

#### 4. CONCLUSION

From the above mentioned results it is concluded that salt stress significantly affected the number

of radicles, fresh/dry weight and chlorophyll contents in *Puccinellia ciliata*. Tiller number, shoot length, total carotenoid,  $\beta$ - carotene, lutein and neoxanthine were triggered by lower salinity doses. Overall, our results indicate that *Puccinellia ciliata* have the ability to survive under 10- 20  $\mu\text{S}/\text{cm}$  salinity stress. It seems that halophytic plants such as Puccinellia could be positively affected by slight saline conditions at early seedling stage, whereas extremely salty-alkaline soil conditions restrict the growth of plants.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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