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Analysis of Variability, Heritability, and Genetic Advance in Yield and Quality Traits of Alfalfa (*Medicago Sativa* L.) Genotypes

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ABSTRACT

The genetic diversity of primary selection material is essential to successful breeding programs and the creation of new cultivars in alfalfa. The present study was carried out during the 2018/2019 and 2019/2020 seasons at the experimental farm of Nubaria National Research Center (NRC), Al-Behaira Governorate, Egypt. Seeds of fifteen alfalfa genotypes were sown in a randomized complete block design (RCBD) with three replications to define the magnitude of variability and the degree of association between the different traits that are important to provide the base for efficient selection for yield improvement. High genetic variations among genotypes were observed in the two successive seasons and across the two years for plant height (cm); number of tillers/ m^2; leafiness %, dry forage yield, crude protein%; crude fiber %; ash% and carbohydrates%, as well as genotype × year interaction variances. G14, G3, and G7 genotypes exhibited the highest values of all traits under study. The values of the genotypic coefficient of variation (GCV %) and phenotypic coefficient of variation (PCV %), heritability, and genetic advance revealed high variation for morphological and quality traits under study. There were positive correlation relationships between dry forage yield and each of plant height, number of tillers/ m^2, crude protein%; crude fiber %; ash% and carbohydrates %, whereas significant negative correlation with leafiness% at both the genotypic and phenotypic levels and the correlation coefficients were all significant in both seasons. Highly positive direct and indirect effects were recorded for number of tillers/m^2, plant height, and quality of dry forage yield. Hence, the selection for these traits has more chance for high-yielding alfalfa evolution among the tested genotypes. Besides that, the genotypes G14, G3, and G7 are the most promising genotypes under calcareous soils conditions and their use in the breeding program of alfalfa.

Keywords: Forage and quality yields, genotype × year, genetic variation, heritability, and genetic advance.

1. Introduction

Alfalfa (*Medicago sativa* L.) is the most important perennial forage legume crop worldwide and its importance as a forage crop due to its high content of protein, vitamins, minerals, important nutritional value, making it ideal crop for animals and dairy production. In addition to its nitrogen fixing performance, it eliminates the need for nitrogen fertilization and adds a beneficial effect in crop rotation, and produces a high yield of biomass from the high-quality feed with easy digestibility. Besides that, quick recovery after cutting and longevity during all periods of exploitation (Avci *et al.,* 2018). Genetic variance of primary election materials is necessary for successful breeding and the creation of new cultivars. For estimates of genetic diversity, criteria such as morphological, agronomic, and physiological characteristics, Alfalfa is distributed worldwide and grown in highly contrasting environments and offers additional advantages as it widely adapts to different climates this wide geographical adaptation enhances genetic variation and provides the opportunity to use diverse gene pools in breeding programs (Moghaddam *et al.,* 2011).

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Tlahig *et al.,* (2017) indicated that the assessment of agricultural morphological traits was of value to achieve a breeding program that focuses on developing new and improved plant species and those agricultural morphological traits are the direct expression of the genetic structure of a genotype.

Arab *et al.,* (2015) studied the seasonal variation in performance for forage yield and quality traits of twenty-two landraces of alfalfa and indicated highly significant differences among the tested landraces. Abo Feteih and Mekhaile (2018) studied variation in twenty-eight genotypes (Hybrids and their parent) and exhibited a wide range of variations for forage yield. Seiam and Farag (2019) suggested that the most promising genotypes for forage yields and protein percentage stability indices compared to other genotypes to be selected for ongoing breeding programs for alfalfa improvement. Johnson *et al.,* (1955) explained the importance of cultivar selection and assessment in terms of quantity and production capacity in any breeding program, and thus cultivars can be introduced to a given local environment. Alfalfa forage quality is closely related to the relative production of leaf and stem components as measured by leaf ratio, but more often as leaf/stem ratio (LSR) and stem nutritional value Johnson *et al.,* (1994). There were positive correlations for crude protein (CP) with leaf/stem ratio and lodging and negative correlations for height and regrowth. Moreover, cleaner production is an important indicator of forage quality (Larson and Mayland 2007).

Variability for agronomic and morphological characteristics of alfalfa is frequently used in breeding programs for developing cultivars with high forage production and quality. The Phenotypic and genotypic coefficients of variation, heritability, and genetic advance in the population as well as the nature of the association between yield and its components are very important indicators in improving traits (Denton and Nwangburuk 2011). This information reflects the genetic merit of the germplasm and is essential in any breeding program, as genetic gains can only be obtained if there is genetic diversity (Costa *et al.,* 2021) and allows simultaneous selection of multiple traits associated with yield (Mahajan *et al.,* 2011).

The heritability estimates of a trait is important in determining its response to selection. Heritability estimates help breeders to allocate the necessary resources effectively to select the desired qualities and achieve the maximum genetic gains with little time and resources (Smalley *et al.,* 2004 and Cruz *et al.,* 2012). Heritability with genetic advance estimates is more reliable and important than individual consideration of the parameters (Nwangburuk and Denton 2012).

The path coefficient analysis is used to detect the amount of direct and indirect effects of causation components on the impact component (Monirifar 2011). As such previous studies, plant breeders can find varieties well qualified with some characteristics using path analysis in the final selection stage for breeding (El-Hifny *et al.,* 2019).

Shanjani *et al.,* (2013) reported that the path-coefficient analysis indicates that dry matter yield mainly depends on plant height, leaf/stem ratio, and tiller numbers when studying the genetic diversity of 27 wild alfalfa populations from seven different countries.

This research was focused on the identification of better-performing genotypes of alfalfa to use in breeding programs. Thence, the main objectives of this work are (i) to study the nature and magnitude of genetic variability of alfalfa genotypes; (ii) to assess phenotypic and genotypic correlations between yield and its components, and (iii) to detect the direct and indirect effects of these morphological traits on dry forage yield.

Materials and Methods

Field Experiment

Fifteen alfalfa genotypes were collected from El-Dakhla oasis (G1, G2, G3, and G4), El-Kharga oasis (G5, G6, G7, G8, and G9), Siwa oasis (G10, G11, G12, G13, and G14) and Ramah-1 variety (G15) were used to study the performance of these genotypes for yield, its components, and quality for further use in plant breeding programmes. Physical and chemical properties of the experimental site are shown in Table (1) at Nubaria National Research Center, Al-Behaira Governorate, Egypt, the farm is located at (30° 30´ 1.4" N, 30° 19´ 10.9 " E, and 21m above sea level) during three years 2018, 2019 and 2020. The genotypes were sown in an augmented design on the second of November 2018. The fifteen genotypes had planted in a randomized complete block design with four replications and were sown into a plot size of 6 m^2 and each genotype was seeded at the rate of 20 kg/fed.

Seeds were inoculated with *Rhizobium meliloti* before seeding. Plots received 60 kg/fed of P_2O_5, 50 kg/fed of potassium sulfate before emergence and 20 kg/fed nitrogen after emergence. The first irrigation was applied seven days after sowing. The following irrigations were applied at ten days in summer and fifteen days in winter. The first cut was taken on February 17th, 2019 and then eight cuts were taken in each season of 2019 and 2020. The data were recorded at each harvest on five guarded plants plot 1 for field performance and chemical composition**.**

Table 1: Physical and chemical properties of the experimental site (mean over seasons).

Particle size distribution	
Sand %	71.80
Silt %	24.60
Clay %	3.60
Texture	Sandy Loam
Field capacity	23.10
Chemical character	
Soil reaction pH (1:2.5)	7.90
Electric conductivity (dsm-1)	0.12
Organic matter (%)	0.23
Calcium carbonate (%)	3.57
Available macronutrients (mg/100g)	
N	15.10
P	13.00
K	21.00
Available micronutrients (ppm)	
Fe	4.47
Mn	2.61
Zn	1.44
Cu	4.00

The following data were recorded as follow

1- Plant height (cm.) was determined by the main stem from the soil surface to the stem tip.
2- Number of tillers/ m^2.
3- Leafiness [leaf weight x 100/ (leaf + stem)] weight on dry basis.
4- Total dry forage yield was calculated from dry matter percentage multiplied by fresh yield (The green plants were chopped manually and weighed on digital weight balance then placed in shad for drying and were shifted to an electric oven at 70 0C for a period till a constant weight was achieved).

Chemical Analysis

The chemical analysis was detected according to the conventional method recommended by the Association of Official Agricultural Chemists (A.O.A.C) on the dried samples at 70 °C for each cut of the first, and second years and combined across the two years to determine:

1. **Crude protein (CP %):** The N-contents of the sample were determined by Kjeldahl N, A.O.A.C (2000) and the recorded value of nitrogen was then multiplied by 6.25 according to Sadasivam and Manickam (1996).
2. **Crude fiber (CF %):** was determined according to the methods described by (Anonymous, 1983).
3. **Ash %:** was estimated by multiplying forage dry yield/fresh forage × 100 (Pearson 1962).
4. **Carbohydrates %:** were determined using phenol sulphuric acid methods according to Dubois *et al.* (1956).

Statistical Analysis

Collected data were subjected to the individual analysis of variance (ANOVA) of randomized complete block design for each one of the two seasons (Gomez and Gomez, 1984). Bartlett's test of variance homogeneity was carried out before the combined analysis (Bartlett, 1937). Mean comparisons were performed using the least significant difference (L.S.D.) at a 5% level of probability. The phenotypic (PCV %) and genotypic (GCV %) coefficients of variability, heritability in the broad sense (h_b^2) and Genetic advance as a percentage of the mean were calculated to compare the extent of the predicted advance of different traits under selection (GA) for the studied traits were calculated according to Kumar *et al.* (1985). Relationships between the traits were studied through genotypic and phenotypic correlation and path coefficient. Genotypic and phenotypic correlation coefficients were calculated for each pair of traits according to Falconer (1989). All correlation coefficients were worked out between all possible combinations of traits. The path coefficients analysis appeared to provide a clue to the contribution of various components of yield to overall fresh or seed yield in the populations under study. It provides an effective way of splitting up the total phenotypic correlation coefficients into direct and indirect effects of the yield components on dry forage yield and helps to understand the relationship among variables based on the general formula (Duarte and Adams 1972). The statistical analysis and the relationships between germplasm were measured by calculating their euclidean distance and complete linkage method as a phenogram using SYSTAT version 7.0. (Wilkinson, 2010).

3. Results and Discussion

3.1. Genetic Variability

Mean performances in Table 2&3 indicated that the 2020 season was more values of all traits than the 2019 season. Data in Table 2 for mean performances revealed the highly significant differences ($P \leq 0.05$) among the investigated genotypes for different traits in both seasons. Data revealed that (G14) genotype exhibited the highest values for the plant height (64.51 and 70.79 cm), the number of tillers/ m^2 (277.57 and 290.48), and dry forage yield (16.92 and 19.39 ton/fed). While, (G10) genotype had the lowest values (42.39 and 49.94 cm) for plant height, (225.45 and 269.63) for the number of tillers/m^2, (32.61 and 36.68%) for leafiness, and (6.89 and 10.36 ton/fed) for dry forage yield in both season, respectively. However, data recorded that (G3) genotype (54.73 and 57.53%) ranked first followed by G14 (53.46 and 55.8 %) for leafiness in both seasons. These results are in agreement with those reported by Jia *et al.* (2018) and Acharya *et al.* (2020). In addition, Annicchiarico *et al.* (2020) found that significant difference among eleven landraces of alfalfa for main stem length; number of stems per plant, and yield, and Badr *et al.* (2020) found that genetic diversity among fifteen alfalfa cultivars from three different countries (Egypt, Australia, and the USA) using analysis of fifteen morpho-agronomic traits. The combined data analysis across the two years revealed that (G14) genotype had the highest for all studies traits except for leafiness % and recorded values of 67.65 cm, 284.02, and 18.16 ton/ fed for plant height, number of tillers/m^2, and for dry forage, respectively.

Table 2: Mean performance of forage yield and yield components of fifteen alfalfa genotypes in first, second years, and combined across the two years.

Genotypes	Plant height (cm)			Number of tillers/ m^2		
	2019	2020	Comb.	2019	2020	Comb.
G1	44.37	51.01	47.69	257.43	270.7	264.06
G2	54.19	61.04	57.62	267.25	280.73	273.99
G3	63.24	69.15	66.2	276.3	288.84	282.57
G4	52.53	59.44	55.99	265.59	279.13	272.36
G5	47.86	55.41	51.64	260.92	275.1	268.01
G6	50.37	57.01	53.69	263.43	276.7	270.06
G7	58.06	65.61	61.84	271.12	285.3	278.21
G8	51.89	58.9	55.4	264.95	278.59	271.77
G9	51.22	57.59	54.41	264.28	277.28	270.78
G10	42.39	49.94	46.17	254.45	269.83	262.14
G11	54.67	61.25	57.96	267.73	280.94	274.33
G12	50.22	56.59	53.41	263.28	276.28	269.78
G13	53.7	60.83	57.27	266.76	280.52	273.64
G14	64.51	70.79	67.65	277.59	290.48	284.02
G15	57.36	63.73	60.55	270.42	283.42	276.92
Mean	53.11	59.89	56.50	266.10	279.59	272.84
L.S.D $_{0.05}$	2.351	3.016	3.182	12.761	10.395	10.971
G.C.V	10.58	11.84	13.77	6.69	6.81	7.56
P.C.V	12.18	12.97	13.98	7.43	8.04	8.96
(H^2)%	92.89	92.82	94.73	88.57	88.92	87.94
GA	53.13	77.89	65.15	331.34	366.98	365.93

Table 2: Cont.

Genotypes	Leafiness %			Dry forage yield (ton/fed)		
	2019	2020	Comb.	2019	2020	Comb.
G1	34.59	37.75	36.17	8.56	11.43	10.00
G2	44.41	47.68	46.05	10.84	13.38	12.11
G3	54.73	57.53	56.13	14.36	16.71	15.54
G4	42.75	46.18	44.47	10.74	13.28	12.01
G5	38.08	42.15	40.12	8.87	11.41	10.14
G6	40.59	43.75	42.17	9.43	11.97	10.70
G7	48.28	51.35	49.82	12.57	15.11	13.84
G8	42.11	45.64	43.88	9.63	12.17	10.90
G9	41.44	44.33	42.89	9.55	12.02	10.79
G10	32.61	36.68	34.65	6.89	10.36	8.63
G11	44.89	47.99	46.44	11.68	14.15	12.92
G12	40.44	43.33	41.89	8.94	11.41	10.18
G13	43.92	47.57	45.75	10.83	13.3	12.07
G14	53.46	55.89	54.68	16.92	19.39	18.16
G15	47.58	50.47	49.03	11.91	14.38	13.15
Mean	43.33	46.55	44.94	10.78	13.36	12.08
L.S.D $_{0.05}$	3.632	4.56	3.771	0.675	0.452	0.631
G.C.V	3.43	3.87	4.76	7.42	16.54	16.87
P.C.V	3.75	4.11	5.28	7.85	16.83	17.41
(H^2)%	91.89	92.77	92.87	90.35	95.97	93.32
GA	41.25	50.37	67.61	505.04	528.87	409.64

However, the (G3) genotype (56.13 %) ranked first followed by G14 (54.68 %) for leafiness. On the other hand, (G10) genotype had the lowest value (46.17 cm) for plant height, (262.54) for number of tillers/m^2, (34.65 %) for leafiness, and (8.63 ton/fed) for dry forage yield followed by (G1) genotype. These results are in harmony with those reported by Wang *et al.* (2018) and Seiam and Farag (2019). Likewise, El-Hifny *et al.* (2019) indicated significant performance and a wide range of adaptability to six alfalfa genotypes. Morphological characters are the external expression of the genotypes. Therefore, it might be used to identify different alfalfa genotypes and help the breeder in selecting the germplasm collections for use in breeding programs.

Table (2) shows the genotypic coefficient of variation (GCV), phenotypic coefficient of variance (PCV), broad sense heritability (h_b^2), and genetic advance as a percent of the mean (GA)for different traits in both seasons. There was wide variation in the characteristic due to the environmental variations (Hand and Crowder, 2017), which leads to different gene expressions and gives a good chance of improvement in alfalfa genotypes. In the first year, the maximum genotypic and phenotypic coefficients of variability were revealed for plant height (cm) (10.58and 12.18%), number of tillers/ m^2 (6.69 and 7.43 %), leafiness (3.43 and 3.75%), and dry forage yield (ton/ fed) (7.42 and 7.85 %), respectively. Heritability (h_b^2%) estimates were generally high for all studied traits and revealed values from 92.89% for plant height to 88.57% for number of tillers/ m^2. The genetic advance (GA) values recorded ranged from 41.25 for leafiness to 505.04 for dry forage yield. Meanwhile, in the second year, the highest genotypic and phenotypic coefficients of variability were recorded for plant height (11.84 and 12.97 %), number of tillers/ m^2 (6.81 and 8.04 %), leafiness (3.87 and 4.11%) and dry forage yield (16.54 and 16.83 %), respectively. Heritability estimates recorded values from 95.97 % for dry forage yield to 88.92% number of tillers/ m^2. Data revealed that values ranged from 50.37for leafiness to 528.87for dry forage yield to genetic advance. Having estimates of genetic parameters for traits is extremely important to identify genotypes that a major or minor variation occurs and know the magnitude of genetic variances. These results are in line with those obtained by Hamd-Alla *et al.,* (2013), Santos *et al.,* (2020) and Costa *et al.,* (2021). The combined data over seasons of genetic parameters also showed highly significant differences for all traits under study. The genotypic and phenotypic coefficients of variability were recorded for plant height (13.77 and 13.98 %), number of tillers/ m^2 (7.56 and 8.96 %), leafiness (4.76 and 5.28 %), and dry forage yield (16.87 and 17.41 %), respectively. The lowest heritability (87.94 %) estimates were recorded in leafiness, while the plant height recorded the greatest value of heritability (94.73%). Genetic advances ranged from 65.15 for plant height to 409.64for dry forage yield. Santos *et*

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al., (2018) reported that with the existence of wide genetic variability and the heritability is high, there is better selection accuracy for traits of interest in a plant breeding improvement program.

3.2. Chemical analysis

The mean performances of chemical analysis of tested genotypes in the two successive seasons, and combined are presented in Table 3. The present investigation revealed a considerable amount of variation for crude protein (CP %), crude Fiber (CF %), ash%, and carbohydrate %in both seasons. The variation in chemical analysis contents has been reported by Lamb *et al.* (2007). Genotype (G14) occupied the highest content of CP with (20.05 and 22.22%), CF (24.79 and 24.89%), and ash (10.91 and 12.60%) in both seasons, respectively, whereas, (G3) genotype ranked first (23.71 and 24.58%) followed (G14) genotype (21.15 and 22.02%) for carbohydrate. On the contrary, (G10) had the lowest values of CP with (12.58 and 14.75%), ash (5.97 and 6.90 %), and carbohydrate (13.68 and 14.55%), while (G1) genotype had the lowest values (19.09 and 20.58%) for CF in both seasons, respectively. Monirifar (2011), Abo-Feteih and Mekhaile (2018), and Tucak *et al.,* (2021) obtained similar results. Data of the combined analysis over the two years recorded that the (G14) genotype had the largest values of CP with (21.14%), CF (24.84%), and ash (11.76%) in both seasons, respectively, whereas, (G3) genotype ranked first (24.15%) followed (G14) genotype (21.59%) for carbohydrate. Contrariwise, (G10) genotype ranked the lowest values of CP with (13.67%), ash (6.44 %), and carbohydrate (14.12%), whilst (the G1) genotype had the lowest value (19.84 %) for crude fiber. These results are with corresponding with those reported by Basbag *et al.* (2009). Besides, Arab *et al.* (2015) reported that the crude protein contents ranged from 19.04- 27.75%, the crude fiber contents ranged from 23.00-32.28%, the ash% contents ranged from 6.27-13.44% and carbohydrate contents ranged from 6.16-25.59 % when studied for quality performances of alfalfa cultivars. Kavut and Avcioglu (2015) found that the crude protein contents varied from 19.83-20.11% and the crude ash contents ranged between 7.50-8.45 % when studied for quality performances of alfalfa cultivars. Turan *et al.* (2017) declared that the variation in forage quality contents of the varieties depends on soil and climatic factors. Also, Sayar *et al.* (2022) reported that the investigated 24 alfalfa genotypes exhibited high total variability for forage quality.

Table 3: Mean performance of forage CP%, CF%, ash%, and Carbohydrates % of fifteen alfalfa genotypes in first, second years and combined across the two years.

	Crude protein%			**Crude fiber %**		
Genotypes	**2019**	**2020**	**Comb.**	**2019**	**2020**	**Comb.**
G1	14.35	16.52	15.44	19.09	20.58	19.84
G2	16.32	18.49	17.41	21.06	22.43	21.75
G3	19.56	21.95	20.76	23.76	25.25	24.51
G4	16.43	18.29	17.36	20.86	22.35	21.61
G5	14.56	16.73	15.65	19.75	21.24	20.50
G6	15.12	17.29	16.21	19.86	21.35	20.61
G7	18.26	20.43	19.35	22.97	24.46	23.72
G8	15.52	17.69	16.61	20.26	21.52	20.89
G9	15.24	17.41	16.33	19.98	21.47	20.73
G10	12.58	14.75	13.67	20.18	21.67	20.93
G11	17.37	19.54	18.46	22.11	23.43	22.77
G12	14.63	16.94	15.79	19.51	21.66	20.59
G13	16.53	17.56	17.05	20.13	21.62	20.88
G14	20.05	22.22	21.14	24.79	24.89	24.84
G15	17.64	19.81	18.73	22.38	23.87	23.13
Mean	16.28	18.37	17.33	21.11	22.52	21.82
L.S.D $_{0.05}$	1.091	1.402	1.765	1.161	1.198	1.342
G.C.V	8.71	9.75	9.08	9.59	10.03	10.92
P.C.V	8.82	9.97	9.92	10.48	10.72	11.61
(H^2)%	86.7	88.17	86.07	88.48	90.95	91.45
GA	167.88	192.64	211.4	65.36	87.45	89.97

Table 3: Cont.

Genotypes	Ash%			Carbohydrates %		
	2019	**2020**	**Comb.**	**2019**	**2020**	**Comb.**
G1	6.30	7.99	7.15	15.35	16.22	15.79
G2	7.18	8.87	8.03	17.63	18.50	18.07
G3	9.88	11.57	10.73	23.71	24.58	24.15
G4	6.98	8.67	7.83	17.53	18.40	17.97
G5	6.34	7.56	6.95	15.66	16.53	16.10
G6	7.77	7.87	7.82	16.22	17.09	16.66
G7	9.09	10.78	9.94	19.36	20.23	19.80
G8	7.11	8.07	7.59	16.42	17.29	16.86
G9	6.10	7.79	6.95	16.34	17.21	16.78
G10	5.97	6.90	6.44	13.68	14.55	14.12
G11	8.23	9.92	9.08	18.47	19.34	18.91
G12	5.63	7.32	6.48	15.73	16.60	16.17
G13	6.25	7.94	7.10	17.62	18.49	18.06
G14	10.91	12.60	11.76	21.15	22.02	21.59
G15	8.50	10.19	9.35	18.70	19.57	19.14
Mean	7.48	8.94	8.21	17.57	18.44	18.01
L.S.D $_{0.05}$	1.012	1.210	1.115	2.154	2.193	2.241
G.C.V	12.14	12.26	13.01	6.14	6.34	6.59
P.C.V	13.03	13.15	13.78	6.57	6.81	7.18
(H^2)%	90.12	90.96	91.24	90.02	87.05	90.86
GA	107.86	125.1	98.74	262.53	286.36	267.13

The genotypic coefficient of variation (GCV), phenotypic coefficient of variance (PCV), broad sense heritability (h_b^2), and genetic advance as a percent of the mean (GA) for different traits in both seasons are presented in Table (3). Narrow differences were obtained between (GCV %) and (PCV %) for all traits may be limited effects of environments on these traits. In the first year, the highest genotypic and phenotypic coefficients of variability were revealed for crude protein% (8.71and 8.82%), crude fiber % (9.59 and 10.48 %), ash% (12.14 and 13.03 %), and carbohydrates % (6.14 and 6.57 %), respectively. Heritability (h_b^2%) estimates were generally high for all studied traits and revealed values from 90.12%for ash% to 86.7% for crude protein%. The genetic advance (GA) values recorded ranged from 65.36 for crude fiber % to 262.53 for carbohydrates %. Also, in the second year, the highest genotypic and phenotypic coefficients of variability were recorded for crude protein(9.75 and 9.97 %), crude fiber (10.03 and 10.72 %), ash (12.26 and 13.15 %) and carbohydrates(6.34and 6.81 %), respectively. Heritability estimates recorded values from 90.96 % for ash to 87.05% for carbohydrates. Data revealed that values ranged from 87.45for crude fiber %to286.36for carbohydrates % of genetic advance. These results are in agreement with those reported by Abd EL-Galil (2007) and Bakheit *et al.* (2011). The genotypic and phenotypic coefficients of the combined data over seasons were recorded for crude protein(9.08and9.92 %), crude fiber (10.92 and 11.61 %), ash (13.01and 13.78 %), and carbohydrates %(6.59 and7.18 %), respectively. Heritability estimates recorded values from 91.45 % for crude fiber to 86.07 % for crude protein. Data revealed that values ranged from 89.97for ash %to 267.13for carbohydrates %of the genetic advance. This agrees with Santos *et al.* (2018) found the values of the genetic parameters for crude protein and crude fiber averages were high, indicating that the differences are mostly determined by genetic causes.

3.3. Phenotypic and genotypic correlation

The phenotypic and genotypic correlation coefficients (r) were calculated using means for yield, yield components, and quality in two seasons and are presented for a clear understanding as shown in Table 4. The magnitude of genotypic correlations (r_g) was higher than those of phenotypic correlations (r_p). When the value of "r_p" was greater than "r_g", it showed that the apparent association of two traits was not only due to genes but also due to the favorable influence of the environment.

In the first season, the dry forage yield showed significant positive correlations with each plant height (r_p=0.912*, r_g=0.954**) and the number of tillers/ m^2 (r_p=0.872**, r_g=0.927**) at both phenotypic and genotypic levels, respectively. While it appeared negative and significant association with leafiness (r_g= -0.862*) at the genotypic level. Significant positive correlations were also detected between dry forage yield ton/ fed and crude protein% (r_p= 0.883*, r_g= 0.942**), crude fiber % (r_p= 0.902*, r_g= 0.938**) as well as carbohydrates% (r_p= 0.917**, r_g= 0.968**) at both phenotypic and genotypic levels, respectively.

Table 4: Correlation coefficients among yield, yield components, and quality estimated over the two years

Characters		Plant height (cm)	Number of tillers/ m^2	Leafiness %	Dry forage yield (ton/fed)
Plant height (cm)	p		0.873*	-0.785^{ns}	0.912*
	g		0.921**	-0.841**	0.954**
Number of tillers/ m^2	p	0.912*		-0.652 ^{ns}	0.872**
	g	0.941**		-0.877**	0.927**
Leafiness %	p	-0.665^{ns}	-0.625 ^{ns}		-0.734^{ns}
	g	-0.733*	-0.816**		-0.862*
Dry forage yield (ton/fed)	p	0.931**	0.894*	-0.912^{ns}	
	g	0.964**	0.932**	-0.943**	
Crude protein%	p	0.887**	0.671^{ns}	0.701*	0.911**
	g	0.958**	0.758**	0.765**	0.963**
Crude fiber %	P	0.861*	0.712^{ns}	-0.862**	0.876**
	G	0.934**	0.789*	-0.932**	0.932**
Ash%	P	0.917**	0.882^{ns}	0.915*	0.882**
	g	0.953**	0.931*	0.968**	0.935**
Carbohydrates %	P	0.897*	0.906^{ns}	-0.854^{ns}	0.896^{ns}
	g	0.958*	0.952**	-0.926*	0.941**

Table 4: Cont.

Characters		Crude protein%	Crude fiber %	Ash %	Carbohydrates %
Plant height (cm)	p	0.855*	0.794**	0.867*	0.792**
	g	0.901**	0.841**	0.912**	0.855**
Number of tillers/ m^2	p	0.637*	0.886^{ns}	0.914*	0.912^{ns}
	g	0.748**	0.927*	0.955**	0.956**
Leafiness %	p	0.735*	-0.896**	0.914*	-0.921^{ns}
	g	0.852**	-0.953**	0.958**	-0.963**
Dry forage yield (ton/fed)	p	0.883*	0.902*	0.912^{ns}	0.917**
	g	0.942**	0.938**	0.951**	0.968**
Crude protein%	p		0.912*	0.906*	0.874**
	g		0.968**	0.926**	0.902**
Crude fiber %	P	0.761*		0.893**	0.921**
	G	0.843*		0.934**	0.957**
Ash%	P	0.905*	0.865*		0.918*
	g	0.947**	0.904**		0.9443**
Carbohydrates %	P	0.878**	0.912**	0.914*	
	g	0.932**	0.936**	0.946**	

** Significant at 1% level of probability, * Significant at 5% level of probability, ns= not significant at P= 0.05.

However, it appeared significant positive correlations with ash% (r_g= 0.951**) at the genotypic level only. These results are in full agreement with Shanjani *et al.* (2013) found a positive relationship between dry matter yield with tiller number and plant height and a negative relationship with leaf/ stem ratio when studying the genetic diversity of 27 wild alfalfa populations. Regarding leafiness%, significant negative correlations were found with plant height (r_g= -0.841**), number of tillers/ m^2(r_g= -0.877**) and carbohydrates% (r_g= -0.963**) at genotypic level only. While with crude fiber % (r_p= -0.896**, r_g= -0.953**) at both phenotypic and genotypic levels, respectively. Number of tillers/ m^2 was significantly and positively correlated with plant height (r_p= 0.873*, r_g= 0.921**), crude protein % (r_p= 0.637*, r_g= 0.748**) and ash %(r_p= 0.914*, r_g= 0.955**) at both phenotypic and genotypic levels, respectively and appeared significant positive correlations at genotypic level only to crude fiber% (r_g= 0.927*), and carbohydrates% (r_g= 0.956**). As for the plant height revealed positive correlations with crude protein% (r_p= 0.855*, r_g= 0.901**), crude fiber % (r_p= 0.794**, r_g= 0.841**), ash% (r_p= 0.867*,

r_g= 0.912**) and carbohydrates% (r_p=0.792**, r_g= 0.855**) at both phenotypic and genotypic levels, respectively. Strbanovic *et al.* (2015) reported significant correlations (P≤0.001) correlation between dry matter yield and tiller length, as well as between dry matter yield and crude protein yield (P≤0.05) which are strong and positive correlations.

In the second year, the dry forage yield showed significant positive correlations with each plant height (r_p=0.931**, r_g=0.964**) and number of tillers/ m^2 (r_p=0.894**, r_g=0.932**) at both phenotypic and genotypic levels, respectively. Further it was negative and significant association with leafiness (r_g= -0.943**) at genotypic level. These results are in line with those obtained by Basafa and Taherian (2009) and Yan *et al.* (2009). Significant positive correlations were also detected between dry forage yield ton/ fed and crude protein% (r_p= 0.911**, r_g= 0.963**), crude fiber % (r_p= 0.876**, r_g= 0.932**) as well as ash% (r_p= 0.882**, r_g= 0.935**)at both phenotypic and genotypic levels, respectively. However, it appeared significant positive correlations with carbohydrates % (r_g= 0.941**) at genotypic level only. Arab *et al.* (2015) stated the highest positive and significant correlations were found among alfalfa genotypes between dry matter weight, number of tillers per m^2, plant height and quality. As regards leafiness%, significant positive correlations were found with crude protein% (r_p= 0.701*, r_g= 0.765**) and ash %(r_p= 0.915*, r_g= 0.968**) at both phenotypic and genotypic levels, respectively. While withcrude fiber % significant negative correlations were found (r_p= -0.862**, r_g= -0.932**)at both phenotypic and genotypic levels, respectively and carbohydrates% (r_g= -0.926*) at genotypic level only. Number of tillers/ m^2 was significantly and positively correlated with crude protein % (r_g= 0.758**), crude fiber% (r_g= 0.789*), ash %(r_g= 0.931*) and carbohydrates% (r_g= 0.952**) at genotypic level only. With respect to plant height revealed positive correlations with crude protein% (r_p= 0.887**, r_g= 0.958**), crude fiber % (r_p= 0.861*, r_g= 0.934**), ash% (r_p= 0.917**, r_g= 0.953**) and carbohydrates% (r_p= 0.897*, r_g= 0.958*) at both phenotypic and genotypic levels, respectively. Riasat *et al.* (2021) recorded the highest significant differences among ten alfalfa genotypes in dry matter yield, plant height, and leaf/ stem ratio. This revealed that the association between these traits was under genetic control, which indicates the predominance of genetic variation in the expression of traits. Maletic *et al.* (2010) highlight a particularly important status phenotypic correlation indicating trends of potential changes under the effect of the applied breeding methods.

3.4. Path-coefficient analysis

Genetic correlation coefficients were divided individually to direct and indirect effects. Direct and indirect genetic effects of plant height (cm), number of tillers/ m^2, and leafiness % of the dry forage yield in two seasons are presented in Table (5). The direct effects were less than one and positive, indicating that the absence of multiline was minimal (Al-Ballat and Al-Araby 2019).

Table 5: Path coefficient analysis of dry forage yield and its components of fifteen alfalfa genotypes in first, and second years.

Trait	Correlation Coefficients		Direct effects		Indirect effect					
					Plant height (cm)		Number of tillers/ m^2		Leafiness %	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
Plant height (cm)	0.954	0.964	0.372	0.489	----	----	0.451	0.402	0.131	0.073
Number of tillers/ m^2	0.927	0.932	0.513	0.624	0.217	0.301	----	---	0.197	0.007
Leafiness %	-0.862	-0.943	-0.216	-0.356	-0.178	-0.284	-0.468	-0.303	---	---
Residual effect	**First year = 0.034**						**Second year = 0.047**			

In the first year, the results indicated that number of tillers/ m^2 (0.513) exerted the highest positive direct effect on dry forage yield followed by plant height (0.372). While leafiness% (-0.216) showed a negative direct effect on dry forage yield. Johnson *et al.* (1994) and Monirifar (2011) found positive direct effects of the number of shoots and plant height on dry yield in alfalfa. Indirect plant height affected dry yield positively and was high on dry forage yield via number of tillers/ m^2 (0.451). It is observed that the indirect effects of number of tillers/ m^2 on dry forage yield through their association with plant height and leafiness% were small (0.217 and 0.197, respectively). Leafiness % affected dry forage yield negatively and indirectly through all the traits. The component of indirect effect was recorded for leafiness % via number of tillers/ m^2 (-0.468). It is recognized that the indirect effect of leafiness% on dry forage yield via plant height (-0.178) was negative and low. Tucak *et al.* (2008) confirm the significance of plant height and number of stems for the indirect selection of alfalfa at early stages.

In the second year, the number of tillers/ m^2 and plant height showed positive direct effects on dry forage yield (0.624 and 0.489, respectively). Whereas leafiness% (-0.356) showed a negative direct effect on dry forage yield. A comparable result was reported by Abdel- Galil (2007) and El-Hifny *et al.* (2019). Its indirect effects of plant height on dry forage yield via number of tillers/ m^2 and leafiness % revealed (0.402 and 0.037) respectively. Whilst the number of tillers/ m^2 on dry forage yield via plant height and via leafiness % was recorded (0.301 and 0.007) respectively for indirect effects. On the other hand, the component of indirect effect for leafiness % was affected negatively and recorded on dry forage yield via plant height and via number of tillers/ m^2 (-0.284 and -0.303) respectively. It could be concluded that the plant height (cm), number of tillers/ m^2 are important traits for the selection of dry forage yield in alfalfa as a result of direct and indirect effects in the path-coefficient analysis. Ibrahim *et al.* (2014) showed the number of shoots m^{-2} and plant height revealed the highest direct or indirect effect on dry yield in the first season, while the leaf/stem ratio gave the most direct or indirect effect on dry yield in the second season. Besides, Santos *et al.* (2020) stated that the plant height was the most prominent direct and indirect effects (0.3041 and 0.2135) respectively on the dry matter percentage of alfalfa.

Popovic *et al.* (2006) stated the direct effect selection for traits detecting quality, as protein content, will not bring about favorable progress, while, the progress can be achieved by indirect improvement of the quality trait. The direct and indirect genetic effects of independent traits on dry forage yield were significant for traits (Table 6). Rotili *et al.* (2001) reported the most significant indicators of alfalfa quality are CP and CF. In the first year, the highest direct effect had positive (0.585) for crude protein% followed by ash% (0.417) and crude fiber % (0.412), while the lowest direct effect was recorded (0.398) for carbohydrates %. This agrees with Johnson *et al.* (1994) who found a positive direct effect for CP and CF with dry weight. Its indirect effects of crude protein% on dry forage yield via crude fiber %, ash %, and carbohydrates % revealed (0.164, 0.098, and 0.095) respectively. Whilst the crude fiber % on dry forage yield via crude protein, via ash% and carbohydrates % recorded (0.175, 0.153, and 0.198) respectively for indirect effects. These results concur with those reported by Lemaire *et al.* (1994). Meanwhile, the ash% on dry forage yield via crude protein, via the crude fiber %, and via carbohydrates % had (0.098, 0.232, and 0.204) respectively and the carbohydrates % on dry forage yield via crude protein, via the crude fiber % and via ash % revealed (0.267, 0.138 and 0.165) respectively.

Table 6: Path coefficient analysis of dry forage yield and quality of fifteen alfalfa genotypes in first, and second years.

Trait	**Correlation Coefficients**		**Direct effects**		**Indirect effect**							
					Crude protein %		**Crude fiber %**		**Ash %**		**Carbohydrates %**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
Crude protein %	0.942	0.963	0.585	0.634	----	----	0.164	0.219	0.098	0.104	0.095	0.006
Crude fiber %	0.938	0.932	0.412	0.465	0.175	0.254	---	----	0.153	0.084	0.198	0.129
Ash %	0.951	0.935	0.417	0.540	0.098	0.117	0.232	0.265	----	----	0.204	0.013
Carbohydrates %	0.968	0.941	0.398	0.543	0.267	0.282	0.138	0.189	0.165	0.073	-----	----
Residual effect	**First year** = 0.143						**Second year** = 0.199					

In the second year, the results indicated that crude protein % with (0.634), crude fiber % with (0.465), ash % (0.540), and carbohydrates % (0.543) revealed a positive direct coefficient effect on dry forage. Regarding the indirect effects, it is noted that crude protein% on dry forage yield via crude fiber %, ash %, and carbohydrates %, revealed (0.219, 0.104, and 0.006) respectively, while the values of crude fiber % on dry forage yield via crude protein, via ash% and via carbohydrates % recorded (0.254, 0.084 and 0.129) respectively for indirect effects. Meanwhile, the ash% on dry forage yield via crude protein, via the crude fiber %, and via carbohydrates % had (0.117, 0.265, and 0.013) respectively and the carbohydrates % on dry forage yield via crude protein, via the crude fiber % and via ash % had

(0.282, 0.189 and 0.073) respectively. These results are in agreement with Monirifar (2011) and El-Hifny *et al.* (2019). Furthermore, the residual effect of the path coefficient analysis of the yield trait was negligible in most of the environments, indicating the presence of there is no other attributes not recorded in the research.

4. Conclusion

Information on the magnitude of genetic variability and the degree of association between the traits would assist plant breeders in choosing the best genotypes; this study showed that there is sufficient genetic variability among the 15 genotypes that can be employed for alfalfa improvement programs for dry forage yield as well as other yield components and quality. It is for number of tillers/m^2, plant height, and quality, which showed a positive correlation and significance with dry forage yield. Results suggest that the G14 genotype of alfalfa achieved superior for getting the highest values for yield, its components, and quality compared to the other genotypes followed by the G3 and G7 genotypes. In contrast, the G10 genotype recorded the lowest values. Generally, the genotypes G14, G3, and G7 are the most promising genotypes under calcareous land conditions and their use in the breeding programs.

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