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Determination of Some Characteristics of Perennial Ryegrass (Lolium perenne L.) Populations Collected from Natural Areas of Eskisehir for Breeding Purposes

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ABSTRACT: *The aim of the research is obtaining material and information for breeding of new varieties which can be used as turf and pasture plant in Central Anatolia and resembling regions. Some of the perennial ryegrass (**Lolium perenne** L.) seeds, collected from natural areas in 2010-2011, send with passport information to the Gene Banks in Ankara and Izmir. The rest of the seeds were grown in greenhouse and then transferred into field. In 2012, 17 populations were identified by observing (according to 1-9 and 1-5 scale) and measuring some characteristics. Then, mean and standard error values of the obtained data were determined. The mean values of the populations in main stem length, main stem thickness, flag leaf length, flag leaf width, number of nodes, internode length, growth pattern, rust resistance, winter resistance, tillering capacity, and leaf color changed between 30.2-48.7 cm, 1.9-2.8 mm, 5.7-10.8 cm, 3.3-4.9 mm, 2.6-3.7, 2.7-6.7 cm, 3.8-8.0, 3.8-7.9, 5.2-8.8, 5.1-9.0, and 3.9-5.0, respectively. The relations between the characteristics were examined by correlation analysis. Furthermore, ploidy levels of some single plants selected from the natural populations for breeding purposes were determined by flow cytometry analysis and all of these plants studied were found diploid.*

Key words: *Perennial ryegrass, **Lolium perenne** L. collection, breeding, correlation, ploidy.*

Eskişehir'de Doğal Alanlardan Toplanan Çok Yıllık Çim (Lolium perenne L.) Populasyonlarında Islah Yönünden Önem Taşıyan Bazı Özelliklerin Belirlenmesi

ÖZET: *Araştırmanın amacı, Orta Anadolu ve benzeri ekolojilerde yürütülen yeşil alan tesisi ve mera ıslah çalışmalarında kullanılabilecek yeni çeşitlerin geliştirilmesi çalışmalarına materyal ve bilgi üretmektir. 2010 ve 2011 yıllarında doğal alanlardan toplanan çok yıllık çim (**Lolium perenne L.**) tohumlarının bir kısmı durak bilgileri ile birlikte Ankara ve İzmir'deki Gen Bankalarına gönderilmiştir. Tohumların kalan kısmı serada fide haline getirildikten sonra araziye aktarılmıştır. 2012 yılında toplam 17 populasyon gözlem (1-9 ve 1-5 skalaları) ve ölçümlere tabi tutularak bazı özellikleri belirlenmiştir. Daha sonra verilerin ortalama ve standart hata değerleri saptanmıştır. Populasyon ortalama değerleri ana sap uzunluğunda 30,2-48,7 cm, ana sap kalınlığında 1.9-2.8 mm, bayrak yaprak boyunda 5,7-10,8 cm, bayrak yaprak eninde 3,3-4,9 mm, ana saptaki boğum sayısında 2,6-3,7 adet, ana saptaki boğum arası uzunluğunda 2,7-6,7 cm, büyüme şeklinde 3,8-8,0, pasa dayanıklılıkta 3,8-7,9, kışa dayanıklılıkta 5,2-8,8, kardeşlenme potansiyelinde 5,1-9,0 ve renkte 3,9-5,0 arasında değişmiştir. Özellikler arasındaki ilişkiler korelasyon analizi ile incelenmiştir. Ayrıca ıslah çalışmaları için seçilen tek bitkilerin ploidi düzeylerinin saptanması amacıyla flow sitometri yöntemi ile analiz gerçekleştirilmiş ve çalışılan tüm bitkilerin diploid olduğu belirlenmiştir.*

Anahtar sözcükler: *Çok yıllık çim, **Lolium perenne** L., toplama, ıslah, korelasyon, ploidi.*

INTRODUCTION

Although flora of Turkey is quite rich in terms of grasses used as turf and pasture plant, imported varieties are used for these purposes in general. There have been difficulties in adapting these varieties to different ecological conditions in the country (Oral and Acıkgoz, 2002). Perennial ryegrass (*Lolium perenne*) is a perennial cool season grass used as turf, pasture and forage plant. As a turf plant, it is widely used in establishing turf areas on sport and golf areas, parks, and gardens. The species germinates easily and covers the planting area quickly. Especially, the genotypes short and with thin leaves are extremely resistant to crushing. Origin of the species is Europe, Temperate Asia and North Africa. The use of imported varieties in turf area establishment in Turkey is an important problem for the country's agriculture and economy. Because turf areas are established at one time with intensive effort and expenditure, these are expected to benefit for a long time (Acıkgoz, 2001). Even in the pasture development studies in Turkey, foreign varieties are being used.

For these reasons domestic varieties are needed which are adaptable to different ecological conditions of the country. Collection and characterization of ecotypes widely found in natural areas of the country is an important resource for the development of ecologically adaptable varieties. It is reported that these natural ecotypes could be used in variety development studies using selection method (Tosun, 1973).

MATERIALS AND METHODS

In this research, there have been presented some observation and measurement data conducted in the ecotypes collected from natural areas to provide sources for breeding studies on turf and pasture type perennial ryegrass for Eskisehir and similar ecologies.

The collected materials were characterized in terms of some properties and then some single plants were selected for breeding purposes. Because ploidy levels should be known in breeding studies with the material collected from natural areas, these plants were subjected to ploidy analysis using flow cytometer method. Although perennial ryegrass is diploid naturally, tetraploid ones are found because of autopoliploidy in natural areas. Rapid and reliable results are obtained with flow cytometer in cases where the ploidy level of a large number of plants, such as plant genetic resources, need to be determined. The method is widely used in determining the DNA content and ploidy level of many turf grass species (Wang *et al*., 2009).

The collection and characterization studies were carried out in 2010, 2011 and 2012 in Eskisehir Province and at the Central Field of Transitional Zone Agricultural Research Institute. Climate data were given in the table below (Table 1). According to long term climate data, the area receives 347 mm precipitation and experiences an average monthly temperature 10.8 °C and 77.7% relative humidity. In the years our research was conducted, precipitation and average relative humidity were higher than long term data.

According to the analysis results of the soil taken from the germplasm area, experimental area is slightly alkaline, clayey, with medium limy, slightly salty, rich in potassium and rich in phosphorus (Table 2).

In the collection study in 2010, single plant seeds were collected from the stations determined for each 10 km considering altitude and direction in Eskisehir Province (Alan, 1986). In addition, they were collected in sheltered areas such as village cemeteries where these plants are concentrated. In the study, the factors such as soil type, soil slope, soil appearance, and their relation with vegetation were considered. Selections also were made according to the characteristics such as general appearance of plant (upright, decumbent etc.), color, and plant height. During the collection, information such as coordinates, altitude, region or local name and distance to the nearest settlement were recorded (Table 3).

The collected seeds divided into 3 parts and one part was sent to the Gene Banks in Izmir and Ankara with the station information data. One part has been preserved in the Institute. The rest of the seeds were grown in greenhouse and the seedlings transferred to field separately for each single plant. There were 20 plants for each single plant (population) collected from natural areas but some were lost due to winter and diseases.

These populations were subjected to the observations and measurements:

Main stem length (cm), main stem thickness (mm), flag leaf length (cm), flag leaf width (mm), number of nodes, internode length (cm), growth pattern (1 oblique - 9 upright), rust resistance (1 susceptible - 9 resistant), winter resistance (1 susceptible -9 resistant), tillering capacity (1 poor - 9 abundant), and leaf color (1 light green - 5 dark green) (Tosun, 1973; Sagsoz, 1974; Tokluoglu, 1979; Kornerup and Wanscher, 1978;Anonymous, 2016).

The obtained data were subjected to statistical analysis and population averages and standard errors were determined. Standard error values were determined to indicate whether there is variation between single plants in population. In addition, a correlation analysis was conducted to determine the relations between the characteristics (Kackar and Harville, 1984; Pearson, 1920).

Table 1. Monthly precipitation, mean temperature and relative humidity in Eskisehir, Turkey.
Çizelge 1. Eskişehir İli aylık toplam yağış, ortalama sıcaklık ve oransal nem değerleri, Türkiye.

Month Aylar	Precipitation (mm) Toplam yağış (mm)			Temperature (^{0}C) Ortalama sıcaklık (C^0)			Relative humidity (%) Ortalama oransal nem (%)		
	2010	2011	2012	2010	2011	2012	2010	2011	2012
January / Ocak	36.0	26.6	58.0	1.5	0.3	-3.6	95.2	97.0	96.8
February / Şubat	42.6	8.9	42.1	4.9	0.1	-5.5	90.5	93.2	97.3
March / Mart	32.6	20.0	56.4	5.9	3.7	1.5	85.5	88.0	87.7
April / Nisan	23.9	56.9	22.1	9.2	7.2	12.0	84.3	91.0	72.6
May / Mayıs	20.7	145.8	80.9	15.2	0.5	14.4	70.4	87.7	83.3
June / Haziran	79.0	9.4	0.0	18.1	16.6	20.0	82.8	84.6	71.6
July / Temmuz	7.4	8.5	5.5	22.0	21.6	22.8	75.4	70.8	68.1
August / Ağustos	0.9	0.0	3.5	24.4	20.0	20.8	66.2	73.5	65.1
September / Eylül	22.5	2.1	0.0	18.2	17.4	18.7	75.8	68.5	66.1
November / Ekim	77.1	57.9	16.1	10.0	8.5	14.2	92.5	83.6	78.1
October / Kasım	7.5	0.0	14.5	9.3	0.8	7.3	81.0	86.8	92.3
December / Aralık	60.4	46.1	73.2	4.2	0.9	2.2	94.1	92.1	95.1
Total / Toplam	410.6	382.2	372.3						
Mean / Ortalama				11.9	8.1	11.0	82.8	84.7	81.2

*Meteorological Station of Transitional Zone Agricultural Research Institute.
*Geçit Kuşağı Tarımsal Araştırma Enstitüsü Meteoroloji İstasyonu.

Table 2. Germplasm area soil analysis.
Çizelge 2. Gözlem bahçesi toprak analizi.

Soil Structure Toprak yapısı	pH	Total Salt (%) Toplam tuz (%)	Lime (CaCO3) (%) Kireç (%)	Organic matter (%) Organik Madde (%)	Phosphorus (kg P_2O_5/da) Alınabilir fosfor (kg P_2O_5/da)	Potassium (kg K_2O/da) Alınabilir potasyum (kg K_2O/da)
clayey killi	7.7	0.153	6.6	1.28	13.0	199.5

*Soil and Water Laboratory of Transitional Zone Agricultural Research Institute.
*Geçit Kuşağı Tarımsal Araştırma Enstitüsü Toprak ve Su Laboratuvarı.

Table 3. Collection site information.
Çizelge 3. Toplama yapılan yöre bilgileri.

Population number Populasyon numarası	District İlçe	Collection site Toplanan yöre	Latitude Enlem	Longitude Boylam	Altitude Yükseklik (m)
42	Sarıcakaya	3 km from Hekimdagı	39.90996 °N	30.62989 °E	1230
43	Han	Center-Cemetery	39.15439 °N	30.86392 °E	1224
44	Sivrihisar	Selimiye Village-Grassland	39.36983 °N	31.29889 °E	843
45	İnonu	Between Asagı Kuzfındık and Yukarı Kuzfındık	39.67896 °N	30.06833 °E	1066
46	İnonu	Between Asağı Kuzfındık and Yukarı Kuzfındık-Village Center	39.67738 °N	30.07262 °E	1075
47	İnonu	Oklubalı Village-Cemetery	39.81717 °N	30.24116 °E	831
48	Sivrihisar	Gerenli-Grassland	39.38882 °N	31.24430 °E	843
49	İnonu	Cemetery	39.81391 °N	30.12539 °E	842
50	Han	Gokcekuyu Village-Cemetery	39.24546 °N	30.83020 °E	1110
51	İnonu	Between Aşagı Kuzfındık and Yukarı Kuzfındık	39.67896 °N	30.06833 °E	1066
110	Merkez	Kargın-Cemetery	39.58781 °N	30.22781 °E	887
111	Gunyuzu	Ertugrul-Cemetery	39.29004 °N	31.59472 °E	863
112	Seyitgazi	Yazılıkaya-In front of the Monument	39.20018 °N	30.71504 °E	1292
113	Merkez	Avdan Village-Cemetery	39.56622 °N	30.49832 °E	1109
114	Tepebası	Taycılar-Cemetery	39.98288 °N	30.91326 °E	1104
115	Merkez	Uludere-Cemetery	39.91566 °N	30.33850 °E	1025
116	Seyitgazi	Bessaray Village-Cemetery	39.43322 °N	30.53410 °E	1028

The selected plants for breeding purposes according to the observations and measurements were subjected to a ploidy analysis using flow cytometer method in 2015. In the method, ploidy levels are determined using the core DNA content of plants (Wang *et al*., 2009). Flow cytometry device in Trakya University, Agricultural Faculty, Field Crops Department was used in the analysis which determines DNA content accurately.

RESULTS

The mean values and standard errors of the characteristics of 17 perennial ryegrass populations are given below (Table 4). As stated before, the number of plants in each population which initially were 20 decreased due to winter and disease damage. At the end of the observations and measurements, the population mean values in main stem length, main stem thickness, flag leaf length, flag leaf width, number of nodes, internode length, growth pattern, rust resistance, winter resistance, tillering capacity, and leaf color changed between 30.2-48.7 cm, 1.9-2.8 mm, 5.7-10.8 cm, 3.3-4.9 mm, 2.6-3.7, 2.7-6.7 cm, 3.8-8.0, 3.8-7.9, 5.2-8.8, 5.1-9.0, and 3.9-5.0, respectively (Table 4).

According to the results of the correlation analysis, there were some remarkable relationships between tillering capacity which is important for turf area and pasture establishment and some other characteristics (Table 5). While there are significant ($p<0.01$) and positive correlations between tillering capacity and internode length (0.642^{**}), tillering capacity and growth pattern (0.789^{**}), tillering capacity and winter resistance (0.980^{**}), there are significant ($p<0.01$) and negative correlations between tillering capacity and main stem length (-0.665^{**}), tillering capacity and number of nodes (-0.841^{**}). Some correlations between winter resistance and some other properties are also notable. While there are significant ($p<0.01$) and positive correlations between winter resistance

and internode length (0.636**), winter resistance and growth pattern (0.731**), there are significant (p<0.01) and negative correlations between winter resistance and number of nodes (-0.883**), winter resistance and main stem length (-0.636**)

As a result of the observations and measurements, some single plants were selected from populations for use in variety development studies. These plants were subjected to a ploidy analysis and they all were found diploid (Table 6).

Table 4. The mean values and standard errors of the perennial ryegrass populations.
Çizelge 4. Çok yıllık çim populasyonlarına ait ortalama ve standart hata değerleri.

*pop. no *pop. num.	plan. num. bit. say.	fll byb	flw bye	msl asu	mst ask	nn asbs	il asbau	c renk	gp bş	rr phd	wr kd	tc kp
42	8	8.0±0.7	3.9±0.4	37.1±2.8	1.9±0.2	2.9±0.2	5.8±0.8	4.4±0.2	6.1±0.3	6.3±0.2	8.8±0.2	8.9±0.1
43	13	8.9±0.7	3.4±0.2	38.3±1.6	2.5±0.1	2.8±0.1	5.6±0.3	3.9±0.1	7.8±0.1	4.8±0.6	8.4±0.1	9.0±0.0
44	4	10.5±0.7	4.8±0.3	36.1±2.5	3.0±0.0	3.0±0.0	5.9±0.7	4.0±0.0	7.8±0.3	6.5±0.3	8.0±0.4	8.5±0.5
45	5	8.0±0.5	3.7±0.2	37.1±4.3	2.2±0.2	3.0±0.0	4.7±0.6	4.0±0.0	7.6±0.2	6.0±0.0	8.0±0.0	9.0±0.0
46	8	7.8±0.7	3.9±0.3	31.8±2.5	2.1±0.1	2.6±0.3	4.3±1.0	5.0±0.0	5.5±0.3	6.0±0.0	8.5±0.2	9.0±0.0
47	7	10.8±1.0	4.6±0.4	37.6±1.8	2.4±0.2	2.9±0.1	4.9±0.4	4.0±0.0	7.1±0.1	5.9±0.3	8.3±0.3	8.7±0.3
48	7	8.4±0.9	3.9±0.3	30.2±2.3	2.1±0.1	2.7±0.2	3.9±0.5	3.9±0.1	6.4±0.4	5.0±0.0	8.3±0.2	9.0±0.0
49	9	7.8±0.8	3.7±0.3	33.4±1.8	2.0±0.0	2.8±0.2	4.8±0.7	4.0±0.0	7.3±0.2	5.8±0.3	8.0±0.0	9.0±0.0
50	5	10.3±1.4	4.0±0.6	41.0±3.3	2.2±0.2	2.6±0.2	6.7±0.4	4.2±0.2	6.2±0.6	3.8±0.5	8.2±0.2	8.8±0.2
51	4	7.9±1.9	3.3±0.3	32.5±2.7	2.8±0.3	2.8±0.3	5.4±0.6	4.0±0.0	8.0±0.0	6.0±0.0	8.0±0.0	9.0±0.0
110	17	8.0±0.7	4.5±0.7	41.9±2.0	2.4±0.1	3.4±0.1	4.3±0.4	3.9±0.1	3.9±0.1	7.9±0.2	5.6±0.2	5.5±0.1
111	14	8.8±0.9	3.4±0.3	39.1±1.6	1.9±0.2	3.1±0.1	3.6±0.3	3.9±0.1	3.9±0.3	7.1±0.1	5.2±0.2	5.1±0.2
112	15	8.5±0.6	4.3±0.1	43.4±1.1	2.1±0.1	3.7±0.1	2.9±0.3	3.9±0.1	3.9±0.2	7.0±0.0	5.4±0.3	5.5±0.2
113	18	5.7±0.4	3.8±0.2	36.5±1.8	2.1±0.1	3.3±0.1	2.7±0.2	4.1±0.1	5.6±0.1	7.0±0.0	5.6±0.2	5.4±0.3
114	20	7.8±0.5	4.9±0.2	48.7±2.1	2.7±0.1	3.6±0.1	4.4±0.5	4.2±0.1	3.8±0.1	7.1±0.1	6.3±0.2	6.0±0.2
115	20	6.8±0.4	4.2±0.2	44.1±1.5	2.3±0.1	3.4±0.2	5.2±0.3	4.2±0.1	5.1±0.1	7.9±0.1	5.8±0.2	6.5±0.1
116	20	8.7±0.2	4.1±0.1	45.1±1.7	2.2±0.1	3.2±0.1	4.5±0.6	4.1±0.1	6.5±0.1	7.0±0.0	5.8±0.2	5.9±0.2

*pop. no: population numbers, plan. num.: the plant number in population, fll: flag leaf length, flw: flag leaf width, msl: main stem length, mst: main stem thickness, nn: number of nodes, il: internode length, c: leaf color, gp: growth pattern, rr: rust resistance, wr: winter resistance, tc: tillering capacity.

* pop. num.: populasyon numarası, bit. say.: populasyon içindeki bitki sayısı, byb: bayrak yaprak boyu, bye: bayrak yaprak eni, asu: ana sap uzunluğu, ask: ana sap kalınlığı, asbs: ana sapta boğum sayısı, asbau: ana sapta boğum arası uzunluğu, renk: yaprak rengi, bş: büyüme şekli, phd: pas hastalıklarına dayanıklılık, kd: kışa dayanıklılık, kp: kardeşlenme potansiyeli.

Table 5. Correlation coefficients-r (n=17).
Çizelge 5. Korelasyon katsayıları-r (n=17).

Character Karakter	msl asu	mst ask	fll byb	flw bye	il asbau	nn asbs	gp bş	wr kd	rr phd	tc kp	c renk
msl	-	0.123	0.001	0.489*	-0.042	0.754**	-0.564*	-0.636**	0.469	-0.665**	-0.142
mst			0.302	0.398	0.383	0.095	0.315	0.130	0.039	0.138	-0.192
fll				0.294	0.507*	-0.348	0.306	0.386	-0.465	0.352	-0.201
flw					0.018	0.460	-0.367	-0.210	0.346	-0.293	0.058
il						-0.528*	0.547*	0.636**	-0.495*	0.642**	0.133
nn							-0.668**	-0.833**	0.802**	-0.841**	-0.286
gp								0.731**	-0.580*	0.789**	-0.103
wr									-0.764**	0.980**	0.294
rr										-0.761**	-0.045
tc											0.232
c											-

**(p<0.01), *(p<0.05).

Table 6. Ploidy analysis results.
Çizelge 6. Ploidi analizi sonuçları.

Population-single plant no Populasyon-tek bitki no	DNA content (pg) DNA içeriği (pg)	Ploidy level Ploidi düzeyi
113-4	5.46	diploid
114-2	5.51	diploid
114-4	5.43	diploid
114-17	5.42	diploid
114-15	5.48	diploid
113-18	5.44	diploid
113-9	5.51	diploid
113-4	5.46	diploid
42-12	5.48	diploid
42-17	5.43	diploid
43-1	5.44	diploid
43-2	5.47	diploid
43-15	5.39	diploid
45-5	5.43	diploid
45-6	5.46	diploid
46-12	5.49	diploid
46-18	5.49	diploid
47-1	5.58	diploid
47-5	5.57	diploid
47-6	5.49	diploid
48-14	5.48	diploid
49-1	5.39	diploid
49-16	5.39	diploid
50-7	5.55	diploid
51-1	5.53	diploid
51-2	5.56	diploid

CONCLUSION

In this collection and characterization study, when the mean and standard error values in the populations are examined, it appears that there is sufficient variation for breeding studies. In addition, according to the results of the correlation analysis, some important relationships were identified in terms of tillering capacity and winter resistance which are important for turf area and pasture establishment. Positive correlations between tillering capacity and internode length (0.642^{**}), tillering capacity and growth pattern (0.789^{**}), tillering capacity and winter resistance (0.980^{**}) and negative correlations between tillering capacity and main stem length (-0.665^{**}), tillering capacity and number of nodes (-0.841^{**}) were statistically significant. Positive correlations between winter resistance and internode length (0.636^{**}), winter resistance and growth pattern (0.731^{**}) and negative correlations between winter resistance and number of nodes (-0.883^{**}), winter resistance and main stem length (-0.636^{**}) were also notable.

The material and information obtained from the collection and characterization studies will be used in variety development studies of turf, pasture and forage type perennial ryegrass (*Lolium perenne* L.) in Transitional Zone Agricultural Research Institute.

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