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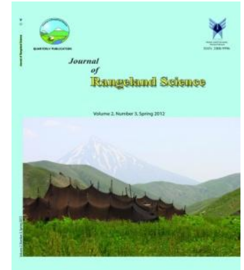

Full Length Article:

Variability and Correlation between the Seed Yield and its Component in Alfalfa (*Medicago sativa* L.) Populations under Dry Land Farming System, Hamadan, Iran

Ali Ashraf Jafari[A], Milad Lak[B], Ghasem Assadian[C]

[A]Prof. Research Institute of Forests and Rangelands, Tehran, Iran. Email: aajafari@rifr-ac.ir. (Corresponding author)
[B]Islamic Azad University, Borujerd Branch, Iran
[C]Agricultural Research Center, Hamadan, Iran

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Abstract. In order to study the variation for seed yield and its components, 200 accessions of alfalfa (*Medicago sativa* L.) were sown as drilled plots, using alpha designs/unreplicated with two repeated entries with in all of 10 blocks under dry land farming system in Kabodarahang Research Station, Hamadan, Iran, during 2010 to 2011. Data were analyzed for descriptive statistics, correlation, regression, path analysis, factor analysis and cluster analysis. Results showed considerable variation for all of traits. The best 20 entries were identified. Among them, Cody, Kerissary, Italian, Australian, Hamadani, Bami, Jirofti and Faridonshahr with average values of 900 to 1250 kg ha^{-1} forage yield and 85 to 107 kg ha^{-1} seed yield were introduced for breeding improved synthetic varieties. Results of correlation analysis showed that DM yield was positively correlated with node number, plant height, stem number, vegetation score, seed yield, and pod weight. Similarly, seed yield had positive correlation with pod weight, DM yield, vegetation score and plant height. Results of path analysis showed that DM yield and pod yield had higher direct and total effects on seed yield. Results of factor analysis were accounted for 77% of total variation for first five factors. Factor 1 which was accounted for 25% of variation was associated with DM yield, seed yield and pod yield. This factor was regarded as productive factor. Factor 2 accounted for 14% of variation was known as stem elongation factor, since it was included for plant height and node number. Based on ward cluster analysis method, 200 entries were divided into 5 groups. Accessions in cluster 4 were averaged well above the overall mean for DM and seed yield. The results of this study indicated that selection of variables in productivity factor (factor1 and cluster 4) could enable breeders to release the desirable increment in forage yield of alfalfa.

Key words: Alfalfa (*Medicago sativa*), Seed, DM yield, Path analysis, Factor analysis, Cluster analysis.

Introduction

Alfalfa (*Medicago sativa*) is originated from Iran and it is one of the most important forage species with cultivated area 600.000 ha with average annual 7200 Kg ha^{-1} DM yield. Alfalfa is grown in arid and semiarid regions for forage and requires large amount of water for high production. Therefore, improvement of alfalfa under dry land farming system could be effective to achieve varieties for profitable production in these zones. Although, drought reduces the herbage yield of all legumes, alfalfa has the greatest yield potential in drought (Peterson *et al.*, 1992).

In alfalfa breeding programs for improved DM yield, it is very important the knowledge of new varieties seed production and its relationships with forage yield. Knowledge of correlations between traits of interest is useful in designing an effective breeding program for a crop. Complex plant characters, such as yield, are quantitatively inherited and influenced by genetic as well as genotype by environment interaction effects. Therefore, selection for seed or DM yield alone may not be the best criterion to improve seed or/and DM production. Hence, it is also important to study correlations between agronomic traits, which may have high heritability, and yield, which has low heritability (Falconer and Mackay, 1996). Moreover, assessment of divergence genetic materials is also vital to success of designed to exploit gene recombination. Strong positive relationships have been found between genetic distance and hetrosis in broad range of crop species (Humphreys, 1991). Measure of genetic distance should have more value to breeding when based on a broad range of traits relevant to breeding objectives.

Mousavi *et al.,* (2010) in study of genetic diversity in 140 domestic and foreign ecotypes of alfalfa, found positive correlations between DM yield with both stem number and plant height. They grouped ecotypes into 8 clusters. However, their cluster analysis did not separate Iranian ecotypes from foreign ecotypes. Bolanos-Aguilar *et al.,* (2002) found positive correlated between seed yield with both DM yield (r=0.94) and pods weight (r=0.91). They suggested that pods seed weight appeared to have a strong genetic association with seed yield. In contrast, Benabderrahim *et al.,* (2009) showed that seed yield is negatively correlated to DM yield (-0.31). Similarly, Huyghe *et al.,* (2001) found no genetic correlation between seed yield and the DM yield suggesting that breeding for a higher seed yield should not decrease the forage production.

Bolaños-Aguilar *et al.,* (2000) showed that seed weight per inflorescence had high correlations with seed yield and suggested the possibility of using the seed weight per pod as a selection criterion. Sengul and Sengul (2006) found significant correlation of seed yield with plant height, pods number, plant height and suggested that plant height may be good selection criteria to improve seed yield of alfalfa cultivars. DM yield were positively correlated with those for plant height, stem number, node number, and vegetation score (Jafari *et al.*, 2003; Davodi *et al*., 2011; Riday and Brummer, 2004). Both stem number and plant height were positively correlated with vegetation score (Davodi *et al*., 2011).

Alfalfa has an important role in forage production in Iran, little breeding work has been done on alfalfa seed production under dry land condition. Rausch (1964) reported a range from 0 to 44 g of seed per plant measured on 1301 individual plants of alfalfa. Bolaños-Aguilar *et al.,* (2002) in evaluation of 12 cultivars of alfalfa at four locations across France found mean seed yield 801 kg ha^{-1}. They obtained the lowest seed yields in the establishing year. Jafari

(2010) in irrigation trial in Karaj, Iran, reported mean seed yield of 700 and 370 kg ha^{-1} for spaced plants and sward.

This research project was conducted to determine the pattern of variation and correlation between seed yield and its components in 200 alfalfa populations under dry land farming system, and to classify the germplasm into similar groups and to identify genetic distance of ecotypes in order to exploit heterosis in the breeding programs.

Materials and Methods

A total of 200 accessions of alfalfa (*Medicago sativa* L.) provided from natural resource gene bank based in research institute of forests and rangelands of Iran were examined. Due to a great number of evaluated accessions, the alpha design was used (Patterson and Williams, 1976). Seeds were sown as drilled plot under dry land farming system using alpha designs/ unreplicated with 2 repeated entries within all of 10 blocks in, Hamadan, Iran, during 2008 to 2010 year.

From total accessions, 155 accessions were domestic and 45 foreign accessions originate from Italy, Hungary, Turkey, France, USA, Kazakhstan, and Australia. In the establishment year no measurement was taken. The evaluation for DM yield and quality traits were carried out in the second year 2009 (Davodi *et al.*, 2011). The data of present study were collected for the only cut in the third year of experiment for the following traits:

1) Plant height (cm) was average of ten measurements taken on random plants as they stood naturally in the field.
2) Stem number was measured as the number of tillers in a 25×25 cm frame.
3) Vegetation score in each plot was recorded visually from 1 to 5, while 1 was very week green cover and 5 was the highest growth rate during growth seasons.
4) Node number was average of ten measurements taken on random stems per plot.
5) Plant diameter (cm) was measured the diameters of five random spaced plants per plot.
6) DM yield was determined by cutting each plot. Then the samples were air dried and DM yield was expressed as Kg ha^{-1}.
7) Pod weight was determined by cutting each plot, then they were air-dried, the pods were separated and weighted as g $plant^{-1}$.
8) Seed yield was determined by cutting each plot, then they were air-dried, threshed and the seed were cleaned and weighted as Kg ha^{-1}.

All of the 200 accessions (unreplicated design with 2 control entries) were adjusted by using the mean yield of the neighboring checks and Agrobase Software (Mulitze, 2004). Phenotypic correlation was determined between traits. Estimation of factor loading was based on data averaged over replications on 12 characteristics of 200 genotypes using MINITAB 16. The numbers of factors were estimated using principal components extraction and varimax rotation method. The factor loading of rotated matrix, the percentage variability explained by each factor and the commonalties for each variable were determined.

Results and Discussion

In order to control the soil fertility gradients, an analysis of variance was made among 10 incomplete blocks (two control varieties). The results showed no significant differences among blocks for all of traits except for DM yield and vegetation score characteristics (Table 1). This indicated that there were similar soil fertility slops for all of 200 entries. However, all of the 200 accessions were adjusted by using the mean yield of the neighboring checks and Agrobase Software (Mulitze, 2004). All of multivariate analyses were made on adjusted data. Results of statistics parameter as (mean, maximum, minimum, standard error, standard deviation and Coefficient of Variation CV%) on average values of morphological traits and seed yield of 200 accessions of alfalfa are shown in (Table 2). The Coefficient of Variation (CV %) was high for Pod weight and Seed yield (Table 2). It was found that DM yield variation ranged from 225 to 1214 Kg ha^{-1} with average values of 554 Kg ha^{-1} (Table 2). Seed yield variation ranged from 0 to 109.1 with mean of 31.03 (Table 1). These values indicate a good variation of DM, seed yield and other agronomic traits in alfalfa genetic resources. The published data for seed yield are higher than present study. For example, Rausch (1964) reported a range from 0 to 44 g of seed per plant (equal to 0 to 440 kg ha^{-1}). Bolaños-Aguilar *et al.*, (2002) in France found mean seed yield of 801 kg ha^{-1} and another trial Bolaños-Aguilar *et al.,* (2000) found the large range of variation for seed yield among 214 genotypes a range of 0.30 to 30.75 g of seed per plant. More recently Jafari (2010) in irrigation trial in Karaj, Iran, reported mean seed yield of 700 and 370 kg ha^{-1} for spaced pants and sward. The reason of lower seed yield in present study is due to dry land farming condition and relatively lower precipitation in the growing season than annual precipitation (330 mm). The means of 20 superior accessions out of 200 accessions for both seed and DM yield are present in (Table 3). Results showed a vast heterogeneity among accessions during seasonal growing under dry land faming system for all of examined traits. Three foreign accessions as Cody, Sarborideh and Kerissary with average values (1215, 1145 and 965 Kg ha^{-1}) and (94.7, 107.6 and 98.9 Kg ha^{-1}) and three domestic accessions as: Bami, Jirofti and 30 year-fridan with average values of (1188, 1024 and 1007 Kg ha^{-1}) and (104.0, 87.1 and 84.5 Kg ha^{-1}) had the highest values of DM yield and seed yield, respectively.

Phenotypic correlations coefficients were positive and significant among DM yield with those for plant height, stem number, node number and vegetation score, pod weight and seed yield. Jafari *et al.*, (2003) and Riday and Brummer (2004) and Davodi *et al.*, (2011) found the same relationships among DM yield with those for plant height, stem number, node number and vegetation score and suggested that these yield components may be good selection criteria to improve DM yield of alfalfa.

The same trends as DM yield were observed for relationships among vegetation score and plant height with the other traits. Both traits were positively correlated with stem number, pod weight, node number and seed yield (Table 4). This result was in agreement with Davodi *et al.*, (2011) that found positive correlations between stem number and plant height and vegetation score.

Seed yield were positively correlated with DM yield, vegetation score, plant height, node number and pod weight (Table 4). Bolaños-Aguilar *et al.*, (2002) found positive correlated between seed yield with both DM yield and pods weight. In contrast, Benabderrahim *et al.*, (2009) found negative correlation and Huyghe *et al.*, (2001) found no correlations, between seed yield and DM yield. Bolaños-Aguilar *et al.*, (2000) and Sengul and Sengul (2006) found positive and significant correlation between seed yield and pods number.

The results of stepwise regression analysis for seed as dependant and other traits as independent variables showed significant effects of pod weight, DM yield, vegetation score, stem number and plant height on seed yield. There was a good agreement between the results of correlation and regression analysis. Results of path analysis for seed yield as dependent variable indicated that DM yield and pod weight had positively direct effect and plant height indirect effect on seed yield. Sengul and Sengul (2006) found significant correlation of seed yield with plant height, pods number. There results of present study indicated that positive direct effect of DM yield and pod weight suggested that these yield components may be good selection criteria to improve seed yield of alfalfa cultivars.

The factor analysis was performed based on principal components extraction and varimax rotation method. Table 6 shows the contribution of different significant characteristics to each factor. The axes represented 77% of total variance for five first factors. Factor 1, which accounted for 25% of variation, was associated with pod weight, seed yield and DM yield. This factor was regarded as productivity factor. Factor 2 which accounted for 14% of variation was named as canopy height factor since it related to node number and, plant height. For the third factor (stem number) forth factor (plant diameter) and fifth factor (vegetation score) important characters.

Based on ward cluster analysis, 200 entries were divided into 5 groups (Fig. 1 and Table 8). Accessions in clusters 4 averaged well above the overall mean for all of traits except stem number and plant diameter. This cluster was ranked as higher for both seed and forage production. The results of cluster analysis did not well group the populations with different geographical origin in the same cluster. Alfalfa is believed to belong to Caucasus region: northeastern Turkey, Turkmenistan and north western Iran; thus, it can be supposed that the European ecotypes have been introduced from Iran and adapted to the climate conditions of Europe countries. In similar results, Tucak *et al.*, (2009) using cluster analysis of 27 populations of alfalfa form different region, in most cases found no agreement between geographical originated areas and grouping in the same clusters. The results of this study indicated that selection of variables in productivity factor (factor 1) could enable breeders to release the desirable increment in forage yield of alfalfa. The main positive traits determining the seed yield in alfalfa growing in dry land farming system were pods weight DM yield followed by plant height.

Table 1. Results of analysis of variance among check genotypes (n=24) to control block soil fertility gradients

S.O.V.	DF	Vegetation Score	Plant Height	Stem No.	Plant Diameter	DM Yield	Pod Weight	Seed Yield	Node No.
Between blocks	11	0.742^{ns}	50.7^{ns}	225^{ns}	22.4^{ns}	42404^{ns}	1899^{ns}	268^{ns}	3.21^{ns}
Error	12	0.856	34.3	106	19.3	46345	2576	296	3.58

ns=there is no significant differences between blocks.

Table 2. Mean, maximum, minimum, standard error, standard deviation and Coefficient of Variation (CV%) on average values of morphological traits and seed yield of 200 accessions of alfalfa

Trait name	Mean	MIN	MAX	SE	SD	CV (%)
DM yield (Kg ha^{-1})	554.0	225.3	1214.9	14.0	217.2	39.2
Vegetation score	2.02	0.62	4.71	0.05	0.76	37.8
Plant height (cm)	28.78	18.4	46.1	0.35	5.46	19.0
Stem number	89.94	61.8	124.7	0.88	13.63	15.2
Plant diameter (cm)	24.61	10.7	36.9	0.35	5.38	21.9
Pod weight (g)	72.65	5.00	400.0	5.36	83.10	94.4
Seed yield (Kg ha^{-1})	31.03	0.00	109.1	1.35	20.94	67.5
Node number	8.10	3.84	13.98	0.14	2.23	27.5

Table 3. Means comparison among 20 superior genotypes of alfalfa for both seed and DM yield out of 200 accessions of alfalfa

Genotypes Name	Origin	DM Yield (Kg ha^{-1})	Seed Yield (Kg ha^{-1})	Vegetation Score	Stem Number	Plant Height (cm)	Node Number	Plant Diameter (cm)	Pod Weight (g.)
Cody	Foreign	**1215**	**94.7**	2.6	**117**	37.6	11.8	23.4	**338.6**
Bami	Bam (IR)	**1188**	**104.0**	2.6	101	**41.1**	11.3	23.9	**384.0**
Sarborideh	Australian	**1145**	**107.6**	**4.3**	**112**	36.6	11.5	20.2	**383.8**
Sabzavar	Sabzavar (IR)	**1085**	56.5	2.6	106	34.1	9.7	20.8	102.3
E075	Italy	**1083**	47.7	2.0	93	39.0	10.7	30.7	63.0
Yazd	Yazd	**1078**	**66.5**	**3.5**	108	**41.3**	11.4	23.0	**138.0**
Kashan	Kashan (IR)	**1055**	**71.2**	**3.5**	103	37.8	10.3	**31.3**	172.9
Jiroft	Jiroft (IR)	**1024**	**87.1**	1.8	90	35.3	9.5	24.8	**267.0**
Local	Iran	**1018**	59.2	3.0	89	34.7	9.5	29.3	98.0
30 year	Fridan (IR)	**1007**	**84.5**	2.8	104	34.5	10.5	**35.7**	**250.2**
M. K	Foreign	**1007**	51.7	**3.6**	**115**	36.7	**12.7**	27.2	71.6
Kerissary	Turkey	**965**	**98.9**	**4.2**	**121**	**42.9**	**12.8**	19.7	**400.0**
Local	Shahroud (IR)	**929**	55.5	2.6	86	27.4	7.0	21.2	90.5
Local	Iran	**898**	63.8	3.0	91	39.5	10.5	21.9	**144.4**
Local	Iran	876	52.9	2.8	100	28.9	8.2	17.8	84.4
West Azar	Iran	874	**85.6**	2.2	102	32.1	11.7	22.2	**332.0**
Cody	Esfahan (IR)	841	**79.7**	2.8	95	31.3	7.2	23.8	**373.0**
Damghan	Iran	841	49.2	2.3	**111**	36.8	8.5	28.8	96.2
Local	Fridan (IR)	840	45.5	3.0	82	**42.0**	11.1	16.5	48.5
Control 1	Iran	815	39.2	2.9	99.6	36.2	10.5	26.3	74.0
Control 2	Iran	763	39.3	2.7	97.0	36.5	11.3	26.4	81.5

The means of genotypes with bold and underlined values are significantly higher than that of controls.

Table 4. Correlation between seed yield and other traits of alfalfa

Traits Name	DM Yield (kg ha^{-1})	Vegetation Score	Plant Height (cm)	Stem Number	Plant Diameter (cm)	Pod Weight (g)	Seed Yield (kg ha^{-1})
Vegetation score	0.62**						
Plant height (cm)	0.72**	0.51**					
Stem number	0.37**	0.56**	0.36**				
Plant diameter (cm)	0.06	0.09	0.03	0.07			
Pod weight (g)	0.46**	0.33**	0.28*	0.13	-0.02		
Seed yield (kg ha^{-1})	0.70**	0.50**	0.45**	0.22	0.01	0.92**	
Node number	0.48**	0.32*	0.66**	0.22	-0.04	0.14	0.25*

*, **= Coefficients of correlation are significant at 5%, 1%, respectively.

Table 5. Results of stepwise regression analysis for seed yield as dependant variables and other traits as independent variables extracted from means of data

Equation Components	Stepwise Regression				
	Step1	Step2	Step3	Step4	Step5
Constant	14.20	-1.53	-2.59	1.72	4.07
Pod weight (g)	0.23	0.19	0.19	0.19	0.19**
DM yield (kg ha^{-1})		0.03	0.03	0.03	0.03**
Vegetation score			1.27	1.87	1.94**
Stem number				-0.06	-0.06*
Plant height (cm)					-0.15*
R square	84.56	92.22	94.35	94.46	94.53

*, **= Coefficients of regressions are significant at 5%, 1%, respectively.

Table 6. Results of path analysis for partitioning of total correlation between seed yield and other important traits into direct and indirect effects

Traits Name	Direct Effect	Indirect Effect Via							Total Correlation
		DM Yield (Kg ha^{-1})	Vegetation. Score	Plant Height (cm)	Stem Number	Plant Diameter (cm)	Pod Weight (g)	Node Number	
DM yield (Kg ha^{-1})	0.35		0.05	-0.01	-0.01	0.01	0.34	-0.01	0.70
Vegetation score	0.07	0.22		-0.01	-0.02	0.00	0.25	-0.01	0.50
Plant height (cm)	-0.02	0.25	0.04		-0.01	0.00	0.21	-0.02	0.45
Stem number	-0.04	0.13	0.04	-0.01		0.00	0.10	-0.01	0.22
Plant diameter	0.01	0.02	0.01	0.01	-0.01		-0.02	0.01	0.01
Pod weight	0.74	0.16	0.02	-0.01	-0.01	0.01		0.01	0.92
Node number	-0.03	0.17	0.02	-0.01	-0.01	0.01	0.10		0.25

Residual error=0.23

Table 7. Factor matrix eiginvalues after varimax rotation and total variance explained for each factor on 8 traits of 200 alfalfa genotypes

Traits Name	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Pod weight	**0.99**	-0.03	0.04	0.02	0.08
Seed yield	**0.90**	-0.10	0.07	-0.01	0.20
DM yield	**0.47**	-0.24	0.16	-0.04	0.28
Node number	0.07	**-0.95**	0.08	0.03	0.10
Plant height	0.18	**-0.50**	0.16	-0.01	0.19
Stem number	0.06	-0.08	**0.96**	-0.03	0.23
Plant diameter	-0.01	0.02	0.03	**-0.98**	0.04
Vegetation score	0.23	-0.13	0.31	-0.05	**0.87**
Variance	2.02	1.15	1.08	1.00	0.99
% of variance	25	14	13	13	12
Cumulative % variance	25	39	52	65	77

The bold underlined values are the most impotent trait for each factor axes.

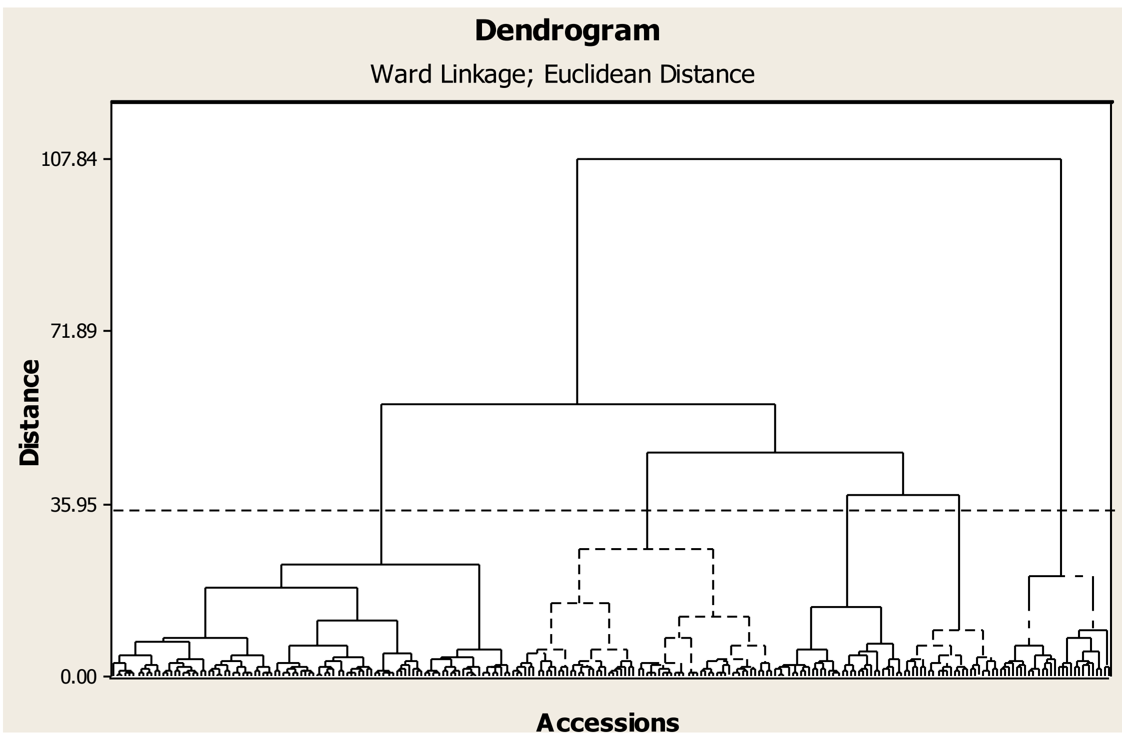

Fig. 1. Dendrogram of 200 accessions of alfalfa by analyzing 8 traits using ward cluster analysis method

Table 8. Means of traits used in classification of the 5 clusters

Cluster No.	DM Yield (kg ha^{-1})	Vegetation Score	Plant Height (cm)	Stem Number	Plant Diameter (cm)	pod Weight (g)	Seed Yield (kg ha^{-1})	Node Number
1	428.7 c	1.65 c	25.38 d	85.0 b	24.56 ab	30.9 c	18.82 d	6.56 b
2	563.8 b	1.78 c	31.02 b	87.3 b	23.20 b	42.9 bc	24.27 cd	10.15 a
3	598.7 b	2.73 b	28.21 c	**106.4 a**	**27.11 a**	62.5 b	31.62 c	7.19 b
4	**950.7 a**	**3.28 a**	**38.38 a**	102.2 a	25.35 ab	**194.6 a**	**68.07 a**	**10.73 a**
5	524.5 bc	1.79 c	26.31 cd	81.1 b	24.45 ab	198.5 a	56.24 b	7.09 b

The means of the each row with the same letters had no differences based on DMRT ($P \leq 0.05$).
The bold underlined values had the higher values in the relevant clusters.

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