Original Research Article

Biological and Agronomic Characterization of Improved Alkali Grass Genotypes (*Puccinelliaciliata*Bor)in (Area of Study)

ABSTRACT

Puccinellia is one of the plants with high performance in unfavourable soil conditions.Fourteen super genotypes developed bythe recurrent selection methodwere grownat three different sowing times in order tocharacterize the alkali grass genotypes for physiological and agronomic traits and to determine the relationships with seed yield(area and moment of study). The interaction between planting time and genotypes was found to be significant for all traits. The seed yield of the 14 Puccinelliagenotypes varied between 28.2 kg ha^{-1} and 96.6 kg ha^{-1} and the grand mean was 61.3 kg ha^{-1}. Genotypes with high yielding were defined as thick-leaved(include the mean +/- standard deviation), large bulliform cells(include the mean +/- standard deviation), high chlorophyll content(include the mean +/- standard deviation), large stomatal area(include the mean +/- standard deviation) and fewer stomata(include the mean +/- standard deviation).Biological characteristics such as stoma area and number, leaf and bulliformstructure and chlorophyll content should be used as selection criteria for production.

Keywords: Leaf anatomy, Puccinellia, planting time, seed yield, superior genotypes.

1. INTRODUCTION

Alkali grass (*Puccinelliaciliata*Bor) is a perennial grass species, widespread on saline and alkali lands under waterlogging conditions.The pH value in the areas where Puccinelliais distributed in the Aydin region of western Turkey varies between 8.91 and 10.83. Boron (5.65-14.47 ppm) and sodium contents (1341-5479 ppm)inthe same areas are at a toxic and very high level, respectively[1]. One of the solutions to salinity, alkalinity and waterlogging is the utilization of tolerant crops such as Puccinellia[2, 3]. Salt-tolerant plants can produce highly edible biomass in arid and salty areas where non-tolerant species cannot grow [4] and these plants constitute an important part of the feeding programs of sheep, goats and camels in arid and semi-arid regions [4, 5].Puccinelliacan easily form a plant in salty soils and this is widely used in the improvement ofare used[6, 7]. Many studies demonstrated the use of Puccinelliaspecies in environmental remediation of heavy metals contamination [8, 9]. Puccinelliaspecies was identified as boron tolerant plants [10, 11] and its potential for boron phytoextraction purposes or pollutionphytomanagement strategies were demonstrated.

Cell differentiation and developmental processes regulated leaf formin unfavourable conditions [12, 13, 14]. Adaxialbulliform cells of Puccinelliaoccur laterally to midrib[15] and these cells are large thin-walled and highly vacuolated on the adaxial epidermis [16, 17]. In rice, which is in the same family with Puccinellia, the leaf becomes rolled by the function of bulliform cells[18]. The Soil Plant Analysis Development (SPAD) chlorophyll meter, proportional to the chlorophyll a + b content, is useda diagnostic tool for the nitrogen status of leaves in a plants[19]. On the other hand, the functional state of the PSII protein complex and the photosynthetic efficiency of the plants is evaluated through Fv/Fm (maximum fluorescence yield of photosystem II)[20]. The most important functions of leavesfor crop production and maintaining plant water balance are related to stomata and anatomy, and the number of stomata varied among plant species and varieties[21].

Puccinellia blooms in April, and seeds ripen in the May-June period. The plants remain dormant over summer and shoot vigorously after the opening rains of autumn. If it is desired to re-plant with seeds, the after-ripening requirement for Puccinellia was more than 90 days under dry storage[7] and optimal germination temperatures for Puccinellia were 30/15 °C[22]. Puccinellia seed is very small, straw-coloured tinged with purple (2 - 2.5 mm long), and ellipsoidal caryopsis; seed yield is approximately 100 kgha^{-1}[23]. The positive and significant correlations between seed yield and leaf colour, length and width were calculatedin *Puccinellianuttalliana* populations from theCanadian Great Plains [24]. Literature searches demonstrated that there arevery few articles on seed yield and leaf characteristics of Puccinellia and the relationshipsamongseed yield and leaf chlorophyll, photosynthetic activity, bulliform cell properties and stomatal behaviour of leaves. Therefore,this study aims at determining the yield and leaf characteristics of the Puccinelliapopulations which we have improved, by creating different climatic conditions with the planting time.

2. MATERIAL AND METHODS

Breeding studies were initiatedto develop superior genotypes by recurrent selection on the *Puccinelliaciliata*populations collected from various regions having waterlogging, saline and severe alkalinity conditions in 2011. Seeds from fourteen populationswere planted in the plastic pots with a length of 50 cm, a width of 18 cm and a depth of 30 cm at three different sowing times (October 1, October 21 and November 16, 2020). There were 50 plants in each pot. Nitrogen (36 kg ha^{-1}) and phosphorus (92 kg ha^{-1}) from di-ammonium phosphate was added at the rate of 200 kg ha^{-1}at pot filling, and the pots were irrigated approximately every 10 days to replenish the lost moisture(refference?).

The climatic data showed that December and January of the experimental year had more precipitationthan the long term, while December, January and February average temperatures are above the average for the long term (Figure 1).This climate trend shows the temperature rise and soil oxygen deficiency under typical waterlogging in the region.These conditions are the adverse conditions that the Puccinellia plant adapts to and resists stress.The lack of precipitation after April indicates that the Puccinellia plant is facing arid conditions after flowering(refference?).

Figure 1. Monthly average temperature and rainfallof the experimental site in 2020 and long-term.

In flag leaves of randomly sampled plants, microscopy observation was carried out to determine the stomata number and size per unit area, bulliform size and leaf thickness (Figures 2 and 3). The same samples were used for SPAD and Fv/Fm measurements.Leaf thickness from leaf cross-sections was measured from Scanning Electron Microscopy's (SEM) with the camera (Leica ICC 50 W). Stomata area, number of stomata (per μm^2), bulliform cell width and length were measured from the abaxial surface in ten stomata by the screening of SEM. Photomicrographs from SEM were captured and digitalized with Vision 4.3. SPAD value was measured with a SPAD meter (SPAD-502, KonicaMinolta Sensing, Inc., Tokyo, Japan). Photosynthetic parameters, Fv/Fm and PI, were recorded with the help of a Chlorophyll Fluorometer,CI-340 from CID Bio-Science. Forty plants in each plot were cut to determine the seed yield, panicle length and branch number in the panicle. Seeds were threshed by hand and weighed. Seed yield per parcel converted to kg ha^{-1}(refference?).

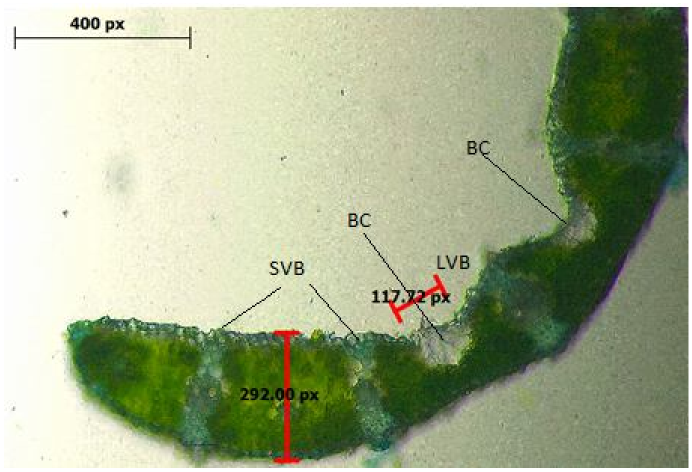


Figure 2. Overview of the transverse section of*Puccinelliaciliata*Bor leaf. BC, Bulliform cell;LVB, Large Vascular Bundle; SVB, Small Vascular Bundle

Figure 3. Stomata cells on the adaxial leaf blade of *Puccinelliaciliata*Bor

Variance analysis was performed among 14 populations and among 3 sowing times for each characteristic according to the split-plot design in randomized parcelswith three replications in the R Studio [31] (v. 4.1.2) using the 'agricolae'package[25; v. 1.3-5]. Phenotypic correlations among characteristics were estimated [26] anda correlogram was created in R Studio using the 'corrplot'package[27; v.0.92]. Heat maps were made in R Studio using the 'heatmap.2' command within the 'gplots' package [28; v. 3.1.1].

3. RESULTS AND DISCUSSION

A heat map was developedto include Puccinelliagenotypes at each planting time for observed characteristics (Figures4, 5 and 6). The heat map for each planting time displays variation among lines based on colour. The blue and red indicate the highest and lowest values for all the traitsrespectively.

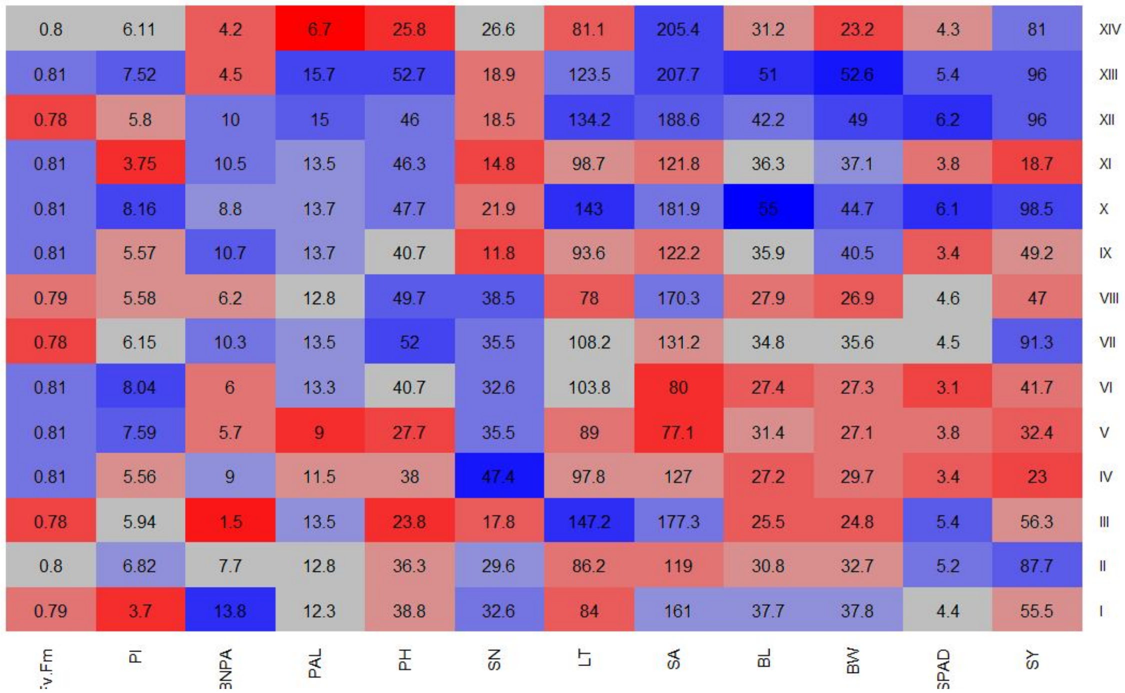
The seed yield of the 14 Puccinelliagenotypes varied between 28.2kg ha^{-1} (genotype VI; Figure 4) and 96.6kg ha^{-1} (genotypeXII; Figure 5),and the grand mean was 61.3kg ha^{-1}. The significant interaction between genotypes and sowing time for seed yield indicated that the genetic potential of Puccinelliagenotypes varied primarily as a result of the environmental conditions during the growing season (Table 1). The high-yielding genotypes such as XIII and XII are more productive at the second planting time (Figure 5), whereas the genotype X had a higher yield in the first planting time (Figure 4). Some genotypes (II and VII) with medium yield are more successful in terms of yield in the first planting time.The number of studies determining the seed yield after growing in the Puccinellia plant is very few. Seed yield of *Puccinelliadistans* species varied between 17.5-25.4 gplant^{-1}by vegetative propagation [29]. Also, the seed yield of *Puccinellianuttallia* was determined as 12.9-33.6 g plant-1 [24].In our study, we determined that the average seed yield was 11.03 mg plant^{-1}. This important difference is due to the harvest year (plant age), tiller number per plant, plant density per unit area and species difference of the perennial Puccinellia plant.

The plant height of Puccinelliagenotypesduring different planting times changed from 23.7 cm to 58.3 cm (Figures4, 5 and 6). Australian flora information defines plant heights as 60 cm for *P. ciliata*[37]. In addition, the plant height of 24 *P. nuttalliana* populations was found70.5-73.5 cm [24]. Plant height was 20-45 cm for *P. teyberi*, 20-50 cm for *P. convolute* and 35-100 cm for *P. festucifomis* [15]. These variations clearly indicated the differences between species and genetic potential. We determined that the highest plant height was recordedin genotypes XIII, XIV, III and Iof second planting time (Figure 5). These values are followed by XI in the third planting time (Figure 6), and XIII and VIII in the first planting time (Figure 4).

Table 1. Analysis of variance for studied characteristics

	SY	SPAD	BW	BL	SA	LT	SN	PH	PAL	BNPA	PI	Fv/Fm
Genotypes (G)	**	**	**	**	**	**	**	ns	**	**	*	**
SowingTime (ST)	ns	ns	ns	ns	*	ns	ns	ns	*	*	ns	ns
G x ST	**	**	**	**	**	**	**	**	**	**	**	**
$LSD_{(0.05)G \times ST}$	2.25	0.06	0.38	0.36	9.58	2.04	0.22	3.95	1.25	1.99	0.06	0.01

*: P = .05; **: P = .01. SY: Seed yield; BW: Bulliform width; BL: Bulliform length; SA: Stomatal area; SN: Stoma number; LT: Leaf thickness; PH: Plant height; PAL: Panicle length; BNPA: Branch number in a panicle; PI:Performance index; Fv/Fm: Maximum quantum efficiency of photosystem II.

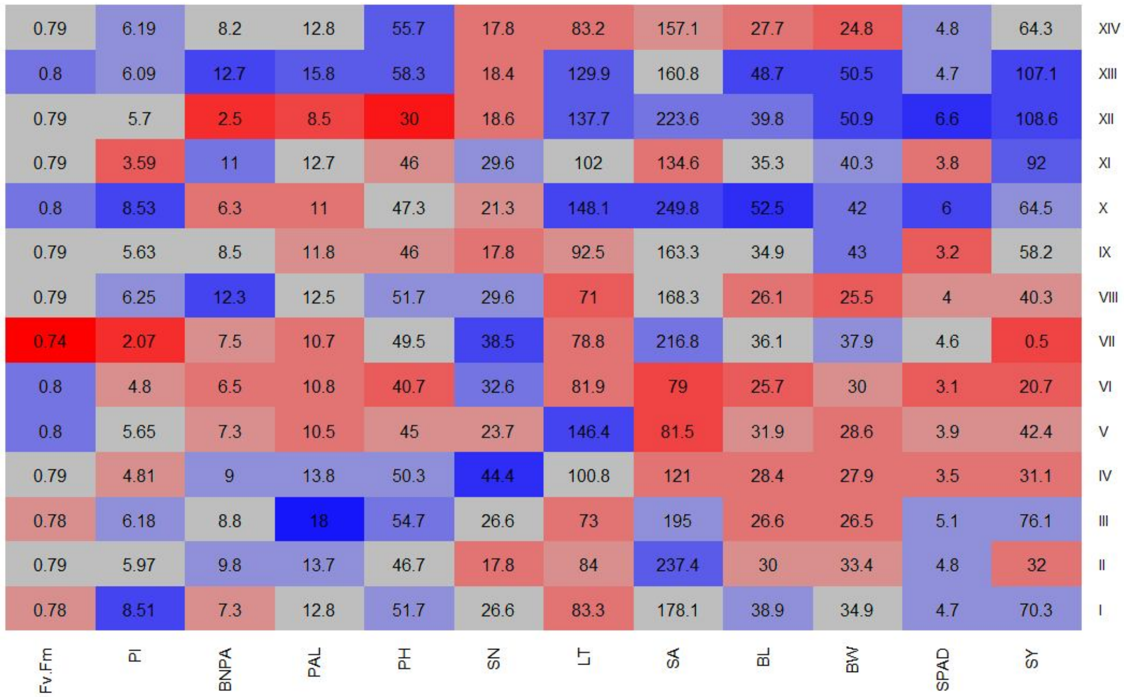


SY: Seed yield; BW: Bulliform width; BL: Bulliform length; SA: Stomatal area; SN: Stoma number; LT: Leaf thickness; PH: Plant height; PAL: Panicle length; BNPA: Branch number in a panicle; PI: Performance index; Fv/Fm: Maximum quantum efficiency of photosystem II.

Figure 4. Mean values for observed characteristics of 14 Puccinelliagenotypes grown in the first planting time.

Panicle lengthwas found between 6.5 cm and 18.0 cm depending on sowing time and genotypes. In studies on the taxonomy of Puccinellia in Croatia by Bogdanovi´c et al. [15], panicle length and branch number in panicle were 5-30 cm and 2-6, respectively. We recorded the highest values in the second planting time of genotypes III and XIII (Figure 4), and in the first planting time of genotypesXIII and XII (Figure 4), respectively. In terms of branch number in panicle, the genotypeXI in the third planting time produced the highest value (Figure 6), whereasgenotypes XIII and XIV in the first planting time had the lowest value (Figure 4).

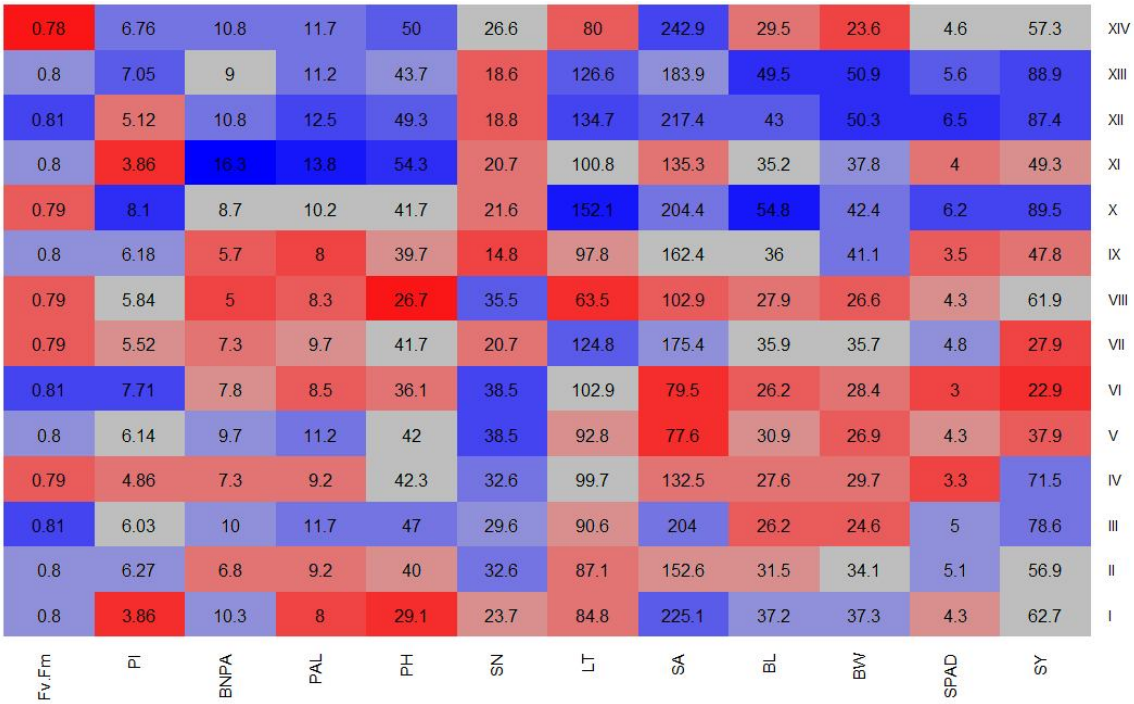
When the general characteristics of the 14 genotypes developed by us are evaluated in terms of yield and yield components,genotypes XII, XIII, X and III with high yielding had tall, long paniculates, and a medium-low number of branches, whereas genotypes V and VI with the lowest yield were shorter in terms of plant height and panicle length. The second planting time is favourable for these characteristics.



SY: Seed yield; BW: Bulliform width; BL: Bulliform length; SA: Stomatal area; SN: Stoma number; LT: Leaf thickness; PH: Plant height; PAL: Panicle length; BNPA: Branch number in a panicle; PI: Performance index; Fv/Fm: Maximum quantum efficiency of photosystem II.

Figure 5. Mean values for observed characteristics of 14 Puccinelliagenotypes grown in the second planting time.

The significant interactions between genotypes and planting times for SPAD and leaf thickness indicated that changing climatic conditions during the growing season depending on the planting time affected the genetic response of the genotypes (Table 1). SPAD values varied from 2.97 to 6.55, and the highest SPAD was recorded in all planting times of the genotypeXII (Figures4, 5 and 6). These values are followed by genotype X in the first (Figure 4) and third planting time (Figure 6).It was demonstrated the significant differences among Puccinelliaspecies for chlorophyll a, b and a:b ratio and the supremacy of the *P. pratensis* [30]. Similarly, the genotype Xhighly performed for leaf thickness in all planting times (Figures4, 5 and 6). Genotype III in first planting time, genotype V in second planting time and genotypeXII in all planting time exhibited performance at the second and third levels.We detected the leaf thickness between 63.5 and 152.1 µm. These values were thinner than the leaf thickness of *P. frigida*(233.3 µm) foundin another study [33].In our study, leaf thickness was determined between genotypes X and XIII, whichattracted attention with their thick leaves and high chlorophyll content.

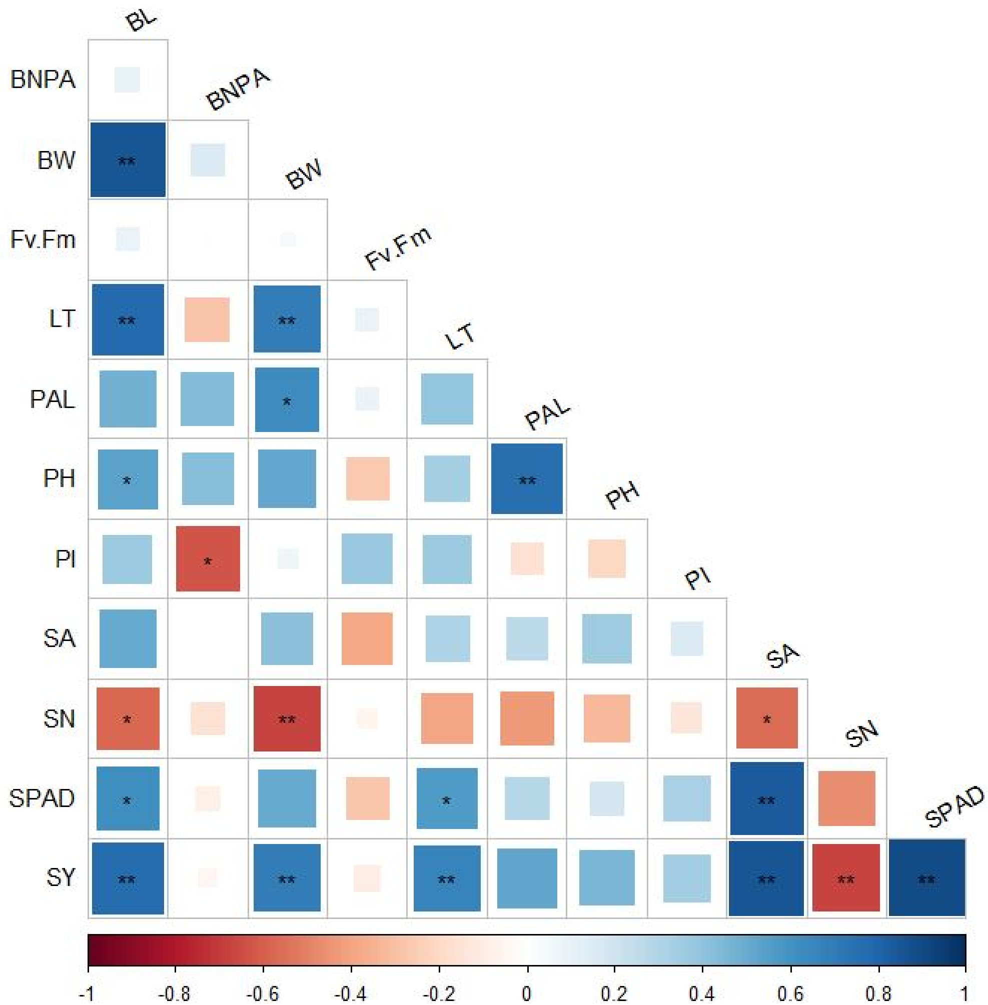


SY: Seed yield; BW: Bulliform width; BL: Bulliform length; SA: Stomatal area; SN: Stoma number; LT: Leaf thickness; PH: Plant height; PAL: Panicle length; BNPA: Branch number in a panicle; PI: Performance index; Fv/Fm: Maximum quantum efficiency of photosystem II.

Figure 6. Mean values for observed characteristics of 14 Puccinelliagenotypes grown in the third planting time.

Fv/Fm (quantum yield) values were found between 0.74 and 0.81. Genotypes IV, V, VI, IX, X, XI and XIII in the first planting time (Figure 4) and genotypes III, VI and XII in the third planting time(Figure 6) exhibited favourable Fv/Fm.Performance index (PI) values ranging from 2.07 to 8.53 were found to be high in all sowing times of the genotype X (Figures4, 5 and 6).

It was expressedthat the thickness of their bulliform cells in *P.poecilantha* and *P. bulbosa*occupied ¼ of the leaf thickness and defined as the type of mesophyll of the abaxial surface [34]. Bulliform cells are often closely associated with xerophyte conditions [35]. When bulliform width and length are examined together, all planting time of genotype XIII for width and all planting time of genotypeX for length exhibited significantly the highest values (Figures4, 5 and 6). The fact that both genotypes have the highest width and length values is an indicator of the large bulliform area in these populations.



*: P = .05; **: P = .01. SY: Seed yield; BW: Bulliform width; BL: Bulliform length; SA: Stomatal area; SN: Stoma number; LT: Leaf thickness; PH: Plant height; PAL: Panicle length; BNPA: Branch number in a panicle; PI: Performance index; Fv/Fm: Maximum quantum efficiency of photosystem II.

Figure 7. Correlations among studied characteristics

Heatmap of correlation coefficients between seed yield, yield components and physiological characteristics were summarizedin Figure 7. The positive and significant correlations of seed yield with bulliform length and width, leaf thickness, stomatal area and SPAD indicated that yielding genotypescould be characterized as having thick leaves, large bulliform cells, and high chlorophyll content, and large stomatal area.On the other hand, the negative and significant correlation between yield and the number of stomata can be explained by the few but large stomata of yielding genotypes. The negative correlations between the number of stomata per mm^2 and bulliform length, width and stomatal area indicated that small bulliform cells and narrow stomatal area increased the number of stomata in Puccinellia. Similarly, higher SPAD values weredue to the increase in bulliformlength, leaf thickness and stomatal area. The fact that the Fv/Fm value did not show a significant correlation with all features and the PI value only has a negative and significant correlation with BNPA was an indication that these two values were not an important character in the absence of stress.The significant and positive correlations of seed yield with leaf colour, length and width calculated in another study [24] indicated the associations between seed yield and leaf characteristics. Similarly, it was statedthat leaf texture could be contributed to the turf quality of perennial ryegrass [36].Our study results along with other studies reveal the importance of leaf characteristics.

4. CONCLUSION

The present study demonstrated thatthe seed yieldrequired establishing a new field of Puccinellia, which was 20.7-108.6 kg ha^{-1} depending on the planting time. The planting time of 21st ofOctober was more favourable for productive genotypes such as XII, XIII, XI and III. Leaf morpho-physiologilogical characteristics can be successfully used as criteria in the selection of productive genotypes, and the breeding of Puccinellia.

REFERENCES

1. Yavaş I, Ünay A, Şimşek S. The evaluation of alkali grass (*Puccinelliaciliata*bor) populations in Aydin province of Turkey. Turkish Journal of Agriculture-Food Science and Technology. 2017;5(8):858-863.

2. Ibrahim M, Akhtar J, Younis M, Riaz MA, Haq MA, Tahir M. Selection of cotton (*Gossypiumhirsutum* L.) genotypes against NaCl stress. Soil Environ. 2007;26:59-63.

3. Ashraf M, Athar HR, Harris PJC, Kwon TR. Some prospective strategies for improving crop salt tolerance. Adv. Agron. 2008;97:45-110.

4. El-Shaer HM. Halophytes and salt-tolerant plants as potential forage for ruminants in the Near East region. Small Ruminant Research. 2010;91:3-12.

5. Squires VR, Ayoub AT. Halophytes as a resurce for livestock and for rehabilitation of degraded lands. Kluwer Academic Publisher. 1994 Dordrecht Boston. London. UK.

6. Hitchcock AS. Manual of the grasses of the united states. 1st Ed. New York: Dover. 1971; 525 p

7. Tarasoff CS, Mallory-Smith CA, Ball DA. Comparative plant responses of *Puccinelliadistans* and *Puccinellianuttalliana* to sodic versus normal soil types. Journal of Arid Environments. 2007;70:403-417.

8. Stiles AR, Bautista D, Atalay E, Babaoğlu M, Terry N. Mechanisms of boron tolerance and accumulation in plants: a physiological comparison of the extremely boron-tolerant plant species. *Puccinelliadistans*. with the moderately boron-tolerant *Gypsophila arrostil*. Environ Sci Technol.2010;44:7089–7095.

9. Padmanabhan P, Babaoğlu M, Terry NA. Comparative transcriptomic analysis of the extremely boron tolerant plant *Puccinelliadistans* with the moderately boron tolerant *Gypsophila arrostil*. Plant Cell Rep. 2012;31:1407–1413.

10. Babaoğlu M,Gezgin S,Topal A, Sade B, Dural H. *Gypsophila sphaerocephala*Fenzl ex Tchihat.: a boron hyperaccumulator plant species that may phytoremediate soils with toxic B levels. Turkish Journal of Botany. 2004;28(3):273-278.

11. Rámila CD, Leiva ED, Bonilla CA, Pastén PA, Pizarro GE. Boron accumulation in *Puccinelliafrigida*an extremely tolerant and promising species for boron phytoremediation. Journal of Geochemical Exploration. 2015;150:25-34.

12. Bowman JL, Eshed Y, Baum SF. Establishment of polarity in angiosperm lateral organs. Trends Genet. 2002;18:134–141.

13. Micol JL, Hake S. The development of plant leaves. Plant Physiol. 2003;131:389–394.

14. Lang Y, Zhang Z, Gu X, Yang J, Zhu Q. Physiological and ecological effects of crimpy leaf character in rice (*Oryzasativa* L.) I. Leaf orientation, canopy structure and light distribution. ActaAgron. Sin. 2004;30:806–810.

15. Bogdanović S, Brullo S, Alegro AL, Rešetnik I, Mitić B. Taxonomic notes on *Puccinelliateyberi* (Poaceae) a critical species of Croatian flora. Biologia. 2012;67(1):71-78.

16. Jane WN,Chiang SHT. Morphology and development of bulliform cells in Arundoformosana Hack. Taiwania Int. J. Life Sci. 1991;36:85–96.

17. Itoh J, Nonomura K, Ikeda K, Yamaki S, Inukai Y, Yamagishi H. Rice plant development: from zygote to spikelet. Plant Cell Physiol. 2005;46:23–47.

18. Zhang JJ, Wu SY, Jiang L, Wang JL, Zhang X, Guo XP, Wu CY, Wan JM. A detailed analysis of the leaf rolling mutant sll2 reveals complex nature in regulation of bulliform cell development in rice (*Oryzasativa* L.). Plant Biology. 2015;17(2):437-48.

19. Yuan Z, Cao Q, Zhang K, Ata-Ul-Karim ST, Tian Y, Zhu Y, Cao W, Liu X. Optimal leaf positions for SPAD meter measurement in rice. Frontiers in Plant Science. 2016;26(7):719.

20. Maxwell K, Johnson GN. Chlorophyll fluorescence—a practical guide. Journal of experimental botany. 2000;51(345):659-68.

21. Dillen SY, Marron N, Koch B, Ceulemans R. Genetic variation of stomatal traits and carbon isotope discrimination in two hybrid poplar families (*Populusdeltoides* 'S9-2'× *P. nigra* 'Ghoy'and*P. deltoides* 'S9-2'× *P. trichocarpa* 'V24'). Annals of Botany. 2008;102(3):399-407.

22. Moravcova L, Frantik T. Germination ecology of *Puccinelliadistans* and *P. limosa*. Biologia. Bratislava.2002;57(4):441–448.

23. Craig A, Nichols P. Victoria department of primary industries - pastures for discharge areas 2022:AG0386. Available from: https://keys.lucidcentral.org/keys/v3/pastures/Html.

24. Liu Y, Coulman BE. Morphological and agronomic variation of *Puccinellianuttalliana* populations from the Canadian Great Plains. Canadian Journal of Plant Science. 2015;95(1):67-76.

25. de Mendiburu F, de Mendiburu MF. Package 'agricolae'. R Package, Version, 1.3. 2019. Available from: https://cran.r-project.org/web/packages/agricolae/agricolae.pdf

26. Steel RGD, Torrie JH, Dickey DA. Principles and procedures of statistics: a biometrical approach. McGraw Hill. New York. USA. 3rd ed. 1997.

27. Wei T, Simko V, Levy M, Xie Y, Jin Y, Zemla JR.Package "corrplot": Visualization of a Correlation Matrix. 2017. Available from: https://cran.r-project.org/web/packages/corrplot/index.html

28. Warnes GR, Bolker B, Bonebakker L, Gentleman R, Huber W, Liaw A, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M, VenablesB, Galili T. Package 'gplots'. Various R programming tools for plotting data. R Package, Version, 3.1.3. 2022. Available from:http://CRAN.R-project.org/package=gplots

29.Elçi Ş, Bostancıoğlu H. Baladevletüremeçifliğindenalinançorakçiminin(*Puccinelliadistans* L.) diploid materyalindemorfolojik, sitolojikvesitogenetikaraştirmalar. 1981. TÜBİTAK, Ankara.

30. Vaziriyeganeh M, Carvajal M, Du N, Zwiazek JJ. Salinity Tolerance of Halophytic Grass *Puccinellianuttalliana*Is Associated with Enhancement of Aquaporin-Mediated Water Transport by Sodium. International Journal of Molecular Sciences. 2022;23(10):5732.

31. R Studio Team. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA 2020. Available from: http://www.rstudio.com/

32. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2020. Available from: https://www.R-project.org/

33. Poblete VE, Campos VI, González LU, Montenegro GL. Anatomical leaf adaptations in vascular plants of a salt marsh in the Atacama Desert (Chile). RevistaChilena de Historia Natural. 1991;64:65-75.

34. Naqinezhad A, Hamedani H. *Puccinelliapoecilantha* (Poaceae) in Iran. Phytotaxa. 2017;313(2):185-94.

35. Grigore MN, Toma C. Bulliform cells. InAnatomical Adaptations of Halophytes 2017 (pp. 325-338). Springer, Cham.

36. Thorogood D. Perennial ryegrass (*Loliumperenne* L.). Pages 75–105 in Casler M. D. and Duncan, R. R., eds. Turf grass Biology, Genetics, and Breeding 2003. John Wiley & Sons. New York, USA.

37. Anonymous. New South Wales Flora Online: *Puccinelliaciliata* Bor. PlantNET. 2014.https://plantnet.rbgsyd.nsw.gov.au/ [Date of access: 04.07.2022]