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Yield related traits in some Persian walnut cultivars: Analysis of genetic and genetic by environment interaction

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Abstract: The most important trait in tree species, including walnut, is the yield. In this study, the effect of genotype and their interaction with year on Nut weight, Kernel weight, Kernel percentage, Fruit set, Nuts number on Scaffold (Canopy) Cross Area (SCA), Nut weight on SCA and Kernel weight on SCA were evaluated on Caspian, Persia, Alvand, and Chaldoran walnut cultivars. The results showed that the effects of year, genotype, and year × genotype interaction on all traits were significant. The results showed that Alvand had the highest number of nuts (41.8 per m^2) and nut weight (472.1 g/m^2) on (SCA). Heritability (H^2_b) for kernel weight and kernel percentage, were estimated 0.75 and 0.80, respectively. The lowest value of H^2_b (0.36) was belong to fruit set. The analyses of genetic and phenotypic correlations between traits showed that, the nut weight had (r_g = 0.31, r_p = 0.27) a moderate correlation with SCA same as kernel weight (r_g = 0.34, r_p = 0.29). The GGE biplot analysis explained most of the existing variations (>90%). The genetic effect (PC_1) for all traits were higher respect to the genetic × environment interaction (PC_2), especially for the kernel percentage (94.4%) and number and weight of nut and kernel on SCA (>90%). The lowest value of the PC_1 was related to the fruit set (65.6%), which indicates the trait was more affected by genetic × environment interactions (21.8%). So, this result showed that the yield-related traits in walnut is highly relevant to environment(year in this study) and evaluation of the new cultivars needs careful attention in this case.

1. Introduction

The accurate identification of genotypes is a basic requirement for appropriate utilization of germplasm in practical breeding programs. The diverse climatic conditions, environment, and their interactions with genetic are the most important factor determining the performance of the cultivars (Fehr, 1987). Therefore, the genetic and environment implies the differential performance of genotypes that rises from the variations in the genotype's sensitivities to the environmental conditions (Rawandoozi *et al.*, 2021). Change of climate not only affect the phenology of tree species, but also affects its production. So, with considering the climate changes and abiotic stresses, walnut production in the world has encountered challenges more than ever before. On the other hand, selection for fruit quality traits is complex; because the most of these traits are often controlled by several loci that are also influenced by the environment (Bliss, 2009). Nut and kernel weight as well as fruit set percentage could be considered as walnut yield components, while the yield efficiency could include nut and kernel weights produced on trunk cross area (TCA) or scaffold cross area (SCA) (Mahmoodi *et al.*, 2015; Dogra *et al.*, 2018; Hassani *et al.*, 2020 b). These traits can be affected by environmental conditions in several ways. For example, the climatic factors affect the receptivity period of walnut pistillate flowers and therefore affect the fruit set percentage and yield of walnut trees (Mariana and Sina Niculina, 2017).

Dogra *et al*. (2018) calculated phenotypic and genetic broad sense heritability of walnut yield related traits. Based on their study the pistillate flower density, fruit set percentage, circumference and cross section of tree trunk showed the highest correlation with the yield. Some walnut trees somewhat show different alternate bearing habits, so the yield is affected by the crop load of the previous year (Mahmoodi *et al*., 2015). Marrano *et al.* (2019) reported that lateral bearing habit have a significant influence on yield of walnuts. Besides the leafing date had high heritability (88%) and was therefore recommended as a reliable character for improvement of new cultivars.

Combining analysis of variance and stability analysis could determine the contribution of genetic, environment and their interactions in traits. In spite of climate change is becoming a bigger challenge every day, determining the genetic and environmental effects can led to understand the response of the cultivars to different environments and select the appropriate cultivars for specific environments and eventually to deal better with changing climate (Bliss, 2009; Rawandoozi *et al*., 2021).

Research with number of genotypes evaluated in different locations and years, makes the genetic × environment analysis a major contest. The GGE biplot analysis is a beneficial tool for data analyzing in multi environment trials (Yan and Tinker, 2006). Rawandoozi *et al*. (2021) estimated the variance components, genetic × environment interaction and heritability of fruit quality related traits in nine peach and nectarine low to medium chill F_1 full-sib families together with their parents in two locations. Based on their research the ripe date and fruit development period had high narrow sense heritability. Fruit weight and shape showed the lower heritability.

Scariotto *et al.* (2013) based on budburst percentage and fruit-bearing shoot formation, evaluated the compatibility and stability of peach genotypes in four years. Arji (2018) investigated the stability of yield components of olive cultivars for three years.

Despite the high priority for data availability regarding the climatic adaptability of walnut cultivars, there is need for a continuous basis research with the newly released cultivars. Therefore, this study is conducted to evaluate the adaptability of some new Persian walnut cultivars to determine the variance components and cultivars adaptability affecting the yield components and yield efficiency traits, especially with increasing the climate change challenge.

2. Materials and Methods

Plant materials and location

Walnut yield component together with the yield efficiency traits in four newly released cultivars (i.e., Caspian, Persia, Alvand, and Chaldoran) (Hassani *et al*., 2020 b) with Chandler and Jamal as reference cultivars, were evaluated in three consecutive years (2015-2017). The cultivars, grafted on Persian walnut seedlings rootstocks, were planted in Karaj in 2006 (35.76031 N, 50.96833 E; elevation: 1240 m a.s.l.; mean annual temperature: 15.8°C; and mean annual precipitation; 247 mm).

Evaluated traits

The data were recorded on yield component traits including nut and kernel weight and fruit set percentage together with the yield efficiency traits such as: nut and kernel weights produced on scaffold cross area (SCA). To estimate the number of pistillate flowers and fruits on experimental trees, pistillate flowers and fruits were counted in sample branches and then were used to predict the whole trees using regression.

To measure Scaffold Cross Area (SCA), the tree's canopy diameter was measured. The SCA was then estimated using the canopy and the circle approximation. For nut and kernel traits, 30 samples in each treatment were evaluated. The tree nut and kernels' yield were obtained from the number of nuts per tree multiplied per average nut and kernel weights. Next, the nut and kernel yield of trees were divided by the corresponding SCA's, for estimating yield efficiencies based on nut and kernel (Hassani *et al.*, 2014). To calculate the fruit set percentage, the fruit number in sample branches were divided by the corresponding number of pistillate flowers.

Statistical analysis

The combined analysis of variances was carried out using general linear model (GLM) procedure. Means were separated by Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD). Phenotypic (σ^2_p), Genetic ($\sigma^2 g$) and genetic × year interaction (σ^2_{gy}) variances were obtained from their corresponding expected mean square in ANOVA table. Heritability in the broad sense (H^2_b) was estimated using the genetic and phenotypic variances (Visscher *et al.*, 2008). The phenotypic and genetic correlations were estimated using the variances and variance-covariance matrices of traits (Dogra *et al.*, 2018; Marrano *et al.*, 2019). The GGE biplot analysis was employed to determine the year and genotype interaction, besides the combining analysis of variances (Yan and Tinker, 2006).

3. Results

Yield-related traits variability and analysis

The descriptive statistics of the traits were reported in Table 1. The nut weight varied from 8-15.3 g with the average of 11.2 g, while the kernel weight average was 5.8 g varying from 3.9-8.3 g. Though the variation in kernel percentage range were 39.4-66.7%. The fruit set average was 48.7%, with a wide range variation (14-82%) in different cultivars. Mean number of nuts on SCA were 26.8 with a range of 2.7-64.7. Moreover, the average of nut and kernel weight on SCA were 295.1 and 157.4 g/m^2, respectively. Nut weight on SCA ranged 28.8-782.9 g/m^2, while the kernel weight on SCA ranged 12.1-409.7 g/m^2. In general, a high variation was observed for the evaluated traits in different cultivars.

The three years combined analysis of variance and genetic variance components for the studied traits are shown in Table 2. The effect of the year was significant on fruit set percent, the nut number on SCA

Table 1 - Descriptive statistics of the traits evaluated in walnut cultivars

Evaluated traits	Min	Max	Range	Mean	Variance
Nut weight (g)	8	15.3	7.3	11.2	2.9
Kernel weight (g)	3.9	8.3	4.4	5.8	1.3
Kernel percentage	39.4	66.7	27.3	52.1	54.5
Fruit set percentage (%)	14	82	68	48.7	264.1
Nut number on scaffold cross area (no./m^2)	2.7	67.4	64.7	26.8	271.6
Nut weight on scaffold cross area (g/m^2)	28.8	782.9	754.1	295.1	29655.1
Kernel weight on SCA (g/m^2)	12.1	409.7	397.6	157.4	9448.5

Table 2 - Combined analysis of variance, genetic variance components and broad-sense heritability (H^2_b) of the traits in six walnut cultivars (2015-2017)

Variance component	DF	Nut weight mean squares	Kernel weight	Kernel percentage	Fruit set percentage	Nuts number on SCA	Nut weight on SCA	Kernel weight on SCA
Year	2	10.3 NS	0.99 NS	16.8 NS	995.4 *	1149.8 *	159004 *	47838 *
Replication (year)	6	1.3	0.18	17.1	142.8	70.1	5671.1	2319.3
Genotype	5	16.2 **	10.9 **	430.9 **	1197.8 *	1056.4 *	141510 *	46238 *
Year x genotype	10	3.17 **	0.71 **	21.8 **	330.3 **	239.9 **	30441 **	10044 **
Error	30	0.47	0.19	6.1	95.3	76.7	7912.8	2300.1
Cv (%)		6.1	7.5	4.7	20.1	22.9	30.1	30.5
H^2_b	-	0.41	0.75	0.80	0.36	0.42	0.44	0.45
SE	-	0.1	0.025	0.015	0.13	0.11	0.104	0.11

**, * and NS show statistical significance at the probability level of 1%, 5% and not significant, respectively.
SCA = Scaffold cross area; Cv = coefficient of variance; H^2_b = broad-sense heritability and SE = Standard error of H^2_b.

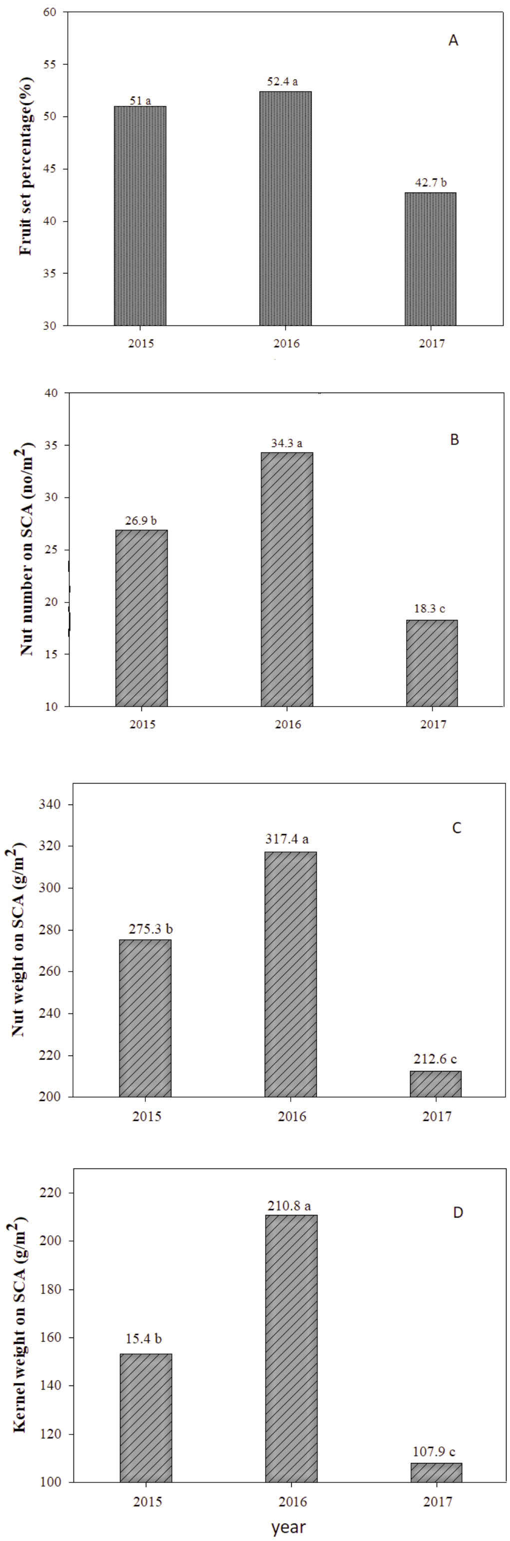

Fig. 1 - Mean comparisons for the effect of years on fruit set percentage (a), nut number on scaffold cross area (SCA) (b), nut weight on SCA (c), and kernel weight on SCA (d).

and the nut and kernel weight on SCA ($P \leq 0.05$). However, it was not significant on nut weight, kernel weight and kernel percent. The effects of genotype and year × genotype interaction was statistically significant in all traits (Table 2).

A high broad-sense heritability (H^2_b) obtained for kernel weight (0.75) and kernel percentage (0.80). The lowest value of H^2_b (0.36) was belong to fruit set (Table 2).

Based on the analysis of results for determining the effect of different years the highest fruit set percentage was observed in 2016 and 2015 with the average of 52.4% and 51%, respectively, although, the lowest fruit set percentage was 42.7% in 2017 (Fig. 1). The highest number of nuts on SCA; and nut and kernel weight on SCA were attained in 2016 with the corresponding averages of 34.3 nuts per m^2; and 317.4 and 210.8 g per m^2.

Evaluation of traits in different genotypes during three experimental years showed that the average of nut weight varied from 9.2 g in Caspian to 13.2 g in Chaldoran. Kernel percentage varied from 42.2% in Chandler to 60.1% in Persia. Fruit set ranged from 33.8% in Persia to 62.7% in Jamal. Furthermore, fruit set was significantly lower in late leafing cultivars and genotypes such as Persia, Chandler, and Caspian (Marrano *et al.*, 2019, Hassani *et al.*, 2020 a) compared with early to medium leafing ones (33.8-43.5% and 55.6-62.7%, respectively) (Fig. 2).

Based on the results a wide range of differences was observed in yield efficiency traits (number of nuts per m^2 SCA and weight of nut and kernel per m^2 SCA). The highest number of nuts per m^2 SCA was observed in Alvand with an average of 41.8 nuts/m^2, while the lowest amount was recorded in Jamal with 9.1 nuts/m^2. Moreover, the highest nut weight on SCA were observed in Alvand with 472.1 g/m^2. Alvand and Chaldoran had the highest kernel weight on SCA with 239.7 and 218.6 g/m^2, correspondingly. The lowest nut and kernel weight on SCA with 108.2 g/m^2 and 50.4 g/m^2 belonged to Jamal (Fig. 2).

Genetic and genetic per environment (GGE) analysis

According to statistically significant interactions between years and genotypes, the studied cultivars showed different responses to years. Analyzing the effect of genotypes and genotype × year interaction on the studied traits have been shown in GGE biplot diagrams in figure 3. In GGE biplot diagrams, the horizontal axis (PC_1) shows the effects of genotypes and the vertical axis (PC_2) shows the interaction of genotype per year (environment). According to the results for nut weight (Fig. 3 a), the PC_1 and PC_2 explained respectively 79.5% and 19.2% of variability, with the

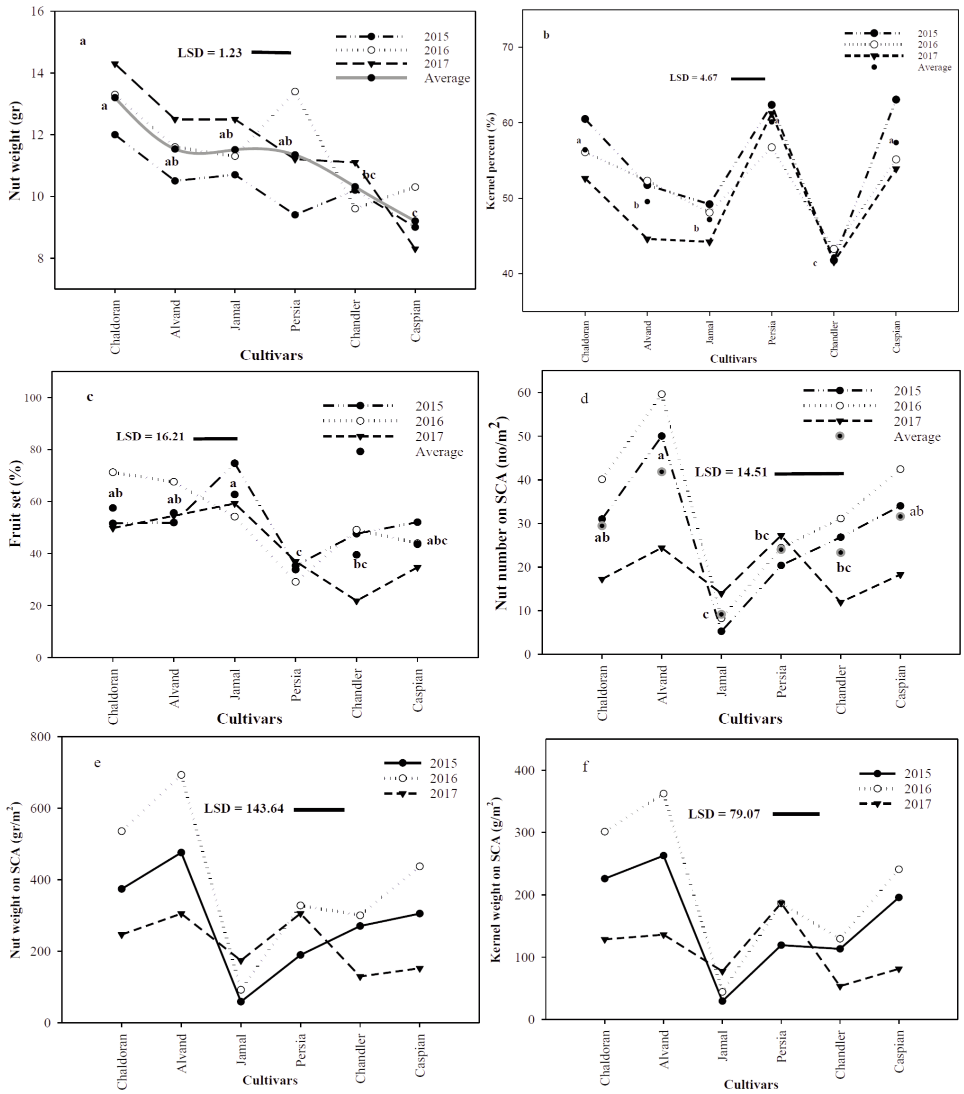

Fig. 2 - Mean comparisons of the effect of the cultivar and year × cultivar on fruit weight (a), kernel percent (b), fruit set (c), number of fruits on SCA (d), fruit weight on scaffold cross area (SCA) (e), and kernel weight on SCA (f) using Duncan multiple range test for genotypes and Least Significant Difference (LSD) test for interaction of year × cultivar.

98.7% of total variability. The biplot 2a was divided into four sectors with a principal sector grouping the years 2015 and 2017, together with Chaldoran and Alvand with higher nut weight. While in the second sector, the year 2016 was grouped with Persia. For the percentage of kernel (Fig. 3 b), the PC_1 and PC_2 explained respectively 94.4% and 4.7% of variability, with 99.1% of total variability in five sectors. The principal sector grouped the years 2015 and 2017. Persia with high kernel percentage was included in this sector. The second sector were grouped the year 2016 together with Chaldoran and Caspian. These cultivars had greater kernel percentage than general average. In figure 3 c the PC_1 and PC_2 explained respectively 65.6% and 21.8% of variability, with 87.4% of total variability about fruit set percentage.

The biplot for fruit set percent was divided into five sectors, too. The principal sector grouped the years 2015 and 2017, and Jamal with higher fruit set. The second sector grouped the year 2016 and Chaldoran. This cultivar had fruit set greater than average. For the fruit number on SCA (Fig. 3 d), the PC_1 and PC_2 explained 94.2% and 5.5% (99.7% of total) of variability correspondingly. The GGE biplot was divided into four sectors. In the principal sector the years 2015 and 2016, and the cultivars Alvand, Caspian and Chaldoran were classified together with higher fruit number on SCA. In the second sector, the year 2017 and Persia were grouped together. Similar results were obtained for nut weight on SCA (Fig. 3 e). For the kernel weight on SCA (Fig. 3 f), the PC_1 and PC_2 explained respectively 90% and 9.4% of variability

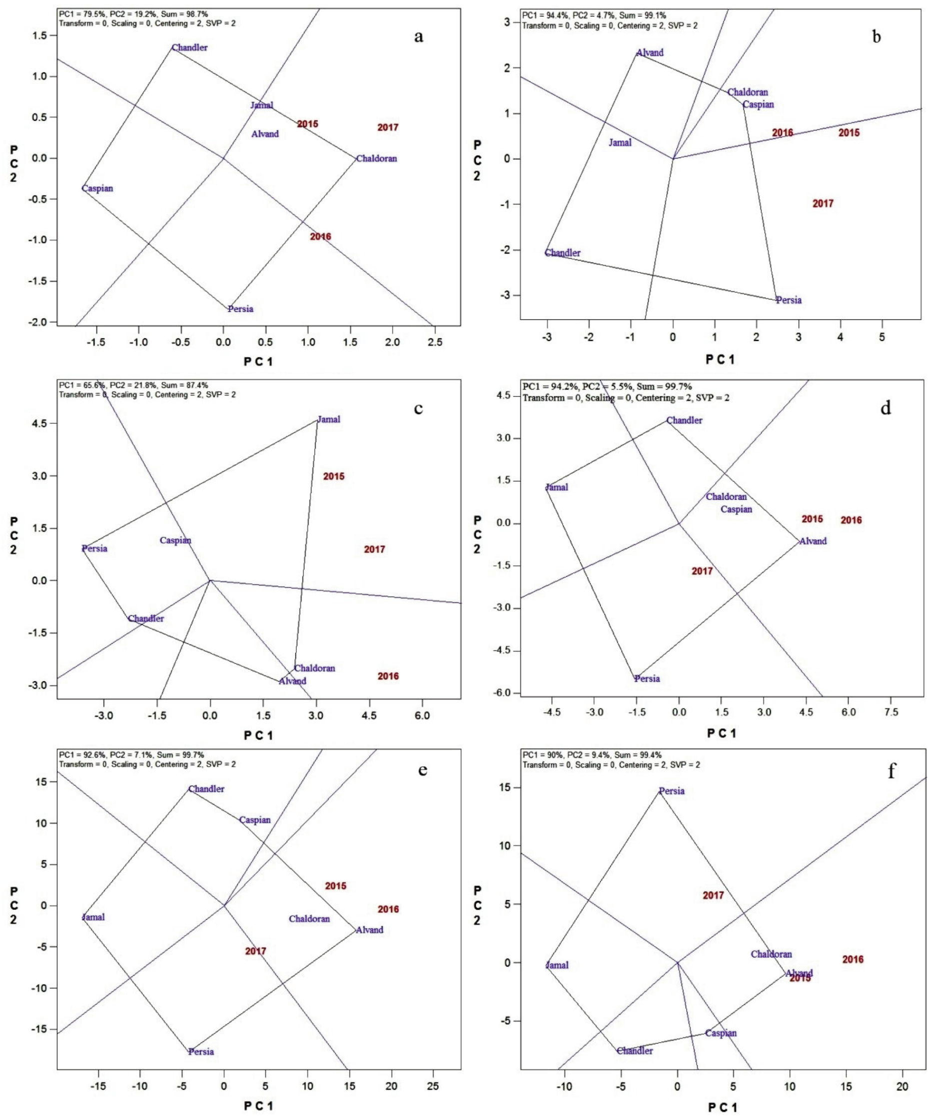

Fig. 3 - Genotype and Genetic × Environment (GGE) biplot of six walnut cultivars over three years (2015-2017) for nut weight (a), kernel percentage (b), fruit set percentage (c), nut number on scaffold cross area (SCA) (d), nut weight on SCA (e) and kernel weight on SCA (f).

and 99.4% of total variability. The GGE biplot for kernel weight on SCA was also divided into five sectors. The principal sector grouped the years 2015 and 2016, and the cultivars Alvand and Chaldoran with higher kernel weight on SCA. The second sector grouped the year 2017 and Persia that had greater kernel weight on SCA.

The figure 4 shows scattering of walnut cultivars based on the yield efficiency traits in a biplot. In figure 4b Chandler, Persia, Chaldoran and Alvand had the highest nut weight on SCA and also nut weight. The same results on figure 4 c with Chaldoran and Alvand which had the highest kernel weight on SCA too. According to figure 4 d the highest fruit set percent and nut weight on SCA also was belong to Alvand and Chaldoran.

Genetic and phenotypic correlations

The genetic and phenotypic correlations between the traits are reported in Table 3. These results showed that the nut weight did not considerably correlate with kernel percentage and number of nuts on SCA. Nut weight had a moderate impact on fruit weight on SCA (r_g = 0.31, r_p = 0.27) and kernel weight on SCA (r_g = 0.34, r_p = 0.29). As expected, a high genetic and phenotypic correlation was observed between the number of nuts on SCA and nut weight on SCA (r_g = 0.95, r_p = 0.95) as well as kernel weight on SCA (r_g = 0.90, r_p = 0.91) (Table 3). Kernel weight on SCA was significantly correlated with most of the traits, but the highest genetic and phenotypic correlations were observed between this trait and fruit weight on SCA (r_g = 0.97, r_p = 0.97).

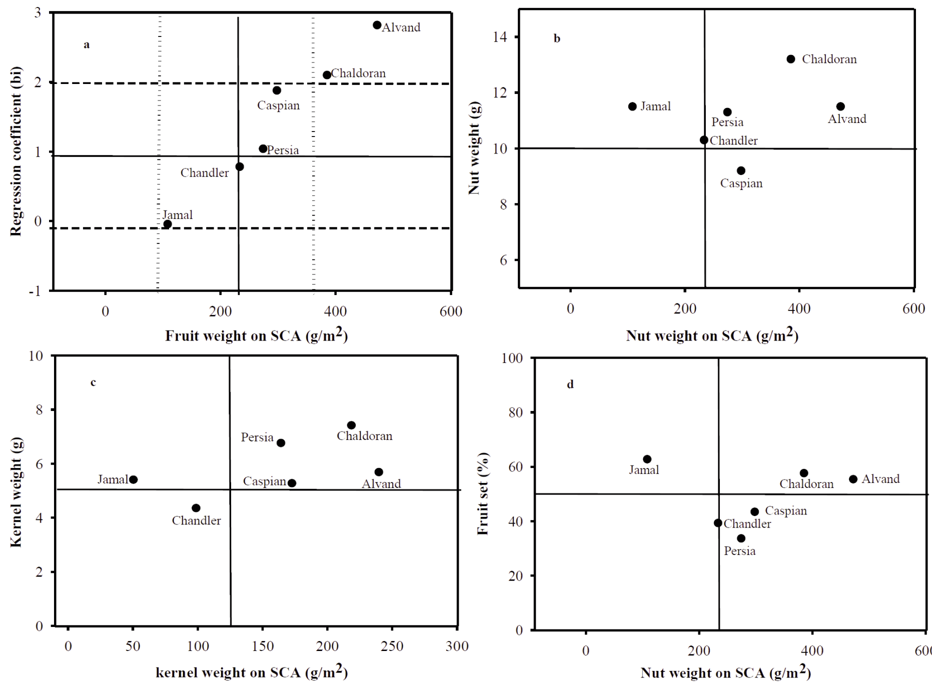

Fig. 4 - Biplot of regression coefficients against average yields of walnut cultivars (The horizontal solid line represents the mean coefficient of regression and the vertical solid line denotes the average fruit weight on scaffold cross area (SCA). The standard error (±1SE) was included and represented by the dotted lines for both yield efficiency and regression coefficients) (a), biplot of nut weight with nut weight on SCA (b), kernel weight with kernel weight on CA (c), and percentage of fruit set with nut weight on SCA (d). Solid lines in graphs show the average of each trait.

Table 3 - Genetic and phonotypic correlations of different traits

		Kernel (%)	Fruit set (%)	Number of nuts on SCA2	Nut weight on SCA	Kernel weight on SCA
Nut weight	G1	0.19	0.72	-0.02	0.31	0.34
	P1	0.12	0.53	-0.03	0.27	0.29
Kernel (%)	G	1	-0.34	0.25	0.31	0.55
	P		-0.26	0.26	0.30	0.52
Fruit set (%)	G		1	-0.29	-0.09	-0.14
	P			-0.12	0.03	0.01
Number of nuts on SCA	G			1	0.95	0.90
	P				0.95	0.91
Nut weight on SCA	G				1	0.97
	P					0.97
Kernel weight on SCA	G					1
	P					

G and P are the genetic and phenotypic correlations, respectively.
SCA = Scaffold cross area.

4. Discussion and Conclusions

The significant effects of year, genetic and genetic × year interaction showed that cultivar and its interaction with environmental conditions as the main determinants of cultivar's adaptability. Therefore, stable and compatible cultivars should be found and introduced for appropriate climate(s) (Rawandoozi *et al.*, 2021). The low fruit set in 2017 caused the fruit production to be significantly lower compared to other two years, while there were no significant differences in pistillate flowers (data not shown).

Understanding the genotype and genetic × environment interaction also is important for increasing the gain in cultivar improvement programs. The genotype's main effect nd especially it's G x E interaction implies the different performance of genotypes across environments that arises from the various sensitivities to the different environments (Rawandoozi *et al*., 2021). For all of the yield related traits, GGE biplot have described most of the existing variations (more than 90 %), which indicates the relative validity of the biplot in explaining the variations of genotypes and genetic × environment interaction (Yan and Tinker, 2006). The effect of genotype in determining the walnut yield efficiency has been demonstrated by Dogra *et al*. (2018). Based on our results high amounts of PC_1 has been recorded for kernel percentage (94.4%) as well as number and weight of nut and kernel on SCA (more than 90%). So, a high and stable production will be expected by selecting cultivars with higher yield efficiencies and more compatible with environmental conditions. The results on nut number and nut weight in cultivars are consistent with the findings of Mahmoodi *et al.* (2016). Fruit set is another important trait affecting the number of nuts in tree (McGranahan and Leslie, 2009; Sarikhani Khorami *et al.*, 2014; Khadivi-Khub *et al.*, 2015). It is clear that, number of pistillate flowers is relatively lower in cultivars with terminal bearing habit compared to lateral bearing ones (McGranahan and Leslie, 2009; Hassani *et al*., 2020 b). In terminal bearing cultivars like Jamal, the higher fruit set usually could compensate the production to some extent.

Among the yield related traits, the lowest value of the PC_1 was belong to fruit set (65.6%), which indicates this trait is more affected by genetic × environment interaction (21.8%). The results of this study present the clear effect of genotype on fruit set (Fig. 2 c) which is consistent with Kumar *et al*. (2005). Due to the fact that the amount of pistillate flowers produced in walnut is lower than pome and stone fruit trees, higher fruit set (50-90%) is necessary in order to produce an adequate yield. In addition to genetic, genetic × environment interaction plays a very important role in pollination and fruit set of cultivars (Cosmulescu *et al.*, 2010; Mariana and Sina Niculina, 2017). According to the results, there was a significant negative correlation between bud break and fruit set (R = -0.54), so that with delayed leafing, the fruit set decreased. It is clear that the late leafing cultivars deal better with late spring frosts (McGranahan and Leslie, 2006; Hassani *et al.*, 2013; Hassani *et al*., 2020 b), but the pollination and fruit set were not the same in late and early leafing walnut cultivars. To obtain a sufficient fruit set, care must be taken regarding producing a sufficient pollen volume with an adequate overlap of pollen-shedding for the receptivity period of pistillate flowers. The response of cultivars could be affected by different climatic conditions in different years especially at leafing time and time of pollination (Cosmulescu *et al*., 2010; Sarikhani Khorami and Vahdati, 2019; Cao *et al.*, 2020). Temperature is one of environmental factors influences the percentage of fruit set by influencing pollination factors, such as pistillate flowers receptivity and pollen-shedding period. In late leafing cultivars the environmental factors such as high temperatures at the pollination time, could lead to lower effective pollination period and pistillate flower receptivity period, that could reduce the fruit set (Ramos, 1997).

High broad-sense heritability relative to kernel weight and kernel percentage also indicated that these traits are less affected especially by genetic × environment interaction. Conversely, low-moderate heritability and high ratio of genetic × environment interaction for fruit set indicated substantial environmental effects on this trait. The heritability values in the present study were somewhat lower than what reported by Eskandari *et al.* (2006), Dogra *et al*. (2018), and Marrano *et al.* (2019).

In terms of yield stability, it seems that the genetics, environment and their interaction could contribute to various characteristics such as: fruit-bearing habit, growth vigor, nut weight, kernel weight, kernel percentage, previous year crop load, pollination, fruit set and late spring frosts (Cosmulescu *et al.*, 2010; Asma, 2012; Sarikhani Khorami *et al*., 2014; Dogra *et al*., 2018; Cao *et al.*, 2020; Hassani *et al.*, 2020 a). Generally, in low-yielding cultivars such as Jamal, year-by-year variations of traits were low. However, they were higher in cultivars with more production such as Chaldoran and Alvand (Hassani *et al.*, 2020 a). Some studies have reported significant alternate bearing in walnut cultivars (Asma, 2012; Hassani *et al*., 2014; Mahmoodi *et al.*, 2016). So, alternate bearing, opposed to genetic stability, is affecting the fruit production trends of walnut cultivars in different years (Amiri *et al.*, 2010). Mahmoodi *et al.* (2015), reported the presence of 2-15% of alternate bearing among different walnut cultivars. In majority of high-yielding cultivars, a heavy crop load is followed by a low fruit production in the subsequent year. Asma (2012) found that the yield is influenced mostly by leafing time, fruit-bearing habit, tree size, nut and kernel weights, and kernel percentage. Moreover, Dogra *et al.* (2018) reported that yield is controlled polygenically and is influenced by environmental conditions. They stated that pistillate flower density, fruit set, trunk section area, trunk circumference, tree height, shoot length, pollen-shedding period, fruit weight, kernel percentage, and shell thickness had affected the yield of walnut trees, which were in part consistent with the findings of the present study.

High variation was observed in yield components and yield efficiency traits with different environments and cultivars, and it was found that genetic by environment interaction are the most important factors determining yield variations. Understanding the contribution of genetics and genetic × environment interaction is very important. The genetic × environment interaction in fruit set was more than other yield-related traits, while the broad sense heritability (H^2_b = 0.36) was the lowest value. Therefore, fruit set is most affected by variation of environmental conditions, so that under undesirable climatic conditions it will be yield determining factor especially in late leafing walnut cultivars. Regarding the nut weight, the effect of genetic by environment interaction was strong while heritability was greatly affected by the environmental conditions. The contribution of genetic × environment interaction on other traits related to yield efficiency was estimated to be less than 10%. Genetic and phenotypic correlation also indicated that nut weight, kernel weight and kernel percentage had a low-moderate correlation with nut and kernel weight on SCA. On the contrary, the nut number on SCA had the highest genetic and phenotypic correlation with nut and kernel produced on SCA. The results showed that in walnuts, that is a nut tree species well adapted to temperate climate, the variability of yield related traits over environments (years), was highly significant. So, the change in climate in one hand and the scarcity of the resources (water and land) on the other hand emphasizes on more accurate evaluation on the new cultivars especially in yield related traits.

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