BIOLOGICAL ACTIVITY OF WALNUT (Juglans regia L.) CULTIVARS AND GENOTYPES IN INNER ANATOLIA

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Abstract- In this study, the fruits of ten seed propagated walnut genotypes pre selected according to it’s better fruit characteristics and high yield capacity were sampled from Nigde province and compared to standard walnut cultivars ‘Sebin’, ‘Kaman’ and ‘Bilecik’ for biological activity of fruits including total phenolic content and antioxidant activity. Total phenolic content of walnut fruits were determined by Folin-Ciocalteu reagent and results expressed as mg gallic acid equivalents (GAE) per 100 g raw walnut fruit. The TEAC (trolox equivalent antioxidant capacity) and FRAP (ferric reducing antioxidant power) assays assessed to estimate total antioxidant capacity. Total phenolic content were found between 1142 (cv. ‘Sebin’) - 1861 (cv. ‘Bilecik’) mg GAE/100 g fresh walnut fruit in cultivars and between 1024 (genotype Nigde-08) - 2014 (genotype Nigde-02) mg GAE/100 g fresh walnut fruit in non-grafted genotypes, respectively. In general seed propagated genotypes exhibited higher antioxidant activity than cultivars in both FRAP and TEAC method. The results showed that there were high variation among genotypes and cultivars in terms of biological activity that is important for future walnut breeding activity to select better parents to obtain high phytochemical content walnut cultivar candidates.

Keywords- Walnut, Biological activity, Fruit properties

I. INTRODUCTION

The Persian walnut (Juglans regia L.) is native to central Asia and grows as a wild or semi-cultivated tree in a wide area from southeastern Europe and the Caucasus to Turkey and Iran, through southern portions of the former Soviet Union into China and the eastern Himalayas1,2. In walnut growing near east Asian countries including Iran, Pakistan, Uzbekistan, Turkmenistan, Azerbaijan and inner and eastern part of Turkey there were high variability in particular the shape and size of fruit, color and thickness of shell and kernel, and shape and size of crown, stem and leaves due to continuous seed propagation of walnut trees for centuries3-8. Walnut trees have been mostly seed propagated across the Turkey, which has resulted in a diverse gene pool of walnut suitable for selection and breeding programs9. However more recently walnuts are grafting on rootstocks in Turkey.

Turkey is one of the native lands of the Persian walnut, which has been cultivated mainly in the inner, northern and eastern Anatolia since ancient times. Numerous non-grafted and cultivated walnut trees are well adapted to different agro-ecological conditions in the country1.

There is great variability among walnut genotypes in particular seed propagated ones grown in different parts of Turkey in terms of fruit characters such as fruit size, shape, kernel weight, kernel ratio, kernel color aril color etc.3,5. These variations are more visible in local genotypes naturally grown in different parts of Turkey5.

Modern objectives in plant breeding may be achieved by the evaluation of traits amongst genetic resources and combination of those in one cultivar. Although, molecular indexes like molecular markers are used already, but these methods are expensive. More over in walnut, because of sufficient varieties and easy application, fruit characteristics might be appropriate for classification9,10. Fruit characters must be recorded for selection of parents and are also the first choice used for describing and classifying the germplasm7,8. Statistical methods including principle components or cluster analysis can be used as useful tools for screening the accessions. In addition, fruit characteristics sometimes have correlation or are associated with characteristics that are difficult to evaluate such as disease susceptibility. Therefore, they may be useful as markers in breeding programs11.

A valuable edible nuts produced by walnut trees are well appreciated because they are enriched with unsaturated fat (linoleic, oleic acid). They also contain other beneficial components like plant protein (e.g. arginine, leucine), carbohydrates (e.g. dietary fibre), vitamins (e.g. vitamin A, E), pectic substances, minerals (magnesium, potassium, phosphorus, sulphur, copper, iron), plant sterols, phytochemicals (phenolic acids, flavonoids, etc.)12,13. For this reason recent years have seen increased interest on the part of consumers, researchers, and the food industry into how walnut fruit can help maintain health; and the role that diet plays in the prevention and treatment of many illnesses has become widely accepted. At the present time, considerable importance is given to walnut fruit, which, in principle, apart from their basic nutritional functions, provide physiological benefits and play an important role in disease prevention or slow the progress of chronic diseases3,10,11.
The present study was designed to evaluate the fruits of walnut cultivars grown in the Nigde province located inner Anatolia for their phytochemical composition.

II. DETAILS EXPERIMENTAL

2.1. Materials and procedures
The study was conducted in Nigde province, Turkey in 2014. Firstly, the spots of naturally grown wild walnuts were identified. The raw walnut fruits were harvested at maturity phase and the harvested fruits were immediately taken into sample cups to prevent moisture loss, transferred to laboratory and stored at -20°C until analyses. In total, 200 fruits from 10 regional genotypes (Nig-01, Nig-02, Nig-03, Nig-04, Nig-05, Nig-06, Nig-07, Nig-08, Nig-09, Nig-10) and 3 commercial cultivars (‘Şebin’, ‘Bilecik’, ‘Kaman’) were collected from the orchards in the district. For each cultivar, the mass of 200 g walnuts was prepared for total phenolic and antioxidant analysis.

2.2. Total phenolic content and antioxidant activity
The samples were chopped in a coffee mill. Total phenolics were measured by the Folin–Ciocalteu reagent mentioned by Linkens and Jackson. A total phenolic extract was prepared by a lightly modified procedure of Anderson et al. The chopped nuts were extracted with a solution of 75% acetone and 25% of 526 µmol/L sodium metabisulfite. The supernatant was pipetted and centrifuged. Then, the extraction solution was evaporated and extracted with hexane. The water soluble phase was used for determining the concentration of total phenolics and antioxidant activity.

The total phenol content of walnut extract was determined according to Singleton et al. with results expressed as mg gallic acid equivalents (GAE) per 100 g raw walnut. The TEAC (trolox equivalent antioxidant capacity) and FRAP (ferric reducing antioxidant power) assays assessed to estimate total antioxidant capacity. FRAP reducing power is expressed as µmol Fe²⁺ per g walnuts and TEAC expressed as µmol TE per g walnuts.

2.3. Statistical Analysis
Statistical analysis of data was done according to SPSS using statistical software. Data were analyzed by Statistical Analysis of Variance (ANOVA) and differences among means were determined for significant at P<0.05 using LSD test.

III. RESULTS AND DISCUSSION
As shown in Table 1, there were statistical differences (p<0.05) between non-grafted walnut genotypes and also between non-grafted walnut genotypes and walnut cultivars in terms of total phenolic content.

Total phenolic content in commercial national cultivars and regional genotypes varied from 1071 mg (cv. ‘Sebin’) to 1683 mg GAE per 100 g nut (cv. ‘Birecik’) and 1024 (Nig-08) to 2014 mg (Nig-02) mg GAE per 100 g walnut, respectively (Table 1). The result indicates that the local genotype Nig-2 revealed the highest content in phenol compounds (2014 mg GAE per 100 g walnut). We also found strong positive correlation between total phenolic content and antioxidant activity (R²=0.88 for between total phenolics and FRAP and R²=0.79 between total phenolics and TEAC). The antioxidant activity of phenolics may be related to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors, their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals and thus, natural antioxidants function as free-radical scavengers and chain breakers, complexers of pro-oxidant metal ions and quenchers of singlet-oxygen formation.

Previously total phenolic content of walnut cultivars from different countries were reported between 1071-2370 mg GAE/100 g nut, which indicates good accordance with our study. The total phenols in walnuts are within the range of fruit such as blueberries (531 mg GAE/100 g), plums (367 mg GAE/100 g), and raisins (1065 mg GAE/100 g).

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants’ colors. They are ubiquitous in all plant organs and are therefore an integral part of the human diet. Phenolics are widespread constituents of plant foods (fruits, vegetables, cereals, olive, legumes, chocolate, etc.) and beverages (tea, coffee, beer, wine, etc.), and partially responsible for the overall organoleptic properties of plant foods.

Several in vitro methods have been developed to assess the total antioxidant capacity of fruits, vegetables and beverages. FRAP and TEAC are widely used methods to determine total antioxidant capacity of plant materials. The FRAP assay is a simple, convenient and reproducible method that was initially developed to measure the plasma antioxidant capacity, but is now widely employed in the...
antioxidant studies of other biological samples, such as food, plant extracts, juices and beverages, etc. The ferric reducing ability (FRAP) of walnut fruit extracts was in the range of 1024-2014µm of Fe²⁺ per g fresh weight (Table 1). Similar to their total phenolic content, regional genotype, Nig-02 walnuts had the highest antioxidant activity with FRAP value of 428µmol Fe²⁺ per g FW. Previously FRAP value of walnuts was found 454 µmol Fe²⁺ per g FW supporting our present results.

The trolox equivalent antioxidant capacity (TEAC) of walnut fruit extracts was in the range of 94-161µm of TE per g fresh weight (Table 1). Similar to their total phenolic content, regional genotype, Nig-2 walnuts had the highest antioxidant activity with TEAC value of 161 µmol TE per g FW. Previously TEAC value of walnuts was found 137 µmol TE per g FW which in accordance with our results.

The TEAC method (trolox equivalent antioxidant capacity) is one of the most used methods for quantifying radicals, which can be scavenged by some antioxidant. It is based on scavenging of the cation radical originated by the one-electron oxidation of the synthetic chromophore 2,2' azinobis(3-ethylbenzothiazoline-6-sulfonate(ABTS•)) to ABTS•++. We found a big diversity among used genotypes and cultivars in terms of antioxidant capacity. It is known that genetics, harvest season, origin, environmental conditions, soil composition, maturity level and the methods of cultivation highly influence the composition of walnuts.

The results of this study indicated that regional and commercial walnut cultivars originated from Turkey has a high antioxidant activity and in particular regional genotype Nig-02 possesses good medicinal potential. A positive relationship between antioxidant activities and total phenolic contents was also observed. The high level of total phenolic in Nig-2 and cv. ‘Bilecik’ indicated high antioxidant activities. This relationship was also reported in previous studies on other fruits.

Human health and nutrition are still one of the most studied and interesting topics. Natural compounds, including those coming from plants, are nowadays under detailed investigation due to their potentially beneficial effects. Further work is required to establish the components in phenolics and flavonoids that may have contributed to the high antioxidant activities so far observed.

CONCLUSIONS

The results indicated that non-grafted walnuts showed significant variations in most of the bioactive content. The study also imply that the richness of walnut germplasm in Turkey. Our study showed that Nig-02 had highest biological activity and thus could be particularly important for future breeding activity. The results described here provide a solid foundation. The data reported in this paper also confirmed that walnuts were a rich source of important nutrients that would be very beneficial to human health.

Table 1. Bioactive content of walnuts

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Total phenolic (mg GAE per 100 g)</th>
<th>TEAC (µmol 1TE per g FW)</th>
<th>FRAP (µmol Fe²⁺ per g FW)</th>
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</thead>
<tbody>
<tr>
<td>NIG-01</td>
<td>1840</td>
<td>155</td>
<td>414</td>
</tr>
<tr>
<td>NIG-02</td>
<td>2014</td>
<td>161</td>
<td>428</td>
</tr>
<tr>
<td>NIG-03</td>
<td>1310</td>
<td>144</td>
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<td>1478</td>
<td>105</td>
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<td>150</td>
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<td>1448</td>
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<td>Bilecik</td>
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<td>25.4</td>
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REFERENCES

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