

### **Physiological and Agronomic Characterization of Improved Alkali Grass Genotypes (*Puccinellia ciliata* Bor)**

#### **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 physiological and agronomic traits and to determine the relationships with seed yield. The interaction between planting time and genotypes was found to be significant for all traits. The seed yield of the 14 Puccinellia genotypes varied between 28.2 kg ha<sup>-1</sup> and 96.6 kg ha<sup>-1</sup> and the grand mean was 61.3 kg ha<sup>-1</sup>. Genotypes with high yielding were defined as thick-leaved, large bulliform cells, high chlorophyll content, large stomatal area and fewer stomata. In conclusion, physiological characteristics such as stoma area and number, leaf and bulliform structure and chlorophyll content should be used as a selection criterion.

**Keywords:** Leaf anatomy, Puccinellia, relationships, 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 Na content (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 easily form a plant in salty soils and this is widely used in the improvement of are used [6, 7]. Many studies demonstrated the use of Puccinellia species in environmental remediation of heavy metals contamination [8, 9]. Puccinellia species was identified as boron tolerant plants [10, 11] and its potential for boron phytoextraction purposes or pollution phytomanagement strategies were demonstrated.

Cell differentiation and developmental processes regulated 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 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 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 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, in this study, we examined the yield and leaf characteristics of the *Puccinellia* populations improved by us by creating different climatic conditions with the planting time.

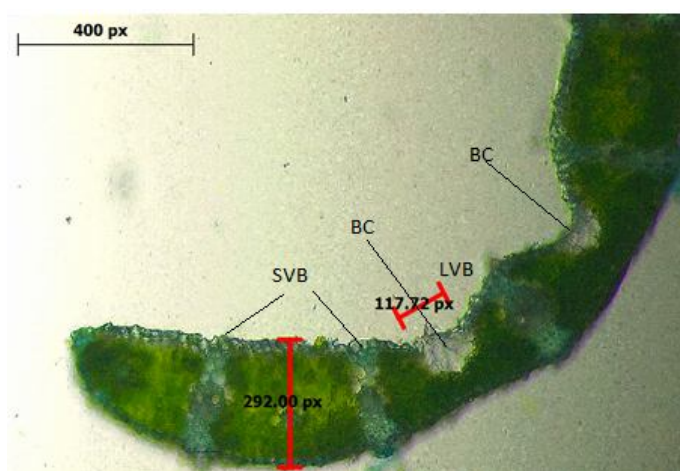
## 2. MATERIAL AND METHODS

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 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<sup>-1</sup>) and phosphorus (92 kg ha<sup>-1</sup>) from di-ammonium 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.

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.

**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, Konica Minolta 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  $\text{kg ha}^{-1}$ .



**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 according to [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 planting time for observed characteristics (Figures 4, 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 traits.

The seed yield of the 14 *Puccinellia* genotypes varied between 28.2 kg ha<sup>-1</sup> (genotype VI; Figure 4) and 96.6 kg ha<sup>-1</sup> (genotype XII; Figure 5), 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 XII 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 *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 planting 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 genetic potential. We determined that the highest plant height was recorded in genotypes XIII, XIV, III and I of 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

	<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 planting 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 planting time of genotypes III and XIII (Figure 4), and in the first planting time of genotypes XIII and XII (Figure 4), respectively. In terms of branch number in panicle, the genotype XI in the third planting time produced the highest value (Figure 6), whereas genotypes 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 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 planting 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	64.3	XIV
0.8	6.09	12.7	15.8	58.3	18.4	129.9	160.8	48.7	50.5	4.7	107.1	XIII
0.79	5.7	2.5	8.5	30	18.6	137.7	223.6	39.8	50.9	6.6	108.6	XII
0.79	3.59	11	12.7	46	29.6	102	134.6	35.3	40.3	3.8	92	XI
0.8	8.53	6.3	11	47.3	21.3	148.1	249.8	52.5	42	6	64.5	X
0.79	5.63	8.5	11.8	46	17.8	92.5	163.3	34.9	43	3.2	58.2	IX
0.79	6.25	12.3	12.5	51.7	29.6	71	168.3	26.1	25.5	4	40.3	VIII
0.74	2.07	7.5	10.7	49.5	38.5	78.8	216.8	36.1	37.9	4.6	0.5	VII
0.8	4.8	6.5	10.8	40.7	32.6	81.9	79	25.7	30	3.1	20.7	VI
0.8	5.65	7.3	10.5	45	23.7	146.4	81.5	31.9	28.6	3.9	42.4	V
0.79	4.81	9	13.8	50.3	44.4	100.8	121	28.4	27.9	3.5	31.1	IV
0.78	6.18	8.8	18	54.7	26.6	73	195	26.6	26.5	5.1	76.1	III
0.79	5.97	9.8	13.7	46.7	17.8	84	237.4	30	33.4	4.8	32	II
0.78	8.51	7.3	12.8	51.7	26.6	83.3	178.1	38.9	34.9	4.7	70.3	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 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 genotype XII (Figures 4, 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 *Puccinellia* species significant for chlorophyll a, b and a:b ratio and the supremacy of the *P. pratensis* [30]. Similarly, the genotype X highly performed for leaf thickness in all planting times (Figures 4, 5 and 6). Genotype III in first planting time, genotype V in second planting time and genotype XII in all planting time exhibited performance at the second and third levels. 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 XIII attracted attention with their thick leaves and high chlorophyll content.

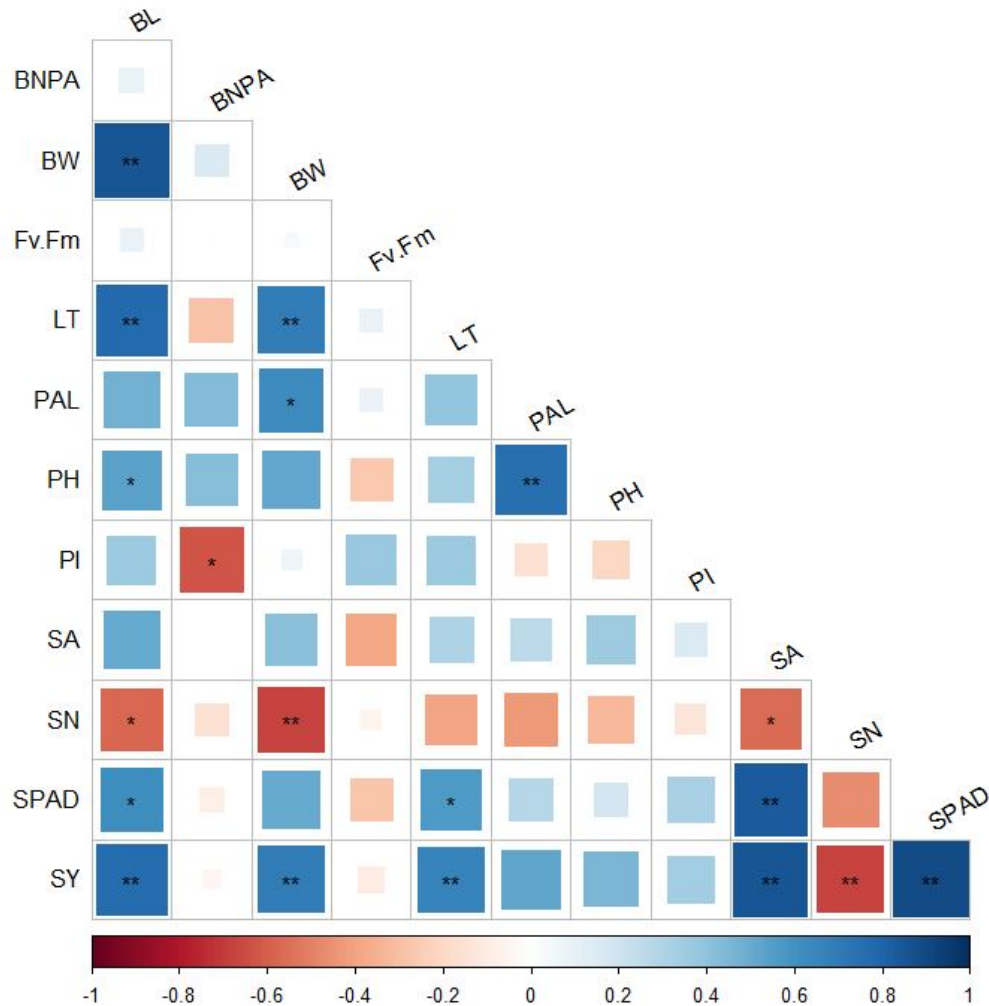
0.78	6.76	10.8	11.7	50	26.6	80	242.9	29.5	23.6	4.6	57.3	XIV
0.8	7.05	9	11.2	43.7	18.6	126.6	183.9	49.5	50.9	5.6	88.9	XIII
0.81	5.12	10.8	12.5	49.3	18.8	134.7	217.4	43	50.3	6.5	87.4	XII
0.8	3.86	16.3	13.8	54.3	20.7	100.8	135.3	35.2	37.8	4	49.3	XI
0.79	8.1	8.7	10.2	41.7	21.6	152.1	204.4	54.8	42.4	6.2	89.5	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 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 (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 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 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.



\*: 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.** A correlogram graph showing correlations among studied characteristics

Heatmap of correlation coefficients between seed yield, yield components and physiological characteristics were summarized in Figure 7. The positive and significant correlations of seed yield with bulliform length and width, leaf thickness, stomatal area and SPAD indicated that yielding genotypes 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 yielding genotypes. The negative correlations between the number of stomata per mm<sup>2</sup> 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 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

The present study demonstrated that the seed yield required establishing a new field of *Puccinellia* was 20.7-108.6 kg ha<sup>-1</sup> depending on the planting time. The planting time of 21 October was more favourable for productive genotypes such as XII, XIII, XI and III. Leaf physiological characteristics can be successfully used as selection criteria in the selection of productive genotypes and the breeding of *Puccinellia*.

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