Minor Olive Varieties from Iran with Promising Nutraceutical Properties

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ABSTRACT

Various nutraceutical properties of olives are ascribed to their different oil compositions and diversity of their active substances. Iran is endowed with great olive diversity which deserves to be studied. Accordingly, nine minor Iranian olive varieties, i.e. Tarom (T) varieties, were studied. Characterization of these minor varieties (T10, T15, T16, T17, T18, T20, T22, T23, T24) along with 3 major Iranian and 4 Mediterranean varieties with 11 chromosomal SSR markers revealed remarkable diversity among them. Most of T varieties had oil and Total Phenolic Content (TPC) comparable to Mediterranean olives. In comparison with Mediterranean varieties, T18 and T22 had higher Oleic Acid (OlA). T24 and T18 contained the highest and the lowest Linoleic Acid (LiA), respectively. T18 exhibited the highest OlA/LiA ratio. T24 was exceptionally phenols-rich variety followed by T20. Radical Scavenging Activities (RSA) results hardly suggested linear correlation between TPC and antioxidant capacity of the examined varieties. Nonetheless, T22 showed Phenol Antioxidant Coefficient higher than Mediterranean samples. Harvest time was influential on LiA content and the (RSA/TPC) ratios. Considering nutraceutical potential, some of T varieties are superior to the prevailing Iranian and Mediterranean varieties, so, they deserve to be introduced to olive improvement programs.

Keywords: Oleic acid/linoleic acid ratio, Phenols Antioxidant Capacity, Radical Scavenging Activity, Tarom varieties.

INTRODUCTION

Iranian traditional medicine is based on food therapy (Nayernouri, 2013; Tavakkoli-Kakhki et al., 2014). Many Iranian famous scholars such as Avicenna and Abu Reyhan Biruni treated patients simply by changing their diets (Shirafkan et al., 2014; Mahdizadeh et al., 2015). As a result, a considerable number of herbs, medicinal plants, and food flavoring have been listed and discussed in Iranian and other Muslim’s traditional medical books (Emami et al., 2012). Interestingly, the study of ancient Iranian medicine and pharmacology texts shows that, in many cases, it was highly recommended for picking a type of plant, fruit, or spice from a particular area (Emami et al., 2012; Mahdizadeh et al., 2015). This discloses the ancient physician’s attention to biodiversity and effects of climate on the nutritional potential of food and consequently their therapeutic properties (Esmaeili et al., 2012).

Olive has a very unique position in Iranian traditional medicine. Muslims on the basis of the Quran consider olive as a sacred fruit and it is seen in many prescriptions. In parallel, the contemporary research testifies

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the nutritional and therapeutic properties of olive. For instance, positive effect of olive products on diseases such as cardiovascular disease and certain cancers have been documented (Cicerale et al. 2010). These properties are ascribed to the diversity of biologically active substances in olives. Studies show that olive oil contains about 230 different compounds (Preedy and Watson 2010) such as oleuropein, which have direct effects on its taste, smell, color, food quality and therapeutic potential (Barbaro, Toietta et al. 2014). This is one of the reasons why studying olive varieties is of crucial importance. Identification and study of talented and enriched olive varieties would not only increase the therapeutic use of olive products but it also helps to boost the fledgling economy of developing countries with appropriate climate for planting such varieties.

Because of the historical attention of Iranians to olive products and the collaborative climate of the country, Iran is endowed with a diversity in olives which offers exciting opportunities for research. As a result of a comprehensive national and international program on Iranian olives, the olive germplasm diversity is being studied (Hosseini-Mazinani et al., 2014; Torkzaban et al., 2015). However, little is known about the nutraceutical and agronomical potential of this valuable germplasm, especially minor varieties. In view of the importance of such information to scientists, olive growers and producers of olive by-products, this research was devoted to the characterization and evaluation of nutraceutical potential of nine minor olive varieties of Iran [Tarom (T) varieties] by measuring their Total Oil Content (TOC), fatty acids composition, Total Phenolic Content (TPC), and Free Radical Scavenging Activity (RSA). These minor varieties were also characterized by nuclear DNA molecular markers to provide all necessary information about their genetic structure, assessing the level of diversity and their relationships with the Iranian and Mediterranean reference cultivars. The ensuing results, in comparison with those of some Mediterranean and prevailing Iranian cultivars, are presented and discussed in this paper.

**MATERIALS AND METHODS**

Gallic Acid (GA), ascorbic acid, n-hexane, and Folin-Ciocalteu reagent were purchased from Merck (Darmstadt, Germany). 1,1-DiPhenyl-2-PicrylHydrazyl (DPPH) was purchased from Sigma Chemical and Biochemical Company (St. Louis, MO, USA). Other chemicals were taken from the authentic samples.

**Plants and Fruit Samples**

About 50 years ago, several autochthonous olive accessions were collected from different geographical regions of Iran and were cultivated in a garden in Hashem-Abad near Gorgan City, Iran. Because of high potential productivity of theses accessions, at least three cuttings of the replicated plants were transferred to Tarom Olive Research Station in Zanjan Province for further studies. But due to their unidentified geographical origin, they were designated as the “T (Tarom) varieties”. Tarom, in North-West of Iran (Latitude 36° 47', Longitude 49° 6' and Altitude of 369 m) with a semi-Mediterranean climate is one of the largest areas of olive cultivation in Iran. Tarom Olive Research Station takes care of various collections of olive trees with more than 200 varieties mainly from Iran and Mediterranean countries.

In this research, three distinct categories of olive varieties were studied. The first group was the Tarom varieties (T) and consisted of nine Iranian olive varieties including T10, T15, T16, T17, T18, T20, T22, T23, and T24. The second group consisted of four Mediterranean varieties including Koroneiki and Arbequina, selected for the relatively high and low content of oleic acid in their oil, respectively (Parvini et al., 2015; Homapour et al., 2016); and Cornicabra and
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Picual, selected for their high phenolic contents (Gómez-Alonso et al., 2002; Beltrán et al., 2004). The third group consisted of three major varieties in Iran including Zard, Mari, and Rowghani. The Mediterranean and the major Iranian varieties were used as references for comparative purposes. All the studied olive trees were nearly 18 years old, reproduced by taking cutting from the mother plant, irrigated and fertilized by a drip system in Tarom Olive Research Station. All the trees sampled were in the same orchard under the same environmental conditions. Three replicated plants were studied. The fruits were picked at 150 and 180 Days After Full Bloom (DAFB) corresponding to different developmental stages of the olive trees. Samples were stored at -20°C on the day of picking.

Genotyping by SSR Markers

Total DNA of the olive leaves was extracted using Gene Elute Plant Genomic DNA Miniprep Kit (SIGMA) according to the manufacturer’s instructions. Polymerase Chain Reaction (PCR) was performed in a total volume of 20 µL, containing 2 ng genomic DNA, 1X supplied PCR buffer (Kawsar Biotech Company, Iran), 200 µM of each dNTPs (Sina Gen Inc. Iran), 0.25 unit of Taq DNA polymerase and 0.2 µM of forward (fluorescently labeled) and reverse primers. Amplifications were performed with PCR System 9600 (Applied Biosystems, Foster City, CA, USA) programmed with denaturation at 94°C for 5 minutes, 35 cycles of 94°C for 20 seconds, annealing temperature at 50°C for 30 seconds and 72°C for 30 seconds, and final extension at 72°C for 7 minutes. The detection of products was done on an automatic capillary sequencer; ABI 3130 Genetic Analyzer (Applied Biosystems/HITACHHI, Tokyo) using fluorescent dyes and fragments size were determined using internal standards.

Genotyping of all the studied tree samples were analyzed by selecting the best 11 ranked chromosomal microsatellites (SSR) loci, which represent the most informative SSRs for olive variety discrimination (Hosseini-Mazinani et al., 2013; Hosseini-Mazinani et al., 2014). Output data were analyzed using GeneMapper 3.7 (Applied Biosystems). Genetic distances between all pair wise combinations of the samples were calculated using Dice coefficients. Grouping of the trees was determined by using the Unweighted Paired Group using Average (UPGMA) Method as well as ordination based on Principal Coordinate Analysis (PCO) (Ingrouille, 1986; Chatfied and Collins, 2013). Cophenetic correlation was also determined for different clustering methods. NTSYS-pc version 2.02 software was used for the statistical analyses (Rohlf, 1990).

Total Oil Content (TOC)

Mesocarp of the fruit sample was weighted then dried at 45-55°C. TOC of the dried sample was determined by TD-NMR analyzer Minispec NMS100 (Bruker Optik GmbH, Ettlingen, Germany) according to the literature (Parvini et al., 2015).

Fatty Acids

Oil was extracted from a certain amount of the dried mesocarp of the olive sample with a soxhlet at 40-60°C for 1 h using n-hexane as the solvent. The extracted oil was transferred into an amber glass bottle and stored in the dark at 4°C for further analysis. The fatty acid composition of the oil samples [Palmetic Acid (PlA), Palmitoleic Acid (PleA), Stearic Acid (StA), Oleic Acid (OlA) and Linoleic Acid (LiA)] was determined by gas chromatography as fatty acid methyl esters according to European Regulations (EEC 2568/91) (REGULATION 1991) using a ACME 6100 Younglin Capillary Gas chromatograph equipped with a flame ionization detector (VICI, Valco, Houston, Texas, USA) and a fused-silica capillary column, 60 m×0.32
mm×0.5 μm film thickness, (Teknokroma, Barcelona, Spain). The injector, detector and oven temperatures were set at 240, 250, and 185°C, respectively. Helium was used as the carrier gas with a linear flux of 1 mL min⁻¹ and a split ratio of 1:50. The experiments were carried out in triplicate and the results were expressed in percent.

**Total Phenolic Content (TPC)**

The fresh mesocarp (3 g) was frozen in liquid nitrogen and ground to fine powder in a porcelain mortar. It was then mixed with methanol (12 mL) and vortexed for 1 minute at 20°C. The resulting mixture was centrifuged (3,500 rpm at 4°C) for 20 minutes. The supernatant was separated and stored in an amber glass bottle at -20°C for further analysis.

Total phenolic content of each extract was determined using Folin-Ciocalteu method (Singleton et al., 1999). Accordingly, different amounts of each sample (10, 30 and 50 μL) were mixed with Folin reagent (100 μL) and water (1.58 mL). The resulting mixture was kept at 20°C for 5 minutes then Na₂CO₃ solution (300 μL, 20% w/w) was added. The resulting mixture was maintained at 20°C in the dark for 2 hours and subsequently readings were acquired with a spectrophotometer reading at 765 nm against the desired blank (methanol). The results were expressed in terms of GA Equivalent (GAE= mg of GA per gram of the fresh sample). A standard curve was constructed using different concentrations (1, 2, 3, 5 and 10 mg μL⁻¹) of GA. All the spectrophotometric examinations were carried out with a UV-VIS ANALYTIK SPECORD-210 (Jena, Germany) spectrophotometer. The results are averages of, at least, three measurements.

**Radical Scavenging Activities (RSA) of the Extracts**

RSA of each extract was determined according to the method of Brand-Williams et al. (1995) with some modifications. The solution of DPPH radicals is purple in color (Mistry and Shah, 2014). Upon the reaction of this solution with an antioxidant, the DPPH radicals are reduced and, as a result, the optical density of the solution at 517 nm decreases. Therefore, 10, 20 and 40 μL of each methanolic extract was added to a mixture of 2.7 mL of methanol and 300 μL of DPPH solution (0.1 mM). The resulting mixture was maintained at room temperature for 10 minutes prior to spectrophotometric measurements. A calibration curve at 517 nm was made with DPPH to calculate the remaining DPPH concentration in the reaction mixture. Antioxidant activity was expressed in terms of inhibition percent and μmol of ascorbic acid equivalent per gram of fresh sample.

**RESULTS AND DISCUSSION**

**Tarom (T) Varieties**

Characterization of T varieties with 11 chromosomal SSR markers revealed considerable variation between them (data not shown). Preliminary agronomical examinations of this collection resulted in selection of 9 varieties (T10, T15, T16, T17, T18, T20, T22, T23, and T24) for further studies. Data obtained from 11 SSR markers were used for elucidating the genetic relationships among the olive varieties (Figure 1). The clustering revealed 4 main groups in which most of the selected T varieties were classified and separated from Mediterranean and Iranian major varieties. Zard variety was separated from the other Iranian varieties and mixed with the T varieties. All Mediterranean varieties (except Koroneiki) were categorized in the same cluster. T20 variety clustered with Mediterranean varieties, while T10 was closer to Iranian varieties (Figure 1). These results which were related to our previous finding (Hosseini-Mazinani et al., 2013) exhibited considerable differences in T
varieties with possible new characters and nutraceutical potential.

**Fatty Acids**

Data of Table 1 indicates that, despite of obvious diversity, TOC of the majority of T varieties is comparable with those of Koroneiki and Arbequina. T18 and T22 had 6 and 12% more OLA than Koroneiki and 4 and 9% more than Arbequina, respectively.

OLA is further elongated and desaturated in human cells. This results in formation of $\omega$-9 fatty acids family which is valuable to our health (Cicerale et al., 2010; Preedy and Watson, 2010). Nonetheless, OLA is not considered as an essential fatty acid since it is anabolized by our cells. In contrast, LIA (18:2, $\omega$-6), similar to other Poly-Unsaturated Fatty Acids (PUFA), is an essential fatty acid which is used as a precursor in eicosanoids biosynthesis (Lewinska et al., 2015; Papackova and Cahova, 2015). With a total oil content similar to Koroneiki, T24 had the highest LIA content, more than three times of that of Koroneiki (Table 1). The TOC of T18 was also similar to Koroneiki and Arbequina but it had the lowest amount of LIA; about 26.2 and 11.5% of that of Koroneiki and Arbequina, respectively.
Table 1. Total Oil Content (TOC) and fatty acid composition of the examined olive varieties at 150 DAFB.

<table>
<thead>
<tr>
<th>variety</th>
<th>TOC (%)</th>
<th>PlA (%)</th>
<th>PleA (%)</th>
<th>SiA (%)</th>
<th>OIA (%)</th>
<th>LiA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T10</td>
<td>41.99</td>
<td>22.55±0.14</td>
<td>1.57±0.06</td>
<td>1.42±0.24</td>
<td>62.09±1.26</td>
<td>12.09±0.55</td>
</tr>
<tr>
<td>T15</td>
<td>44.58</td>
<td>17.38±0.13</td>
<td>0.86±0.01</td>
<td>1.26±0.10</td>
<td>73.49±0.58</td>
<td>8.12±0.77</td>
</tr>
<tr>
<td>T16</td>
<td>36.30</td>
<td>19.82±0.35</td>
<td>3.34±0.06</td>
<td>2.36±0.01</td>
<td>76.56±0.39</td>
<td>5.21±0.16</td>
</tr>
<tr>
<td>T17</td>
<td>46.87</td>
<td>14.68±0.27</td>
<td>1.06±0.03</td>
<td>1.50±0.10</td>
<td>81.46±1.00</td>
<td>0.96±1.36</td>
</tr>
<tr>
<td>T18</td>
<td>46.01</td>
<td>16.12±1.00</td>
<td>0.76±0.08</td>
<td>2.33±0.60</td>
<td>45.33±1.04</td>
<td>24.19±0.37</td>
</tr>
<tr>
<td>T20</td>
<td>16.64</td>
<td>26.19±1.00</td>
<td>3.34±0.06</td>
<td>1.26±0.10</td>
<td>73.49±0.58</td>
<td>2.68±0.25</td>
</tr>
<tr>
<td>T21</td>
<td>47.69</td>
<td>21.21±1.01</td>
<td>1.68±0.15</td>
<td>1.15±0.10</td>
<td>70.75±0.94</td>
<td>5.20±0.18</td>
</tr>
<tr>
<td>T22</td>
<td>45.76</td>
<td>29.43±1.01</td>
<td>5.50±0.23</td>
<td>3.50±2.13</td>
<td>51.45±8.77</td>
<td>12.30±2.18</td>
</tr>
<tr>
<td>Koconeiki</td>
<td>44.88</td>
<td>18.47±1.70</td>
<td>1.26±0.24</td>
<td>4.77±0.33</td>
<td>75.06±2.83</td>
<td>3.57±0.57</td>
</tr>
<tr>
<td>Arbequina</td>
<td>50.80</td>
<td>18.75±4.50</td>
<td>1.65±0.67</td>
<td>1.22±0.06</td>
<td>70.04±9.39</td>
<td>7.58±4.49</td>
</tr>
<tr>
<td>Zard</td>
<td>37.93</td>
<td>17.49±0.79</td>
<td>1.16±0.01</td>
<td>1.56±0.37</td>
<td>70.14±4.18</td>
<td>9.51±0.44</td>
</tr>
<tr>
<td>Mari</td>
<td>43.53</td>
<td>20.69±4.24</td>
<td>2.01±0.58</td>
<td>1.43±0.36</td>
<td>67.04±8.06</td>
<td>8.98±3.65</td>
</tr>
<tr>
<td>Rowghani</td>
<td>49.83</td>
<td>21.51±0.91</td>
<td>1.81±0.05</td>
<td>2.12±0.00</td>
<td>57.46±5.56</td>
<td>16.37±0.10</td>
</tr>
</tbody>
</table>

*a TOC is the weight of oil to weight of the dried fruit in percent and the percent of each constituent was calculated from the ratio of the weight of the constituent to TOC.

There is evidence suggesting possible correlations between LiA-rich diets and cardiovascular disorders as well as cancer incidents (Osendarp, 2011). On the contrary, results of different researches disclosed effects of OlA-rich diets such as Mediterranean diet on prevention of cardiovascular disease, hyper-tension, and cancer development (Cicerale et al., 2010; Preedy and Watson, 2010). Although the details of the mechanisms through which these effects are exerted have remained to be elucidated, the key role of OIA in reduction of fatty acids and cholesterol biosynthesis has been demonstrated (Gnoni et al., 2010). Therefore, the OIA/LiA ratio is an important index for evaluation of nutraceutical potential of olive oils.

There are only 3 T varieties with higher OIA/LiA ratios than that of Koconeiki (Figure 2-A). It is worth noticing that T18 exhibited very high ratio, more than 4 times, as compared to that of Koconeiki, at 150 DAFB. However, this difference decreased to 2.87 times at 180 DAFB. Similarly, T22 achieved very high OIA/LiA ratio at 180 DAFB. OIA/LiA changes from 150 to 180 DAFB were mostly due to LiA changes rather than OIA. This means that postponing harvest time from 150 to 180 DAFB did not change much the OIA content of the fruits, about 3% for most of T samples (Table 1-S, Supplementary document).

It should also be mentioned that according to Figure 1, among T collection, T17 is the closest one to Mediterranean olives. Reviewing data of Tables 1 and 1-S indicates that there is a considerable similarity between the TOC and oil composition of T17 and Koconeiki. Interestingly, T17 obtained an OIA/LiA ratio of 21.72 at 180 DAFB while it was 20.58 for Koconeiki at 150 DAFB.

**TPC and RSA**

Phenolic content is a determinant factor of nutraceutical potential of olives. Not only are phenols biologically active substances, they are also able to terminate free radicals. For instance; anti-inflammatory properties of oleuropein (Omar 2010) and its effect on counteracting lipid accumulation in a mouse model of non-alcoholic fatty liver disease as well as its antioxidant potential have been demonstrated (Omar 2010; Barbaro et al. 2014). Data in Table 2 shows that TPC of most of T varieties and the controls are comparable. T24 seems to be an
exceptionally phenol-rich variety with 3.8 and 2.3 times more phenols than Cornicabra and Picual, respectively, at 150 DAFB. T20, which is located among Mediterranean varieties in phylogenetic dendrogram (Figure 1), can also be considered a phenol-rich variety with 2.1 and 1.3 times more phenols than Cornicabra and Picual, respectively. Table 2 indicates that postponing the fruit picking time from 150 to 180 DAFB decreased the TPC of all the examined varieties. This may be due to the participation of free phenols in the biosynthesis of macromolecules such as lignins during ripening time (Singh et al., 2010).

Although it is rather difficult to explain mechanisms through which the nutraceutical effects of a phenolic compound are exerted, it is possible to quantify antioxidant capacity of phenols via measuring their RSA. Table 2 hardly suggests a linear correlation between the phenolic content and antioxidant capacity of the examined varieties. T20, with TPC higher than T18 and T22, had lower RSA at 150 DAFB. Similarly,
Cornicabra, with 40% lower TPC than Picual, showed about 40% higher RSA. This is not surprising though as the various phenolic substances exhibit different RSA due to the structural reasons (Cai et al., 2004).

The ratio of RSA result to TPC (DPPH/Folin) is called Phenol Antioxidant Coefficient (PAC). This indicates how important are phenols of a sample from antioxidant point of view (Hodzic et al., 2009). According to Figure 2-B, at 150 DAFB, most of T varieties had lower PAC values than those of the controls, but, at 180 DAFB, some of T varieties showed an increase. T22 had a PAC value higher than Cornicabra and Picual, i.e. 290 and 35%, respectively, at 180 DAFB. A similar increase was observed for T22 with respect to its OlA/LiA ratio at 180 DAFB while there was not much change in its OlA content (Tables 1 and 1-S). This means that the best harvest time for T22 is 180 DAFB. In contrast, delaying fruit picking to 180 DAFB did not increase remarkably the PAC value of T18, while it obviously affected its OlA/LiA ratio (Figure 2-A). It is important to note that the PAC value of T18 was very similar to that of Picual and higher than many T varieties at 150 DAFB. Therefore, the right harvest time for T18 could be 150 DAFB if the fruit size and the oil content are satisfactory.

**T Varieties versus the Commercial Iranian Varieties**

Zard, Mari, and Rowghani are three prevailing olive varieties in Iran. Data of Tables 1 and 1-S suggest that they reached higher TOC and OlA content at 180 DAFB. Their TOC and OlA content values fall somewhere close to those of Koroneiki and Arbequina (Table 1-S). But, from OlA/LiA ratio point of view, they are far behind the selected T varieties (Figure 3-A) mainly because of their high LiA content. As a matter of fact, Rowghani can be considered as a LiA-rich variety (Tables 1 and 1-S).

Among the prevailing Iranian varieties, Rowghani showed the highest TPC at 150 DAFB, but it was still about 34% less than that of T24 (Table 2). Although Zard and Mari showed TPC comparable with those of Mediterranean varieties Cornicabra and Picual, at 150 DAFB, they exhibited different RSA. This is well perceived from Figure 3-B. Zard obtained the lowest PAC value as it exhibited lower RSA at 150 DAFB. However, delaying the harvest time to 180 DAFB increased its PAC value so that it became comparable to those of Picual and T18.

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**Figure 3.** Normalized (A) OlA/LiA ratios and (B) PAC values of selected T varieties in comparison with controls and prevailed varieties in Iran at 150 (dark columns) and 180 (bright columns) DAFB. Also, Koroneiki (Cont1), Arbequina (Cont2), Cornicabra (Cont3) and Picual (Cont4).
Finally, because of significant variation in Iranian olive germplasm, even in minor varieties, and considering auspicious results related to their nutritional parameters of oil, these varieties seem to be promising varieties that could be applied in olive improving programs in the country.

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زنوتیپ های محیط زیتون ایران با خواص دارویی و تغذیه ای امیدبخش

چکیده

خواص متعدد و فراوان دارویی و تغذیه ای زیتون وابسته به نوع و شکل دهنده روغن آن است. بر اساس مطالعات انجام شده، ایران یکی از کشورهای خارج از حوزه مدیریت زرم ایرانی و منوع زیتون می باشد. در این پژوهش کیفیت روغن و ترکیبات فلی 9 زنوتیپ محیطی T10, T15, T16, T17, T18, T20, T22, T23, T24 زیتون موجود در استان تحقیقات زیتون طارم به نام های T22, T23, T24 و چهار رقم مهم میان میانهای ای مورد مطالعه قرار گرفتند. بررسیی ها نشان داد که برخی از زنوتیپ های گروه T (T18, T24) دارای حبکی ای بوده و نسیب اولینک اسید به لیولیک اسید بودند. حبکی باعث داشت آهذ است که رابطه RSA/TPC حبکی اسید با اتصال آکسیژن به زنوتیپ های محیطی ایران (T24 و گروه ( RSA دارای ارتباط غذایی و دارویی بسیار بالایی می باشد که به بهره وری می شود از این زنوتیپ ها در طرح توسعه زیتون کشور به عنوان ارقام امیدبخش استفاده شود.