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Genetic Analysis of Path Coefficients of Some Traits in Relation with Tolerance to Alfalfa Leaf Weevil (*Hypera postica* Gell.)

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ABSTRACT

Alfalfa, as a fodder plant in Hamedan, is attacked by alfalfa leaf weevil (*Hypera postica* Gell.). In this study, 76 alfalfa populations in the germplasm of Bu-Ali Sina University farm were investigated randomly. Traits SPAD, number of larvae, percentage of damage, height of the plant in the time of damage and harvest, percentage of dry matter, and traits of the dry and fresh yield of forage were studied in this research. The results showed that there is a correlation between the number of larvae trait and damage percentage (r = 0.733**), SPAD and damage percentage (r = 0.292*), forage yield and plant height at the time of damage (r = 0.512**), dry fodder yield and plant height at the time of damage (r = 0.314**), the yield of wet and dry fodder (r = 0.754), the percentage of dry matter with the yield of fodder (r = 0.316**), and the dry yield of fodder with the percentage of dry matter (r= 0.332**). Step-by-step regression for the wet yield of fodder as a function variable showed that traits of the dry yield of fodder, percentage of dry matter, and plant height in the damage stage were entered into the model, respectively, and justified the most changes in fodder performance with a cumulative explanation coefficient of 93.53%, while other studied traits had no significant effect on the model. According to the entered traits (forage dry yield traits, dry matter percentage, and plant height in the damage stage) in the regression model, path analysis was done to determine causal relationships affecting. Path analysis of traits under study on wet forage yield revealed that it had the most direct and indirect effects on dry forage yield and matter percentage, respectively. This result was confirmed by the high correlation between wet fodder yield and dry fodder yield.

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Introduction

Alfalfa increases soil nitrogen content and generally improves soil fertility due to deep infiltration systems, accumulation in soil, and rapid decomposition of root biomass (Skuodiene *et al.*, 2023; Kusvuran *et al.*, 2014; Laki´c *et al.*, 2019). Alfalfa (*Medicago sativa* L.) (2n = 4x = 32) is one of the most important forage plants due to its unique characteristics and plays an important role in providing the forage needs of the country (Arbab, 2006). In the study of different alfalfa populations, agro-biological traits and statistical analysis were used properly (Skuodiene *et al.*, 2023). Alfalfa is another agricultural product that Hamedan province ranks second in the country for its production. There is a consensus that Iran is the central region of the geographical origin of alfalfa, but its importance, abundance, and distribution are in the western region of the country, including Hamadan province. Importantly, the effect on agricultural land improvement through land ventilation, rotation, drainage, and increase of soil organic matter and nitrogen is considered another characteristic of alfalfa (Karimi, 1990; Osborn *et al.*, 1997). The nativeness of this plant has made Iran to be significant in terms of beneficial and harmful insects of alfalfa. Many pests feed on different parts of alfalfa, among them, the leaf weevil (*Hypera postica* Gell.) is the most important damaging species to which alfalfa is sensitive and vulnerable (Arbab and Macknil, 2000; Chandra, 2010). Alfalfa leaf weevil is one of the most important pests of alfalfa (the first-degree pest of all alfalfa-producing areas of Iran), which is sometimes capable of destroying more than 90% of the first crop of alfalfa. This pest has a high density in most of the alfalfa fields in Hamadan province and the main damage is done by the third and fourth instar larvae (Khanjani, 2009). Forage yield is the most important characteristic of forage plants, which is a quantitatively complex trait and is influenced by many morphological and physiological characteristics; so selection based on yield is not only dependent on yield but yield components should also be considered. The relationship between forage yield and morphological characteristics is an issue that needs further investigation (Farshadfar *et al.*, 2014). Diversity and selection are the two main pillars of any reform program, and the selection depends on the presence of desirable diversity in terms of the goal and is under investigation (Naroierad *et al.*, 2006). In fact, breeding is based on variety and selection, and genetic variation increases the breeder’s field of activity and choice for selection and other breeding operations (Abouzari Gazafrodi *et al.*, 2006). The study of genetic diversity is important not only for the organization and protection of plant materials but also for the phenomenon of heterosis and the production of hybrid seeds. Correlation between traits is useful in planning and evaluating multi-breed programs; in other words, selecting a trait is done when there is information about how it affects other traits that are of interest and importance, and knowledge of the existence of a correlation between important traits makes the interpretation of the previous results easier and provides the basis for planning effective plans in the future, as well as the correlation between important and less important traits, multi-racial specialists in indirect selection. For important traits, it helps through less important traits that are easier to measure (Saki Nejad and Seyed-Mohammadi, 2011). Unilateral selection for agricultural traits without considering other traits will not have favorable results. Therefore, in breeding programs, attention should be paid to the correlation between traits (Kakaei *et al.*, 2013).

Many researchers have used correlation analysis in their reports (Rezaei *et al*., 2011 in alfalfa; Asilan and Hajiloei, 2010 in alfalfa; Kakaei *et al.*, 2010 in alfalfa; Sengul, 2002 in alfalfa). Considering that the correlation is not always perfect, it is possible to use a parameter that introduces the relationship between the variables with more power. This parameter is the regression analysis that studies the nature of the relationship between the variables; in fact, multiple regression analysis is the broadest statistical tool that is used to model and estimate the value of a quantitative variable or response, according to its relationship with one or more quantitative variables (independent or predictor variables) (Farshadfar, 2014). In order to know the relationships between variables, several researchers have used regression analysis in their studies (Rezaie *et al.*, 2011 in alfalfa; Jabbari *et al.*, 2011 in barley; Zarei *et al.*, 2013 in wheat). Breeders deal with correlated traits in their breeding programs. The degree of influence of one variable on another variable can be quantitatively expressed. Path analysis is a method that was first introduced by Wright (1921). In fact, path analysis is a special type of multivariate analysis that has a closed system of variables that have a linear relationship. A closed system of variables means that each variable in the system is both one of the main factors of the system and a linear combination of the variables in the system (Choukan, 2008). Different traits present in alfalfa of different regions (domestic and abroad) examine the correlation between traits to make the selection process efficient and to find out how the studied traits are related to pest resistance and their genetic affinity through determining the correlation coefficient and regression formed the other goals of this research. Finally, with the help of the results of the regression and correlation analyses, the relationships between the traits included in the regression model are studied with the help of causality analysis.

Material and methods

Plant material and experimental design

Seventy-six ecotypes of alfalfa from different geographical origins were evaluated in this study (Table 1). The experiment was carried out in the research farm of the Bu-Ali Sina University in the Dastjerd region with an altitude of 1810 meters from sea level and with a geographical length of 48º, 88' and geographical width of 34º, 54'. The field trial was arranged in a completely randomized design. Each line grown included two meters. Sowing had previously been done in an appropriate land as a good homogenous experiment material. The stand had been irrigated every 9 days due to the conventional irrigation after spring rainfall in the region. Data for studied characteristics were recorded in different growth stages and weeds were mechanically controlled by hand. In this experiment, a completely randomized design (evaluation germplasm) was used in two replicates for 150 ecotypes and genotypes of alfalfa as germplasm, 76 ecotypes were studied for statistical studies. Each experimental unit consisted of a 2.00 meters row with 50 cm row spacing. No insecticide was used in the field as routine spraying operations in the region were made. A natural pest infection in the field happened in early spring (depending on the ecological conditions of the study area).

Phenotypic data collection and statistical analysis

During the study, eight phenotypic traits were observed. The following traits were collected on all individual plants of each ecotype:

The number of larvae per plant (Larvae): In three steps of the seven-day trial and each step of one-third of the experiment, the number of larvae per plant was counted by placing a white paper on the foot and shaking limbs.

Plant height at ten percent flowering time (He1): At 10% germination time of flowering plants up to five centimeters from the top of the collar, a few plants from each plot were recorded by measuring the height and the average plant height in centimeters.

Plant height since of damages (He2): In the third week of losses by pests, plant seedlings to five centimeters measured from the top of the collar, a few plants from each plot were recorded by measuring the height and the average plant height in centimeters.

Chlorophyll content (SPAD): The SPAD device (Minolta, Japan) was used to measure the size of the SPAD plant infestations in the first and third weeks.

Dry matter yield: For this purpose, 250 grams of fresh forage were weighed after being air-dried in an oven at 75℃ for 24 h, and typically, the dry weight was generalized in terms of grams of the test plots.

Percent dry matter (PDM): To calculate forage yield, it was multiplied by 100 and divided by percent more performance, respectively.

Damage amount (Score): Considering the shoots wounding and estimating relative damage, the amount of damage was determined by scoring from zero to nine so that zero for 1%-10% and 9 for 91%-100% damage caused by larval feeding.

Evaluation of this character was carried out three times, each time after seven days. In order to reduce the experimental error during scoring, evaluating the amounts of damage to shoots was done by a sampler person while he was standing in the direction of sun radiation.

After data transformation, the quality data were changed to quantity data as the percent of damage amounts.

Fresh forage yield: Alfalfa shoots of each ecotype were cut off from 5cm above the top of the scalar when the flowering percentage was at 10%. The forages were harvested from the middle 1-meter length in the middle experimental unit and weighed in grams.

Table1. List of alfalfa (*Medicago sativa*) ecotypes evaluated in the present study.

Code (Row)	Name population	Type/species	Code (Row)	Name population
1	Hamedan 29	cultivar/sativa	39	Telantic 34044
2	Bahar-e- Hamedan 28	cultivar/sativa	40	Devipoe 34630
3	Hamedan Ala 31	cultivar/sativa	41	Dupuits
4	Hamedani 87	cultivar/sativa	42	Hamedani 32
5	Hamedani 106	cultivar/sativa	43	Tak boteh 51
6	Hamedani 54	cultivar/sativa	44	Lahontan
7	Afghani 81	cultivar/sativa	45	Eur
8	Sarab 16	cultivar/sativa	46	Karaj2122
9	Calyfornia 19	cultivar/sativa	47	Kodi 108
10	Harati 26	cultivar/sativa	48	Bami 86
11	Tafresh 42	cultivar/sativa	49	Bami 103
12	Feez 48 (Fc32666)	cultivar/sativa	50	Bami 6
13	Feez (varragensee)	cultivar/sativa	51	Renjer 107
14	Feex (Vermal)	cultivar/sativa	52	Yazdi 105
15	Yazdi 40	cultivar/sativa	53	Yazdi 41
16	Shiraz 9	cultivar/sativa	54	Yazdi 37
17	Trnambda soued	cultivar/sativa	55	Yazdi 38
18	Mahali Shapor	cultivar/sativa	56	Yazdi 91
19	Mahali marandi	cultivar/sativa	57	Lutece 81
20	Mahali bami	cultivar/sativa	58	Kerisari Torkish 2122
21	Sun loez America 288	cultivar/sativa	59	Tak Bute 58
22	Mahali Hamedan	cultivar/sativa	60	Tak Bute 2
23	Hamedani 292	cultivar/sativa	61	Tak Bute 48
24	Yazdi	cultivar/sativa	62	Famenin Hamedan 18
25	Talent 2	cultivar/sativa	63	Luxor
26	Lahontan policross America	cultivar/sativa	64	Zabol 15
27	Ladak	cultivar/sativa	65	Topoa Abad Rezaeei 9
28	Policross Hamedan	cultivar/sativa	66	Mahalat 25
29	Policross bam 10	cultivar/sativa	67	Dehnavar Nahavand 13
30	Naragamet	cultivar/sativa	68	Khozestan 12
31	Etlantic 1	cultivar/sativa	69	Feize 46
32	Argantin	cultivar/sativa	70	Feize 43
33	Mahalo khoe	cultivar/sativa	71	Khoram Abad 10
34	Grem-e- America	cultivar/sativa	72	Famenen Hamedan 80
35	Policross shiraz 8	cultivar/sativa	73	Karim Abad Rezaeeie 24
36	Ardabili	cultivar/sativa	74	Kerisary
37	Rezaeeh	cultivar/sativa	75	Motafareghe Hamedan 27
38	Vernal	cultivar/sativa	76	Hamadani (Control)

Results and discussion

Stepwise regression analysis for wet forage yield: In many cases, one variable is affected by several variables; in these cases, the extended mode of correlation and simple regression is used for more than two variables (Choukan, 2008). Stepwise regression was used in order to determine the traits with the greatest effect on the economic performance trait and to determine the contribution of each of these traits to the variance of the total performance (Table 4). Based on the result, traits forage dry yield dry matter percentage, plant height at the time of damage, and the number of larvae were respectively entered into the regression model and in total, explained 93.53% of the changes in the economic performance traits (forage yield).

Based on the result, other studied traits had no significant effect on the model. The findings showed that the first three traits entered the model; this indicates the important effect of these traits on the yield of forage. In the study of correlation and regression related to leaf weevil in alfalfa germplasm, results revealed that the stepwise regression analysis for the fresh yield of forage as a function variable showed that dry yield traits of forage, the percentage of dryness, height of the plant at the 10% flowering stage, and the number of larvae were entered into the model, respectively and explained the most changes in the yield of forage with a cumulative explanation coefficient of 89.29% (Kakaei and Mazaheri Laqab, 2013). The final model of the equation of the regression line is presented, Y= 361.622+2.391X1-12.568X2+5.406X3.

The variance inflation factor for the variables entered in the model was also calculated, and this value is given to the variables entered in Table 3. This value for all the variables in the model is less than 10, which indicates the absence of collinearity between the existing variables.

Table 2 shows the results of the analysis of variance regression. The significance of the multiple linear regression analysis of variance indicated the linear relationship between the independent variables and the dependent variable; in other words, the yield of forage increased linearly with the increase in the values of the independent variables.

Table2. The results of stepwise regression variance analysis for the fresh yield of forage

Sources of variation	df*	Sum of squares	Mean of squares	F-test
regression	7	3410082.317	487154.617	151.506**
Error	68	218648.209	3215.415	
Total	75	3628730.526		

* Degrees of freedom

Table3. The results of the variance inflation factor for the variables included in the model.

Sources of variation	RC[1](B)	SIRC[2] (β) (direct effects)	t	VIF
Constant	361.622		4.843**	
Dry yield of forage	2.391	0.923	26.425**	1.358
Dry matter percentage	-12.568	0.602	17.749**	1.280
Height of the plant at the damage stage	5.406	0.098	2.897*	1.263

1= Regression Coefficients; 2= Standardized Incomplete Regression Coefficient

Table 4. Step-by-step regression results of the dry yield of forage as the dependent variable and other evaluated traits as independent variables

Characteristics	Intercept	Regression coefficients[1]				Cumulative coefficient[2]
		(1)	(2)	(3)	(4)	
(1) Fresh yield of forage	206.9	1.95				56.20**
(2) Dry matter percentage	561.3	2.5	-13.28			92.58**
(3) Plant height at the time of damage	361.6	2.39	-12.57	5.4		93.26**
(4) Number of larvae	385.5	2.40	-12.63	5.5	-1.50	93.53**

1= Regression coefficients for traits in different stages of entering variables; 2= Cumulative coefficient of explanation; * and **: Significant at 5% and 1% probability levels, respectively.

Correlation analysis

Knowing the relationship between forage performance and its components will certainly help improve the efficiency of a breeding program with appropriate selection criteria. In correlation analysis in genetic studies, the correlation between two or more traits is very useful. The relationship between two traits that were observed and measured is a phenotypic correlation. Phenotypic correlation is estimated from the observed phenotypic values of two traits on a number of people (Choukan, 2008).

The results of the correlation analysis between traits (Table 5) showed that the characteristics of the fresh yield of forage and dry yield of forage (r = 0.754) had a significant correlation; if the amount of fodder yield increases, the yield of dry fodder will also increase on the same basis. Moreover, as it is clear from the correlation analysis in Table 5, the dry matter percentage trait has a significant correlation with the characteristics of the fresh yield of forage (r = -0.316) and dry yield of forage (r = 0.332).

The fresh yield of forage traits (r= 0.512*) and subsequently dry yield of forage traits (r= 0.314*) have a positive and significant correlation with height trait at the time of damage because forage yield increases with the increase in height as the plant increases yield by maintaining (recovery) itself and increasing longitudinal growth and finally height. Additionally, a positive and significant correlation is observed between the SPAD trait and rating trait compared to freshness (r = 0.512*) at the probability level of 1% (r =

0.733). Therefore, with the increase in freshness, the amount of chlorophyll in the aerial parts of the plant, and the height, the tendency of the pest to feed on the relevant population's increases and a lot of damage is imposed on the host. This is the mechanism of preference of the pest to the host. Many researchers separately reported the effects of damage caused by pest infestation and feeding on the reduction of forage yield (Khanjani, 2004; Mazaheri-laqab, 1991; Jamshidi Golan, 2012). One study investigating some eggplant genotypes reported a positive and significant correlation (r = 0.349**) between the amount of SPAD and the preference of *Tuta absoluta* to lay eggs in the field (Shararbar, 2014).

Table 5. Double correlation coefficients between investigated traits in 76 alfalfa populations.

DM[1]	NL[2]	DP[3]	PD[4]	SPAD	FF[5]	DF[6]	HF[7]	DM[1]
Number of larvae	1							
Damage percentage	0.733**	1						
Plant height at the time of damage	0.89	0.20	1					
SPAD	0.173	0.292*	0.112	1				
Fresh yield of forage	0.064	0.073	0.512**	0.173	1			
Dry yield of forage	0.107	0.133	0.314**	0.129	0.754**	1		
Height of the plant at the time of 10% flowering	0.078	0.084	-0.062	-0.025	-0.081	-0.062	1	
Dry matter percentage	-0.042	0.010	-0.208	-0.040	-0.316**	0.332**	0.018	1

1= Dry matter percentage; 2= Number of larvae; 3= Damage percentage; 4= Plant height at the time of damage; 5= Fresh yield of Forage; 6= Dry yield of forage; 7= Height of the plant at the time of 10% flowering; * and **: Significant at 5% and 1% probability levels, respectively.

Path analysis of traits

The main goal of most alfalfa breeding programs is to increase yield and improve quality. Since it is not possible to achieve genetic improvement beyond the limit set by the genes present in a population, the selection of available germplasm in a breeding program is a priority for any breeder (Popovic *et al*., 2006). Based on the order of importance of traits and step-by-step regression, four traits were selected and subjected to causality analysis. According to Table 6, the dry yield of forage has a direct effect of 0.927 on the fresh yield of forage, and the total correlation of this trait with a fresh yield of forage was found to be 0.754**. Moreover, the dry matter percentage trait has a direct effect of -0.603 on the yield of forage and the total correlation of this trait with the yield of forage is -0.316. The height of the plant at the time of damage also has a direct effect of -0.097 on the fresh yield of forage and the total correlation of this trait with the fresh yield of forage is -0.512. Additionally, the number of larvae has a direct effect of -0.057 on the fresh yield of forage and the total correlation of this trait with yield forage is 0.064, and the residual effects were insignificant.

Table 6. The amount of direct and indirect effects of traits on the dry yield of forage based on correlation coefficients of traits

Characteristics	DE1	IE2				TC3
		(1)	(2)	(3)	(4)	
(1) Fresh yield of forage	0.927		-0.209	0.03	-0.005	0.754**
(2) Dry matter percentage	-0.603	0.698		-0.02	0.002	-0.316**
(3) Plant height at the time of damage	0.097	0.291	0.125		-0.045	0.512**
(4) Number of larvae	-0.057	0.099	0.025	0.086		0.064

1= Amount of direct effects through traits; 2= Amount of indirect effects through traits; 3= Total correlation with a fresh yield of forage; * and **: Significant at 5% and 1% probability levels, respectively.

Fig. 1 shows the relationship between the dependent variable and the independent variable. Moreover, Table 6 demonstrates the amount of direct and indirect effects of traits on the dry yield of forage based on trait correlation coefficients. In research on the Varamin cotton cultivar and investigating the causal analysis of the effects of some cotton traits on cotton seed weight, the merits of such studies were examined (Kakaei and Kahrizi, 2009). Many researchers have used path analysis to study the relationships between traits (Monirifar, 2006; Tadele *et al*., 2022).

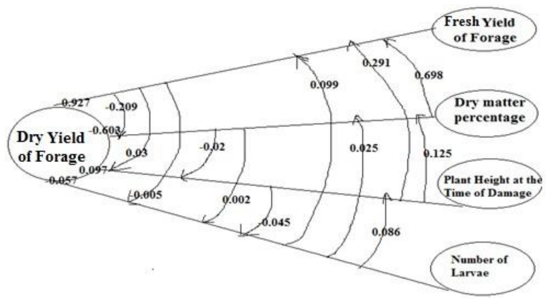

Fig. 1. Relationships between the dependent variable and the independent variable.

Conclusion

Alfalfa is one of the forage plants in the world, especially in Iran. Alfalfa (*Medicago sativa* L.) is one of the most important perennial forage crops for creating effective diets for livestock producers. The improvement of forage crops largely depends on the availability of diverse germplasm and their efficient use (Mervat *et al*., 2022). Based on the general results of the path analysis of the studied traits, it can be concluded that the traits such as fresh yield of forage and plant height at the time of damage had the greatest direct and positive effect, and the traits of the percentage of dry matter and the number of larvae had the greatest direct and negative effect on the yield of dry forage. In general, the selection based on the characteristics of the fresh yield of forage and the height of the plant at the time of damage is useful in order to increase the forage yield. Summarizing the results of the investigation of the three mechanisms of antixenosis, antibiosis, and tolerance on the studied populations showed that the local populations of Bami, Hamadani 106, and Kadi 108 were promising populations with the occurrence of levels of all three resistance mechanisms, and their cultivation is recommended considering other agronomic and technical points to deal with alfalfa leaf weevil damage. Therefore, these lines can be used in commercial types if their superiority continues. In addition, it can be used in the programs of improvement and development of new alfalfa lines and species tolerant to climate change conditions in Iran.

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Conflicts of interest

The authors declare no conflicts of interest.

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