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AMMI Analysis of Yield Performances of Argan Genotypes (*Argania spinosa* L. Skeels) over Disparate Years and Preselection of Promising Trees

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Abstract Argan tree (Argania spinosa L. Skeels) is an emerging oil species of great social and economic interest in Morocco. The thriving national and international demand for its oil increased the anthropomorphic pressure on its forests, slowing the natural regeneration of the natural stands. In order to alleviate the pressure on the species and to satisfy the growing demand for its oil, the domestication of the argan tree and its cultivation should be urgently implemented. High and stable yield over years is an extremely important trait in argan tree selection and should be among the main objectives of its domestication programs. This study aimed to follow-up the production of 122 argan genotypes preserved *in situ*, at Admine reserve of the Horticultural Complex of Agadir in southwestern Morocco, over three contrasted years. The trees bearing fruits for in any year of the study varied from 50 to 73%. The 52 genotypes with a regular annual production were evaluated for their productivity and yield stability (in number of fruits and almond weight) using AMMI (Additive Main effects and Multiplicative Interaction) analysis. The analysis of variance showed a major genotype-to-year interaction (GYI) effect; the genotype too, had a significant influence, while the year had a negligible effect. Data analysis revealed different responses of each individual between years and subsequently the complexity of this trait. The first multiplicative component of the interaction accounted for 67 and 60 % of the sum of squares for fruit density and almond yield respectively. The AMMI1 and AMMI2 biplots, and the AMMI stability value were used to discriminate genotypes with superior and stable fruit yield. The analysis also enabled the identification of potentially productive trees in some specific environmental conditions. Thus, allowing the pre-selection of 12 promising genotypes to constitute the genetic basis of stable and highly productive argan cultivars. Such genotypes need a confirmation of their aptitude for cultivation through further investigation.

Keywords *Argania spinosa*, Yield stability, AMMI model, GY interaction, Selection

1. Introduction

Argan tree (*Argania spinosa* L. skeels) is a multipurpose tree that belongs to the Sapotaceae family [1]. This species is endemic to southwestern Morocco, where it occupies an approximate area of 952,000 ha [2, 3]. Its main product is the oil extracted from its almonds, rich in unsaturated fatty acids (80%) and γ-tocopherol, highly prized for its therapeutic, cosmetic, and culinary uses [4]. *Argania spinosa* plays an important socioeconomic and environmental role in the region. According to the Moroccan independent institution for exports control and coordination, the amount of exported argan oil increased from nearly 100 tons in 2004 to over 1,000 tons in 2015.

Despite the importance of the species as an emerging alternative oil species, its natural stands are subject to a constant degradation due to the overexploitation, desertification, and farms expansion over its forests [5]. Given the situation, the domestication and establishment of argan orchards can help to decrease the anthropomorphic pressure on its forest and fulfill the strong demand of its oil.

Being a wild forest species, argan trees have a vast underlying genetic diversity, making the genotype environment interaction (GEI) very significant for most traits, which reduces the general association between the genotype and phenotype and make the selection process very difficult [6-9]. In the literature, very few studies have addressed the productivity and selection in argan tree [10, 11], and to our knowledge, none have addressed the yield stability issue.

Targeting stable and performant genotypes can be more consistent on a better understanding of the GEI pattern. The

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analysis of variance (ANOVA) is able to identify the GEI as a source of variation but cannot analyze its intrinsic effects, while the principal component analysis (PCA) cannot present the additive effects for genotype or environment. The Additive Main effects and Multiplicative Interaction (AMMI) model is a hybrid analysis that provides a visual inspection and interpretation of the GEI components [12]. It separates the additive main effects for genotypes and environments using a regular ANOVA analysis and then analyses the interaction effect using the multiplicative model provided by the PCA [12, 13].

The aims of this study are to (i) assess the general productivity of the studied argan genotypes, (ii) assess the yield's stability of genotypes not presenting an apparent alternate bearing (a year with no production) using the AMMI analysis and to (iii) determine those with both high and stable yields through the contrasted years of the study intended for future argan selection and breeding program.

2. Material and Methods

2.1. Study Site and Plant Material

The study was carried out during three cropping years (from 2013/14 to 2015/16), in the Admine natural reserve placed within the Horticultural Complex of Agadir in upper southwest Morocco. The reserve extends over 28 ha and is located at 9°28'35"W, 30°21'58"N and at an average elevation of 30 m above sea level. The average annual rainfall in the region is 300 mm (2000-2016), the average high temperature varies between 19 and 37°C, and the average low temperature varies from 6 to 21°C. Occasionally, the area experiences winds from the Sahara, which can increase the temperature above 40°C.

During the study period, the average seasonal temperatures were comparable between years except for winter 2016, where it was slightly higher than the two previous years (Table 1). Regarding the precipitation levels, 2015 was the wettest year with 314 mm, and with most of the rain (280 mm) occurring during autumn. For 2014 and 2016, both years had slightly more than 100 mm rainfall with the most (78 mm) occurring during winter in 2014 and autumn in 2016 (Table 1).

The studied argan trees were sampled randomly in a parallel diversity assessment and morphological characterization study [9]. The total number of evaluated trees was 122; all were mature (over 25 years old) and vigorous with no apparent disease symptoms.

2.2. Yield Estimation

To compare between trees with different sizes and shapes, the yield was estimated per m² of the productive surface of the tree. For each tree, the total number of fruits present in a 0.25 m² (0.5 m x 0.5 m) of the external tree surface was counted. Then the average density was calculated over 12 values/tree. After fruit ripening, 3 batches of 100 fruits were collected from each tree to estimate the 100 fruits weight and its components, the weight of pulp, shell and almond of 100 fruits. The final yield, used to estimate the productivity and to compare between the different genotypes, was expressed in gram of almonds per external tree surface m².

2.3. Data Analysis

Rate of productive trees, maximum, minimum, mean and coefficient of variation (CV) were computed for both fruit density and for almond weight of 100 fruit for every year of the study, to visualize the effect of the year over the overall productivity of the sampled trees.

Table 1. Climatic Data (Rainfall and Temperature) per Season of Admine Reserve from Cropping Year 2013/14 to 2015/16

Cropping Year code	Season	Rainfall (mm)	Max Temp (°C)	Avg Temp (°C)	Min Temp (°C)
2014	Sep-Nov 13	2	26.70	20.42	14.27
	Dec-Feb 13/14	78	21.84	14.57	7.53
	Mar-May 14	71	25.69	19.20	12.61
	Jun-Aug 14	0	30.12	23.41	17.03
2015	Sep-Nov 14	280	27.62	21.67	15.84
	Dec-Feb 14/15	16	20.40	13.31	6.42
	Mar-May 15	40	24.73	18.21	11.70
	Jun-Aug 15	14	30.58	24.14	18.00
2016	Sep-Nov 15	78	27.56	21.06	14.67
	Dec-Feb 15/16	25	24.27	16.18	8.06
	Mar-May 16	23	23.52	17.71	11.76
	Jun-Aug 16	0	30.44	24.40	18.49

Max Temp, Avg Temp, Min Temp: Maximum, Average and Minimum temperatures respectively, the higher value for a season between years is Bolded

Fruit density and almond yield data of the studied genotypes not presenting an apparent alternate bearing tendency (a year without fruit production), were analyzed by the AMMI model to adjust the additive genotype and environmental effects by the analysis of variance. In addition, the adjustment of the multiplicative effects of the G×Y interaction (GYI) was performed by principal component analysis. The AMMI model used in the analyses was as follows [13]:

$$Yij = \mu + g_i + e_j + \sum_{n=1}^{N} \lambda_k \gamma_{ik} \delta_{jk} + \rho_{ij} + \varepsilon_{ij} \qquad (1)$$

where Y_{ij} is the fruit density or almond yield of the i^{th} genotype in the j^{th} year, μ is the overall mean, g_i and e_j are the fixed genotype effects and environmental deviations, respectively, λ_k is the eigenvalue of the Principal Component Analysis axis k, γ_{ik} and δ_{jk} are genotype and environmental factors, respectively, of the singular vectors associated with λ_k from the interaction matrix, ρ_{ij} is the residual of GYI, and N is the number of principal components retained.

The AMMI Stability Value (ASV) is used to quantify the stability of the genotypes based on the two first Interaction Principal Component Axis and was calculated using the formula developed by Purchase [14]:

$$ASV = \sqrt{\left[\frac{SS_{IPCA1}}{SS_{IPCA2}}(IPCA1_{score})\right]^2 + (IPCA2_{score})^2} \qquad (2)$$

where, SSIPCA1 is Sum of Squares of interaction principal component axis 1, SSIPCA2 is Sum of Squares of interaction principal component axis 2, IPCA1 is the first interaction principal component axis and IPCA2 is the second interaction principal component axis.

Descriptive statistics and the ASV were computed using the excel spreadsheet, while the ANOVA and AMMI analysis were performed using the software R, version 3.3.2.

3. Results and Discussion

The average fruit density was higher in 2014 and 2016 (90.15 and 85.56 fruits/m² respectively), while it was significantly lower (54.79 fruits/m²) in 2015 (Table 2). This decrease in the fruit density was caused by the falling of fruits observed after storm rains, which took place in autumn 2014. Those storms caused a natural thinning, which decreased the fruit density, but helped the improvement of the almond weight. Almond weight reached 30 g/100 fruits in 2015 whereas in 2014 and 2016 it was 21.85 and 25.72 g/100 fruits respectively (Table 2). Such behavior has been reported in other species like avocado, where the excess of water caused premature fruit dropping [15]. In fact, it have been found that the primary effect of flooding is the depletion of soil's oxygen which usually affects the yield of many species [16, 17].

The highest rate of fructifying trees was that of 2015 with 72.95% of the total population, while 2016 presented the lowest rate with only 50.82% of trees producing fruits (Table 2). This high rate in 2015 could be due to the 2014 spring rainfall, which helped the development of new stems and floral buds, and also to the following autumn rainfall that sustained the fruits growth. The difference in the number of productive trees among years could also depend on the alternative bearing ability of the tree genotype, which generally relies more on the endogenous nutritive components or on the flowering-fructification cycle length, in fact it is attested in argan that the period from flowering to fructification stages can vary from 10 to 16 months [18]. Generally, in long cycles, fruit development and flower formation overlap which, probably affect both the development of the old fruits and the ability to form new flow buds. In fact, it has been found in many studies that the presence of developing fruits can inhibit subsequent formation and growth of new ones, because of the difference in sink strength, or the dominance of a one development processes over the other [19, 20].

Table 2. Average, Maximum, Minimum, and CV of Fruit Density of Fructifying Genotypes and Almond Weight of 100 Fruits, and the Rate of Fructifying Trees in the Sample per Year, from 2014 to 2016

	2014	2015	2016
Average fruit density of fructifying trees	**90.15**	54.79	85.56
Maximum fruit density	**255.17**	244.25	234.80
Minimum fruit density	**10.42**	2.75	4.41
CV of fruit density of productive trees (%)	65.06	**85.67**	67.80
Average almond weight of 100 fruit (g)	21.84	**30.73**	25.72
Maximum almond weight of 100 fruit (g)	35.60	55.11	**58.00**
Minimum almond weight of 100 fruit (g)	4.75	**15.10**	6.98
CV of almond weight of 100 fruit (%)	29.66	30.44	**40.53**
Rate of fructifying trees (%)	59.02	**72.95**	50.82

Either hypothesis, (alternative bearing or sink effect) deserve to be studied focusing on the water's effect on argan flowering and fructification capabilities, and the possible relationship between phenological cycles and productivity.

Among the 122 studied trees, 18.85% did not produce any fruit during the 3 experimental years, while 21.31, 17.21 and 42.62% fructified once, twice and 3 times respectively. To identify the more stable and productive trees, the AMMI analysis included only the genotypes that produced fruits during all the 3 years of the study.

The analysis of variance (ANOVA) showed significant ($p \leq 0.001$) genotype (G) and GY interaction (GYI) effects on fruit density and almond yield, whereas, the year's effects (Y) was not significant for both traits (Table 3).

The GYI was highly significant for both traits; it explained 70.18 and 66.85% of the total sum of squares (SS) of respectively the fruit density and almond yield, while the genotype, gathered respectively only 23.32 and 32.77% of total's SS. A large GYI and genotype SS coupled with a low year contribution indicate the substantial disparity that exist between trees' behavior over the years. The large size of the studied sample and its high level of genetic diversity emphasize these differences. Anandan, Eswaran [21] reported a similar effect on rice on which the environment effect was very low due to the high number of genotypes used in the study.

In addition, the AMMI analysis over the three years showed that the first interaction principal component (IPCA1) captured more than half of the GYI SS, for both, fruit density (66.97%) and almond yield (59.92%), while the second axis captured the remaining GYI SS. This indicates that the IPCA1*Yield (AMMI1) biplot describes sufficiently the behavior of the Argan genotypes. The IPCA1 value regenerated through this model was higher than those reported in other studies on other species [22-24]; furthermore, IPCA1 had a sum of squares greater than that of the genotype and the year. This high IPCA1 value is mainly due to the limited number of the years, which simplify the capture of the variability in one axis.

The AMMI1 fruit density biplot (IPCA1-to-fruit density) is illustrated in figure 1. The year 2015, with the highest precipitation, was the less productive (64.89 fruits/m²), whereas 2014 presented the highest yield (99.57 fruits/m²) (Figure 1). This low fruit density in 2015 could be mainly related to the premature fruits falling noticed after heavy rains, which were also reported on other species by several authors [15, 25]. In fact, Bower and Cutting [15] reported that an excess of water reduces the yield and the fruit quality because a reduction in available root oxygen and the promotion of root rot conditions. In fact, in our case, during 2015 cropping year, 75% of the total precipitations occurred during only 10 days. The fruit fall can also be the result of the rainfall pattern, where it has been reported that high precipitation at specific growth stages can cause fruit drop [26].

The most interactive years with the IPCA1 are 2014 (IPCA1 score of -16.87) and 2016 (IPCA1 score of 13.59). Consequently, they are the most discriminant for assessing the stable genotypes. Twenty genotypes among 52 have a fruit density above average. Z2G3a, Z4D6a, Z1G7b, and Z1F1a were the most yielding, with only Z1F1a showing a good stability, whereas Z2E3a, Z1G4a, and Z1F5a presented the lowest fruit density, but were all stable (Figure 1).

In general, the most productive genotypes show a lower fruit yield stability; in fact, the 7 more unstable accessions have a fruit yield above average. Such behavior can be explained by the fact that in most cases, high average yield is the result of high and low yields in contrasting years rather than moderate yields over the years. While differences between annual yields of unstable low yielding genotypes are inferior to the differences between productive genotypes annual yields.

Regarding the almond yield's GY interaction, the AMMI1 biplot indicates that unlike in fruit density, all the 3 years' yields were close to the average and interactive. The cropping year 2014 presented the highest interaction (IPCA1 score of -9.05, Figure 2). The effect of the premature fruit drop was less prominent on almond yield, because of the almond weight gain observed in presence of water. In 2014, the most yielding genotypes were Z1G7b (58.58 g/m²), Z1H7a (52.35 g/m²) and Z1G6a (51.04 g/m²) while Z1G3a and Z4E6a were the less productive trees with respectively 0.88 and 2.43 g/m². In 2015, Z4D6a and Z2C1a were the most productive genotypes with respectively 92.91 and 63.61 g/m², while in 2016 the highest yielding entries were Z4C4a (76.57 g/m²) and Z4D6a (60.11 g/m²) (Table 4).

The AMMI1 biplot show that 25 from 52 genotypes have an almond yield above average (20.76 g/m²); Z4D6a has expressed the most important almond yield (52.54 g/m²), but was the most unstable, followed by Z2C1a (39.22 g/m²), Z4C4a (38.25 g/m²) and Z2B2a (33.08 g/m²). The genotypes with the lowest yields were Z2B2b, Z4E6A, and Z2E3a (Figure 2). Twenty-six genotypes were stable (IPCA1 between -1 and 1), among which 11 have an almond yield above average. The most stable genotypes are Z1B8a (IPCA1 score of 0.01), Z2E3a and Z1D2a (IPCA1 score of 0.05) and Z4B3a (IPCA1 score of 0.08), while Z2B2a (33.08 g/m²), Z1G5a (30.30 g/m²), Z4D6b (29.94 g/m²) and Z1B8a (27.97 g/m²) were the most productive among the stable genotypes.

Table 3. Average, Maximum, Minimum, and CV of Fruit Density of Fructifying Genotypes and Almond Weight of 100 Fruits, in Addition to the Rate of Fructifying Trees per Year, From 2014 to 2016

		Fruit density (fruit/m²)				Almond Yield (g/m²)			
Source	**DF**	**SS**	**MS**	**F-Obs**	**%SS**	**SS**	**MS**	**F-Obs**	**%SS**
Year (Y)	2	127336	63668	3.80^{+}	6.49	571	286	0.30^{ns}	0.38
Rep / Y	9	150957	16773	33.04***	--	8648	961	35.63***	--
Genotype (G)	51	457363	8968	17.66***	**23.32**	49329	967	35.86***	**32.77**
GYI	102	1376381	13494	26.58***	**70.18**	100616	986	36.58***	**66.85**
IPCA1	**52**	**921824**	**17727**	**34.92*****	**66.97**	**60287**	**1159**	**42.99*****	**59.92**
IPCA2	**50**	**454556**	**9091**	**17.91*****	**33.03**	**40329**	**807**	**29.91*****	**40.08**
Residuals	459	233044	508			12379	27		

DF = Degree of freedom; SS = Sum Square; MS = Mean Square; IPCA = Interaction Principal Component Axis; ***, **, * significant at 0.1%, 1% and 5% respectively; ns = Non-significant.

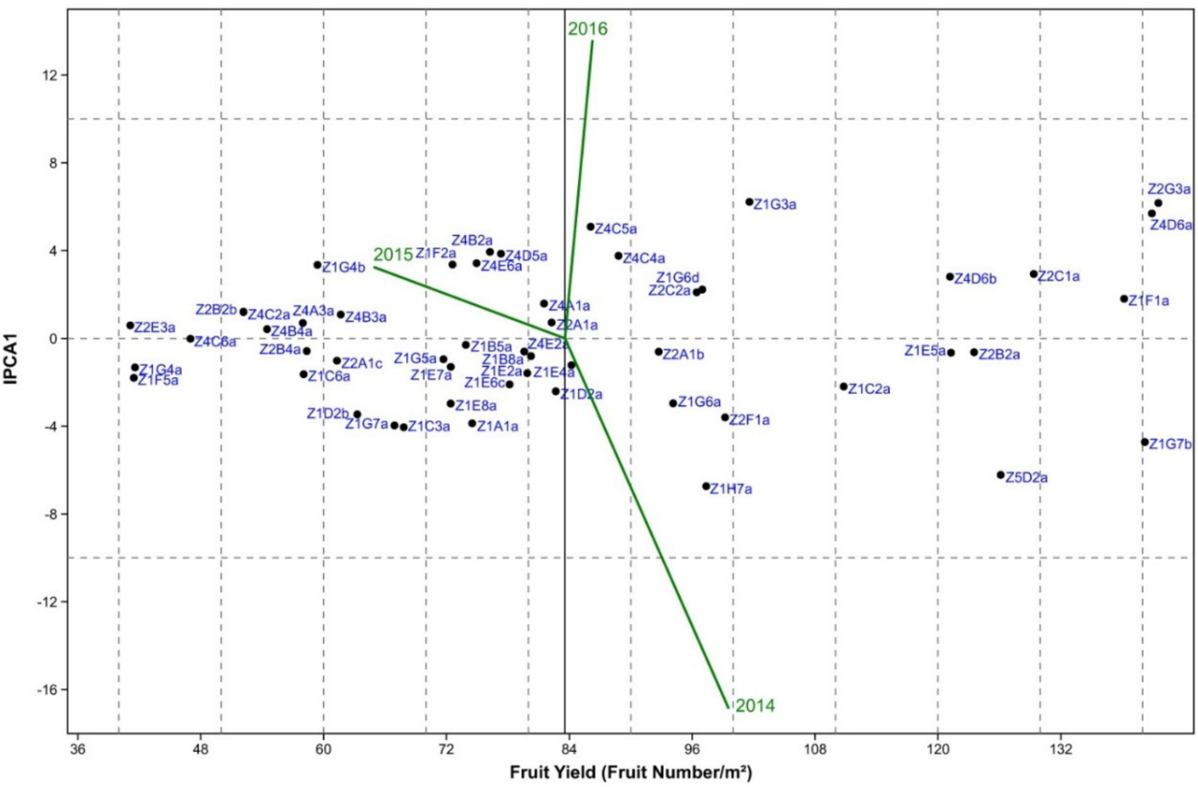

Figure 1. Interaction principal component axis (IPCA1) against average fruit yield (fruit number/m²) biplot of 52 argan genotypes and three years

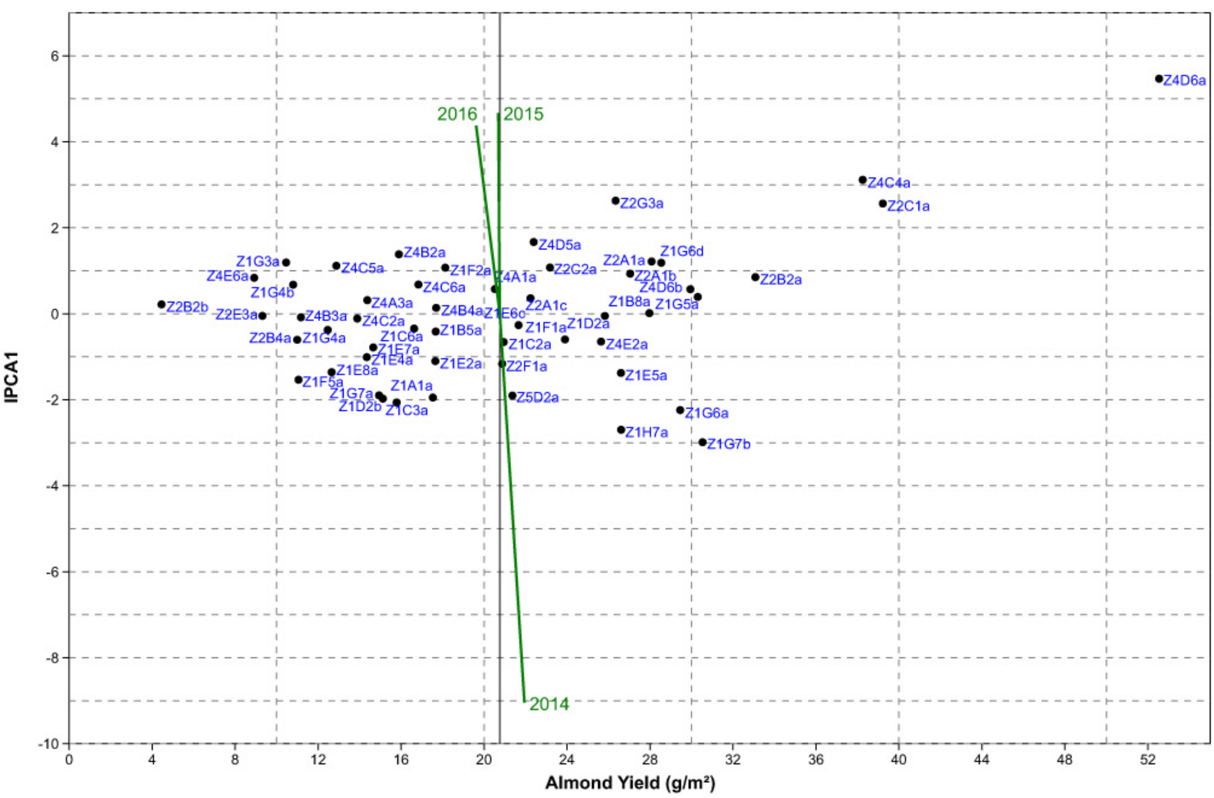

Figure 2. Interaction principal component axis (IPCA1) against average almond yield (g/m²) biplot of 52 argan genotypes and three years

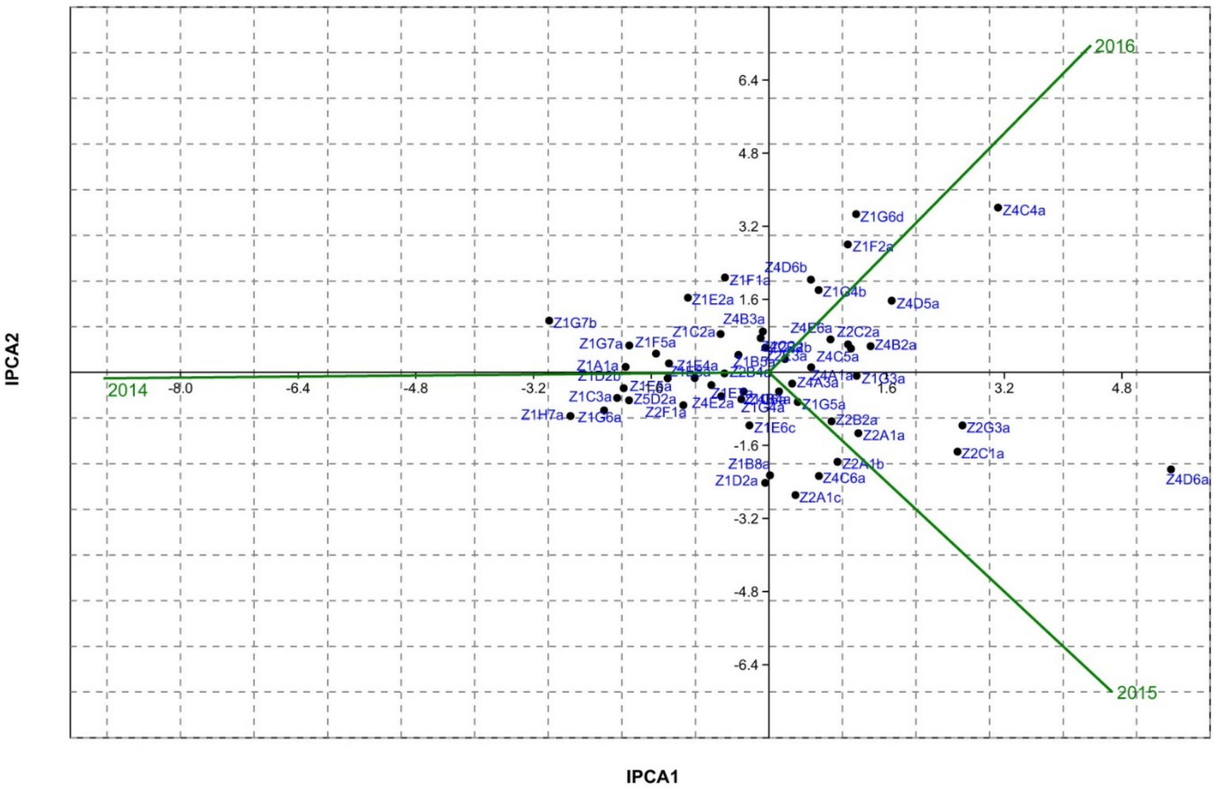

Figure 3. IPCA1 against IPCA2 biplot for almond yield (g/m²) of the 52 genotypes versus three years

Based on the almond yield AMMI2 biplot, and unlike the AMMI1 biplot, 2015 and 2016 were the most interactive years based on their high interaction scores on both IPCA1 and IPCA2 (Figure 3). Relative to the first IPCA, 2014 was more interactive and opposed to 2015 and 2016. On the other side, on IPCA2, 2014 was non-interactive, while 2015 and 2016 were equally interactive and opposed to each other (Figure 3).

Sixteen genotypes were ranked stable (IPCA1 and IPCA2 between -1 and 1), Z2E3a, Z2B2b, and Z4B4a presented the highest stability by being plotted near the biplot origin (Figure 3). However, Z1B8a and Z1D2a, considered very stable, according to the AMMI1, were ranked in fact unstable based on the AMMI2 biplot because of their high instability in IPCA2 score. Changes in some genotypes behavior between AMMI1 and AMMI2 biplots were also reported in other studies, such as in passion fruit and cowpea [23, 27]; they are mainly due to the increase of accuracy of the AMMI2, which combines information from both first IPCAs. Among stable genotypes, only Z1G5a (30.30 g/m²), Z4E2a (25.65 g/m²), and Z1C2a (20.95 g/m²) have an almond yield above the average.

In order to rank argan trees in terms of their yield stability, AMMI Stability Value (ASV) can be used as a criterion that combines IPCA1 and IPCA2 upshots. Genotypes with a low ASV score have better stability and large adaptation across environments; high score productive trees would have high adaptation to a specific environment. The genotypes annual almond yield and average yield above the global average yield, and their ASV scores are reported in Table 4. As on the scatter plot, among the productive genotypes, Z1G5a has the best stability (ASV = 0.87), followed by Z4E2a (1.10) and Z1E6c (1.23), whereas, Z4D6a (8.44), Z4C4a (5.89) and Z1G7b (4.61) were more sensitive to the environment (Table 4).

From the previous analysis and considering the genotypes' list in table 4, 3 groups with a significant potential need to be tested for their aptitude confirmation. The first group gathered five genotypes, Z1G5a, Z2B2a, Z4D6b, Z2A1a and Z1B8a, whose average almond yield (AAY) is 29.87 g/m². Those trees have better adaptation and could be intended to both irrigate and rainfed growing conditions. Those genotypes combine between good almond yield and stability; they presented significant yield on a rainy year and also high fruit density stability resulting from their better resistance to premature fruit drop.

The second group, made of Z4D6a, Z4C4a and Z2C1a, is highly unstable, but has a higher average almond yield (AAY= 43.34 g/m²) than the first group, especially the Z4D6a genotype (52.54 g/m²); Their instability is probably a disadvantage, but over time their elevated yield once a while might raise their average yield over long periods. Such genotypes should be planted under irrigation conditions, as they produced higher yields the second and third years of the study.

The third group includes unstable genotypes (Z1G7b, Z1G6a, Z1G6d and Z1H7a) that has an AAY equal 28.78 g/m² but which could produce very high yields (over 50g/m²) once a while. Such genotypes still need further characterization studies in orchard environment and under controlled irrigation to outline the optimal conditions that stabilize their production.

Moreover, the three groups have a potential for any breeding program that aims to select superior cultivars. However, the remaining germplasm still needs more yield evaluation for their agronomic potential appreciation.

Table 4. Almond Yield per Year (AY), Average Almond Yield (Avg), and AMMI Stability Value (ASV) of Genotypes having an Almond Yield Above Average

Genotype	AY14	AY15	AY16	Avg	ASV
Z1G5a[1]	28.04	36.64	26.22	30.30	0.87
Z4E2a	32.77	26.25	17.91	25.64	1.10
Z1E6c	25.43	28.53	11.04	21.66	1.23
Z1C2a	27.96	11.96	22.92	20.95	1.29
Z2B2a[1]	26.73	44.52	27.98	33.08	1.66
Z2C2a	14.55	23.86	31.12	23.18	1.72
Z2F1a	32.70	20.44	9.47	20.87	1.88
Z1E5a	40.26	21.05	18.49	26.60	2.06
Z4D6b[1]	25.66	18.34	45.82	29.94	2.20
Z2A1a[1]	18.47	43.05	22.71	28.08	2.25
Z1B8a[1]	29.37	43.79	10.75	27.97	2.25
Z1F1a	30.18	6.51	35.01	23.90	2.26
Z2A1b	20.10	45.09	15.94	27.04	2.40
Z1D2a	27.85	42.50	7.13	25.82	2.42
Z2A1c	20.57	42.72	3.42	22.24	2.74
Z5D2a	39.85	16.75	7.50	21.37	2.91
Z4D5a	8.25	19.14	39.79	22.39	2.95
Z1G6a[3]	51.04	24.79	12.53	29.45	3.45
Z1G6d[3]	18.48	9.76	57.39	28.54	3.89
Z2G3a	3.90	46.73	28.39	26.34	4.10
Z1H7a[3]	52.35	20.68	6.79	26.61	4.15
Z2C1a[2]	17.48	63.31	36.87	39.22	4.21
Z1G7b[3]	58.58	8.61	24.42	30.53	4.61
Z4C4a[2]	10.72	27.47	76.57	38.25	5.89
Z4D6a[2]	4.60	92.91	60.11	52.54	8.44

Maximum values are underlined, 1,2 and 3 designate the genotypes that belong to the defined selection groups 1, 2 and 3 respectively

4. Conclusions

Almond yields data analysis of 122 argan trees in the natural reserve of Admin under natural conditions during the 2014-2016 three-year period showed that the rate of trees bearing fruits varied from 50 to 73%. This investigation study suggests also that argan yield variation is mainly influenced by the genotype-to-year interaction. The study of different yield component, showed that the importance of fruit yield is not always reflected in almond yield, under contrasting environments both traits can be negatively correlated in some genotypes.

The AMMI analysis showed that more the genotype is productive, the more its chance to be stable decreases. The use of AMMI model facilitates the selection of stable and high yield genotypes; such genotypes deserve further yield evaluation under orchard conditions.

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