

### **Biological and Agronomic Characterization of Improved Alkali Grass Genotypes (*Puccinellia ciliata* Bor) in the Aegean region of Turkey**

#### **ABSTRACT**

*Puccinellia* is one of the plants with high performance in unfavourable soil conditions. Fourteen super genotypes developed by the recurrent selection method were grown at three different sowing times in order to characterize the alkali grass genotypes for biological and agronomic traits and to determine the relationships with seed yield in the Aegean region of Turkey in 2020. The interaction between sowing time and genotypes was found to be significant for all traits. The seed yield of the 14 *Puccinellia* genotypes varied between 28.9 kg ha<sup>-1</sup> and 90.2 kg ha<sup>-1</sup> according to the average of 3 sowing times, and the grand mean was 61.3 kg ha<sup>-1</sup>. Genotypes with high yielding were defined as thick-leaved (123.3±12.1), large bulliform cells (42.4±5.2), high chlorophyll content (5.7±0.3), large stomatal area (199.6±5.9) and fewer stomata (20.9±1.3). In conclusion, biological characteristics such as stoma area and number, leaf and bulliform structure and chlorophyll content should be used as selection criteria for production.

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

#### **1. INTRODUCTION**

Alkali grass (*Puccinellia ciliata* Bor) is a perennial grass species, widespread on saline and alkali lands under waterlogging conditions. The pH value in the areas where *Puccinellia* is 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) in the 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]. *Puccinellia* can be grown in saline soils and can be used for reclamation of these soils [6, 7]. Many studies demonstrated the beneficial role of *Puccinellia* species in environmental remediation of heavy metals contamination [8, 9]. *Puccinellia* species were identified as boron tolerant plants [10, 11] and its potential for boron phytoextraction purposes or pollution phytomanagement strategies was demonstrated.

Cell differentiation and developmental processes regulate leaf form in unfavourable conditions [12, 13, 14]. Adaxial bulliform cells of *Puccinellia* occur 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 used as a diagnostic tool for the nitrogen status of leaves in a plant [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 leaves for crop production and maintaining plant water balance are related to stomatal 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 kg ha<sup>-1</sup> [23]. The positive and significant correlations between seed yield and leaf colour, length and width were calculated in *Puccinellia nuttalliana* populations from the Canadian Great Plains [24]. Literature searches demonstrated that there are very few articles on seed yield and leaf characteristics of *Puccinellia* and the relationships among seed 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 *Puccinellia* populations which we have improved, by creating different climatic conditions with the sowing time.

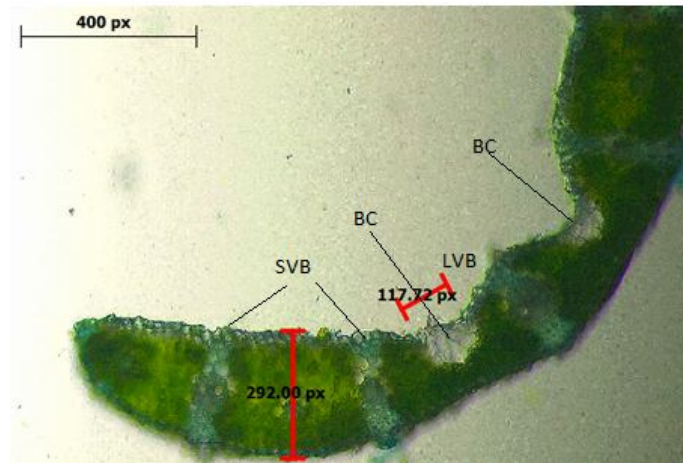
## 2. MATERIAL AND METHODS

Breeding studies were initiated to develop superior genotypes by recurrent selection on the *Puccinellia ciliata* populations collected from various regions having waterlogging, saline and severe alkalinity conditions in 2011. Seeds from fourteen populations were planted in 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) in research and experiment fields of Aydin Adnan Menderes University, Agricultural Faculty (37°75' N and 27°75' E). There were 50 plants in each pot. Nitrogen (36 kg ha<sup>-1</sup>) and phosphorus (92 kg ha<sup>-1</sup>) from diammonium phosphate was added at the rate of 200 kg ha<sup>-1</sup> at pot filling, and the pots were irrigated approximately every 10 days to replenish the lost moisture [38].

The climatic data showed that December and January of the experimental year had more precipitation than 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 [1].

**Figure 1.** Monthly average temperature and rainfall of 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  $\mu\text{m}^2$ ), 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  $\text{kg ha}^{-1}$  [23].



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



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

Variance analysis was performed among 14 populations and among 3 sowing times for each characteristic according to the split-plot design in randomized parcels with 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] and a 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 developed to include *Puccinellia* genotypes at each sowing time for observed characteristics (Figures 4, 5 and 6). The heat map for each sowing time displays variation among lines based on colour. The blue and red indicate the highest and lowest values for all the traits, respectively.

The seed yield of the 14 *Puccinellia* genotypes varied between 28.9 kg ha<sup>-1</sup> (genotype VI) and 90.2 kg ha<sup>-1</sup> (genotype XIII) according to the average of 3 sowing times, and the grand mean was 61.3 kg ha<sup>-1</sup>. The significant interaction between genotypes and sowing time for seed yield indicated that the genetic potential of *Puccinellia* genotypes 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 all sowing times (Figure 5), whereas the genotype X had a higher yield in the first and third sowing time (Figure 4). Some genotypes (II and VII) with medium yield are more successful in terms of yield in the first sowing time. The number of studies determining the seed yield after growing in the *Puccinellia* plant is very few. Seed yield of *Puccinellia distans* species varied between 17.5-25.4 g plant<sup>-1</sup> by vegetative propagation [29]. Also, the seed yield of *Puccinellia nuttallia* was determined as 12.9-33.6 g plant<sup>-1</sup> [24]. In our study, we determined that the average seed yield was 11.03 mg plant<sup>-1</sup>. 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 *Puccinellia* genotypes during different sowing times changed from 23.7 cm to 58.3 cm (Figures 4, 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 found 70.5-73.5 cm [24]. Plant height was 20-45 cm for *P. teyberi*, 20-50 cm for *P. convolute* and 35-100 cm for *P. festuciformis* [15]. These variations clearly indicated the differences between species and environment. We determined that the highest plant height was recorded in genotypes XIII, XIV, III and I of second sowing time (Figure 5). These values are followed by XI in the third sowing time (Figure 6), and XIII and VIII in the first sowing time (Figure 4).

**Table 1.** Analysis of variance for studied characteristics

	<b>SY</b>	<b>SPAD</b>	<b>BW</b>	<b>BL</b>	<b>SA</b>	<b>LT</b>	<b>SN</b>	<b>PH</b>	<b>PAL</b>	<b>BNPA</b>	<b>PI</b>	<b>Fv/Fm</b>
Genotypes (G)	**	**	**	**	**	**	**	ns	**	**	*	**
Sowing Time (ST)	ns	ns	ns	ns	*	ns	ns	ns	*	*	ns	ns
G x ST	**	**	**	**	**	**	**	**	**	**	**	**
LSD <sub>(0.05)G x ST</sub>	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.

0.8	6.11	4.2	6.7	25.8	26.6	81.1	205.4	31.2	23.2	4.3	81	XIV
0.81	7.52	4.5	15.7	52.7	18.9	123.5	207.7	51	52.6	5.4	96	XIII
0.78	5.8	10	15	46	18.5	134.2	188.6	42.2	49	6.2	96	XII
0.81	3.75	10.5	13.5	46.3	14.8	98.7	121.8	36.3	37.1	3.8	18.7	XI
0.81	8.16	8.8	13.7	47.7	21.9	143	181.9	56	44.7	6.1	98.5	X
0.81	5.57	10.7	13.7	40.7	11.8	93.6	122.2	35.9	40.5	3.4	49.2	IX
0.79	5.58	6.2	12.8	49.7	38.5	78	170.3	27.9	26.9	4.6	47	VIII
0.78	6.15	10.3	13.5	52	35.5	108.2	131.2	34.8	35.6	4.5	91.3	VII
0.81	8.04	6	13.3	40.7	32.6	103.8	80	27.4	27.3	3.1	41.7	VI
0.81	7.59	5.7	9	27.7	35.5	89	77.1	31.4	27.1	3.8	32.4	V
0.81	5.56	9	11.5	38	47.4	97.8	127	27.2	29.7	3.4	23	IV
0.78	5.94	1.5	13.5	23.8	17.8	147.2	177.3	25.5	24.8	5.4	56.3	III
0.8	6.82	7.7	12.8	36.3	29.6	86.2	119	30.8	32.7	5.2	87.7	II
0.79	3.7	13.8	12.3	38.8	32.6	84	161	37.7	37.8	4.4	55.5	I
Fv/Fm	PI	BNPA	PAL	PH	SN	LT	SA	BL	BW	SPAD	SY	

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 *Puccinellia* genotypes grown in the first sowing time.

Panicle length was 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 Bogdanović 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 sowing time of genotypes III and XIII (Figure 4), and in the first sowing time of genotypes XIII and XII (Figure 4), respectively. In terms of branch number in panicle, genotype XI in the third sowing time produced the highest value (Figure 6), whereas genotypes XIII and XIV in the first sowing 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 panicles, 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 sowing time is favourable for these characteristics.

0.79	6.19	8.2	12.8	55.7	17.8	83.2	157.1	27.7	24.8	4.8	53.3	XIV
0.8	6.09	12.7	15.8	58.3	18.4	129.9	160.8	48.7	50.5	4.7	85.8	XIII
0.79	5.7	2.5	8.5	30	18.6	137.7	223.6	39.8	50.9	6.6	85	XII
0.79	3.59	11	12.7	46	29.6	102	134.6	35.3	40.3	3.8	47.3	XI
0.8	8.53	6.3	11	47.3	21.3	148.1	249.8	52.5	42	6	88.6	X
0.79	5.63	8.5	11.8	46	17.8	92.5	163.3	34.9	43	3.2	46.8	IX
0.79	6.25	12.3	12.5	51.7	29.6	71	168.3	26.1	25.5	4	60	VIII
0.74	2.07	7.5	10.7	49.5	38.5	78.8	216.8	36.1	37.9	4.6	27	VII
0.8	4.8	6.5	10.8	40.7	32.6	81.9	79	25.7	30	3.1	22.3	VI
0.8	5.65	7.3	10.5	45	23.7	146.4	81.5	31.9	28.6	3.9	37.3	V
0.79	4.81	9	13.8	50.3	44.4	100.8	121	28.4	27.9	3.5	71.3	IV
0.78	6.18	8.8	18	54.7	26.6	73	195	26.6	26.5	5.1	77.3	III
0.79	5.97	9.8	13.7	46.7	17.8	84	237.4	30	33.4	4.8	56	II
0.78	8.51	7.3	12.8	51.7	26.6	83.3	178.1	38.9	34.9	4.7	61.3	I
Fv/Fm	PI	BNPA	PAL	PH	SN	LT	SA	BL	BW	SPAD	SY	

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 *Puccinellia* genotypes grown in the second sowing time.

The significant interactions between genotypes and sowing times for SPAD and leaf thickness indicated that changing climatic conditions during the growing season depending on the sowing time affected the genetic response of the genotypes (Table 1). SPAD values varied from 3.00 to 6.60, and the highest SPAD was recorded in all sowing times of genotype XII (Figures 4, 5 and 6). These values are followed by genotype X in the first (Figure 4) and third sowing time (Figure 6). It was demonstrated the significant differences among *Puccinellia* species for chlorophyll a, b and a:b ratio and the supremacy of the *P. pratensis* [30]. Similarly, genotype X had a high performance for leaf thickness in all sowing times (Figures 4, 5 and 6). Genotype X was followed by genotype III in the first sowing time, genotype V in the second sowing time and genotype XII in all sowing times. We detected the leaf thickness between 63.5 and 152.1  $\mu\text{m}$ . These values were thinner than the leaf thickness of *P. frigida* (233.3  $\mu\text{m}$ ) found in another study [33]. In our study, leaf thickness was determined between genotypes X and XII, which attracted attention with their thick leaves and high chlorophyll content.

0.78	6.76	10.8	11.7	50	26.6	80	242.9	29.5	23.6	4.6	57.3	XIV
0.8	7.05	9	11.2	43.7	18.6	126.6	183.9	49.5	50.9	5.6	88.9	XIII
0.81	5.12	10.8	12.5	49.3	18.8	134.7	217.4	43	50.3	6.5	87.4	XII
0.8	3.86	16.3	13.8	54.3	20.7	100.8	135.3	35.2	37.8	4	49.3	XI
0.79	8.1	8.7	10.2	41.7	21.6	152.1	204.4	54.8	42.4	6.2	89.5	X
0.8	6.18	5.7	8	39.7	14.8	97.8	162.4	36	41.1	3.5	47.8	IX
0.79	5.84	5	8.3	26.7	35.5	63.5	102.9	27.9	26.6	4.3	61.9	VIII
0.79	5.52	7.3	9.7	41.7	20.7	124.8	175.4	35.9	35.7	4.8	27.9	VII
0.81	7.71	7.8	8.5	36.1	38.5	102.9	79.5	26.2	28.4	3	22.9	VI
0.8	6.14	9.7	11.2	42	38.5	92.8	77.6	30.9	26.9	4.3	37.9	V
0.79	4.86	7.3	9.2	42.3	32.6	99.7	132.5	27.6	29.7	3.3	71.5	IV
0.81	6.03	10	11.7	47	29.6	90.6	204	26.2	24.6	5	78.6	III
0.8	6.27	6.8	9.2	40	32.6	87.1	152.6	31.5	34.1	5.1	56.9	II
0.8	3.86	10.3	8	29.1	23.7	84.8	225.1	37.2	37.3	4.3	62.7	I
Fv/Fm	PI	BNPA	PAL	PH	SN	LT	SA	BL	BW	SPAD	SY	

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.

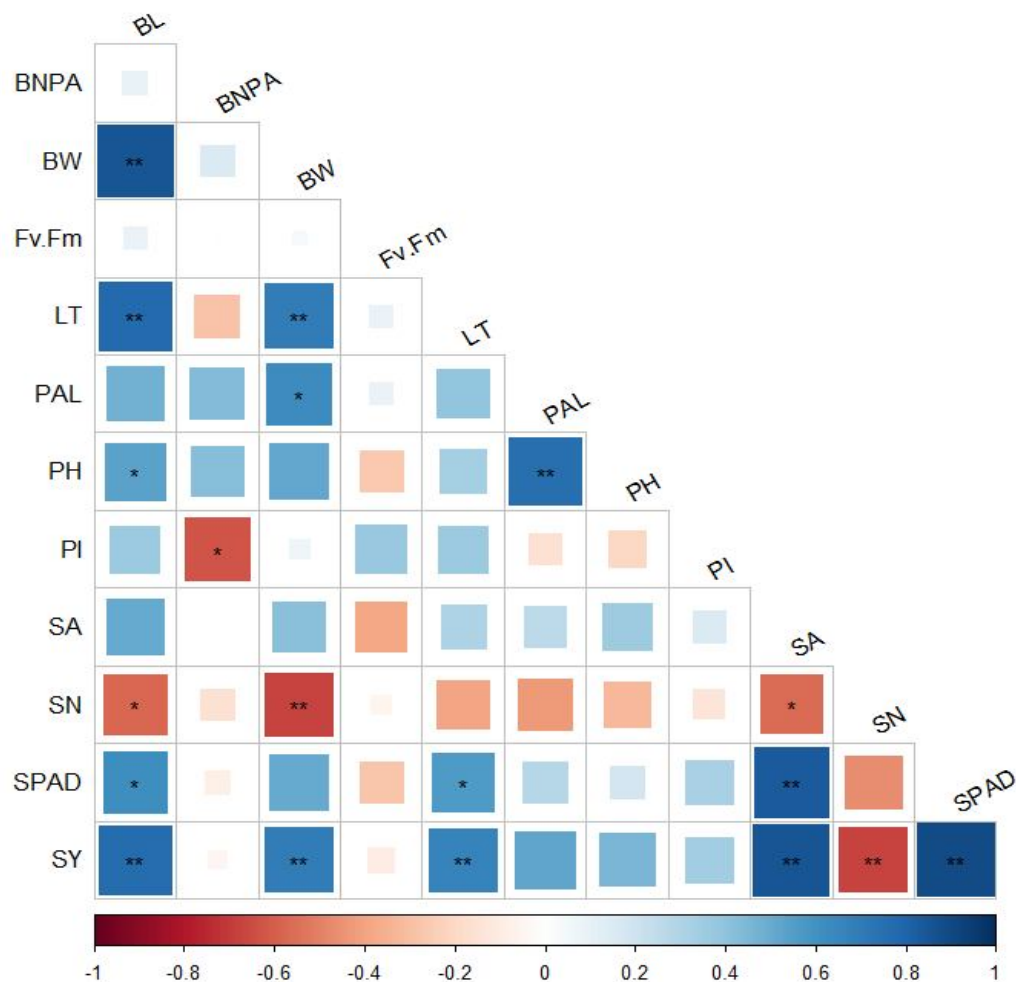
**Figure 6.** Mean values for observed characteristics of 14 *Puccinellia* genotypes grown in the third sowing time.

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 sowing time (Figure 4) and genotypes III, VI and XII in the third sowing 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 (Figures 4, 5 and 6).

It was expressed that the thickness of their bulliform cells in *P. poecilantha* and *P. bulbosa* occupied  $\frac{1}{4}$  of the leaf thickness and was 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 sowing times of genotype XIII for width and all sowing times of genotype X for length exhibited significantly the highest values (Figures 4, 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.

Figure 7 presented the characters significantly and positively correlated with seed yield in genotypes XIII, XII, X and III with high yielding. It was clearly seen that genotypes XIII, XII and X had superior values for seed yield and all characters, and this group is followed by genotype III.

Figure 7. Characters significantly and positively correlated with seed yield for productive genotypes such as XIII, XII, X and III.



\*: 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 8. Correlations among studied characteristics

Heatmap of correlation coefficients between seed yield, yield components and biological characteristics were summarized in Figure 8. The positive and significant correlations of seed yield with bulliform length ( $R^2=0.857$ ) and width ( $R^2=0.835$ ), leaf thickness ( $R^2=0.714$ ), stomatal area ( $R^2=0.746$ ) and SPAD ( $R^2=0.788$ ) indicated that genotypes having high yield could 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 genotypes with high yield. The negative correlations between the number of stomata per  $\mu\text{m}^2$  and bulliform length, width and stomatal area indicated that genotypes having small bulliform cells and narrow stomatal area exhibited more number of stomata in *Puccinellia*. Similarly, higher SPAD values were due to the increase in bulliform length, 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 stated that 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

Genotypes with superior seed yield and biological characteristics were identified in the present study. The seed yield was changed from 18.7 to – 98.5 kg ha<sup>-1</sup> depending on the sowing time. The sowing time of the 21<sup>st</sup> of October was more favourable for productive genotypes such as XIII, XII, X and III. The suggested genotypes and sowing time can be used successfully for *Puccinellia* cultivation. In addition, leaf morphological and biological 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 (*Puccinellia ciliata* bor) populations in Aydın 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 (*Gossypium hirsutum* 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 resource 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 *Puccinellia distans* and *Puccinellia nuttalliana* 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 *Puccinellia distans* 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 *Puccinellia distans* 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 *Puccinellia frigida* 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 crimp leaf character in rice (*Oryza sativa* L.) I. Leaf orientation, canopy structure and light distribution. *Acta Agron. Sin.* 2004;30:806–810.
15. Bogdanović S, Brullo S, Alegro AL, Rešetnik I, Mitić B. Taxonomic notes on *Puccinellia teyberi* (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 *Arundo formosana* 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 (*Oryza sativa* 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 (*Populus deltoides* ‘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 *Puccinellia distans* 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 *Puccinellia nuttalliana* 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, Venables B, 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. Bala devlet üreme çifliğinden alınan çorak çiminin (*Puccinellia distans* L.) diploid materyalinde morfolojik, sitolojik ve sitogenetik araştırmalar. 1981. TÜBİTAK, Ankara.
30. Vaziriyeganeh M, Carvajal M, Du N, Zwiazek JJ. Salinity Tolerance of Halophytic Grass *Puccinellia nuttalliana* 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). *Revista Chilena de Historia Natural*. 1991;64:65-75.
34. Naqinezhad A, Hamedani H. *Puccinellia poecilantha* (Poaceae) in Iran. *Phytotaxa*. 2017;313(2):185-94.
35. Grigore MN, Toma C. Bulliform cells. In *Anatomical Adaptations of Halophytes 2017* (pp. 325-338). Springer, Cham.
36. Thorogood D. Perennial ryegrass (*Lolium perenne* 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: *Puccinellia ciliata* Bor. PlantNET. 2014. <https://plantnet.rbgsyd.nsw.gov.au/> [Date of access: 04.07.2022]
38. Bouma, T. J., Koutstaal, B. P., Van Dongen, M., & Nielsen, K. L. (2001). Coping with low nutrient availability and inundation: root growth responses of three halophytic grass species from different elevations along a flooding gradient. *Oecologia*, 126(4), 472-481.