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Study of Correlations betweenHorticultural Traits and Variables Affecting Kernel Percentage of Walnut (*Juglans regia* L.)

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Abstract

Studies of physiological diversity and its effect on the expression of traits in plants are used in breeding programs as a selective tool with the aim of selecting special attributes for creating a new product. In this study, the correlation coefficients among 17 horticultural traits of 34 genotypes from 11 different places of Semnan province were examined. A significant positive correlation was observed between fruit weight and kernel weight, susceptibility to blight and leaf abscission, leafing date and female flower emergence and between kernel percentage, kernel weight and kernel plumpness. Furthermore, a significant negative correlation existed between harvest time and tree vigor, fruit shape and fruit weight and kernel percentage with shell thickness. The most important characteristics that had a direct impact on kernel percentage were shell thickness and kernel plumpness. The attributes that were effective on fruit ripening were tree form, catkin emergence time and shell thickness. However, the residual effects suggested that there might be other important determinant traits for harvest date, which were not considered in this study.

Keywords: Correlation, Kernel percentage, Path analysis, Walnut.

Introduction

Walnut production of Iran in 2012 was 450,000 tons, which is second in walnut production in the world (FAO 2012). The presence of old walnut trees in different regions of Iran, use of different traditional agriculture methods in walnut growing based on native knowledge, great variety of nutritional, medicinal consumption and many decorative uses of walnut tree in wood industry, existence of different and classified local names for Persian walnut based on ripening steps of fruit in different Iranian cultures, and special customs and traditions for harvest time, suggest a long history of walnut cultivation in Iran (Papoli *et al*. 1995).

There are several areas with favorable climate for walnut cultivation on the northern edge of Semnan province, Iran including: Shahmirzad, Toyehdarvar, Astaneh and Ahoovano and some valleys of Shahroud city, including the walnut orchards in Semnan province. Most walnut orchards in Semnan province are seed - borne, which causes great variation in size and shape of fruit, thickness of shell and kernel quality, which are important for breeding programs (Aslantas, 2006; Zeneli *et al*., 2005).(Fig. 1)

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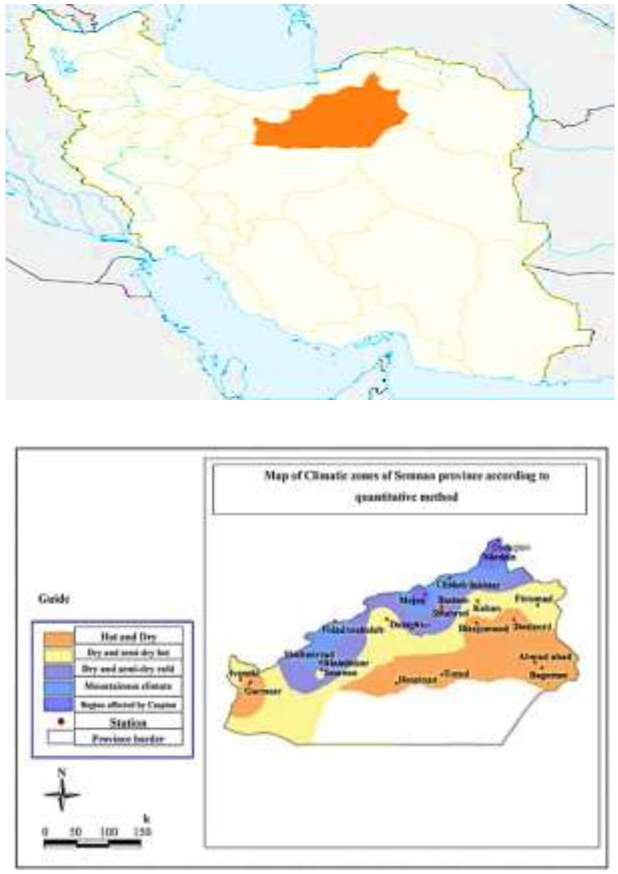

Fig 1.Climatic map of Semnan province, Iran.

An ideal walnut cultivar should have traits such as late leafing, both terminal and lateral bearing, minimum loss of female flowers, high yielding (more than 6 million tons of fruit per hectare) and large fruit, bright and fleshy kernel, kernel percentage more than 50% and moderate resistance to pests and diseases (Mc Granahan and Leslie, 1990).

Many factors such as tree age, area agro ecology, number of female flowers, fruit set, fruit size and kernel percentage also affect total production of walnut (Akça, 1997).

Breeding to achieve new varieties of walnuts through hybridization is difficult and time-consuming. Therefore, appropriate methods based on climate adaptation, precocity, high yield, fruit and kernel quality and resistance to plant diseases, and diversity are more practical (Coates, 2012; Michailides *et al*., 2012). Many researchers around the world selected high quality fruits from seed - borne populations of walnut (Germain, 1997; Sharma and Das, 2003; Cosmulescu and Botu, 2012).The new objectives in breeding plant can be achieved by evaluating valuable traits among the genetic resources and providing a combination of them in a cultivar. Although, molecular markers such as isozymes and DNA - based marker can be used for breeding programs, these methods are expensive. In addition, in walnut, there are many varieties. The use of morphological criteria for classification maybe more appropriate (Asadian and Pieber, 2005; Arzani *et al*., 2008; Karimi *et al*., 2009).

Morphological characteristics that are recorded for parental selection is the first choice used to describe and classify germplasm. Morphological traits may be correlated with each other or with traits that are difficult to assess, such as resistance to disease. Thus, they may be used as useful markers in breeding programs (Karimi *et al*., 2009).

Determining the relationship between fruit quality and yield in a similar situation ensures an effective breeding program which facilitates an appropriate choice consistent with the purpose.

However, considering this possibility that the correlation between two variables maybe influenced by other variables prevents the use of correlation coefficient alone at the beginning of selective work.

Awareness of the relationship between characteristics of kernel and fruit and other traits of tree can be a useful guide in selective breeding program of walnut. The first attempt to study Persian walnut genotypes was made by Atefi (Arzani *et al*., 2008; Atefi, 1990). Further evaluation was also conducted in Iran (Arzani *et al*., 2008; Eskandari *et al*., 2006).

Atefi (1990) reported that there is a strong positive correlation between yield and tree diameter and height, but correlation between yield and nut weight was negative. Altitude positively associated with cold tolerance but has a negative correlation with yield. Forde and McGranahan (1996) reported a negative correlation between tree height and yield. In reported correlations about yield and protogynous or protandrous flowering habits, conflicting reports have been published (Amiri *et al.*, 2010).

Hansche *et al*. (1972) found a strong positive correlation between lateral bearing habit and yield. Germain (1997) found that lateral bearing habit was moderately correlated with early leafing, tree architecture, and precocity. Solar *et al*. (2001) also found that trees with lateral flowering habit developed flowers earlier in the spring and had better yield potential than terminal bearing habit. Leafing date was correlated with flower timing as well as tree vigor, branching density, height of tree, and trunk diameter. Tree vigor was positively correlated with tree height, trunk diameter, tree form, branching density, and fruit bearing type. Arzani *et al*. (2008) reported there were significant correlations between nut weight and nut length (0.57), nut width (0.68), nut thickness (0.67), kernel weight (0.75), and shell thickness (0.32); whereas there were a negative correlation between shell thickness and kernel ratio (-0.34), Results of the correlation between nut weight and shell thickness (0.375) and also between shell thickness and kernel ratio (-0.639) were in accordance with the results reported of Ghasemi *et al.* (2012). Furthermore, Ghasemi *et al*. (2012) reported a significant positive correlation (0.863) between nut weight and kernel weight. However, no relationship between nut weight and kernel ratio was found in their studies. Sen (1985) obtained a significant negative correlation between shell thickness and kernel percentage. Arzani *et al*. (2008) found no correlation between geographic location and biodiversity. Akca and Sen (1997) found that nut and kernel weights and kernel to shell ratio in protogynous varieties were greater than those of protandrous types.

In all studies, the correlation coefficients did not explain the cause and relationships effect in cultivars, because the correlation between two variables may be dependent on a third variable. Using path analysis provides an acceptable interpretation of the observed correlation by modeling of reason and relationships effect between variables, making it possible to analyze correlation coefficient of variables in the form of variance and covariance. As a general statistical method, path analysis is created to analyze the causes and effects. In correlation between variables, a path coefficient is a partial regression coefficient that is standard and measures the direct effect of a predictor variable on the response (dependent) variable (Mohammadi *et al*., 2003). That helps separating correlation coefficient in to direct effects (path coefficients) and indirect effects (effects that shown through other independent variables) (Mohammadi *et al*., 2003).

Unexplained effects are treated as residual effects. Path analysis has been used by plant breeders to understand the relationship between productivity and its components in various crop plants (Kang *et al*., 1983). This technique, however, is rarely used in the plant population biology. One report on its application in walnut has been published by Amiri *et al*. (2010). The aims of this study were to determine the correlations among agronomical traits of walnut in landraces of Semnan Province, Iran, and to use cause (path) analysis to study the influence of other horticultural traits on kernel percentage and harvest date.

Materials and Methods

Thirty four native walnut genotypes were selected from planting and growing areas of walnuts in Semnan province including: Shahmirzad (1984 M35.79 N, 53.36 E), Abarsaj (36.57 N, 54.91E 1750 M), Armeyan (36.36 N, 55.40 E 1452M), Nam-e Nik (37.12N, 55.68E, 1589M) and walnut planting areas around Damghan, including: Āstāne (36.27N, 54.09E1480M), Dibaj (36.44 N, 54.23 E, 1835), and TuyehDarvar (36.02 N, 53.85 E, 2017M) villages.

These areas have a climate that consists of a cold, wet winter and cool, semi-dry summer. Since all selected trees were seed – borne, the traits showed high diversity. Some pomological and phonological characteristics of mature trees were recorded. Seventeen horticultural traits were evaluated based on Biodiversity International (IPGRI, 1994) descriptors (Table 1), and Pearson's coefficient of correlation was calculated. Two main traits, kernel percentage and harvest date, were analyzed as dependent variables and traits influencing them were detected by stepwise regression (Draper and Smith, 1998). Based on the results of the stepwise regression analysis for kernel percentage, this trait was selected to use as a dependent variable in the path analysis, and the traits that had a linear relationship with kernel percentage in stepwise regression, including shell thickness and kernel plumpness, were considered as exogenous variables. Correlation analysis and stepwise regression were performed using SPSS (Version 16.0) software.

Table1. The Horticultural traits evaluated and their scaling in 34 walnut genotypes of Semnan Province.

The traits	Scaling
1. Tree form	Upright (1), moderately upright (3), moderately spreading (5),spreading (7)
2. Tree vigor	Very weak growth (1) to very vigorous (9)
3. Flowering habit	Terminal bearing (1), less than 25% lateral bearing (3), 25% to 50% lateral bearing (5), more than 50% lateral bearing (7)
4. Leafing date	Genotypes ranked from 1 to 8 in relation to the average walnut leafing date within a location: greater than 26 d before average (1) to 21 to 25 d after average (8)
5. Harvest date	Very early (1) to extremely late (7)
6. Leaf drop date in fall	Early (3), moderate (5), late (7)
7. Blight susceptibility	Five classes: from very low (1) to very high (5)
8. Cold susceptibility	Low damage (1), moderate damage (3), extensive damage (5)
9. Nut weight in grams	From 1 (less than 8.5 g) to 9 (greater than 15.5 g)
10. Nut shape	Round (1), triangular (2), broadly ovate (3), ovate (4), short trapezoid (5), long trapezoid (6), broad elliptic (7), elliptic (8)
11. Shell texture	Smoothest (1) to roughest (9)
12. Difficulty in extracting	Very difficult (1) to very easy (9)
13. Shell thickness	Very thin (1), thin (3), moderate (5), thick (7), very thick (9)
14. Kernel weight in grams	From 1 (less than 3.5 g) to 7 (5.5 to 6.49 g)
15. Kernel Plumpness	Shriveled (3) to very plump (9)
16. Kernel color	Brown (1), amber (3), light amber (5), light (7), and extra light (9)
17. Kernel percentage	Ratio of kernel weight to nut weight *100

Results

Correlation coefficients

Correlations between horticultural traits are shown in Table 2. Tree form showed positive correlation with vigor (0.683), so that more vigorous trees have more extensive form. Harvest time is influenced by the vigor and therefore fruits of stronger trees can be harvested earlier (-0.367). Opening time of female flowers had a significant positive correlation with leafing date (0.992), while the kernel extraction of vigorous trees (-0.444) was more difficult than weak trees. Trees with lateral bearing habit showed more sensitivity to blight (0.363). Furthermore, trees with leaves falling late (0.361) and fruits having thinner shell (0.369-) showed higher sensitivity to blight.

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Shell texture had a significant positive correlation with leafing date (0.427) and opening time of female flowers (0.423) but it showed a significant negative correlation with leaf drop date (-0.417). Fruit shape and fruit weight (-0.350) and fruit shape and kernel weight (-0.324) showed a significant negative correlation but fruits with greater weight had increase kernel weight (0.563). Kernel extraction from fruits with plump kernel (-0.444) was very difficult. A negative correlation (-0.531) existed between kernel percentage and shell thickness, while correlations between kernel percentage and kernel plumpness (0.448) and kernel percentage and kernel weight (0.363) were positive.

Table 2. Correlation coefficients (r) for characteristics of native walnut genotypes in Semnan province of Iran

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20
1. Tree form	1.000																		
2. Tree vigor	.683**	1.000																	
3. Flowering habit	.090	.091	1.000																
4. Leafing date	.090	.091	-0.62	1.000															
5. Female flowering	.089	.091	-0.62	.992**	1.000														
6. Catkin flowering	-.100	-.112	-0.185	.339	.336	1.000													
7. Harvest date	-.367*	-.165	-0.161	-.161	-.160	.334	1.000												
8. Leaf abscission	-.014	.087	0.105	-.224	-.229	.058	.082	1.000											
9. Blight susceptibility	.112	.114	0.363*	-.078	-.077	-.230	-.201	.361*	1.000										
10. Cold susceptibility	-.311	-.124	-0.170	-.170	-.168	.125	.219	.084	-.211	1.000									
11. Nut weight	-.091	.046	0.034	-.023	-.028	.096	.142	.048	-.208	.245	1.000								
12. Nut shape	.054	.054	0.099	.099	.105	-.098	-.225	.068	.247	-.218	-.350*	1.000							
13. Shell texture	-.226	-.234	-0.142	.427*	.423*	.263	-.055	-.417**	-.265	.189	.200	.028	1.000						
14. Difficulty in extracting kernel	-.163	-.444**	-0.090	-.090	-.089	.122	-.017	-.314	-.112	-.044	-.140	-.049	.246	1.000					
15. Shell thickness	-.096	-.247	-0.252	-.252	-.244	-.105	.288	-.280	-.369*	.079	-.267	-.024	.097	.249	1.000				
16. Kernel weight	-.210	-.024	-0.113	.145	.144	.302	.242	.204	-.248	.271	.563**	-.324*	.246	-.210	-.271	1.000			
17. Plumpness	.006	.228	0.139	.139	.138	.265	-.098	.234	-.072	-.047	.000	.221	-.138	-.444**	-.269	.152	1.000		
18. Kernel color	-.194	-.192	-0.085	-.269	-.260	-.078	.152	-.119	.022	-.006	-.062	.010	.051	.194	.290	-.102	.029	1.000	
19. Kernel percentage	-.031	.072	-0.068	.074	.073	.288	-.032	.252	.082	-.055	-.025	.071	-.224	-.280	-.531**	.353*	.448**	-.192	1.000

*, **Significant at 0.05 and 0.01 P levels, respectively; others are non-significant.

Stepwise regression and path analysis

Stepwise regression for harvest date indicated its use as a dependent variable in path analysis with tree form, catkin flowering date, and shell thickness treated as exogenous variables. After calculating correlation coefficients of kernel percentage and harvest date that were considered as dependent variables, important independent variables were also determined by stepwise regression (Tables 3 and 4), and direct effects of each independent variable on the dependent variables were calculated. Stepwise regression showed that more than half of the kernel percentage changes are justified with shell thickness and part of the remaining changes with kernel plumpness trait. About the harvest date, -0.346, 0.353 and 0.321 percent of changes are explained by tree form, catkin flowering time and shell thickness respectively. As shown in Table 3, the first variable entered into the model, was shell thickness, and in the second step, kernel plumpness entered into the model.

Table 3. Stepwise regression between kernel percentage (dependent variable) and other characteristics (independent variables) of native walnut genotypes in Semnan province

Step	Inserted variable	b	R^2	Partial R^2
1	Shell thickness	-0.552**	0.283**	-0.552
2	Shell thickness	-0.433**	0.427**	-0.493
	Kernel plumpness	0.414**		0.476

*, **Significant at 0.05 and 0.01 P levels, respectively; others are non-significant.

Table 4. Stepwise regression between date of harvest (dependent variable) and other characteristics (independent variables) of native walnut genotypes in Semnan province

Step	Inserted variable	b	R^2	Partial R^2
1	Tree form	-0.380*	0.118**	-0.380
2	Tree form	-0.370*	0.199**	-0.392
	Catkin flowering date	0.321*		0.347
3	Tree form	-0.347*	0.286**	-0.394
	Catkin flowering date	0.356*		0.402
	Shell thickness	0.323*		0.370

*, **Significant at 0.05 and 0.01 P levels, respectively; others are non-significant.

Discussion

Most research studied the correlation between horticultural traits carried out in different geographic regions. Therefore, separating environmental effects of different climates, geographic and climatic information of tree growing regions are not easily possible. However, the correlation between traits with regard to the inheritance of traits is important in breeding of crops. Positive correlation between vigor and tree form (0.683) indicates that the stronger trees have more ability for branching and widespread development. These results are in agreement with the results reported By Ehteshamnia *et al.* (2010). Moreover, significant negative correlation between tree form and harvest time (-0.367) indicates that fruits in widespread trees, ripe earlier than other trees which can be explained by more exposure of widespread trees to light and greater power of trees in ripening of their fruits. Due to the high inheritance of harvest time trait (0.85), there is good coordination between two desirable traits for breeding goals. Although earlier fruit harvest and the occurrence of sooner leaf fall reduce sensitivity to autumn frosts.

Significant negative correlation between tree vigor and the difficulty of kernel extraction (-0.444), and also between kernel plumpness and difficulty of kernel extracting (-0.444), show vigorous trees produce more plump kernels. This view can be explained in that the outer dimensions of nuts and core of stone fruits fix earlier than achievement of final dry weight and considering the impact of growth vigor on kernel size can explain more penetration of plump kernel to shell after fixation of final fruit size.

In this study, significant positive correlation was also observed between the sensitivity to blight and flowering habit (0.363), in that trees with lateral fruiting habit are more susceptible to blight, which is consistent with findings of Amiri *et al.* (2010).

A positive correlation between sensitivity to blight and leaf abscission time (0.361) can explained in that there is greater chance for increase in infection of both leaves surfaces and number of leaves.

A significant positive correlation existed between kernel weight and fruit weight (0.563), which is in accordance with the findings of Amiri *et al.* (2010), Eskandari *et al.* (2006) and Sharma and Sharma, (2001). Hansche *et al.* (1972) also reported that there was a positive correlation between fruit weight and kernel weight.

A significant negative correlation observed between kernel percentage and shell thickness (-0.531) is confirmed in the studies of Norouzi *et al.* (2013), Eskandari *et al.* (2006), Amiri *et al.* (2010), Bayazit (2012) and arzani *et al.* (2008).

Furthermore, Ehteshamnia *et al*. (2010) reported that kernel size, kernel plumpness, difficulty of kernel extraction, and shell thickness are factors that affect kernel percentage, in which the first three factors have direct effect and the fourth of them has an inverse relationship.

We found a significant positive correlation between kernel percentage and kernel weight (0.353) and kernel plumpness (0.448). Our results are in agreement with Amiri *et al*. (2010) and do not correspond with findings of Eskandari *et al*. (2006). In the regression model for kernel percentage, shell thickness entered into the model in first step, and in second step, kernel plumpness entered into the model. Effects of these variables on the kernel percentage are almost equal and can be an explanation for half of the effects. In the research of Amiri *et al*. (2010), shell thickness entered into the model in the first step, and in next step, difficulty extraction of kernel entered into the model. Stepwise regression model for crop ripening date showed that tree form, catkin emergence time, and shell thickness are traits that entered into model, and each of them justified about one-third of the effect on the dependent variable.

In conclusion, two different keys traits were identified in this study: shell thickness and kernel plumpness. They are the most important variables for calculating kernel percentage and can be used in breeding studies. Path analysis also showed that tree form, anthesis time and shell thickness had direct and positive impacts on harvest date.

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