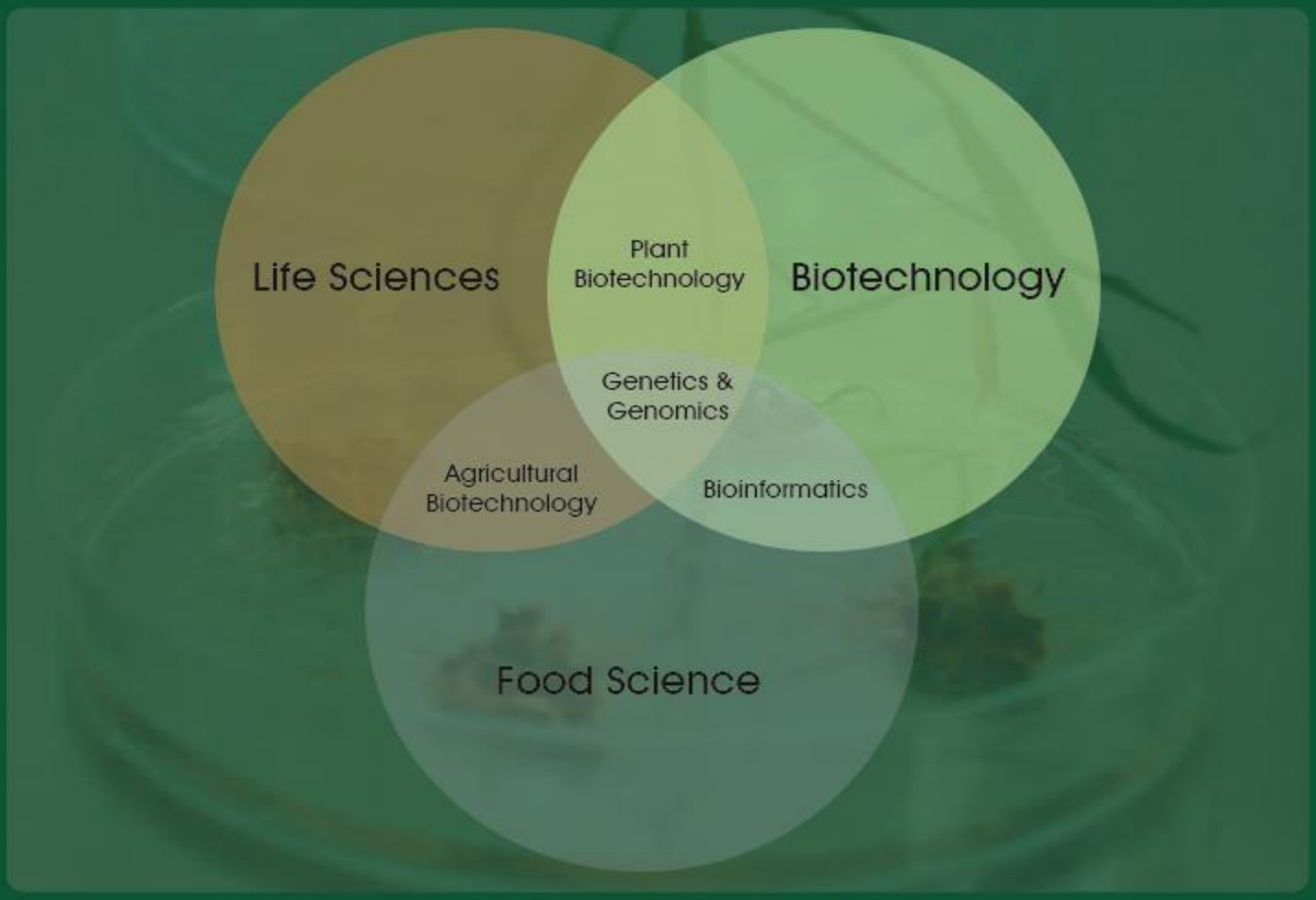
International Journal of Life Sciences and Biotechnology

e-ISSN:2651-4621



Volume/Cilt:2 **2019** **Issue / Sayı:2**

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"International Journal of Life Sciences and Biotechnology" dergisi Türkiye'de Yaşam Bilimleri, Biyoloji, Biyoteknoloji, Biyomühendislik, Ziraat Bilimleri, Gıda Biyoteknolojisi ve Genetik alanlarındaki ilgili araştırmacılara, kurum ve kuruluşlara teorik ve pratik uygulamalarda katkı sağlamayı, tarafsızlık ilkesi ve bilim etiği ilkelerine bağlı kalarak çözüm temelli, yenilikçi ve katma değeri olan çalışmalara odaklanan, günceli ve geleceği tartışan çalışmaların yayınlanmasını hedeflemektedir.

Bu düşüncelerle dördüncü sayısını yayınladığımız "International Journal of Life Sciences and Biotechnology" dergisini, makaleleri ile onurlandıran akademisyenlere, Fikir / Görüş / Katkı / Eleştirileri ile değerlendirme süreçlerine katkılarından dolayı hakem ve yayın kurullarında yer alan kıymetli bilim insanlarına yürekten teşekkür ediyoruz. Bir sonraki sayıda görüşmek ümidiyle…

10. 08. 2019
Editör
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From The Editor;

Dear Readers and Authors,

As “International Journal of Life Sciences and Biotechnology”, we are pleased and honored to present the fourth issue of the journal. "International Journal of Life Sciences and Biotechnology" is an international double peer-reviewed open access academic journal published on the basis of research-development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, We are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in fourth issue of "International Journal of Life Sciences and Biotechnology". Hope to see you in the next issue…

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Güzelavrat Otu (*Atropa belladonna* L.) Genel Özellikleri

Merve Gün[1]*, Selim Aytaç[1]

ÖZET

Atropa belladonna sistematik olarak *Solanaceae* ailesinde yer alan çok yıllık otsu bir bitkidir. Çiçekleri yeşilimsi mor, yaprakları oval, meyveleri siyah renkte ve parlaktır. Güzelavrat otu; *atropin, skololamin, belladonnin, hyosiyamin, apoatropin* gibi önemli alkoloidleri kapsar. En önemli kimyasal maddesi ise *atropin*' dir. İçerdiği etken madde 1809 yılında yalıtılmış ve 1819 yılından sonrada alkaloid olarak sınıflandırılmıştır. En yüksek alkaloid oranı yeşil yapraklarında ve meyvelerinde olmasına rağmen bitkinin bütün kısımları alkaloid içerir. Birçok kodekste alkaloid oranının %0,3'ün üstünde olması istenir. Güzelavrat otu (*atropa)*, kapsadığı etken maddelerden dolayı eskiden bu yana birçok ülkede ekilen bir bitkidir, esas alkoloid olan atropinin sentetik olarak elde edilen türevleri eczacılıkta önemli bir yere sahiptir. Ülkemizde de *Atropa belladonna* sahip olduğu tropan alkaloidleri ile bitkisel kökenli ilaçlar arasında önemli bir yere sahiptir.

MAKALE GEÇMİŞİ
Geliş 11.02.2019
Kabul 18.05.2019

ANAHTAR KELİMELER
Atropa belladonna, alkaloid, etken madde

General Features of Deadly Nightshade's (*Atropa belladonna* L.)

ABSTRACT

Atropa belladonna is a perennial herbaceous plant that is systenatically found in Solanaceae family. The flowers are greenish purple, the leaves are oval, the fruits are black and bright. Deadly nightshade includes important alkaloids suvh as atropine, skololamine, belladonnaine, hypocaine,apoatropine. The most important chemical substance is atropine. İts active ingredient was isolated in 1809 and later classified as alkaloid by 1819. All parts of the plant contain alkaloids although the highest alkaloid ratio is in the green leaves and in the fruites. İt is the desirable that the ratio of several coteche alkaloids is not less than %0,3. Deadly Nightshade is a plant that has been planted in many countries since ancient times due to ıts active ingredients and the synthetically derived derivatives of atropine whis is the main alkaloid have important place in pharmacy. In our country *Atropa belladonna* has an important place among tropane alkaloids and herbal medicines.

ARTICLE HISTORY
Received 11.02.2019
Accepted 18.05.2019

KEY WORDS
Deadly nightshade, alkaloids, active ingredient

Giriş

Son yıllarda bitkilerde bulunan etken maddeler üzerinde yoğun çalışmalar yürütülmektedir. Bunun nedenlerinden bazıları, tedavide kullanılan bazı sentetik ilaç hammaddelerinin sağlık üzerinde yan etkiler oluşturması veya bitkilerden elde edilen hammaddelerin sentetik olarak elde edilememesi tıbbı bitkilerdeki etken maddelerin önemini arttırmaktadır. Genel bir kanı olarak bitkisel droglar birden fazla etkiye sahipken sentetik ilaçların tek yönlü etki yaptığı

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düşünülmektedir. Günümüzde endüstrinin gelişmesiyle birlikte yeniden doğal kaynaklara yöneliş artmış ve bu yönde talepler ortaya çıkmıştır. Dünya Sağlık Teşkilatı'nın yapmış olduğu bir araştırma da tıbbı amaçla faydalanılan bitki sayısının yirmi bin kadar olduğunu ortaya çıkarmıştır [22]. Ülkemizde ise on bin kadar bitki türü içerisinde 3649 kadarının endemik bitki olduğu görülmüştür [2, 4, 21]. Ülkemiz iklim ve coğrafik yapı sebebiyle tür çokluğuna sahip olmasından dolayı Avrupa'nın en zengin bitki varlığına sahip olduğu halde ilaç sanayilerinin ihtiyaç olan hammaddelerinin % 70'den fazlası ithal edilmektedir [1]. Alkaloid ve türevleri ise bu ithalatta önemli yer tutmaktadır. Buna karşılık alkaloid içeren *Atropa* ve *Datura* gibi bitkiler ülkemizde doğal olarak bulunmakta ve kültür çalışmaları yapılmış ve yapılabilmektedir.

Güzelavrat Otu Hakkında Genel Bilgiler

Solanaceae (Patlıcangiller) familyasından olan *Atropa belladonna* (Güzelavrat otu) ülkemizde Karadeniz Bölgesi'nde, Toroslarda, Kırıklareli, Bolu, Balıkesir, Adana ve Hatay gibi illerimizde yabani olarak yetişmekte olup [35-41], Dilber otu, İt üzümü, Ayı çileği (Trabzon), Kurt böğürtleni (Kastamonu), Yidin (Giresun), Siyah üzüm, Şeytan vişnesi-kirazı, Yabani tütün (Hamsiköy-Trabzon) gibi isimlerle anılmaktadır [40]. Dünya genelinde ise Avrupa (Avustralya, Ukrayna, ve Arnavutluk), Kuzey Afrika (Cezayir, Fas) Batı Asya (İran, Türkiye) İtalya, Fransa İngiltere ve Amerika doğal olarak yetişmekte olup Avrupa'da, Amerika'da ve Hindistan ile Pakistan'ın da bir kısmında tarımı yapılmaktadır [15, 41, 42].

İsmini Yunan mitolojisinde yer alan kaderi belirleyen üç tanrısından kader ipini kesen Atropos'dan alır. Bella –Donna ise İtalyanca güzel kadın demektir. 16 yy. kadınlar, özellikle İtalyan kadınları, göz bebeklerini büyüterek daha efsunlu bir bakış oluşturmak için meyvelerinin suyunu gözlerine damlatmışlardır [31]. Diğer yandan içerdikleri alkaloitlerin zehirli olmasından dolayı birçok Roma imparatorunun öldürülmesinden; İskoç süikasçileri olan Anglo-Saxson'ların zehirlenerek öldürülmesine kadar birçok suikaste kullanılmıştır [25]. Bunun dışında halüsinolejik etkiye sahip olmasından dolayı büyücülükte de kullanılmıştır [26]

Güzelavrat otunun bütün bitki kısımlarında alkaloid bulunmasına rağmen en fazla kullanılan kısmı yapraklarıdır.

Atropa; göz muayenelerinde, kalp hastalıklarında kullanılmaktadır [12]. Merkezi sinir sisteminde etkilidir. Ter ve mide ifrazatını hafifletici, spazm giderici ve ağrı kesici olarak kullanılmaktadır [6]. İdrar yolları ve safra kesesi rahatsızlıklarında, romatizma ağrılarının dindirilmesinde, boğmaca, sigara içmeden meydana gelen astım (nefes darlığı) üzerine de etkilidir [41]. Güzelavrat otu iltihaplanmaları önlemede, Parkinson hastalığında, böbrek ve

safra taşlarında, hipertansiyon ve hipotansiyonda kullanılır [41-43]. Ayrıca *Atropa* halk hekimliğinde de mide rahatsızlıkları için çay olarak, astımda sigara şeklinde kullanılır. Yara ve şişliklere yapraklar lapa şeklinde kullanılır. Ancak *Atropa* çok zehirli bir bitki olduğu için çok küçük miktarlardaki doz aşımı bile zehirlenmelere sebep olmaktadır bu nedenle evde kullanımı tercih edilmez.

Morfolojik Özellikleri

Atropa belladonna yaklaşık 1.5-2 metreye kadar boylanabilen toprak seviyesinin biraz üstünden itibaren dallanma gösteren, morumsu renkli saplara sahip çok yıllık çalımsı bir bitkidir [44]. Yaprakları; tütün yapraklarına benzeyip oval, belirgin damarlara sahip, alt yüzeyleri tüylü olup, uzunlukları 7-25 cm arasında değişen yapraklar alternatif veya düzgün karşıt çiftler olup bir yaprak diğerinden daha büyüktür [33-43, 44]. Çiçekleri; 2-3 cm uzunluğunda olup -yaprak koltuğunda tek, çan şeklinde ve morumsu renkte olup çiçeklerin dip kısımları sarı renklidir. Bitki yaz boyunca çiçeklidir. Göze çarpan sarı anterleri vardır. Anterler stigma etrafında toplanmıştır. Çilek küçük kümelenmelerde asılı durur, olgunlaşmadığı zaman yeşil, olgunlaştığında siyah/koyu mor ve parlaktır [32 - 43, 44]. Meyveler-çilekler tatlı, koyu renkli, mürekkep benzeri meyve suları içerir. Çilek çapı 1,5-2 cm olup 2 hücrelidir [9]. Tabanda yıldız şeklinde 5 loplu kaliks bulunmaktadır. Bu bitkinin tohumları oldukça küçük olup, bin tohum ağırlığı 1 gram civarındadır. Uzunluğu 1,5-1,8 mm, genişliği 1-1,5 mm kadardır. Tohumlar bir tarafa hafif eğimli, yumurtamsı şekilli, sert kabuklu, yüzeyi ağımsı yapıda, mat veya hafif parlaktır [11].

Kimyasal Özellikleri

Atropa belladonna modern tıpta büyük bir öneme sahip olup, eczacılık endüstrisi için yaygın olarak yetiştirilmektedir. Tıbbı uygulamalar için değerli ve suikastçiler tarafından ürkütücü bir kullanım şekline sahip çeşitli alkaloidleri içerirler [43]. Bitkinin tüm kısımları tropan alkaloitleri içerir ve bitkinin kökü genellikle en zehirli kısımdır, ancak bu bir numuneden diğerine değişebilir [25]. Total alkaloidi oluşturan en önemli maddeler; *hyosiyamin, atropin, skololamin, apoatropin ve belladonnin*'dir. Ancak esas madde *L- Hyosiyamin* (*atropin*) olup total alkaloidin % 95 kadarını oluşturmaktadır [12, 41]. Bitki toksik etkiye sahiptir. Deride tahrişe sebebiyet verip alerji oluşturabilir [43]. 1 gram kuru yaprak ya da 10 adet meyve tüketimi zehirlenmeye yol açabilir [41]. Yapılan bir araştırmada, köklerinde toplam alkaloid oranı % 1.25, yapraklarında % 0.91 olarak bulunmasına [28] rağmen, olgun meyve ve

yaprakları en yüksek alkaloid içeriğine sahiptir [16]. Kodekslerde total alkaloid oranının yaprakta %0.3'ün üstünde olması istenir [28].

İklim ve Toprak İstekleri

Güzelavrat otu 90-180 metre yükseklikte ve 50-55 derece kuzey enlemleri arasında yetişir, bununla birlikte yeterli gölgenin sağlandığı iyi drene olmuş kireçli topraklarda deniz seviyesine kadar inebilir [44]. Nemli-ılıman iklimlerden çok kurak iklimlere kadar yetişir ancak nemli toprakları tercih eder [31]. Ilıman iklim bitkisi olan Güzelavrat otu koru ve orman açıklıklarında doğal olarak yetişir [41]. Yazları ılık ve kurak olan güneşli yerlerde alkaloid içeriği daha yüksektik [43]. Çiftlik gübresi veya sodyum nitrat – Thomas fosfatı ve Kainit gübre karışımının kullanımı mahsulde gözle görülür şekilde artış sağlamaktadır [44].

Tarımı

Geniş alanlarda tarımı yapılan Güzelavrat otu tohum ve kök saplarından elde edilir. Tohumlar genellikle martın ilk yarısına kadar ekilir. Ancak dormansinin bulunması sebebiyle çimlenmenin bazı durumlarda yaklaşık olarak 3 ay gibi bir süre sürmesi sebebiyle çiftçiler ekim için kök saplarını tercih ettiklerini bildirmişlerdir [45]. Belli bir yüksekliğe gelen fideler 1.5 metre sıra üzeri ve 60-90 cm sıra üzeri mesafe ile tarlaya şaşırtılır [43]. İlk yıl bitkiler genellikle 1.5 metreye kadar uzar ve eylül ayına kadar çiçeklenir. Yalnızca yapraklar ve üst kısımları toplanır ve sonraki yıllarda aşırı dallanmayı engellemek için 75-90 cm yukardan kış aylarında seyreltme yapılır. İkinci yılında bitkiler haziran ayında çiçeklenme zamanı kesilir ve mevsim şartlarının iyi olduğu durumlarda bitki ikinci mahsul için eylül ayına kadar yeniden hasata gelir [46]. Kuru sıvı ekstreler, tentür, merhem, bandaj ve gliserin hazırlıkları şeklinde işlenmek için çiçeklenme zamanında bitkinin tamamı kesilir ve kurutulur. Kökleri için hasat ise ağustos ayında iki üç yılda bir sökülerek yaşlı bitkiler kaldırılır ve benzer metotlar kullanılarak işlenir [43]. Dikimle beraber 8 kg saf olarak Azot ve taban gübresi olarak fosfor, dikimden dört hafta sonrada azot tekrar üst gübre olarak verilir [27].

Verim

Atmosferik koşullar belladonna'nın alkaloid içerikleri üzerinde belirgin bir etki gösterir. En yüksek alkaloid yüzdesi güneşli ve kurak mevsimde yetiştirilen bitkilerden elde edilir. Mayıs ve hazirandaki güneşli günlerde alkaloid oranı %0,68 olurken, güneş ışığının yetersiz olduğu durumlarda alkaloid oranı %0.34 olarak bildirilmiştir. İngiliz yetiştiriciler toprağa tomas fosfatı uygulamasının üçüncü yıldaki bitkilerde ve kuru yapraklarda %0,84 gibi iyi sonuçlar elde

etmişlerdir [46]. Yaprak hasatında kurutma sonucu 7-8 kg yaş yapraktan 1 kg kuru yaprak elde edilir.

Yapılan Çalışmalar

Güzelavrat otu tohumları fiziksel ve fizyolojik dormansiye sahiptir. Kazıma veya hormon uygulamalarıyla tohum kabuğunda değişiklik meydana gelir ve çimlenme özelliği geliştirilebilir [45]. Bununla ilgili yapılan çalışmalarda, Bhat ve Dhar, (1971) Güzelavrat otu tohumlarında bir dormansinin olduğunu ve bunun tohum kabuğunda bulunan inhibitör maddelerden ileri geldiğini, çimlenme alternatif sıcaklık koşullarında birkaç hafta alabileceğini, ancak gibberellik asit kullanımı ve farklı uygulamalarla ile hızlandırılabileceğini belirtmiştir. Nitekim bununla ilgili olarak yapılan çalışmalarda; Dachler ve Pelzmann, (1989) tohumların buzdolabında nemli ortamda sekiz hafta tutulmasının ve fidelik veya kasalara sonbaharda ekimin yapılıp, kış şartlanın geçirmelerinin yararlı olacağını, Ruminska ve ark. (1978) gibberellik asit ile muamele edildiğinde çimlenme oranının % 2'den, % 81'e çıkacağını, Arslan ve ark. (1994) yaptığı çalışmalarda ise gibberellik asit uygulamasıyla çimlenmenin % 65, gibberellik asit + soğuk uygulamasıyla birlikte ise % 75'e kadar artacağı belirtilmiştir.

Su ürünleri yetiştiriciliğinde gerek hastalıkların tedavisinde, gerekse büyütme amaçlı kullanılan antibiyotiklere oluşturdukları zararlı etkilerinden dolayı birçok ülkede kısıtlanma getirilmesiyle [36] bitki ekstraktlarının kullanımı ve fitoterapik çalışmalar uygulanmaya başlanmıştır [37]. Bu çalışmalarla ilgili kullanılan bitkilerden biriside *Atropa belladonna* olup [34], Trichodina ve Costia gibi parazitlere karşı balıkların trasportasyonunda güzel avrat otu tavsiye edilebilmektedir [38].

Organofosfatlar (OF) dünyada özelikle tarımsal faaliyetlerin fazla olduğu ülkelerde yaygın olarak kullanılan böcek öldürücü maddelerdir. OF ile meydana gelen zehirlenmelerde ise güzelavrat otunun etken maddesi olan atropin içerikli atropin sülfat ilaçları kullanılmakta olup; artmış salgı, göz bebeklerinin küçülmesi, akciğer bronşlarında kasılma, kusma, ishal, terleme ve idrar tutama gibi sorunları tedavi edici özelliktedir [24]. Ayrıca gastroenteroloji, üroloji, kadın doğum ve nöroloji gibi pek çok klinikte ve acil kliniklerinde yaygın olarak kullanılan ilaçlar antispazmodik ilaçlar olup, bu ilaçlar içinde en çok tercih edilen antikolinerjiklerin etken maddelerinden biride *Belladon* alkaloidleri (*atropin, hiyosiyamin ve simetropium*)'dir [21].

Türkiye Sağlık Bakanlığı'ndan alınan bilgiye göre etken maddesi atropin ve atropin sülfat olan 0,25 mg, 0,5mg ve 1mg ampul şeklinde ruhsatlı 19 ilacımız bulunmaktadır [39].

Sonuç ve Öneriler

Tüketici bilincinin artması sonucunda insanların doğala yönelmesi ile birlikte bitkisel içerikli ürünlere olan talep her geçen gün artmaktadır. Ülkemiz de çok sayıda tıbbı ve aromatik bitki bulunmakta ve bunlardan bazıları doğadan toplanarak ihracatı yapılmakta bu ise toplanan bitkilerin doğadan kaybolmasına sebep olmaktadır. Ülkemiz ise tüm bu doğal çeşitliliğe rağmen ihtiyaç duyulan ilaç hammaddesinin büyük bir kısmını ithalatla karşılamaktadır [1].Yapılan çalışmalar ise bize doğal ortamda yetişen bitkilerin kültüre alınabildiğini ve istenilen kodeks değerlerinde etken maddeler elde edildiği göstermiştir.

Güzelavrat otu (*Atropa belladonna L.*) de ülkemizde doğal olarak yetişen ve içerdiği önemli etken maddeler ile çok değerli olan bir bitkidir. Etken maddesi, özellikle atropin, sentetik olarak elde edilse bile etki yönünden doğal elde edilenler daha çok tercih edilmektedir. Güzelavrat otundan ticari olarak etken maddelerin elde edilmesi için kültüre alınma çalışmalarının arttırılması, etken maddenin saflaştırılması ve ihtiyaç duyulan sektörlere hammadde sağlar duruma gelmesi için konu ile ilgili çalışmalara önem verilmesi gerekmektedir. Diğer taraftan ülkemiz doğal florasında bulunan *Atropa* türlerinin ilaç hammaddesi olarak kullanılabilme çalışmalarına yön ve destek verilmesi doğal kaynak kullanımı açısından faydalı olabilecektir.

Kaynaklar

1. Anonim, VI. Bitkisel İlaç Hammaddeleri Toplantısı Bildiri Kitabı,16-19 May, Ankara. 1996.
2. Arslan, N., Doğal Ekonomik Bitkilerin Korunması. Tarım ve Köy İşleri Bakanlığı Dergisi, 1992. 74(3): p. 17 -19, Ankara.
3. Arslan, N., et al., Farklı Uygulamaların ve Gibberellik Asit Dozlarının Güzelavrat Otu (*Atropa belladonna L.*) Tohumlarının Çimlenmesi Üzerine Etkileri. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 1994. 3(2) p. 3-4.
4. Başer, K.H.C., Biyolojik Zenginlikler ve Kalkınma. Türkiye Çevre Sorunları Vakfı Yayını, 1988. P.76-93, Ankara.
5. Bayram, E., et al., Tıbbı ve Aromatik bitkiler Üretiminin Artırılması Olanakları.
6. Baytop, T., Türkiye'de Bitkilerle Tedavi. İ.Ü. Eczacılık Fakültesi Yayınları, 1984. No:40, İstanbul.
7. Bhat, B.K. and A.K. Dhar., Seed Dormancy in *Atropa belladonna L.* Indian J. Agric. Sci, 1971. 41(9): p. 761-764.
8. Birinci, S., Doğu Karadeniz Bölgesi'nde Doğal Olarak Bulunan Faydalı Bitkiler ve Kullanım Alanlarının Araştırılması. Yüksek Lisan Tezi, Çukurova Üniversitesi, 2008, Adana.
9. Blamey, M. and C. Grey Wilson, Illustrated Flora Britain Northern Europe, 1989. ISBN-13: 978-0340401705.
10. Bown, D., The Royal Hortıcultural Society Encyclopedia Of Herbs and Their Uses, 2002.
11. Brouwer, W. and A. Stahlin., Handbuch der Samenkunde, 1975. DLG, Stuttgart.
12. Ceylan, A., Tıbbi Bitkiler III. E.Ü. Ziraat Fakültesi Yayınları, 1994. No.509, İzmir.
13. Çetinkaya Ethemoğlu, F.B., et al., *Atropa belladonna* (Güzelavrat Otu) Yaprakları Pişirilirse Ne Olur ? Antikolinerjik Toksik Sendrom Olgusu. Anestezi Dergisi, 2017. 25: p. 87-89.
14. Dachler, M. and H. Pelzmann, Heil-und Gewiirzpflanzen, 1989.'Osterreichischer Agrarverlag. Wien
15. Davis, P.H., Flora of Turkey and The East Aegean Islands, 1978.

16. Demir, C., et al., *Atropa belladonna* ile Zehirlenme: Bir Olgu Sunumu. Van Tıp Dergisi, 2006. 13(2): p. 61-63.
17. Demirhan, A., et al., *Atropa belladonna* (Güzelavrat Otu) Meyvesi İle İlişkili Antikolinerjik Toksit Sendrom Bir Olgu Sunumu, 2013. Türk J. Anaesth Reanim, 41: 226-8.
18. Demirtürk, Y., Tıbbi Bitkilerimizin değerlendirilmesi, Tarım Orman ve Köy İşleri Bakanlığı Dergisi, 1990. 53(4): p.12-16, Ankara.
19. Faydaoğlu, E. and M.S. Sürücüoğlu, Günümüzde Tıbbı ve Aromatik Bitkilerin Kullanılması ve Ekonomik Önemi. Kastamonu Üniversitesi, Orman Fakültesi Dergisi, 2011. 11 (1): p. 57-67.
20. Gül, V., Rize Yöresine Ait Tıbbi ve Aromatik Bitkilere Genel Bir Bakış. Iğdır Üniversitesi, Fen Bilimleri Enstitüsü Dergisi, 2014. 4(4): p. 97-107.
21. Güner, A., Türkiye Bitkileri Listesi Damarlı Bitkiler. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmalı Derneği Yayını, 2012. Flora Dizisi 1, İstanbul.
22. Kara, H. and M.T. Göktaş, Antispozmodik İlaçlar. Genel Gastroenteroloji 19/4. Yıldırım Beyazıt Üniversitesi, Farmakoloji Ana Bilim Dalı, Ankara.
23. Kırıcı, S., Farklı Ekolojik Koşullarda *Atropa* Türlerinde Verim ile Morfogenetik ve Ontogenetik Varyabilitenin Araştırılması. Doktora Tezi, Ç.Ü. Fen Bilimleri Enstitüsü (Basılmamış), 1991. Adana.
24. Kozacı, N. and Y. Gökel, Organofosfat Zehirlenmelerinde Pralidoksimin Farklı Doz Uygulama Şekillerinin Etkinliği Ve Yan Etkilerinin Klinik Karşılaştırılması. Acil Tıp Anabilim Dalı Uzmanlık Tezi, Çukurova Üniversitesi Tıp Fakültesi, 2006. Adana.
25. Kumar, V., Dogu Uttar Pradesh'deki Convolvulaceae ve Solanaceae'da Floristik Çeşitliliğin Ekonomik Önemi ve Koruma Stratejileri Üzerine Çalışmalar. Doktara Tezi Bölüm 4. Botanik Bölümü. 2013, V.B.S. Üniversitesi.
26. Lee, MR., Solaneceae 4: *Atropa belladonna*, Deadly Nightshade. J.R Coll Physicions Edinb, 2007. 37(7): p.77-84.
27. Özgüven, M., S. Kırıcı, and B. Şener, Toroslar 'da (Pozantı) Farklı *Atropa* Türlerinin Yetiştirilme Olanakları Ve Alkaloid İçerikleri Üzerinde Araştırmalar. VI. İlaç Hammaddeleri Toplantısı Bildiri Kitabı, 1986. Gazi Üniversitesi No:113
28. Özgüven, M., B. Şener, and S. Kırıcı, Çukurova Koşullarında Bazı *Atropa* Türlerinin Drog Verimleri ve Alkaloit içerikleri Üzerinde Bir Araştırma. V. Bitkisel İlaç Hammaddeleri Toplantısı Bildiri Kitabı, 1987. p: 161-163, 15-17 Kasım 1984, Ankara.
29. Rita, P. and K.D. Animesh, An Updated Overview on *Atropa belladonna L.* . Internatıonal Research Journal Of Pharmacy, 2011. 2(11): p.11-17. ISSN 2230 – 8407.
30. Ruminska, A., K. Suchorska, and Z. Weglarz, Effect of Gibberellik Acid on Seeds Germination of Some Vegetable and Medicinal Plants Acta Horticulturae, 1978. 78: p. 131-136.
31. Stewart, A., Wicked Plants: The Weed That Killed Lincoln's Mother and Other Botanical Atrocities, 2009. American Horticultural Society Book Award (2010). ISBN: 1565126831 (ISBN13: 9781565126831). 236s.
32. Turner, N.J. and A.F. Szczawinski, Common Poisonous Plants and Mushrooms of North America, 2003. ISBN 13: 9780881921793. 311s.
33. Uzun, S., A. Uzun, and S. Akbulut, Trabzon ve Çevresi Zehirli Bitkileri. KTÜ Orman Fakültesi, 1. Uluslar Arası Odun Dışı Orman Ürünleri Sempozyumu, Bildiriler Kitabı, 2006. 1-4 Kasım, Trabzon.
34. Yigitarslan, K.D., et al., Alabalıklarda Fitoterapi Uygulamaları. Türk Bilimsel Derlemeler Dergisi, 2011. 4 (1):63-68.
35. Zeybek, N., Farmasötik Botanik. E.Ü. Eczacılık Fakültesi Yayınları, 1985. No:1, İzmir.
36. Parlat, SS., et al., Düşük Protein İçerikli Rasyonlara Prebiyotik veya Antibiyotik Katkısının Japon Bıldırcınlarının (Coturnix coturnix japonica) Besi Performansına Etkisi. Selçuk Üniversitesi, Ziraat Fakültesi Dergisi, 2002. 16 (30):38–42.
37. Çetin, T., and G. Yıldız, Esansiyel yağların alternatif yem katkı maddesi olarak kullanımı. Yem Magazin Dergisi, 2004. 12(38): 41–47
38. Sarıeyyüpoğlu, M., and M. Köksal, Balık Hastalıkları Tıbbi Bitkilerle Tedavi ve Kontrolünün Araştırılması, 1995.
39. Titck. Türkiye Ruhsatlı İlaçlar Listesi 2019; Available from: https://www.titck.gov.tr/ (Erişim Tarihi: 10.02.2019)

40. https://www. Tübives.com.tr (Erişim tarihi: 20.12.2018)
41. Asımgil, A., Şifalı Bitkiler Kitabı. Timaş Yayınları, 1993, İstanbul.
42. N.S, A.R., and P. MP., Studies on the Organogenesis of *Atropa belladonna* in *in-vitro* Conditions. İnternational Journal Of Biotechnology and Bioengineering Research, 2013.
43. Bown, D., Encyclopedia Of Herbs and Their Uses. The definite A-Z guide to herbs. The Royal Horticultural Society, 2002. Revised Edition 2002. Dorling Kindersley Limited 80 Strand, London WC2R ORL A Penguin Company.
44. A Modern Herbal/ Nightshade, Deadly-Botanical.com. http://www.botanical.com/botanical/mgmh/n/nighde05.htlm (Erişim Tarihi:02.04.2019)
45. N.S. Rani, A., and P. MP., İn-Vitro Studies on the Germination of *Atropa belladonna* Seed Under Different Condition. International Journal of Science and Research, 2012.
46. Rita, P., and D.K. Animesh, An Updated Overview On *Atropa belladonna L*. İnternational Research Journal Of Pharmacy, 2011. ISSN 2230-8407.

Derleme/Review

Meral, U.B., Ayçiçeği (*Helianthus annuus* L.,) Bitkisinin Önemi ve Üretimine Genel Bir Bakış International Journal of Life Sciences and Biotechnology, 2019. 2(2): p. 58-71.

Ayçiçeği (*Helianthus annuus* L.,) Bitkisinin Önemi ve Üretimine Genel Bir Bakış

Ülkü Binboğa Meral[1]

ÖZET

Astreceae familyasının bir üyesi olan ayçiçeği; dünyanın en önemli yağlı tohumlarından birisidir. Tüketim bakımından soya yağından sonra ikinci sırada yer almaktadır. Hem yemeklik hem de tıbbi amaçla kullanılmaktadır. Tek yıllık bir kültür bitkisidir. Anavatanı Kuzey Amerika'dan önce Avrupa'ya daha sonra tüm dünyaya yayılmıştır. İçerdiği yüksek oranda tekli ve çoklu doymamış yağ asitleri, vitaminler, minerallerden dolayı çok tercih edilen yemeklik yağ olarak kullanımının yanı sıra bol proteinli bir hayvan yemi olarak çiftliklerde de tercih edilir. Yağı çıkarılmış tohumlar kuşyemi olarak kullanılırken, sapları yakacak olarak kullanılır, çevreye dost enerji hammaddesidir. Ayrıca, yapısında bol miktarda bulunan lif besin değerini artırır. İçeriğindeki maddeler tıbbi açıdan da oldukça değerlidir. Hücre yenileyici, kolesterol dengeleyici bir kalp dostudur. Antioksidan özelliği yüksek, kanser önleyici, mantar, iltihap ve mikrop önleyicidir. Bu çalışmada güncel bilgilerden yararlanılarak ayçiçeği üretiminde karşılaşılan problemlerden bahsedilmiştir. Türkiye'de her geçen yıl üretiminde bir artış görülse de artan nüfusun ihtiyacına karşılık verilememiştir. Ayçiçeği üretiminin artmasına yönelik bazı çözüm önerilerine yer verilmiştir.

MAKALE GEÇMİŞİ
Geliş 05.03.2019
Kabul 18.05.2019

ANAHTAR KELİMELER
Helianthus annuus L., ayçiçeği, yağlı tohumlar, üretim

An Overview of Importance and Production of Sunflower (*Helianthus annuus* L.,)

ABSTRACT

Sunflower, a member of the family Astreceae, is one of the most important oil seed crops in the world. It ranks second after soybean oil in terms of consumption, and it is used for both edible and medicinal purposes. North America is its centre of origin, from there it spread to Europe and then to the whole world. It is an environment-friendly source of energy and it is preferred in animal farms due to protein rich feed and, it is also used as fodder to fatten animals, while the seeds are used as bird feed, and the stems are used as raw material for making bio fuel. It is rich fiber in its structure that increases its nutritional value. The ingredients are cell regenerative, cholesterol balancing and heart friendly that makes it very valuable for medicinal use. It has high antioxidant, anti cancer, antifungus, anti inflammation and anti microbial properties. The problems encountered in sunflower production by using the current information are mentioned and reviewed in this study. Although there is a rise in annual production of sunflower in Turkey, this does not to respond to the needs of fastly growing local population. Some solutions for increasing sunflower production are included in this review.

ARTICLE HISTORY
Received 05.03.2019
Accepted 18.05.2019

KEY WORDS
Helianthus annuus L., sunflower, oily seeds, cultivation

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Ayçiçeği (*Helianthus annuus* L.,)

Ayçiçeği; Asterales takımından, Asteraceae familyasının bir üyesi olan *Helianthus* cinsinden *Helianthus annuus* L. (Şekil 1), olup, tek yıllık bir bitkidir. *Helianthus* cinsi 51 türe ve 19 alt türe sahiptir. Bunlardan 14'ü tek yıllık, 37'si çok yıllıktır ve farklı kromozom sayısına sahiptirler [1, 2]. *Helianthus* cinsine dahil türlerde temel kromozom sayısı n=17 olup, diploid, tetraploid ve hexaploid türleri de bulunmaktadır [3, 4, 5]. Türlerin büyük bir çoğunluğu süs bitkisi olup, bunlardan iki önemli tür; *Helianthus annuus* ve *Helianthus tuberosus* gıda amaçlı olarak kültüre alınmaktadır.

Günümüzün en önemli yağ bitkilerinden biri olan ayçiçeğinin (*Helianthus annuus* L.) anavatanı Kuzey Amerika olarak bilinmektedir [6]. İ.Ö. 3000'li yıllarda üretilmeye başlanmıştır. 1500'lü yıllarda İspanya'ya daha sonra tüm Avrupa ülkelerine dağılmıştır. Önceleri dekoratif amaçla kullanılmaya başlanmış, 1700'lü yıllarda, Avrupa'da tercih edilen bir yağ bitkisi olmuştur. Türkiye'ye 1. Dünya Savaşı'ndan sonra Romanya ve Bulgaristan'dan gelen göçmenler tarafından getirilip, yayıldığı düşünülmektedir. İlk olarak Trakya'da başlayan üretimi daha sonra Türkiye'nin her tarafına az veya çok yayılmıştır [5, 7, 8].

Şekil 1 Ayçiçeği (*Helianthus annuus* L.,) bitkisi çiçeğinin şematik görüntüsü

Ayçiçeğinin morfolojik özellikleri çoğunlukla toprak iklimine bağlıdır. Ayçiçekleri, deniz seviyesinde ve 3.000 m yüksekliğe kadar değişik birçok bölgede yetişmektedir. Olgunluk çağında, bu bitkilerin çeşitli fenotipik varyasyonları bulunmaktadır. Boyları 1-1,4 m arasında değişir fakat genellikle 1.6 m'dir. Dallı ya da dalsız, pürüzsüz ya da tüylü olabilmektedir [9].

Ayçiçek yağı, yağ oranı %40 - 52 arasında değişen *H. annuus* bitkisinin tohumlarından elde edilen yağdır. Ayçiçek yağı, %15 doymuş, %85 doymamış yağ asidi içermekte, doymamış yağ asitlerinin %14-43'ünü oleik asit, %44-75'ini linoleik asit oluşturmaktadır. Linoleik asit, yağın doygunluğunu azaltmakta, hazmını ve kana geçmeyi kolaylaştırmaktadır. Ayrıca, linoleik asit hücre zarının yapısına katılmakta ve kolesterolü düşürmektedir. Yağlarda linoleik asit miktarının fazla olması kaliteyi artırmaktadır. En fazla % 0.7'sini de linolenik asit oluşturmaktadır [10]. Ayçiçek yağının yağ asitleri bileşimi tablo 1'de verilmektedir.

Tablo 1 Ayçiçek yağının yağ asitleri bileşimi [11]

Yağ Asidi	**Zincir uzunluğu**
Laurik	(12:0)
Miristik	(14:0)
Palmitik	(16:0)
Palmitoleik	(16:1)
Stearik	(18:0)
Oleik	(18:1)
Linoleik	(18:2)
Linolenik	(18:3)
Araşidik	(20:0)
Behenik	(22:0)

Ayçiçek yağında; sekonder metabolitler olarak %0.025-0.31 hidrokarbonlar, %0.542-0.584 steroller, %0.008-0.044 vakslar gibi ekolojik ve tıbbi olarak büyük rolleri olan sabunlaşmayan maddeler bulunmaktadır. Bunlar böcek öldürmek, mikrop ve iltihap oluşumunu önlemek gibi biyolojik özelliklere sahiptirler [12].

Ayçiçeği yağının besin değerinin zeytinyağına eşit olduğu ifade edilmektedir. Titre bulanma derecesi 17-20^{o} C, donma derecesi -17 ile -18^{o} C'dir. Donma derecesinin düşük olması da ayçiçeği yağına bir avantaj sağlamaktadır [10, 13]. Ayçiçeği yağının bazı karakteristik özellikleri tablo 2'de verilmiştir.

Tablo 2 Ayçiçek yağının bazı karakteristik özellikleri [14]

Özellikler	**Değerler**
Özgül ağırlık (25°C)	0,915-0,919
Kırılma indeksi (25°C)	1,472-1,474
İyot sayısı	125
Sabunlaşma sayısı	188-194
Sabunlaşmayan madde	(%) 1,5

Ayçiçeği, yapısında bol miktarda karbonhidrat, protein, vitamin ve mineral maddeler bulundurmaktadır. Ayrıca, 64 g ayçiçeği tohumu, 370 kcal enerji, 7 g lif ve 12 g protein, 17 mg E vitamini, 4,5 mg niacin (vit B3), 0,5 mg B6 (pridoksin), 4,5 mg pentothenic asit, 151 mg folik asit, 44.8 mg kalsiyum, 2.45 mg demir, 82.5 mg magnezyum, 1.15 mg bakır, 739 mg fosfor, 544 mg potasyum, 1.9 mg sodyum ve 3.4 mg çinko ve 51 mg selenyum içermektedir [15].

Ayçiçeğinin Kullanım Alanları

İnsanlık tarihinin bilinen en eski medeniyetlerden olan Mısır, Çin, Hindistan ve Yunan uygarlıklarında bitkisel ilaçların çeşitli hastalıkların tedavisinde kullanıldığını gösteren kanıtlar bulunmuştur [16]. Dünya sağlık örgütünün tahminlerine göre, dünya nüfusunun %70-80'i özellikle gelişmiş ülkelerde yaşayan insanların bitkisel ilaçlarla tedaviyi tercih ettikleri ve sağlıklarını bu şekilde korudukları belirtilmiştir [17, 18].

Ayçiçeği tohumları, tıbbi tedavilerde doğal bir kolesterol düşürücü olarak kullanılmaktadır. Yapılan çalışmalarda görülmüştür ki ayçiçeği tohumlarının bağışıklığı kuvvetlendirmek, kolon ve prostat kanserini önlemek, cildin elastikiyetini artırmak, kalbi korumak gibi özellikleri vardır [15]. Mantar enfeksiyonlarını önlemek, iltihabi oluşumları durdurmak gibi durumlarda tedavi amaçlı kullanımı yaygınlaşmaktadır [19, 20]. Babcock, (21) tarafından yapılan bir çalışmada da ayçiçeğinin zengin E vitamini içeriğinden dolayı önemli bir antioksidan olduğu belirtilmektedir. Yüksek E vitamini içeriği kolesterolü dengelemekte, yüksek tansiyonu düşürmekte, kalp hastalığı ve cilt hastalıkları riskini azaltmaktadır [21]. Bağırsak, mesane, prostat kanserine karşı koruyucudur, yüksek selenyum içeriği meme kanserini önlemede rol oynamaktadır. Tiroid fonksiyonlarını düzenlemek, DNA hasarını gidermek ve kan şekerini dengelemek gibi alanlarda da etkili olmaktadır [22]. Ayçiçeği tohumları çok iyi bir E vitamini, kolin,

lignin, betain, arjinin ve phonelik asit kaynağıdır [23, 24]. Bu bileşenler romatizma, idrar yolu enfeksiyonları, karaciğer hastalıkları ve mide ülserlerinde tedavi amaçlı olarak kullanılmaktadır [25, 26].

Yeni tedavi yöntemleri geliştirilmesine rağmen dünyadaki en önemli ölüm nedenlerinden biri kanserdir. Kanser önleyici birçok ilacın yüksek maliyetli olması ve kemoterapi sırasında birçok yan etkilerinin görülmesi ucuz ve güvenilir alternatif yöntemler araştırma ihtiyacını doğurmuştur [27, 28].

Ayçiçeğinin kafeik, klorogenik ve ferulik asit gibi metabolitleri yüksek anti-oksidandır ve anti mutajenik özelliğe sahiptir. Mutajenlerin metabolik aktivitelerini bloke ederek mutajenleri elimine eder ve serbest radikaller üretir [29, 30]. Çalışmalarda gözlenmiştir ki ayçiçeği yağının kansere karşı yüksek koruyucu özelliği bulunmaktadır [31]. Protein üretim döngüsünde ayçiçeğinin doğal tripsin inhibitörü (SFT1) özelliği, meme kanserini, modifiye edilmiş formunun ise diğer tip kanserleri önlediği görülmüştür [32]. Ayçiçeği yağının sıvı ekstrakının derinin içine uygulanmasıyla deride gelişen kötü huylu tümör oluşumunu ve lenf nodüllerinin çoğalmasını önlemek amacıyla tıbbi tedavilerde kullanıldığı belirtilmiştir [33]. Yine ayçiçeği yağının yapısında bulunan en önemli elementlerden biri olarak kabul edilen P- kumarinin de antioksidan özelliğe sahip olduğu ve kanser hücrelerinin çoğalmasını önlediği belirtilmiştir [34, 35]. Tannin yaygın olarak birçok bitkide bulunan ve ayçiçeği tohumlarından da elde edilen diğer bir anti kanser özelliğe sahip metabolik bileşendir. Kanser hücrelerini ölüme teşvik ederken normal hücrelerin büyümesini durdurmaz [36, 37].

Ligno-selülozik bir madde olarak ayçiçeği kabuğu, fermentasyon yoluyla şeker üretiminde, bazı kimyasallara ve değerli bileşiklere dönüşümde oldukça ucuz bir kaynaktır. Örneğin ayçiçeği kabuğunun asit veya selülaz enzimleriyle hidrolizi, kabukta şeker oranını arttırarak besin değerinin artmasını ve böylece maya için gerekli maddelerin oluşumunu sağlar [38].

Ayçiçeği kabukları saman ile aynı oranda sıvı emme kapasitesine sahiptir bu nedenle sığır yetiştiriciliğinde saman yerine altlık olarak kullanılabilir. Ayrıca bu kabuklar kereste endüstrisinde dolgu ve yalıtım maddesi ve paketleme materyali olarak da kullanılır. Ayçiçeği kabuğunun bir diğer kullanım alanı da, özellikle de bazı türlerde kabuğunda bulunan antosiyanin olarak bilinen kırmızı boya maddesi nedeniyle gıdalarda doğal

katkılı boya olarak kullanılmasıdır. Gıda sektöründe kullanılan yapay renklendiricilerin yan etkileri nedeniyle, kırmızı ve pembe renk veren doğal antosiyanin kaynakları oldukça önemlidir. Çiçek yaprakları da sarı boya yapımında kullanılmaktadır. Yenilebilir çiçekleri salata yapımında tercih edilmektedir. Tohumları ham, kavrulmuş veya un şeklinde tüketilmektedir [12].

E vitamini sadece fotosentetik organizmalar tarafından sentezlenen; hücre koruyucu ve besleyici, biyolojik aktivitesi yüksek önemli bir antioksidandır [21]. En önemli antioksidan α-tocopherol'dür, ayçiçeği en iyi α-tocopherol kaynağı olarak bilinmektedir [39] . İçerdiği toplam tocopherol'lerin %95'i α-tocopherol'dür. Ayçiçeği sahip olduğu tekli ve çoklu doymamış yağ asitleri içeriğinden dolayı yüksek kaliteli bir yağdır ve özellikle kalp hastaları tarafından tercih edilmektedir [40].

Oleik asidin yüksek oksidatiflik performansı, düşük stearik asit ve çoklu doymamış yağ asidi miktarı, bu yağı kozmetik, ilaç, deterjan, yüzey aktif madde ve kimyasal sentez gibi endüstriyel uygulamalar için uygun hale getirmektedir [41, 42]. Oleik asitçe zengin ayçiçeği yağları LDL (low density lipoprotein)'yi ve kolesterolü düşürerek koroner kalp rahatsızlıklarını azaltmaktadır [43]. Saini ve arkadaşları (23) tarafından yapılan bir çalışmada ayçiçeğinin geleneksel olarak yemeklik yağ ve çeşitli hastalıkların tedavisinde kullanıldığına değinilmiştir. Yaraları hızla iyileştirdiği, böbrek, göğüs ve akciğer hastalıklarında, romatizma ve astım tedavisinde etkili bir tedavi aracı olduğunu belirtmişlerdir [44]. Benzer bir çalışmada da Amerikalı yerlilerin kurutulmuş ayçiçeklerinin yakılmasıyla oluşan dumanı ve çiğnenmiş yumru köklerini yılan ve akrep ısırıklarında kullandıklarını, çiçeklerinden elde edilen çayın akciğer hastalıklarında, sıtma ve sarıhumma tedavisinde, yaprak ve tohumlarının da idrar ve balgam söktürücü olarak kullanıldığı belirtilmektedir [19].

Ayçiçeği yemeklik yağ sanayinde olduğu kadar, kimya, kozmetik, boya, motor yağı, biyodizel, hidrolik yağ, sabun, cila ve plastik sanayinde de önemli bir hammaddedir. Dedio, (37), Ayçiçeği tohumlarının yağı alındıktan sonra geriye kalan küspesinin çok besleyici bir hayvan yemi olduğunu, tohumlarında %17-18 oranında protein bulunduğunu ifade etmiştir. Küspede yüksek oranda (kabuklu %32,3; kabuksuz %46,8) protein ve %1-7 arasında yağ bulunmaktadır. Ayçiçeği küspesi, karma yem sanayinin en önemli hammaddesi olduğu kadar doğrudan doğruya besi ve süt sığırları için de zengin bir besin kaynağıdır. Yeşil yem bitkisi olarak genç devrelerde hayvanlara verilebileceği gibi, silaj

yapılarak da yedirilebilmektedir [22]. Ayçiçeği küspesinde bulunan proteini oluşturan önemli amino asitler tablo 3'te, kabuklu ve kabuksuz ayçiçeği küspesinin bileşimi tablo 4 'te verilmektedir [3].

Tablo 3 Ayçiçeği Küspesinde Bulunan Önemli Amino Asitler

Amino asitler	**Oransal değerler**
Arjinin	10,38
Lösin	7,11
Treonin	5,33
Fenilalanin	5,25
Valin	5,21
İsolösin	4,28
Lisin	4,03
Histidin	2,67
Metionin	2,16

Tablo 4 Kabuklu ve kabuksuz ayçiçeği küspesinin bileşimi [45]

Bileşim	**Kabuksuz Ortalama (%)**	**Kabuklu Ortalama (%)**
Nem	15,7	10,8
Ham yağ	1,1	4,9
Ham protein	49,5	19,6
Hazmolabilir protein	45,0	16,3
Azotsuz öz maddeler	28,6	27,0
Ham selüloz	5,4	35,9
Ham kül	5,9	5,6
Kalsiyum	0,26	-
Fosfor	1,22	-

Birçok araştırma sonuçlarına göre ayçiçeği yağı; oldukça yüksek biyolojik aktivitelere sahip mikrop, mantar ve tümör oluşumunu önleyici çeşitli alkoloid, flavanoid, uçucu yağ ve terpenoid gibi antioksidanlar içermektedir [46].

Ayçiçeğinin karasal ve sucul ortamlarda biyoremidasyon amacı ile kullanımı yaygınlaşmaktadır. Mahmood (47)'un çalışmalarına göre bu yöntem, son yıllarda geleneksel yöntemlere alternatif olarak ortaya çıkan, düşük maliyetli, çevre dostu, dikkat çekici bir yöntem olarak dikkat çekmektedir. Karasal ve sucul ortamları kirleten ve besin zinciri yoluyla insan sağlığını, tarım ürünlerinin yetişmesini, büyümesini, üremesini ve fotosentez hızını olumsuz etkileyen Cd, Pb, Zn gibi ağır metallerin, endüstriyel, evsel ve kanalizasyon atıklarının, suni gübre kalıntıları gibi kirleticilerin giderilmesinde

kullanılmaktadır [47, 3]. Günümüzde fosil yakıtların giderek azalması, çevre kirliliğinin artmasından dolayı çevreye dost alternatif enerji kaynaklarına ihtiyaç artmaktadır. Smith ve arkadaşlarına (42) göre, biyoyakıtlar sadece alternatif bir enerji kaynağı olmakla kalmamakta, aynı zamanda net sera gazı emisyonlarını ve iklim değişikliğini de azaltmaktadır [48]. Ayçiçeği önemli bir süs bitkisi, biyodizel üretiminde son derece elverişli, çevreye dost enerji hammaddesidir [49].

Türkiye'de Ayçiçeği Üretim Miktarı

Ayçiçeği, Türkiye'de en fazla ekim alanı ve üretime sahip yağlı tohumlu bitkidir. Tohumunda bulunan yüksek orandaki yağ (%40-55) birim alandan elde edilen yağ miktarının yüksek olmasını, ayçiçek yağının kalitesinin de yüksek olmasını sağlarken; dolayısıyla bu yüksek kaliteli ayçiçek yağı, üretim talebinin artışını sağlamaktadır [50]. Bitkisel yağ üretimimizin %69'u, toplam sıvı yağ tüketimimizin yaklaşık %84'ü, toplam yağ kullanımının ise %32'si ayçiçeğinden karşılanmaktadır. Dünyada ve Türkiye'de ayçiçeği çerezlik olarak da yetiştirilmekte ve tüketilmekte olup, dünya ayçiçeği üretiminin % 2,6'sı çerezlik olarak tüketilmektedir. Türkiye'de 2017 yılı ayçiçeği üretiminin % 8,37'si çerezlik olarak üretilmiştir. Tablo 5'te Türkiye ayçiçeği ekim alanı (da)-üretim (ton)-verim (kg/da) görülmektedir.

Tablo 5 Türkiye Ayçiçeği Ekim Alanı (da)-Üretim (ton)-Verim (kg/da) [51]

YIL	Ayçiçeği Tohumu (Yağlık)			Ayçiçeği Tohumu (Çerezlik)			Toplam	
	Ekilen Alan (da)	Üretim Miktarı (ton)	Verilen kg /da)	Ekilen Alan (da)	Üretim Miktarı (ton)	Verilen kg /da)	Ekilen Alan (da)	Üretim Miktarı (ton)
2004	4.800.000	800.000	167	700.000	100.000	143	550.000	900.000
2005	4.900.000	865.000	177	760.000	110.000	145	5.660.000	975.000
2006	5.100.000	1.010.000	198	754.000	108.000	143	5.854.000	1.118.000
2007	4.857.000	770.000	159	6.897.778	84.407	126	5.546.778	854.407
2008	5.100.000	900.387	177	700.000	91.613	133	5.800.000	992.000
2009	5.150.000	960.300	186	690.000	96.825	140	5.840.000	1.057.125
2010	5.514.000	1.170.000	212	900.000	150.000	167	6.414.000	1.320.000
2011	5.560.000	1.170.000	210	997.000	165.000	166	6.557.000	1.335.000
2012	5.046.160	1.200.000	238	1.000.000	170.000	170	6.046.160	1.370.000
2013	5.202.600	1.380.000	265	895.239	143.000	160	6.097.839	1.523.000
2014	5.524.651	1.480.000	269	1.049.925	157.900	152	6.574.576	1.637.900
2015	5.689.950	1.500.000	264	1.163.224	180.700	155	6.853.174	1.680.700
2016	6.167.800	1.500.000	244	1.033.281	170.716	166	7.201.081	1.670.716
2017	6.813.976	1.800.000	264	982.241	164.385	168	7.796.217	1.964.385

Türkiye'de ekimi yapılan yağlı tohumlu bitkiler içinde ekim alanı ve üretim bakımından birinci sırayı ayçiçeği almaktadır. Günümüzde Türkiye'de üretimi yapılan bitkisel yağların yaklaşık yarıdan fazlası ayçiçeğinden elde edilmektedir. Türkiye'deki ayçiçeği ekim alanlarının %73'ü Trakya-Marmara, %13'ü İç Anadolu, %19'u Karadeniz, %3'ü Ege ve %1'i Doğu ve Güneydoğu Anadolu Bölgesi'nde olmaktadır. İç Anadolu Bölgesi, Marmara Bölgesi'nden sonra ayçiçeği ekimi bakımından ikinci sırada yer almaktadır. Son yıllarda ayçiçeği ekimi Akdeniz Bölgesi'nde de yaygınlaşmıştır. Tablo 6'da Türkiye'de illere göre ayçiçeği üretim miktarları gösterilmiştir. Türkiye'nin ayçiçeği dış ticaret verilerine göre 2017 yılında 595.501.657 ton ayçiçeği ihracatı yapılırken, 2.266.261.247 ton ayçiçeği ithal edilmiştir. 2002-2018 yıllarında ithalat %499 oranında artmış ve bu ithalat karşılığında 15.646.602.900 dolar yabancı ülkelere ödenmiştir [51]. Tablo 7'de Türkiye'nin ayçiçeği dış ticareti verileri gösterilmiştir.

Tablo 6 Türkiye'de İllere Göre Ayçiçeği Üretim Miktarı (ton) [51]

İller	2013		2014		2015		2016		2017	
	Miktar	%	Miktar	%	Miktar	%	Miktar	%	Miktar	%
Tekirdağ	211.671	13,90	260.753	15,92	267.012	15,89	283.838	16,99	368.125	18,74
Konya	266.775	17,52	268.751	16,41	217.634	12,95	212.312	12,71	263.008	13,39
Edirne	175.857	11,55	258.568	15,79	226.573	13,48	222.064	13,29	244.655	12,45
Adana	100.677	6,61	89.565	5,47	134.361	7,99	166.524	9,97	195.225	9,94
Kırklareli	146.682	9,63	165.206	10,09	188.998	11,25	170.278	10,19	193.784	9,86
Çorum	47.739	3,13	38.297	2,34	53.189	3,16	59.069	3,54	75.157	3,83
Tokat	47.096	3,09	33.740	2,06	41.593	2,47	39.306	2,35	41.549	2,12
Eskişehir	43.101	2,83	35.520	2,17	29.281	1,74	30.553	1,83	39.993	2,04
Samsun	33.018	2,17	27.652	1,69	39.083	2,33	35.546	2,13	38.253	1,95
Balıkesir	27.837	1,83	26.483	1,62	30.609	1,82	30.555	1,83	37.923	1,93
Bursa	27.471	1,80	30.463	1,86	31.548	1,88	37.764	2,26	37.382	1,90
Deniz	37.263	2,45	45.996	2,81	36.144	2,15	32.155	1,92	32.900	1,67
Karaman	21.015	1,38	19.632	1,20	20.504	49,30	16.485	0,99	22.979	1,17
Kayseri	15.092	0,99	14.035	0,86	19.853	6,80	20.361	1,22	19.676	1,00
Doğu İller	321.706	21,14	323.239	19,73	344.318	26,42	313.906	18,78	353.776	18,00
TOPLAM	**1.523.000**	**100,00**	**1.637.900**	**100,00**	**1.680.700**	**100,00**	**1.670.716**	**100,00**	**1.964.385**	**100,00**

Tablo 7 Türkiye'nin Ayçiçeği Dış Ticareti [51]

Yıl	İhracat		İthalat	
	Miktar (kg.)	Değer ($)	Miktar (kg.)	Değer ($)
2000	42.893.190	26.832.227	906.286.860	173.534.129
2001	26.625.626	18.573.820	369.698.839	111.915.467
2002	13.643.377	12.411.361	282.002.462	101.632.461
2003	32.848.795	31.171.805	748.844.214	220.089.550
2004	23.173.358	26.984.456	911.807.466	261.641.461
2005	32.362.753	38.572.072	976.706.499	332.169.143
2006	110.054.531	108.399.368	1.197.809.190	399.206.153
2007	42.909.003	63.336.363	1.133.437.679	464.165.382
2008	106.667.700	195.035.170	1.071.522.041	1.060.612.784
2009	125.539.491	146.920.775	1.114.162.358	755.439.439
2010	100.252.941	159.636.909	1.353.011.001	739.472.698
2011	237.813.638	419.915.999	1.944.182.465	1.360.886.520
2012	312.896.951	517.970.359	2.225.829.216	1.617.458.389
2013	378.980.656	596.458.932	1.804.946.446	1.533.902.073
2014	697.815.573	899.887.431	2.158.859.600	1.820.246.817
2015	655.423.289	758.138.271	1.938.171.830	1.539.607.490
2016	655.698.940	758.138.271	1.902.405.500	1.447.545.246
2017	595.501.657	687.038.049	2.266.261.247	1.203.486.976
2018	343.028.037	391.757.295	1.688.541.702	789.040.318

Dünya Ayçiçeği Üretimi

Ayçiçeği tarımı dünyada en fazla Ukrayna, Rusya ve Arjantin'de yapılmaktadır. Bu ülkeler, 2016 yılında dünya üretiminin %58,38'i gerçekleştirmişlerdir. Tablo 8 'de ülkelerin ayçiçeği üretim miktarları gösterilmiştir. Türkiye'nin ise dünya ayçiçeği üretimindeki payı 2016 yılında %3,53'tür [51].

Tablo 8 Ülkelerin Ayçiçeği Üretimi (Ton) [51]

Ülkeler	1961	1970	1980	1990	2000	2010	2015	2016	2016 %
Ukrayna					3.457.400	6.771.500	11.181.120	13.626.890	28,78
Rusya					3.918.549	5.344.821	9.280.296	11.010.197	23,26
Arjantin	585.000	1.140.000	1.650.000	3.900.000	6.069.655	2.232.034	3.158.290	3.000.367	6,34
Çin	61.000	70.000	909.700	1.338.736	1.954.141	2.298.000	2.698.113	2.587.422	5,47
Romanya	481.000	769.587	800.600	556.242	720.871	1.262.926	1.785.771	2.032.340	4,29
Bulgaristan	301.000	406.887	379.950	388.560	425.369	1.536.321	1.699.228	1.873.677	3,96
Türkiye	96.700	375.000	750.000	860.000	800.000	1.320.000	1.680.700	1.670.716	3,53
Macaristan	109.964	95.509	455.915	683.706	483.649	969.718	1.556.976	1.534.959	3,24
ABD	17.000	85.785	1.697.000	1.032.000	1.607.730	1.240.830	1.326.180	1.204.170	2,54
Fransa	12.201	56.830	245.400	2.430.000	1.833.062	1.640.837	1.186.913	1.189.832	2,51
Diğer Ülkeler	5.152.799	7.046.118	6.767.761	11.516.315	5.279.104	6.836.168	8.815.732	7.614.466	16,08
TOPLAM	**6.817.064**	**10.045.716**	**13.656.326**	**22.705.559**	**26.549.550**	**31.453.155**	**44.369.319**	**47.345.036**	**100,00**

Ayçiçeği Tarımında Yaşanan Sorunlar ve Çözüm Önerileri

Türkiye'de yağlı tohum üretimi yeterli değildir. Türkiye'de bitkisel sıvı yağ tüketiminin önemli bir kısmını yağlık ayçiçeğinden elde edilen ayçiçek yağı oluşturmaktadır. Yurtiçinde tüketilen 900 bin ton civarındaki ayçiçek yağının sadece 500-550 bin tonu ülkemiz üretiminden karşılanmaktadır [51].

Türkiye ayçiçeği tarımı için uygun ekolojik koşullara sahip olmasına rağmen ayçiçeği ekim alanlarımız arttırılamamaktadır. Ayçiçeği bitkisi kazık kök yapısıyla kuraklığa toleranslı bir bitki olsa da yazlık bir bitki olması ve bu mevsimde de yeterince yağış düşmemesi sonucu oluşan kuraklık, dekardan alınan verimi oldukça düşürmektedir [10]. Bütün koşullar iyi olsa dahi, ayçiçeğinde bir defa elle yabancı ot kontrollü yapılması faydalıdır. En etkili mücadele yöntemi, elle yapılan olmasına rağmen iş gücünün pahalı olması nedeniyle herbisitler tercih edilmektedir. Ancak, bilinçsiz kullanılan herbisitlerin ayçiçeğinin çimlenmesinden başlayarak, bütün aşamalarında olumsuz etkilerde bulunduğu ve bu olumsuzlukların verimi etkilediği, beklenilen fayda yerine zararlar ortaya çıkardığı, yapılan çalışmalarla belirlenmiştir [39, 40].

Ayçiçeği üretiminde hedeflenen miktarlara ulaşabilmenin yolu; önemli oranda birim alandan alınan tane ve yağ verimlerinin artırılması için yüksek verimli kaliteli tohumluk

kullanımının yaygınlaştırılması, çeşitli araştırmalarla teknik uygulamaların yerinde ve zamanında yapılması ile mümkün olacağı düşünülmektedir [10].

Çok fazla iş gücü gerektirmesi, tohum çimlenmesinde karşılaşılan olumsuzluklar, küresel iklim değişikliği, azalan toprak ve su kaynakları göz önüne alındığında klasik ıslah yöntemleriyle üretimde istenilen hedefe ulaşmak oldukça zordur. Bu sorunun çözülebilmesi için, ilk önce ayçiçeği verimini arttırmak üzere biyoteknolojik teknikler geliştirilerek, ayçiçeği üretiminde yaygın olarak görülen hastalıklara, orobanş ve mildiyö gibi zararlılara dayanıklı türlerin geliştirilmesi gibi çalışmalar yoğunlaştırılmalıdır [51]. Bununla birlikte çiftçilere bu alanda gerekli eğitimin verilmesinin yararlı olacağı düşünülmektedir [52].

Kaynaklar

1. Shaimaa, Khalil, Bassita A.Hussein and Ebtissam Hussein. Silver nitrat is essential for successful reneration of Egiptian sunflower (Helianthus annuusL.) ev.Giza 102. International Journal of Advanced Research. 2005. 3(9):p.506-512.
2. Inoka and Nilanthi Dahanayake, Effect of plant growth regulators on micro propagation of sunflower (Helianthus annuus L.) International Journal of Scientific and Research Publications. 2015. 5(1):ISSN 2250-3153.
3. Lahaye, L., et al., Technological processes of feed manufacturing affect protein endogenous losses and amino acid availability for body protein deposition in pigs. Animal Feed Science and Technology., 2004. 113:141–156.
4. Verleyen, T., et al., Gas chromatographic characterization of vegetable oil deodorization. Distillate J. Chromatography A., 2001. 921:277- 285.
5. Arıoğlu H., Adana : Yağ Bitkileri Yetiştirme ve Islahı. ÇÜ Ziraat Fakültesi Yayınları: NO 2045,37-43.
6. Anonymous, <http://en.wikipedia.org/wiki/Sunflower_oil>, retrieved 22 February 2011.
7. Incekara F., Adana : EÜ Ziraat Fakültesi Yayınları, 1972. s.49-57.
8. Ilsulu, K., İstanbul : Yağ Bitkileri ve Islahı EÜ, Çağlayan Kitabevi, 1973. s.286-290.
9. OECD Consensus Documents, 2006. Safety Asessment of Transgenic Organisms, Vol:1, OECD Publishing.
10. Kolsarıcı Ö., et al., Ankara : Yağlı Tohumlu bitkilerin üretim projeksiyonları ve üretim hedefleri IV. Teknik tarım kongresi bildiri kitabı, 1995. cilt.1, 467-483.
11. Warner, K., et al., 2003.
12. Aschenbrenner A. K, et al., Spring O.İdentification and characterization of two bisabalone synthases from linear glandular trichomes of sunfower (Helianthus annuus L.,) Phytochemistry, 2016. 124, 29-37.
13. Anjum FA., et al., Nutritional and trerapeutic potential of sunflower seeds: a review, British Food Journal; 2012. 114:544-552.
14. Gunstone, F.D., and Padley, F.B., Lipid Technologies and Applications Marcel Dekker Pub., New York. Compositions of Sunflower NuSun, (Mid-Oleic Sunflower) and High Oleic Sunflower Oils, The Codex Committee on Fats and Oils, 1997.
15. Sunflower seeds nutritioanal information available at www.nutritiondata.self.com.

16. Gaidhani, S. N, et al., In vitro anticancer activity of standard extracts used in ayurveda. Phycog. Mag. 5, 2009. pp:425–429.
17. WHO Traditional Medicine Strategy 2002-2005, Document WHO / EDM/TRM, 2002.1, WHO; Geneva; 2002, pp:11.
18. Kaskoniene, V.; et al., 2009. Quantitative and qualitative determination
19. Vogt TM, et al. Serum selenium and risk of prostate cancer in U.S. blocks and whites. Int J Cancer; 2003. 103(5):664-70.
20. Sukarevich VM, Shvertsova NN, Prodon SI, Malkov MA., Use of flour from sunflower oil cake in the biosynthesis of antigungal antibiotics. Antibiotici; 1977. 22(4):297-301.
21. Babcock, J. (2017, November 04). Sunflower Seeds: Benefits, Nutrition & Recipes. Retrieved April 06, 2018, from https://draxe.com/sunflower-seeds/.
22. Al-Shamma, L. et al., Effect some of sunflower genotypes oil on some pathogenic bacteria. Iraqi. J. Sci., Vol. 51 (4), 2010. pp:565–570.
23. Saini, S. and Sharma, S. Helianthus annuus (Asteracea) Review. Inter. J. Phar. Profess. Res., Vol. 2(4), 2011. pp:465–470.
24. Odabaşoğlu F, et al., Beneficial effects of vegetable oils (com, olive and sunflower oils) and alpha-tocopherol on anti-inflammatory and gastrointestinal profiles of indomethacin in rats. Eur J Pha. 2008.
25. Gürbüz B., et al., Ayçiçeği Tarımı, Hasad Yayıncılık ve Reklamcılık Ltd. Şti., Ege Basım 96-97. 6. Holliday, R. and Philips, K.,Health benefits of the sunflower kernel. Cereal Foods, World., 46, 2003. pp:205–208.
26. Zhang, X.C., et al, Purification and characterization of an inhibitor of a cathepsin B-like proteinase from sunflower seed. J. of Enzyme Inhibition and Medicinal Chemistry., 21 (4), 2006. pp:433–439.
27. Holliday, R. and Philips, K. Health benefits of the sunflower kernel. Cereal Foods, World., 46, 2001, pp:205–208.
28. Desai, A. G. ; Qazi, G. N. ; Ganju, R. K. ; El-Tamer, M. ; Singh, J. ; Saxenam A. K. ; Bedi, Y. S., Taneja, S. C. and Bhat, H. K., Medicinal plants and cancer chemoprevention. Current Drug Metabolism., 9, 2008., pp:581–591.
29. Ubied, H. N., Study of the anticancer effects of Olea europea leaves crude extracts (in vitro and in vivo). M. Sc. Thesis. College of Medicine. Kufa University. 2006.
30. Raja, A. S., et al., Caffeic acid as an inhibitor of DMBA induced chromosomal breakage in mice assessed by bone marrow micronucleus test. Mutat. Res., 124, 1983. pp:247–253.
31. DeLeonardis, A., Macciola, V. and Di Rocco, A. 2003. Oxidative stabilization of cold-pressed sunflower oil using phenolic compounds of he same seeds. J. Sci. Food. Agri., 83 (6), pp:523–528.
32. Giada, M., et al., Antioxidant capacity of the striped sunflower (Helianthus annuus L.) seed extracts evaluated by three in vitro methods. International Journal of Food and Nutrition., 60 (5), 2009. pp:395–401.
33. Mylne, S. J.; et al., Albumins and their processing machinery are hijacked for cyclic peptides in sunflower. Nature Chemical Biology., 7, 2011. pp:257–259.
34. Silva, L. A., et al., Sunflower seed oil – Enriched product can inhibit elrlich solid tumor growth in mice. Chemotherapy, 52, 2006. pp:91-94.
35. Loe, K. T., Essential moiety for antimutagenic and cytotoxic activity of hedergenin monodemosides and bidesmosides isolated from the stem bark of Kalapanox pictus. Planta Med., 66, 2000. pp:329–332.
36. Siger, A. ; Nagala-Aalucka, M. and Lampart-Szczapa, E. 2008. The content antioxidant activity of phemolic compounds in cold – pressed oils. Journal of Food Lipids., 15, pp:137–149.
37. Dedio, W., The Biology of Helianthus Annuus L. (Canada, Canadian Food Inspection Agency, Plant Biosafety Office). Retrieved April 06, 2018, from http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-9408, 2015.

38. Kaya Y., Edirne: Ayçiçeği Kabuğunun Değerlendirilmesi ve İçeriği, Trakya Tarımsal Araştırma Entitüsü.
39. K. Kumar, S.n.Mandal, S.P Deshpande, S.P. Mehtre.,Assessment of molecular diversity of CMS, maintainer and restorer lines of sunflower (Helianthus annuus L.) using RAPD, ISSSR and SSR markers, Journal of Crop and Weed,13 (2) : 95-101, 2017.
40. Gaipel K., Song X., and Socher M. L., Induction of a photomixotrophic plant cell culture of Helianthus annuus L and optimization of culture conditions for improved α-tocopherol production. Apl microbiol Biotechnol 2014. 98:2029-2040 .
41. Mohamed, S., Boehm, R., and Schnabl, H., Stable genetic transformation of high oleic Helianthus annuus L. genotypes with high efficiency, Plant Science, 171, 2006., 546-554.
42. Smith, S.A., King, R.E. and Min, D.B., Oxidative and thermal stabilities of genetically modified high oleic sunflower oil, Food Chem., 102, 2007. 1208-1213.
43. Flagella, Z., et all., Changes in seed yield and oil fatty acid composition of high oleic sunflower (Helianthus annuus L.) hybrids in relation to the sowing date and the water regime, Europ, 2002.
44. Saini S. and Harma S. Helianthus Annuus (Asteracea): A Review. International Journal of Pharma Professional's Research; 2011., 2(4):465-470.
45. Dwivedi A AND Sharma G., A review on Heliotropism plant: Helianthus annuus L., The journal of Phytopharmacology 2014. 3(2):149-155.
46. Küçükyavuz, O., 1996. Ayçiçek Yağı Üretim Tesisi Sanayi Profili, Ankara.
47. Mahmood Z.F., and Ibrahim K.M., Evaluation of Helianthus annuus L.,. Tolerance For Zinc In Vıtro Post Combination With Naphthalene Acetic Acid and Benzyl Adenine, Wrld Journal Of Experimental Biosciences. Volume 5, No: 1: 14-18, 2017.
48. Okuyama, T., et al., Studies on the antitumor-promoting activity of naturally occurring substances II. Inhibition of tumor enhanced phospholipids metabolism by umbelliferous materials. Chem.. Pharma. Bull., 38, 1990., pp:1084–1086.
49. Bawadi, H. A., et al., Inhibition of Caco-2 colon MCF-7 and Hs578T breast and DU 145 prostatic cancer cell proliferation by water soluble black bean condensed tannins. Cancer Lett., 218, 2005. pp:153–162.
50. Catherine, N. K., Antioxidant and antidiabetic properties of condensed tannins in acetonic extract of selected was and processes indigenous food ingredient, from Kenya. Journal of Food Science., 76 (4), 2011. pp:560–567.
51. Türkiye İstatistik Kurumu, TÜİK. Bitkisel üretim istatistikleri veri tabanı. 2015; Available from: http://tuikapp.tuik.gov.tr/bitkiselapp/bitkisel.zul.
52. Gedikoglu H., Ayçiçeği neden stratejik ürün olmalı? Yağlı Tohumlu Bitkiler Konferansı, 14 Eylül 2017, İstanbul, BYSD.

Araştırma makalesi/ Research article

Doğan, M., Farklı Benzil Amino Pürin ve Gibberellik Asit Kombinasyonunun Rotala rotundifolia (Buch-Ham. ex Roxb) Koehne'nın Mikroçoğaltımı Üzerine Etkisi. International Journal of Life Sciences and Biotechnology, 2019. 2(2): p. 72-79.

Farklı Benzil Amino Pürin ve Gibberellik Asit Kombinasyonunun *Rotala rotundifolia* (Buch-Ham. ex Roxb) Koehne'nın Mikroçoğaltımı Üzerine Etkisi

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ÖZET

Rotala rotundifolia (Buch-Ham. ex Roxb) Koehne geneneksel tıp sisteminde hastalıkların tedavisinde kullanılan önemli akuatik tıbbi bitkidir. Bu çalışmada, 6-benzil amino purin (BAP) ve gibberellik asit (GA_3)'in farklı kombinasyonlarını içeren kültür ortamında *R. rotundifolia*'nın boğum eksplantlarından *in vitro* sürgün rejenerasyonu araştırılmıştır. Kültür ortamlarında ilk sürgün oluşumları 10 gün sonunda gözlenmiştir. Sekiz hafta sonunda deneme sonlandırılmış ve rejenerasyon verileri alınmıştır. Sürgün rejenerasyon oranı %72,22-100,00 arasında sıralanmıştır. %100 sürgün rejenerasyon oranı 0,25-0,75 mg/L BAP + 0,25 mg/L GA_3 içeren MS ortamında kaydedilmiştir. Eksplant başına maksimum sürgünler (18,38 adet) ve en uzun sürgünler (2,36 cm) 0,25 mg/L BAP + 0,25 mg/L GA_3 eklenmiş kültürlerde elde edilmiştir. Çoğaltım ortamında sürgünlerin köklenmesi nedeniyle ayrıca köklendirme çalışması yürütülmemiştir. Rejenere sürgünlerin akvaryum ortamına alıştırılması başarıyla sağlanmıştır. Bu rapor, tıbbi bitki *R. rotundifolia*'nın çoklu üretimini sunması bakımından önemlidir. İleride bu bitki ile yürütülecek sekonder metabolit üretimi ve gen aktarım çalışmalarına yardımcı olabilir.

MAKALE GEÇMİŞİ
Geliş 09.05.2019
Kabul 10.06.2019

ANAHTAR KELİMELER
Boğum eksplantı, doku kültürü, sürgün rejenerasyonu, BAP

The Effect of Different Combinations of Benzyl Amino Purine and Gibberellic Acid on Micropropagation of *Rotala rotundifolia* (Buch-Ham. ex Roxb) Koehne

ABSTRACT

Rotala rotundifolia (Buch-Ham. ex Roxb) Koehne is an important aquatic medicinal plant for the treatment of diseases in the traditional medicine system. In this study, *in vitro* shoot regeneration from the nodal explants of *R. rotundifolia* in culture medium containing different combinations of 6-benzyl amino purine (BAP) and gibberellic acid (GA_3) was investigated. First shoot formation was observed after 10 days in culture medium. At the end of eight weeks, the trial was completed and regeneration data were obtained. Shoot regeneration rate is listed as 72.22-100.00%. 100% shoot regeneration rate was recorded in MS medium including 0.25-0.75 mg/L BAP + 0.25 mg/L GA_3. The maximum shoots per explant (18.38) and the longest shoots (2.36 cm) were obtained in cultures with 0.25 mg/L BAP + 0.25 mg/L GA_3. No rooting study was carried out due to the rooting of the shoots in the multiplication environment. The acclimation of regenerated shoots to the aquarium environment has been successfully achieved. This report is important in that it offers multiple production of medicinal plant *R. rotundifolia*. In the future, it can help with the production of secondary metabolites and gene transfer studies.

ARTICLE HISTORY
Received 09.05.2019
Accepted 10.06.2019

KEY WORDS
Nodal explant, tissue culture, shoot regeneration, BAP

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Giriş

Rotala rotundifolia (Buch-Ham. Eski Roxb) Koehne (Lythraceae) Asya'da yayılış gösteren sucul bir bitkidir [1]. *R. rotundifolia* geneneksel tıp sisteminde hastalıkların iyileştirilmesinde şifali bitki olarak kullanılmaktadır. Özellikle eklem ağrısının tedavisi ve romatizmal tedavisinde tercih edilmektedir [2]. Ayrıca *R. rotundifolia*'nın akuatik ekstrelerinin en fazla antioksidan kapasiteye barındırdığı bildirilmiştir [3].

Bitkiler süsleme, tıbbi ve diğer birçok amaç için doğal ortamlarından aşırı şekilde toplanmaktadır. Bu durum bitki türlerinin neslinin yok olmasına ve bu bitki türleri ile beslenen diğer canlı türlerinin ölmesine neden olabilimektedir. Bu nedenle, önemli bitki türlerinin büyük ölçekli çoğalmalarını ve korunmalarını sağlayacak etkili çoğaltma yöntemlerinin geliştirilmesine acil bir ihtiyaç vardır.

Normal koşullarda tatlı su bitkilerini yetiştirme teknikleri eşeyli ve eşeysiz olmak üzere ikiye ayrılır. Eşeyli üretimde yapay polenleme ve tohumla üretimden yararlanılmaktadır. Yapay polenleme, kendini dölleyemeyen türlerde uygulanan bir yöntemdir. Döllenme olayı, bir fırça yardımıyla alınan polenlerin diğer bitkinin çiçeğine taşınmasıyla sağlanabileceği gibi olgunlaşmış anterleri bulunan çiçeklerin koparılarak diğer çiçeğin stigmasına sürtülmesi ile de sağlanabilir. Tohumla üretim, bitkinin normal hayat döngüsünde bulunan bir olaydır. Diğer üretim şekli eşeysiz üretimdir. Bu yöntem ticari olarak en yaygın kullanılan yöntemdir. Çeşitli şekillerde yapılabilmektedir. Örneğin; ana bitkiden kesilen parçaların toprağa dikilmesiyle, rizoma sahip bazı su bitkilerinde, rizomlar üzerinde yeni çıkan bitkilerin kesilip toprağa aktarılmasıyla ve çiçek açmayan bazı su bitkilerinde ana bitkinin yapraklarından yeni bitkilerin gelişmesiyle elde edilebilmektedir [4].

Geleneksel üretim yöntemlerinin aksine bitki üretiminde yaygın şekilde biyoteknolojik bir yöntem olan doku kültürü teknikleri kullanılmaya başlanmıştır. Geleneksel tekniklerle çoğaltma ile ilişkili zorluklar, doku kültürü teknikleri kullanılarak aşılabilir [5]. Bitki doku kültürü teknikleri, özellikle nadir ve nesli tükenmekte olan bitki türlerinin hızlı bir şekilde çoğaltılması için kolay ve güvenilir yöntemler olarak kabul edilir [6-9].

Bu çalışmada, 6-benzil amino purin (BAP) ve gibberellik asit (GA_3)'in farklı kombinasyonlarını içeren kültür ortamında *R. rotundifolia*'nın boğum eksplantlarından *in vitro* sürgün rejenerasyonu araştırılmıştır.

Materyal ve Metot

R. rotundifolia Konya'da (Türkiye) yer alan akvaryumculardan temin edilmiştir. Bitkinin yüzey sterilizasyonu işlemleri %20 hidrojen peroksit (H_2O_2) ile 10 dk etkileşime sokularak başarılmıştır. Ardından bitki parçaları üzerinde H_2O_2'i uzaklaştırmak için 3 kez 5'er dk süre ile durulama yapılmıştır. Bu bitki parçalarında boğum eksplantları izole edilerek bitki büyüme düzenleyici içermeyen Murashige ve Skoog (MS) temel besin ortamına transfer edilmiştir [10]. Çoğaltım çalışmalarında, bu kültür ortamında büyüyen 4 haftalık sürgünler kullanılmıştır.

In vitro çoğaltım çalışmalarında boğum eksplantları izole edilerek %3 sukroz ve 0,05-1,25 mg/L BAP ve 0,25 mg/L GA_3 kombinasyonlarını içeren sıvı MS besin ortamı kullanılmıştır. Besin ortamın pH'ı otoklavlanmadan önce 1N NaOH ve 1N HCl ile 5.8 ± 0.1'e ayarlanmıştır. Otoklavda 120°C'de 20 dk boyunca (118 kPa atm) steril edilmiştir. Bütün kültürler, beyaz floresan lambalar altında 16 saat ışık fotoperyodunda (5000 lux), 24±1°C'de bekletilmiştir.

Çoğaltım ortamında sürgünlerin köklenmesi nedeniyle ayrıca köklendirme çalışması yürütülmemiştir. Kültür ortamında üretilen bitkiler üzerinde bulunan besin ortamları uzaklaştırılmış ve musluk suyu ve kum içeren akvaryuma alınmıştır (16 saat ışık; 23°C).

Kültür ortamındaki dataların analizi için SPSS 21 for Windows programı kullanılmıştır. Post Hoc testlerinden ise Duncan seçilmiştir. Yüzde veriler için arksin transformasyon uygulanmıştır [11].

Bulgular ve Tartışma

In vitro sürgün rejenerasyonu için *R. rotundifolia*'nın boğum eksplantları 0,05-1,25 mg/L BAP ve 0,25 mg/L GA_3 kombinasyonlarını ihtiva eden sıvı MS besin solüsyonunda kültüre alınmıştır. Benzer şekilde boğum eksplantlarından başarılı sürgün rejenerasyonları daha önce Mishra ve ark. [12], Dogan [13], Reddy ve ark. [14], Subbaiyan ve ark. [15] ve Warakagoda ve ark. [16] tarafından da bildirilmiştir. İlk sürgün çıkışları 10. günde gözlenmiştir. Sekiz hafta sonunda deneme sonlandırılmış ve kültür ortamlarındaki boğum eksplantlarından çıkan çoklu sürgün oluşumları kaydedilmiştir. Sürgün rejenerasyon verileri için varyans analizi uygulanmıştır (Tablo 1).

Tablo 1 0,25 mg/L GA_3 ve farklı BAP konsantrasyonlarının *R. rotundifolia*'nın boğum eksplantlarından sürgün rejenerasyonuna ait varyans analizi

V.K.	S.D.	Sürgün Rejenerasyon Oranı (%)		Eksplant Başına Sürgün Sayısı (adet)		Sürgün Uzunluğu (cm)		Kök Oluşturan Eksplant oranı (%)	
		K.O.	F	K.O.	F	K.O.	F	K.O.	F
Ortam	5	361,18	2,60^{ös}	42,91	13,95**	0,95	80,01**	2395,09	8,62**
Hata	12	138,91	-	3,08	-	0,02	-	277,74	-
Genel Toplam	17	-	-	-	-	-	-	-	-
** p<0,01 düzeyinde önemli, ^{ös}Önemsiz.									

Tablo 2. incelendiğinde, sürgün rejenerasyonu oranı bakımından ortamlar arasında istatistiksel olarak anlamlı bir farklılık bulunmazken, kök oluşturan eksplant oranı, sürgün uzunluğu ve eksplant başına sürgün sayıs bakımından istatistiksel olarak önemli bir farklılık bulunmuştur (p<0,01). Bu farklılığın anlamlılık seviyesi için Duncan testi uygulanmıştır (Tablo 2).

Sürgün rejenerasyon oranı %72,22-100,00 arasında sıralanmıştır (Tablo 2). %100 sürgün rejenerasyon oranı 0,25-0,75 mg/L BAP + 0,25 mg/L GA_3 içeren MS ortamında kaydedilmiştir. %72,22 sürgün rejenerasyon oluşumu 1,25 mg/L BAP + 0,25 mg/L GA_3 eklenmiş kültürlerde kaydedilmiştir. Genel olarak çok yüksek hormon kombinasyonu ve çok düşük hormon kombinasyonları eksplantların sürgün rejenerasyonları için olumlu bulunmamıştır. Benzer şekilde *Ceratophyllum demersum* L.'un sürgün ucu ile yürütülen çalışmada sürgün rejenerasyon frekansları yüksek ve düşük BAP içeriğinde azalmıştır Emsen ve Dogan [17]. *Limnophila aromatica* (Lamk.) Merr.'nın boğum ve boğum arası eksplantları 0,10 mg/L GA_3 ve 0.05-1.60 mg/L Tidiazuron kombinasyonunu içeren MS besin ortamında kültüre alınmış ve en yüksek ve en düşük hormon kombinasyonlarında sürgün rejenerasyon yüzdeleri düşüş göstermiştir [18]. Benzer sonuçlar *Ipomoea purpurea* (L.) Roth'nın boğum eksplantlarında da tespit edilmiştir [19]. BAP ve GA_3 kombinasyonlarını içeren MS besin ortamlarında eksplant başına sürgün sayısı 8,16-18,38 adet arasında değişmiştir (Tablo 2). En fazla sürgün oluşumu 18,38 adet ile 0,25 mg/L GA_3 + 0,25 mg/L BAP eklenmiş kültürlerde (Şekil 1a,b,c), ardından da 17,10 adet ile 0,25 mg/L GA_3 + 1,25 mg/L BAP eklenmiş kültür ortamında tespit edilmiştir. Düşük sayıda sürgünler ise 0,25 mg/L GA_3 + 0,75 mg/L BAP eklenmiş kültürlerde (8,16 adet), ardında da 0,25 mg/L GA_3 + 0,05 mg/L BAP'lı ortamda belirlenmiştir (12,64 adet).

Tablo 2 0,25 mg/L GA_3 ve farklı BAP konsantrasyonlarının *R. rotundifolia*'nın boğum eksplantlarında sürgün rejenerasyonuna etkisi

Büyüme Düzenleyicileri (mg/L)		Sürgün Rejenerasyon Oranı (%)	Eksplant Başına Sürgün Sayısı (adet)	Sürgün Uzunluğu (cm)	Kök Oluşturan Eksplant Oranı (%)
BAP	**GA_3**				
0,05	**0,25**	88,89 [ös]	12,64b	1,08c	83,33a
0.25	**0,25**	100,00	18,38a	2,36a	66,66a
0.50	**0,25**	100,00	15,27ab	1,75b	55,55ab
0.75	**0,25**	100,00	8,16c	1,51b	44,44ab
1,00	**0,25**	88,89	14,34ab	1,077c	16,66b
1,25	**0,25**	72,22	17,10a	0,83c	11,17b

Aynı sütundaki farklı harfler istatistiksel olarak farklılıkları göstermektedir (p<0.01)
[ös] Önemsiz

Veriler incelendiğinde, sürgün sayılarının hormon kombinasyonlarının etkisiyle önemli ölçüde etkilendiği fakat sürgün sayısının hormon kombinasyonları ile düzenli bir artış veya azalış göstermediği görülmüştür. Benzer şekilde farklı horman konsantrasyonlarının etkileri ile sürgün sayıları üzerinde değişimler *Lysimachia nummularia* L. [20], *Echinops kebericho* [21], *Pongamia pinnata* [22], *Picea abies* (L.) H. Karst [23] ve *Pogostemon erectus* (Dalzell) Kuntze [24] bitkilerinde de bildirimiştir.

Şekil 1 0,25 mg/L GA_3 ve farklı BAP dozlarını ihtiva eden sıvı kültür ortamında *R.rotundifolia*'nın boğumlarından rejenere sürgünler; Sekiz hafta sonra **(a,b,c)** 0,25 mg/L BAP + 0,25 mg/L GA_3 eklenmiş kültürlerde boğum eksplantından çoklu sürgünler

Kültür ortamlarında sürgün uzunlukları 0,83-2,36 cm arasında değişmiştir. En uzun sürgünler (2,36 cm) 0,25 mg/L BAP + 0,25 mg/L GA_3 eklenmiş kültürlerde, en kısa sürgünler ise 1,25 mg/L BAP + 0,25 mg/L GA_3 eklenmiş kültürlerde elde edilmiştir. Bitki büyüme düzenleyicilerin 0,25 mg/L'den fazla kullanılması sürgün uzunluğunu olumsuz etkilemiştir. Sürgün sayısı ve sürgün uzunluğu bakımından en uygun hormon kombinasyonları BAP ve

GA_3'in eşit oranda (0,25 mg/L) kullanıldığı ortam olarak kaydedilmiştir. Dogan [25] *R. rotundifolia*'nın sürgün ucu ve boğum eksplantları doku kültürü ile üretim için 0,05-1,25 mg/L Kinetin ve 0.25 mg/L GA_3 eklenmiş büyütme ortamına aktarılmış ve en uzun sürgünleri sürgün ucu eksplantlarında 0,75 mg/L Kinetin + 0.25 mg/L GA_3'lı kültürlerde, boğum eksplantlarında 0,50 mg/L Kinetin + 0.25 mg/L GA_3'lı kültürlerde elde etmiştir. Bu sonuçlar değerlendirildiğinde aynı bitki türlerinde bile farklı hormonlar bitki üzerinde farklı etkiler gösterebilmektedir. Kök oluşturan eksplant oranı %11,17-83,33 arasında sıralanmıştır. En yüksek kök oluşumu (%83,33) BAP'ın en düşük oranda kullanıldığı ortamda (0,05 mg/L), en düşük kök oluşumu (%11,17) BAP'ın en yüksek oranda (1,25 mg/L) kullanıldığı kültür ortamlarında kaydedilmiştir.

Çoğaltım ortamlarında bitkilerden kök oluşturmaları nedeniyle ayrıca köklendirme çalışmaları yürütülmemiştir. Rejenere sürgünler üzerinde bulunan besin ortamı çeşme suyu ile dikkatlice uzaklaştırıldıktan sonra akvaryum ortamına aktarılmıştır. Dört hafta sonunda rejenere bitkilerin dış koşullara alıştırılması sağlanmıştır.

Sonuç ve Öneriler

Son zamanlarda ekonomik olarak değerli bitkilerin çoklu üretimi için doku kültürü teknikleri yaygın şekilde kullanılmaktadır. Bu çalışmada, farklı BAP ve GA_3 kombinasyonlarını içeren kültür ortamında *R. rotundifolia*'nın *in vitro* hızlı ve çoklu üretimi başarıyla sağlanmıştır. En fazla eksplant başına sürgünler ve en uzun sürgünler 0,25 mg/L BAP ve 0,25 mg/L GA_3 kombinasyonunda elde edilmiştir. Bu rapor tıbbi bitki *R. rotundifolia*'nın doku kültürü koşullarında çoklu üretimine yardımcı olabilir ve böylece bu bitkiden değerli sekonder metabolitlerin fazlaca üretilmesine olanak sağlayabilir. Ayrıca doğal ortamlarından toplanmasının önüne geçilerek biyoçeşitliliğe katkı sağlayabilir. Bu çalışma sonuçları, tıbbi ve süs amaçlı kullanılan *R. rotundifolia*'nın büyük ölçekli üretimine yardımcı olabilir. Ayrıca bu bitki ile yürütülecek genetik mühendisliği çalışmalarına katkı yapabilir.

Teşekkür

Bu çalışma, Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK) tarafından 2130190 numaralı proje ile desteklenmiştir. Desteklerinden dolayı TÜBİTAK'a teşekkür ederim.

Kaynaklar

1. Bhowmik S, M. Saha, and B.K. Datta, Extended Distribution of *Rotala rotundifolia* (Buch.-Ham. ex Roxb.) Koehne (Lyhthraceae) from India. NeBIO, 2012. 3: p. 48-50.
2. Tan, Q.G., et al., Megastigmane-type Compounds from *Rotala rotundifolia*. Chinese Journal of Natural Medicines, 2009. 7: p. 187-189.
3. Ho, Y.L., et al., *In vitro* Antioxidant Properties and Total Phenolic Contents of Wetland Medicinal Plants in Taiwan. Botanical Studies, 2012. 53: p. 55-66.
4. Cirik, Ş., S. Cirik, ve M. Conk-Dalay, Su Bitkileri II (İçsu Bitkilerinin Biyolojisi, Ekolojisi, Yetiştirme Teknikleri). E.Ü. Su Ürünleri Fakültesi Yayınları, No:61, 2011.p. 4-150
5. Sivanesan, I. et al., Micropropagation of *Cotoneaster wilsonii* Nakai-a Rare Endemic Ornamental Plant. Plant Cell, Tissue and Organ Culture (PCTOC), 2011. 105: p. 55-63.
6. Joshi, P., and V. Dhawan, Axillary Multiplication of *Swertia chirayita* (Roxb. Ex Fleming) H. Karst., a Critically Endangered Medicinal Herb of Temperate Himalayas. *In Vitro* Cellular & Developmental Biology-Plant, 2007. 43(6): p. 631-638.
7. Sivanesan, I., and B.R. Jeong, Direct Shoot Regeneration from Nodal Explants of *Sida cordifolia* Linn. *In Vitro* Cellular & Developmental Biology-Plant, 2007. 43(5): p. 436-441.
8. Offord, C.A., and J.L. Tyler, *In vitro* Propagation of *Pimelea spicata* R. Br (Thymelaeaceae), an Endangered Species of the Sydney Region, Australia. Plant Cell, Tissue and Organ Culture (PCTOC), 2009. 98(1): p. 19-23.
9. Gonçalves, S., L. Fernandes, and A. Romano, High-Frequency *In Vitro* Propagation of the Endangered Species Tuberaria Major. Plant Cell, Tissue and Organ Culture (PCTOC), 2010. 101(3): p. 359-363.
10. Murashige, T., and F. Skoog, A Revised Medium For Rapid Growth And Bioassays With Tobacco Tissue Cultures. Physiological Plantarum, 1962. 15: p. 473-497.
11. Snedecor, G.W., and W.G. Cochran, Statistical Methods. The Iowa State University Press, 1997. Iowa, USA.
12. Mishra, A.K., et. al., Factors Affecting the Efficiency of *In Vitro* Regeneration from Seedling-Derived Nodal Explants of *Nyctanthes arbor-tristis* L. and Evaluation of Genetic Fidelity. Plant Biosystems, 2019. p. 1-9.
13. Dogan, M., Multiple Shoot Regeneration Via Indirect Organogenesis from Shoot Tip and Nodal Meristem Explants of *Ceratophyllum demersum* L. Journal of Animal and Plant Sciences, 2019. 29(2): p. 568-577.
14. Reddy, R., M. Nakhooda, and S. Shaik, Germination Studies And *In Vitro* Propagation of *Momordica balsamina* L. Using nodal explants and zygotic embryos. South African Journal of Botany, 2018. 115: p. 328-328.
15. Subbaiyan, B., M. Visveshwari, and V. Thangapandian, An Efficient Indirect Regeneration And Multiple Shoots Formation from Nodal Explant of *Ceropegia juncea* Roxb. International Journal of Conservation Science, 2017. 8(3): p. 529-536.
16. Warakagoda, P. S., S. Subasinghe, and M.T.K Gunasekare,. *In vitro* Clonal Propagation of *Coscinium fenestratum* (Gertn.) Colebr.(Weniwel) Through Nodal Explants. Journal of the National Science Foundation of Sri Lanka, 2017. 45(2): p. 133-141.
17. Emsen, B. and M. Dogan, Evaluation of Antioxidant Activity of *In Vitro* Propagated Medicinal *Ceratophyllum demersum* L. Extracts. Acta Scientiarum Polonorum-Hortorum Cultus, 2018. 17(1): p. 23-33.
18. Dogan, M., *In Vitro* Shoot Regeneration of *Limnophila aromatica* (Lamk.) Merr. from Nodal and Internodal Explants. Iğdır Universiry Journal of the Institue of Science and Technology, 2018. 8(3): p. 77-84.
19. Acemi, A., Comparative Analysis Of The Effects of Chitosan And Common Plant Growth Regulators on *In Vitro* Propagation of *Ipomoea purpurea* (L.) Roth from Nodal Explants. *In Vitro* Cellular & Developmental Biology-Plant, 2018. 54(5): p. 537-544.
20. Dogan, M., *In Vitro* Micropropagation from Nodal Explants of the Medicinal Plant *Lysimachia nummularia* L.. KSU Journal of Agriculture and Nature, 2018. 21(6): p. 875-881.
21. Enyew, M., and T. Feyissa, *In Vitro* Shoot Regeneration from Leaf Explants of *Echinops kebericho*: an Endangered Endemic Medicinal Plant. Plant Biosystems, 2019. 153(2): p. 199-204.

22. Tan, S.N., C.S. Tee, and H.L. Wong, Multiple Shoot Bud İnduction and Plant Regeneration Studies of *Pongamia pinnata*. Plant Biotechnology, 2018. 35: p. 325-334.
23. Varis, S., K., Klimaszewska, and T. Aronen, Somatic Embryogenesis and Plant Regeneration from Primordial Shoot Explants of *Picea abies* (L.) H. Karst. Somatic Trees. Frontiers in plant science, 2018. 9: 1551.
24. Dogan, M., *In Vitro* Rapid Propagation Of An Aquatic Plant *Pogostemon erectus* (Dalzell) Kuntze. Anatolian Journal of Botany, 2019. 3(1): p. 1-6.
25. Dogan, M., Multiple Shoot Regeneration from Shoot Tip and Nodal Explants of *Rotala rotundifolia* (Buch-Ham. ex Roxb) Koehne. Anatolian Journal of Botany, 2017. 1(1,2): p. 4-8.

Research article

Ozata, E., Evaluation of Fresh Ear Yield And Quality Performance In Super Sweet Corn. International Journal of Life Sciences and Biotechnology, 2019. 2(2): p. 80-94.

Evaluation of Fresh Ear Yield And Quality Performance In Super Sweet Corn

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ABSTRACT

Aim of this study is determine to fresh ear yield and quality characters of sweet corn varieties which Vega, Challenger, Sentinel, HYRIX 39, and HYRIX 53 in two location in Turkey. The research was conducted on randomised blocks design with four replication in Bafra and Tekkekoy location. The experiment was caried out evaluate the yield and quality performance of some sweet corn genotypes as well as demonstrate unfamiliar supersweet corn concept in Turkey. In the research were obserwed some yield component and quality characters such as flowering time, plant length, first cob length, grain yield, cob diameter, cob length, number of rows in the cob, amount of grains in row, number of marketable cobs per plant, wet cob harvest time, wet cob yield, grain cob ratio, grain moisture, dry grain yield. It was found that sweet corn genotype are significantly different in terms of yield and quality characteristics. The highest wet cob yield was 24.238 t ha^{-1} in Hybrix 39 super sweet. Super sweet corn has an important potential in sweet corn cultivation due to high yield of dry grains and its high maintenance rate of sugar raito in the long term after harvesting. Statistical analysis showed that the effect of genotype and the environment, as well as their interactions, had a significant impact on the yield of sweet corn hybrids. The presented results have demonstrated the poerformance of some sweet corn in Turkey. This work is intended to inspire similar studies using other sweet corn varieties and also to encourage the wide-scale production sweet corn in Turkey.

ARTICLE HISTORY
Received
17 May 2019
Accepted
25 June 2019

KEYWORDS
Fresh ear yield, grain yield, super sweet corn, quality and cob length

Introduction

Sweet corn [*Zea mays* (L.) var. *saccharata*] is cultivated plant for human consumption and it is a raw or processed material of the food industry throughout the world. It is popular with the consumer for its unique taste, pleasant flavour and sweetness. Sweet corn plays an important role in the human diet because of its health-promoting nutritional characteristics. The nutritional value of sweet corn kernels is related to the content of water (72.7%) and the total content of solid parts (27.3%). Solid parts include hydrocarbons (81%), proteins (13%), lipids (3.5%) and others (2.5%). Starch is the dominant hydrocarbon component [1]. Sweet corn kernels are moderately high in calories in comparison to other vegetables. Corn features high-quality phyto-nutrition profile

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comprising of dietary fibre, vitamins, and antioxidants in addition to a reasonable proportion of minerals. Sweet corn contains a significant amount of lutein, zeaxanthin and other carotenoids [1]. Sweet corn is widely consumed as a healthy food since it is rich in carotenoids.

The world sweet corn cultivation area is 1444037 ha, production is 18841356 tons, and yield is 13.029 tha^{-1}. Moreover, worldwide sweet corn producing area ocure that Africa (32.3%), European Union Countries (27.7%), America (24,7%) and Asia (12,6%), respectively. European Union countries (52.4%), then America (29.5%), Africa (9.1%) and Asia (6.9%), respectively, are the top producer's countries in terms of production of sweet corn. Similar to production, the European Union Countries with 24.745 tha-1and the United States with 15.552 t/ha-1 have the highest yield. Nowadays, its biggest producers are Ukraine (6967780 t), USA (3838630 t), Croatia (1283068 t), and Mexico (898793 t). Moreover, the USA and UK are the most significant exportercountries. Sweet corn is consumed as a human food in the worldwide, which approximately 21% of total production [1]. Sweet corn production has dramaticaly increased by the 250% in the last 30 years [1]

The sweet corn is divided into three groups according to the sugar content and its storage ability. The first group is named as a sugar corn. Sugar corn after the grain maturation, it is necessary to harvest corn spadix and sequentially process or consume by 24 hours. When reached this stage, the content of sugars very quickly decreases and they are converted to starch [1]. The second group is tagged as SE (sugary enhanced). SE corn is more slowly convert sugars to starch after harvest is slow. The corn spadix is possible to storage about three days after harvest in good taste quality [1]. The third group is named as sh-2 (super sweet, shrunken) and spadix has the highest sugar content, and its storage ability after harvest is about seven days in cold conditions. After that, the grain quality is decreased. The grain starts to shrivel because of water lack, despite preserving sweet taste. This variety group is the most preferred variety by producers and consumers [1].

The consumption of sweet corn as fresh in the form of canned or frozen food is increasing extremely quick in all over the world. Although sweet corn is produced with contract farming model intensively in Aegean, Marmara and the west part of Turkey, demand for sweet corn is not reached by domestic production in Turkey. Turkey's frozen-canned sweet corn imports in 2016 are approximately 8 thousand tons. In addition, although the

value of imports is approximately $ 10 million, the cost of export is about $ 0,95 million [1] Turkey sweet corn yield potential is close or higher than world avarage statistics. Since Turkey is a country that exports most of its agricultural products abroad, there is the potential to move from importer to exporter position with the increase in sweet corn production areas. The research carried out to evaluate the yield, yield component and quality of widely planted sweet corn genotypes and supersweet genotypes at ecological conditions in Bafra and Tekkekoy locations in Turkey.

Materials and Methods

Experimental site and material

The field trials were conducted on Bafra and Tekkeköy location in Blacksea Agricultural Research Institution (Bafra; 41^{O} 36'N, 35^{O} 55' E; Tekkeköy 41^{O}.13 N', 36^{O} 30' E; 16 m evaluation Bafra, E; 7 m evaluation) during to 2018 cropping seasons as a double crop. The sweet corn varieties in the research and their some characteristics are presented Table 1.

Table 1 Sweet corn varieties and their some charectiscs

Variety Name	Sweet corn	Ear colour	Origin	Growing period (days)
Vega	Super Sweet (Sh2)	Yellow	USA	70-80
Challenger	Super Sweet (Sh2)	Yellow	USA	80-85
Sentinel	Super Sweet (Sh2)	Yellow	Australia	80-85
HYRIX 39	Super Sweet (Sh2)	Yellow	Thailand	120-130
HYRIX 53	Super Sweet (Sh2)	Yellow	Thailand	120-130

The soil of the experimental area is clayed-loamy and little alkaline. Total salt and the amount of takable phosphor were low, but the plant was rich in terms of nutrition elements and potassium and lime, but low in terms of organic matter (Table2).

Table 2 Some properties of study soil *

Parameter	Bafra	Tekkeköy	
Soil texture (%)	66	68	Clay Loam
pH	7,2	7,4	Slightly alkaline
P_2O_5 (kg ha^{-1})	25,2	25	Very Low
K_2O (kg ha^{-1})	940	920	High
Organic Matter (%)	1,76	1,7	Low
$CaCO_3$ (%)	6,76	7,5	Medium
EC (%)	0,054	0,061	Nonsaline

*(Samsun, Blacksea Agrıcultural Research Institute, Soil Department Laboratory, Analyze Number:183,2018)

Winter and summer in Samsun are temperate and rainy, which is a type of a Black Sea climate. The mean monthly air temperature during the research period (May- October) was 15.9 - 23.8 C^o in Tekkekoy location, whereas it was 17.4 - 24.6 C^o in the Bafra location. Total rainfall was 317.7 mm in Tekkekoy and 208.6 mm in Bafra during the growing periods in 2018. In addition, the average relative humidity was ranged from77.5-80.0 % in Tekkekoy location, 74.8-78.6% in Bafra location (Table 3, Table 4).

Whilistmoisture and temperature values relatively had the close values each other in cultivating season (2018) differed from long term averages. Average temperatures in Bafra location was measured 1-1.5 °C higher than long term averages. Significant differences were measured in total rainfall and distribution of rainfall into months.

Table 3 The climate conditions in 2018 and long term in Tekkekoy location (1970-2018)*

Months	Mean temperature (ºC)		Relative humidity (%)		Precipitation (mm)	
	Long Term	2018	Long Term	2018	Long Term	2018
May	15,9	16,9	77,5	79,9	49,0	65,2
June	20,1	21,0	77,5	78,0	45,4	57,5
July	23,4	23,8	77,5	77,5	32,0	37,5
August	23,8	24,0	78,5	78,8	40,1	72,0
September	20	20,5	79,5	80	51,7	85,5

Table 4 The climate conditions in 2018 and long term in Bafra location (1970-2018)*

Months	Mean temperature (ºC)		Relative humidity (%)		Precipitation (mm)	
	Long Term	2018	Long Term	2018	Long Term	2018
May	15,4	17,4	78,6	78,5	46,3	15,3
June	20,1	21,8	74,5	76,5	44,9	38,4
July	22,8	24,6	72,5	74,8	29,9	23,5
August	22,7	24,5	73,9	77,5	44,4	50,0
September	19,2	20,9	76,3	76,5	58,5	81,6

* X Meteorologi Regional Directorate Data

The experiment treatments

The experiment was conducted on a Randomized Complete Block design with four replications in the main crop separately. Seeds were sown by hands as spreading two seeds in per growing bed, and every plot had four lines, and plot area was 14 m^2 plot dimensions inter-rows was 70cm and within rows was 18cm and the length of plot 5m. When the plants reached knee-deep (40-50cm) in the experiment, the weak one from two plants in the growing bed was thinned. Irrigations were applied with drum irrigation systalk and earthing up was applied with hoeing regularly [1]

The dressing was made as pure 80 kg ha^{-1} P_2O_5 and 200 kg N ha^{-1} totally per hectare according to soil analysis. All phosphorised manure and 80 kg ha^{-1} of nitrogenous manure were given at the cultivating period as bottom fertiliser, the rest of the nitrogenous was given when the plants became 4-6 leafed (V4-V6 phase), reached approximately 40-50cm. Two lines in the middle were harvested for fresh ear yield another two lines in the middle were harvested for dry grain yield. Corn was sowed in one day at both locations on 12 May 2018. Wet cob harvests between the dates of 4-21 August 2018, the dry crops were harvested between 18 September 2018 and 16 November2018. Wet cob was harvested when the grain moisture of the cobs is 70-75 %, while dry grain was harvested when grain moisture was close to 20%. Dried grain yield per decare was calculated after the grain moisture was adjusted to 15%. During the growing period, some cultural practices such as weed control, irrigation, pesticide and fungicide were applied at the proper time intervals for each experiment.

Data collection and measurement

Besides, the number of days for %50 flowering, plant height, Phenological and morphological observations taken during the research were made based on technical order of agricultural values evaluation testings by Ministry of Agriculture and Forest [1]. Data obtained from the research were subjected to the variance analysis [1] using JMP 7.0 statistical software with Split Plot Design. Obtained data were statistically processed by the two-factorial analysis of variance, where the factor A was genotype and the factor B environment. Least Significant Differences (LSD) test was used to compare the treatments at the probability level of 0.05.

Results and Discussion

There are a statistically significant difference (p>0.05) between location, genotype and location genotype interaction among sweet corn genotypes. Flowering times are avarege 52.7 and 58.4 days Bafra and Tekkeköy location, respectively. Among the genotypes, the earliest flowering time was observed in Sentinel super sweet, as 47 days and the latest flowering time was observed in Hyrix 39 super sweet corn. Differences in flowering times between locations are thought to be caused by climatic conditions. The average temperature values between sowing and flowering period in Bafra location were relatively 1-1.5 C^{o} higher 1-1.5 than Tekkeköy location (Table 5). The reason for the difference in flowering time among genotype is the genetic structure. As Hyrix 39 and Hyrix 53 sweet corn genotypes were tropical origins, they showed later flowering. Many researchers have reported that there are differences between genotypes in their studies [1].

Table 5 The flower days and plant height belonging to some sweet corn variety in location

Variety	**% 50 Flower (day)**						**Plant height (cm)**					
	TKKY		**BAFRA**		**Av.**		**TKKY**		**BAFRA**		**Av.**	
HYRIX 39	72,5	a	62,8	b	67,5	***A***	316,3	a	305,0	ab	310,6	***A***
HYRIX 53	72,0	a	63,0	b	66,0	***A***	298,8	b	305,0	ab	304,2	***A***
VEGA	50,0	c	45,8	ef	48,1	***B***	221,3	c	185,0	d	201,9	***B***
SENTINEL	48,8	d	45,3	f	47,0	***C***	228,8	c	173,8	d	201,3	***B***
Challenger	48,5	d	46,5	e	47,5	***BC***	225,0	c	181,3	d	203,1	***B***
Mean	58,4	A	52,7	B			258,0	A	230,0	B		
CV (%)	1,42						3,54					
LSD (Location)	1,05	F(L)	628,84	P(L)	<,0001		LSD (L)	38,25	F(L)	30,95	P(L)	0,0001

There is a statistically significant difference (p>0.05) between the average plant height of the sweet corn genotypes and location, the interaction genotype and location ×genotype. The average plant height at Tekkekoy location as 258 cm was measured higher than Bafra (230cm). Among the genotypes, the highest height was measured in Hyrix 39 sweet corn as 310.6 cm, and the shortest height was measured in Sentinel variety as 201.3 cm. The differences in plant height between the locations are thought to be caused by climatic conditions. The total precipitation in the area of Tekkekoy is 313.7 mm and is about 100 mm more rainfall than Bafra location. The differences in plant height among genotypes are due to the genetic structure of the species. Hyrix 39 and Hyrix 53 sweet corn genotypes are tropical species, so it stands out as tall and quite fast growing sweet corn species. Many researchers have reported that there are differences between genotypes in their research [1].

Table 6 The grain/ear ratio and grain moisture belonging to some sweet corn variety in location

Variety	**Grain/Ear ratio (%)**					**Grain moisture (%)**					
	TKKY	**BAFRA**		**ORT**		**TKKY**		**BAFRA**		**ORT**	
HYRIX 39	81,7	82,6		82,2	**B**	22,3		22,7		22,5	**AB**
HYRIX 53	82,0	82,3		81,9	**B**	22,9		22,9		22,9	**A**
VEGA	82,5	81,4		82,1	**B**	19,9		22,3		21,3	**AB**
SENTINEL	82,6	82,9		82,8	**AB**	20,1		21,4		20,7	**B**
CHALLENGER	82,4	85,6		84,0	**A**	20,1		22,0		21,0	**AB**
AVERAGE	82,2	83,0				21,1	B	22,2	A		
CV (%)	1,7					6,17					
LSD (Location)	-	F(L)	5,88	P(L)	0,05	LSD (L)	0,72	F(L)	6,63	P(L)	0,04
LSD (Variety)	1,44	F (V)	2,81	P (V)	0,05	LSD (V)	1,34	F (V)	4,56	P (V)	0,01
LSD (L×V)	-	F (LXV)	2,50	P (LXV)	0,07	LSD (LXV)	ns	F (LXV)	1,20	P (LXV)	0,34

The rate of grain tocobs is a critical selection criterion for breeders, and it is desired to be 80% or more. Among the genotypes, the grain/cob ratio was measured from the highest Challenger sweet corn genotype with 84% and from the lowest, Hyrix 53 corn species 81.9%. The grain/cob ratios are directly related to the grain length among genotypes, and there can be differences between genotypes. The findings of the research are consistent with the studies, and the differences between genotypes have been reported by many researchers [1].

There was a statistically significant difference (p>0.05) between grain moisture of sweet corn varieties and the location and genotypes. The Bafra location with an average rate of

21.1% of grain moisture was measured relative lower than the Tekkekoy location (22.2%). Among the genotypes, the highest amount of grain moisture was measured in Hyrix 53 super sweet corn with 22.9%, while the lowest amount of grain moisture in the Sentinel super sweet corn with 20.7%. Differences in grain moisture between locations are thought to be caused by climatic conditions. The average temperature values during the growing period of Bafra location were 1-1.5 C higher than Tekkeköy location. The differences in grain moisture between genotypes are due to the fast drying time between grain bonding and harvest and total temperature requirement. Many researchers have reported that there are differences between genotypes in their research [1].

Table 7 The number of row per ear and number of kernel per ear belonging to some sweet corn variety in location

Variety	Number of row per ear					Number of kernel per ear					
	TKKY	**BAFRA**		**ORT**		**TKKY**	**BAFRA**		**ORT**		
HYRİX 39	18,0	19,5		18,8	a	36,8	46,5		41,6		
HYRİX 53	17,0	19,0		17,3	a	34,3	41,0		36,8		
VEGA	16,0	16,5		16,0	b	38,5	38,3		42,0		
SENTİNEL	16,5	16,0		16,3	b	38,8	36,8		37,8		
CHALLENGER	15,5	16,5		16,0	b	39,5	39,0		39,3		
AVERAGE	16,6 B	17,5 A				37,6	40,3		39,5		
CV (%)	7,62					12,24					
LSD (Location)	0,88	F(L)	7,83	P(L)	0,03	LSD (L)	-	F(L)	20,5	P(L)	0,052
LSD (Variety)	1,34	F (V)	7,26	P (V)	0,00	LSD (V)	-	F (V)	18,7	P (V)	0,074
LSD (LXV)	---	F (LXV)	1,08	P (LXV)	0,38	LSD (LXV)	-	F (LXV)	5,89	P (LXV)	0,068

It is found that statistically significant differences ($p>0.05$) between row number in cob, location and genotypes among sweet corn varieties. The average number of rows in cob in Bafra location was measured 17.5 rows/cobs, and it is higher than Tekkekoy location (17.5 rows/cobs). It was measured that Hyrix 39 super sweet corn variety has the highest number of rows in the cob as 18.8 rows/cob and Challenger has the lowest amount of grain in the row as16 grain/rows. The number of rows in the cob is under the influence of genetic structure and varies according to the genotypes. The findings obtained from the experiment show similarities and differences with the studies [1]. These differences are thought to be caused by genotypes.

Table 8 The ear diameter and ear length belonging to some sweet corn variety in location

Variety	Ear diameter (mm)			Ear length (cm)		
	TKKY	BAFRA	ORT	TKKY	BAFRA	ORT
HYRİX 39	47,0	50,8	48,9	21,3	23,3	22,3
HYRİX 53	44,8	47,3	45,8	20,3	21,3	20,8
VEGA	46,8	50,3	48,3	21,3	22,5	21,9
SENTİNEL	49,8	46,0	47,9	21,3	21,0	21,1
CHALLENGER	47,5	46,3	46,9	22,0	21,8	21,9
AVARAGE	47,2	48,1	47,5	21,2	22,0	21,6
CV (%)	7,03			9,65		

LSD (Location)	-	F(L)	1,06	P(L)	0,34	LSD (L)	-	F(L)	2,38	P(L)	0,17
LSD (Variety)	-	F (V)	0,97	P (V)	0,43	LSD (V)	-	F (V)	0,60	P (V)	0,66
LSD (LXV)	-	F (LXV)	1,91	P (LXV)	0,14	LSD (LXV)	-	F (LXV)	0,38	P (LXV)	0,81

There was no statistically significant difference in cobs height between the location and genotypes of sweet corn genotypes ($p > 0.05$). The average height of the cobs among genotypes ranged between 20.8-22.3 cm. Breeders working on sweet corn take into account the cob height as an important selection criterion. The sweet corn consumed as fresh is preferred 18 cm and above for being marketable. The findings obtained from the experiment show similarities and differences with the studies [1]. These differences are thought to originate from genotypes and cultural processes such asfertilisation and irrigation.

Table 9 The first ear height and number of row per ear belonging to some sweet corn variety in location

Variety	First Ear height (cm)						number of row per ear					
	TKKY		BAFRA		Av.		TKKY		BAFRA		ORT	
HYRİX 39	165,0	a	122,5	c	143,8	a	661,5	bc	906,8	a	780,5	a
HYRİX 53	137,5	b	122,5	c	135,0	b	582,3	c	779,0	b	638,4	b
VEGA	72,5	d	61,3	d	66,3	c	616,0	c	631,1	c	672,0	b
SENTİNEL	72,5	d	52,5	f	62,5	c	639,4	c	588,0	c	613,4	b
CHALLENGER	70,0	de	66,3	de	68,1	c	612,3	c	643,5	c	628,0	b
AVARAGE	103,5	A	85,0	B			622,3	B	709,7	A	666,5	b
CV (%)	12,88						13,51					

LSD (Location)	17,72	F(L)	37,00	P(L)	0,001	LSD (L)	13,04	F(L)	10,55	P(L)	0,017
LSD (Variety)	7,66	F (V)	222,20	P (V)	<,0001	LSD (V)	92,82	F (V)	5,09	P (V)	0,004
LSD (LXV)	14,48	F (LXV)	7,75	P (LXV)	0,001	LSD (LXV)	131,26	F (LXV)	4,06	P (LXV)	0,011

There is no statistically significant difference in cob diameter between the location and genotypes of different sweet corn genotypes ($p>0.05$). The average diameter of the cobs was varied between 45.8-48.9 mm among the genotypes. The breeders working on sweet corn take into account cob diameters as an essential selection criterion in parallel with the height of the cob. The sweet corn consumed as fresh, it is preferred to be 38 mm and above for being marketable. The findings obtained from the experiment show similarities and differences with the studies. [1] These differences are thought to originate from genotypes and cultural processes (sowing frequency, fertilisation).

There is a statistically significant difference in first cob height between the location, genotype and location x genotype interactions in different sweet corn genotypes ($p> 0.05$). Among the genotypes, the first cob heights were measured from Hyrix 39 sweet corn with the highest average height of 143.8 cm and from Sentinel variety with the lowest average height of 62.5 cm. Since sweet maize is usually harvested by hand, the varieties, which do not give cob from the bottom or top, are ideal. The findings obtained from the experiment show similarities and differences with the studies [1]. These differences are thought to be caused by differences in genotypes and cultural processes.

There is a statistically significant difference in different genotype in the number of rows in cob between location, genotype, and location x genotype interactions ($p> 0.05$). In genotypes, the highest the average amount of grain in the cob was measured in Hyrix 39 with 780.5 grain/ cob, while the lowest the average number of grain in the cob was measured in Sentinel with 613.4 grain/cob. There is a significant positive correlation in the number of grains in the cob, the number of rows in the cob and the number of grain in the row. The findings obtained from the experiment show similarities and differences with the studies [1]. These differences are thought to be caused by differences in genotypes and cultural processes.

Table 10 The fresh ear yield and dry ear yield belonging to some sweet corn variety in location

	Fresh ear yield (kg/ha)						Dry ear yield (kg/ha)					
Variety	**TKKY**		**BAFRA**		**ORT**		**TKKY**		**BAFRA**		**ORT**	
HYRİX 39	18413	c	30064	a	24238	***A***	3884	bc	5522	s	4703	***A***
HYRİX 53	18480	c	22786	b	20173	***B***	3536	cd	4300	b	3841	***B***
VEGA	12843	f	16961	d	14995	***C***	2669	ef	3376	d	3014	***C***
SENTİNEL	12193	f	14200	e	13196	***D***	2439	f	2801	ef	2620	***D***
CHALLENGER	12643	f	15743	d	14193	***C***	2513	ef	2881	e	2697	***D***
AVERAGE	14914	B	19951	A			3008	B	3776	A		
CV (%)	5,16						8,75					
LSD (Location)		F(L)	157,80	P(L)	<,0001		62,42	F(L)	37,92	P(L)	0,00	
LSD (Variety)	93,73	F (V)	220,77	P (V)	<,0001		30,59	F (V)	72,71	P (V)	<,0001	
LSD (LXV)		F (LXV)	35,06	P (LXV)	<,0001		43,21	F (LXV)	6,14	P (LXV)	0,00	

There is a statistically significant difference in the fresh cob yield of different sweet corn genotypes between the location, genotype and location x genotype interactions ($p > 0.05$). It was measured that the highest average fresh cob yield is in Hybrid 39 with 24.238 tha-1, whereas the lowest fresh cob yield is in the Sentinel with 13.196 tha-1. In terms of locations, the highest yield was obtained at Bafra location with 19.951 tha-1. Hyrix 39 and Hyrix 53 varieties, which have the tropical origin, are more productive than early varieties because they are late flowering. There is a positive correlation between maturity groups and yield in corn breeding. The findings obtained from the experiment show similarities and differences with the studies. These differences are thought to be caused by genotypes and climatic variations. The grain proportion, weight and length of ears belong to basic parameters influencing the total yield of cultivated sweet corn varieties. This fact was presented in the research works of several authors which stated significant impact of variety to the yield quantity of sweet corn [1].

There is a statistically significant difference in the dry grain yield of different sweet corn genotypes between the location, genotype, and location x genotype interactions ($p > 0.05$). It was measured that the highest average dry grain yield is in Hybrid 39 with 4.703 tha-1, whereas while the lowest dry cob yield is in the Sentinel with 2.620 tha-1. In terms of locations, higher yield was obtained at Bafra location with 3.776 tha-1. The dry grain yields of Hyrix 39 and Hyrix 53 were found to be high in parallel with the yields of the wet cob. Although sweet corn is mostly consumed fresh, dry grains of sweet corn are also consumed as snacks after different processes. As well, the yield per unit area is an

essential factor for the producers who produce sweet corn as a snack. The findings obtained from the experiment show similarities and differences with the studies [1]. These differences are thought to be caused by genotypes and climatic variations.

Table 11 The number of marketable ears, days to fresh maturity and Days to dry maturity belonging to some sweet corn variety in location

Variety	**number of marketable ears**			**Days to fresh maturity**			**Days to dry maturity**		
	TKKY	**BAFRA**	**ORT**	**TKKY**	**BAFRA**	**ORT**	**TKKY**	**BAFRA**	**ORT**
HYRİX 39	1,8	2,0	1,9	99,8	102,0	100,9	154,0	154,3	154,1
HYRİX 53	1,6	1,8	1,7	99,8	102,0	100,5	154,0	154,3	154,2
VEGA	1,1	1,2	1,2	82,5	84,0	83,1	125,5	126,0	125,9
SENTİNEL	1,0	1,0	1,0	82,0	84,3	83,1	125,5	125,8	125,6
CHALLENGER	1,1	1,2	1,2	82,5	84,0	83,3	125,8	127,8	126,8
AVARAGE	1,3	1,5	1,4	89,3	91,3	90,2	137,0	137,6	137,3

The number of cobs in crop, fresh cob harvest dates and dry grain harvest times of different sweet corn genotypes are of great importance in terms of production. The number of cobs in the crop is directly related to the yield. The sweet corn has the potential to show the second cob in general similar to that of flint corn. Since the sweet corn is mostly produced for fresh consumption purposes, the number of marketable cobs obtained by evaluating the cob height with the cob diameter is of great importance. The highest number of marketable cobs per plant were collected from Hyrix 39 and Hyrix 53 varieties with 1.9 and 1.7, respectively.

One of the essential elements in sweet corn cultivation is to provide products to the market as early grown. The wet cob harvest times are of great importance for the producers. Super sweet corn varieties in the temperature group were determined to be grown on average between 80-85 days, while tropical varieties were determined to be grown on average between 99-102 days. In addition, dry grain harvests were also determined as 125 days in temperature group and 155 days in tropical varieties under Samsun conditions.

Conclusion

In the study, five sweet corn varieties were agronomicaly tested in two locations where Samsun, Turkey ecological condition. Vega variety, which is in the early maturity group, came to the forefront in terms of the number of marketable cobs and the yield of wet cob. It was determined that two tropical origin sweet corn varieties have a very significant advantage in terms of the high wet cob yield and the number of marketable cobs. It was noted that the remaining part after the wet cob harvest could have a significant silage feed potential. Especially, Hyrix 35 and Hyrix 53 have an advantages for silage feed because of number of leaves and higher plant length compare the other varieties. It is essential for the sweet corn breeders that their genetic pool has to copmrise advantages both the fresh ear yield and silage feed potential. The presented results have demonstrated the fresh ear yield of sweet corn is significantly influenced by genotype. It may have affected metrological conditions in different environments and the interaction of those two factors.

References

1. Szymanek, M., Processing of sweet corn, trends in vital food and control engineering. Ayman Amer Eissa (Ed.). Intech. Available at http://www.intechopen.com/books/trends-invital- food-and-control engineering/processing-of-sweet-corn.,2012.
2. Junpatiw, R., et al.,. Effects of steaming,boiling and frozen storage on carotenoid contents of various sweet corn cultivars International Food Research Journal, 2013. 20(5): p. 2219-2225.
3. FAO., Food and Agriculture Organization of the United Nations. Crop productıon of maizereen. Http://www.fao.org/faostat/en/#data/QC, 2016. Date Accessed:15.04.2019
4. Salunkhe, D, K., and Kadam, S, S., Sweet corn Handbook of vegetable science and technology, production, composition, storage and processing. 1998. New York: Marcel Dekker, 742 p. ISBN 0-8247- 0105-4.
5. Sıngh, I., Langyan, S., and Yadava, P., Sweet Corn and Corn-Based Sweeteners. In Sugar Technology, vol. 16, 2014. no. 2, p. 144– 149. ISSN 0972-1525.
6. FAO, Turkey ımport and export sweet corn frozen and sweet corn prep or preserved. http://www.fao.org/faostat/en/#data/TP, 2016. Date Accessed:15.04.2019.
7. Kırtok, Y., Mısır Üretimi ve Kullanımı, 1998. Kocaoluk yayınevi, İstanbul.
8. Anonymous. Tarımsal değerleri ölçme denemeleri teknik talimatı-Mısır. https://www.tarimorman.gov.tr/BUGEM/TTSM/Belgeler/Tescil/Teknik%20Talimatlar/S%C4%b1cak%20%C4%b0klim%20Tah%C4%b1llar%C4%B1/MISIR_TEKNIK_TALIMATI.pdf, 2018.
9. Düzgüneş , O., et al., Araştırma ve Deneme Metotları (İstatistik Metotları II), 1987. Ankara Üniv. Zir. Fak.Yay. 1021, Ders Kit.: 285, Ankara, 381 s.
10. Turgut, İ., and Balcı, A., Bursa koşullarında değişik ekim zamanlarının şeker mısırı (*Zea mays L. Var. Saccharata Stur*t.) çeşitlerinin taze koçan verimi ile verim öğeleri üzerine etkileri. Uludağ Üniv. Zir. Fak. Dergisi, 2002. 16(2):79-91.

11. Öktem, A,. and Öktem, G,A., Bazı şeker mısır genotiplerinin (*Zea mays L. Var. Saccharata Sturt*) harran ovası koşullarında verim karakteristiklerinin belirlenmesi. Uludağ Üniv. Zir. Fak. Dergisi 2006. 20(1):33-46.
12. Egesel, C, et al.,. Bazı şeker mısır (*Zea mays L. Var. Saccharata Sturt*.) genotiplerinin verim ve bitkisel özelliklerinin incelenmesi. Türkiye VII. Tarla Bitkileri Kongresi, 25-27 Haziran 2007. Erzurum. S:206- 209.
13. Sönmez, K., et al,. Bazı Şeker Mısır Çeşitlerinin (Zea mays saccharata Sturt) Bitki, Koçan ve Verim Özellikleri. SDÜ Ziraat Fakültesi Dergisi, 2013.8(1):28-40.
14. Özata, E., Geçit, H,H., and Ünver İkincikarakaya, S., Orta karadeniz ekolojik koşullarında şeker mısırda (Zea mays saccharata Sturt.) Değişik ekim sıklıkları ve dozlarının verim öğelerine etkisi. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 2016, 25 (Özel sayı-1) p:74-80.
15. Kahrıman, F., et al., Şeker mısırda (*Zea mays saccharata Sturt.*) Farklı tohumluk miktarlarının koçan verimi üzerine etkisi. Türkiye VII. Tarla Bitkileri Kongresi, 25-27 Haziran 2007, Erzurum.
16. Özata E, and Kapar, H., Determination of yield and quality of some hybrid dent corn (Zea mays indentata Sturt) genotypes under Samsun conditions, 2013. Tarım Bilimleri Araştırma Dergisi. 6 (2): 19.
17. Özata E, and Kapar, H., Bazı atdişi hibrit mısır (Zea mays indentata Sturt) genotiplerinin Samsun koşullarında kalite ve performanslarının belirlenmesi. Tarım Bilimleri Araştırma Dergisi 2014 Vol.7 No.2 pp.1-7
18. Özata, E., et al.,. Atdişi hibrit mısır adaylarının ana ürün koşullarında performanslarının belirlenmesi. Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 2013. 3(1): 91-98.
19. Gençtan, T., Uçkesen, B., Tekirdağ koşullarında ana ürün ve ikinci ürün şeker mısır (*Zea mays saccharata Sturt.*) yetiştirme olanaklarının araştırılması. Türkiye 4. Tarla Bitkileri Kongresi 17-21 Eylül 2001. Tekirdağ.
20. Bozokalfa, M,K., Eşiyok, D., and Uğur, A., Ege bölgesi koşullarında ana ve ikinci ürün bazı hibrit şeker mısır (Zea mays L. Var saccharata) çeşitlerinin verim, kalite ve bitki özelliklerinin belirlenmesi. Ege Üniv. Ziraat Fak. Dergisi, 2004.41 (1): 11-19.
21. Sönmez, K., et al., Bazı şeker mısır çeşitlerinin (Zea mays saccharata Sturt) bitki, koçan ve verim özellikleri. Süleyman Demriel Üniversitesi Ziraat Fakültesi Dergisi, 2013. 8(1):28-40
22. Esiyok, D., and Bozokalfa, K,M., The effects of sowing and planting dates on yield and some agronomic properties of sweet corn (*Zea mays L. Var. Saccharata*). The Journal of Agricultural Faculty 2005. 42(1):35-46.
23. Egesel, C.Ö., et al,. Bazı şeker mısır (*zea mays l. var. saccharata sturt*.) genotiplerinin verim ve bitkisel özelliklerinin incelenmesi. Türkiye VII. Tarla Bitkileri Kongresi. 25-27 Haziran 2007. Erzurum. S:206- 209.
24. Öktem, A. and Öktem, G, A., Bazı Şeker Mısır Genotiplerinin (*Zea mays L. Var. Saccharata Sturt)* Harran ovası koşullarında verim karakteristiklerinin belirlenmesi. Uludağ Üniv. Zir. Fak. Dergisi, 2006. 20(1):33-46.
25. Banotra M., et al., Producvity potensial of sweet corn cultıvars under different planting time in subtropical foothills of N-W Himalayas. Internaitonal Journal of Basic Applied Agrıcultural Research Vol. 13 (Special Issue) November, 2015. p.331-334.
26. Koçak, M., and Köycü, C., Samsun ekolojik şartlarında bazı tatlı mısır çeşitlerinde verim, verim öğeleri ve bazı kalite özelliklerine azotlu gübrelemenin etkisi üzerinde bir araştırma. Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi, 1994. 9(2):83-94,
27. Turgut, I., Effects of plant population and nitrogen doseson fresh ear yield component of sweet corn (Zea mays L. Var. Saccharata Sturt.) Grown under Bursa conditions. Turkısh Journal Agriculture Foresty, 2000. 24:341-347.

28. Esiyok, D., Bozokalfa, K,M., and Ugur, A., Determination of yield quality and some plant characteristic of some sweet corn (Zea mays L. Var. Saccharata) varieties in different locations. The Journal of Agricultural Faculty, 2004. 41(1):1-9.
29. Olsen, J.K., Blight, G,W., and Gillespie, D., Comparation of yield, cobcharacteristics and sensory quality of six supersweet corn cultivarsgrown in a ubtropical environment. Australian Journal of Experimental Agriculture, 1990. 30(3).387-393.
30. Nagy, J., Effect ot sowing date on the yield and guality of maize hyrids with different growing seasons. Acta Agronomica Hungarica, 2009. 57 (4);389-399.
31. Khan, ZH., et al., Phenology and yield of sweet corn landraces influenced by planting dates. Sarhad Journal of Agriculture, 2009. 25 (2); 153-157.
32. Hassan, A., Effect of plant population density on yield and yield components of 8 Egyptian maize hybrids. In Bulletin of the Faculty of Science of Cairo University, vol. 51, 2000, no. 1, p. 1–16. ISSN 0526-8613.
33. ShafI, M., et al.,Effect of planting density on phenology, growth and yield of maize (Zea mays L.). In Pakistan Journal of Botany, vol. 44, 2012, no. 2, p. 691–696. ISSN 0556-3321.
34. Eskandarnejad, S., Effect of row spacing and plant density on yield and yield components of Sweet corn (Zea mays L. Saccharata) varieties. In Advanced Crop Science, vol. 3, 2013, no. 1, p. 81–88. ISSN 2329-8863.
35. Anapalli, S, S., et el., Simulating planting date effects on corn production using Rzwqm and Ceres-Maize models. Agron. Journal, 2005. 17(97): 58-71.
36. Dekhane, S,S., and Dumbre, R,B., Influence of different sowing dates on plant growth and yield of hybrid sweet corns. Adv. Res. J. Crop Improv., 2017. 8 (2) : 191-194, Doı : 10.15740/ Has/Arjcı/8.2/191-194.

Research article

Cevher-Keskin, B., Quantitative mRNA Expression Profiles of Germin-Like and Extensin-Like Proteins under Drought Stress in Triticum aestivum. International Journal of Life Sciences and Biotechnology, 2019. 2(2): p. 95-107.

Quantitative mRNA Expression Profiles of Germin-Like and Extensin-Like Proteins under Drought Stress in *Triticum aestivum*

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ABSTRACT

Drought stress can severaly damage plant growth and the most important factor in the reduction of wheat yield in cultivated areas. Development of new methodologies to improve wheat productivity and quality under drought conditions have a primary importance. Extensin-like (*TaExtLP*) and Germin-like Protein (*TaGLP*) transcripts were selected from our RNAseq data for their relation with defense mechanism. We aim to show the expression patterns of these genes in drought tolerant (Gerek 79 and Müfitbey) and non-tolerant *T. aestivum* (Atay) cultivars under drought stress conditions using qRT-PCR technique. Extensin is the most abundant proteins present in the cell wall of higher plants and has an important role in plant defense through strengthening the cell wall and preventing tissue damage. GLPs are involve in different biological processes; e.g., disease resistance and superoxide scavenging metabolism. We established different mRNA expression regulation of Extensin like and Germin-like mRNAs in root and leaf tissues of tolerant and non-tolerant *T. aestivum* cultivars under drought stress. We observed GLP transcript was significantly up-regulated (5 fold) in 4 h drought- stressed root tissues of tolerant cultivar Gerek and then decreased in 8 h. On the other hand, there was no dramatic difference in leaf tissue of each cultivar. Extensin-like gene up-regulation was approximately 6 and 3.5 fold in 4 h stressed root tissues of tolerant cultivars. In leaf tissues, different expression pattern was observed in tolerant and non-tolerant cultivars. Drought stress caused to up-regulation (4 fold) in 4 h stressed leaf tissues of tolerant cultivar. On the contrary, down-regulation (4 fold) was identified in non-tolerant stressed leaf tissues. These results suggest that overexpression of Extensin-like gene under drought stress conditions may enhance drought tolerance. The qRT-PCR results from root and leaf tissues from 3 different cultivars were in agreement with our previous RNAseq data. This is the first report shows the expression profiles of these defense proteins under drought stress conditions in *T. aestivum*.

ARTICLE HISTORY
Received
17 May 2019
Accepted
25 June 2019

KEYWORDS
T. aestivum,
extensin-like protein,
germin-like protein,
drought,
qRTPCR

Introduction

Bread wheat, *Triticum aestivum* L. is one of the main fundamental crops for many countries including Turkey. Drought is the major factor affecting wheat yields throughout the world however; it is more problematic factor for wheat agriculture in arid regions

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including central and eastern Anatolian regions of Turkey. Especially in central Turkey, yield losses could reach up to 80% in some years (http://www.turkstat.gov.tr). Therefore, development of new methodologies to improve wheat productivity and quality under drought conditions has a primary importance. Improvement in wheat breeding for drought tolerance is difficult because of the complexity of quantifying and measuring drought traits.

In higher plants, drought stress causes physiological changes, including loss of turgor, reduced leaf water potential and osmotic adjustment [1, 2]. Turgor pressure is a crucial factor for cell growth regulation. The management of cell enlargement depends on the cell wall extensibility [3, 4]. Water stress causes a low turgor pressure and then leads regression of growth by reducing cell extensibility and cell expansion [5]. From our previous studies, drought-stress related genes were identified in *Triticum aestivum* (bread wheat) cultivars under drought conditions by RNASeq technology (Illumina HiSeq2000) [6]. The selection of most promising drought stress tolerant and sensitive genotypes was performed by slow drought treatment experiments with three biological replications for 12 bread wheat cultivars. Three of them were selected as drought tolerant (Gerek 79 and Müfitbey) and non-tolerant (Atay-85) bread wheat cultivars based on the results of the physiological and biochemical analyses [6]. From RNAseq data, we selected differently expressed Extensin-like protein (*TaExtLP*) and Germin-like (*TaGLP*) proteins important for the defense mechanism in biotic stress [7, 8, 9]. In this report, we aimed to investigate mRNA expression profiles of these genes in the root and leaf tissues of different *T. aestivum* cultivars under drought stress conditions. As a member of the family of Hyp-rich glycoproteins (HRGPs), extensin is the most abundant protein group present in the cell wall of higher plants and regulated developmentally in a tissue-specific [7, 8]. They play an important role in plant defense through strengthening the cell wall, preventing tissue damage, enabling attachment of symbiotic organisms or limiting the pathogen invasion and propagation. Germins and GLPs were firstly reported in wheat as a specific marker for the start of germination [9]. Dwarfism induction, cell morphology changes and disease increase (sheath blight and blast fungal) were observed in GLP1 downregulated in transgenic *O. sativa* [10].

We aim to illuminate the mRNA expression profiles of Extensin-like and Germin-like proteins under shock dehydration stress conditions in showing different drought tolerance *T. aestivum* cultivars. QRT-PCR analyses were performed for these defense proteins in the root and leaf tissues under normal and two different dehydration stress conditions.

Materials and Methods

Growth conditions

Three *T. aestivum* cultivars Gerek 79 and Müfitbey (drought-tolerant) and Atay 85 (non-tolerant) were used in this study. Seeds were surface sterilized with 70% alcohol and 30% sodium hypochlorite and pre-germinated in Petri dishes for 10 days at 4°C in the dark. Seedlings were transferred to 10 L plastic pots containing moistened perlite after the germination and grown in a plant growth room under 16/8 h; temperature 22-18°C; relative humidity 60%. Seedlings that at the same developmental stage were moved to hydroponic (continuously aerated ½ Hoagland's solution) culture, renewed every 3 days, and grown under controlled conditions in the plant growth room. Plants were removed from the hydroponic culture at the age of four leaf stage and treated dehydration shock stress for 4 h and 8 h under the same temperature and light conditions. From 3 wheat varieties (2 drought tolerant and 1 non-tolerant) root and leaf tissues were harvested and frozen with corresponding controls and stored -80°C until RNA isolation [11].

RNA extraction and cDNA synthesis

Total RNA extraction was performed by using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA) from 3 different *T. aestivum* cultivars according to the manufacturer's instructions. Extracted high-purity total RNAs from 4 h and 8 h drought stressed and corresponding control root and leaf tissues were measured by nanodrop (Thermo). RNA samples were treated with DNase I (10 U) (Roche, GmbH, Germany) for removing residual genomic DNA and purified following to the method described previously [12]. The integrity of RNA samples was controlled by running on 1% formaldehyde agarose gel and first-strand cDNA synthesis was performed to the manufacturer's (Roche High Fidelity cDNA synthesis kit) instructions in a 20 µl final volume containing 5 µg total RNA, 200 U MMLV RTase, 100 pmol oligo-dT (18 mer), 15 pmol dNTPs, and 20 U RNase inhibitor [12].

Quantitative real-time PCR

qRT-PCR reactions contained 75–200 ng of the cDNA as a template, 10 pmole of each primer, 12.5 µl SYBR Green (Roche FastStart Universal SYBR Green Master, Rox) in a total volume of 25 µl. QRT-PCR experiments were carried out in 96 well polypropylene plates and performed in triplicate for each sample with IQ5 System (BioRad, Hercules, USA). The following standard thermal profile was employed: After 95°C for 5 min for polymerase activation, amplification and quantification cycles (45 times) 95°C for 30 s, 55°C for 1 min. Melting-curve analysis was carried out for the specificity of the primer pairs after 45 amplification cycles (55-95°C). Housekeeping gene β-actin was used (AY663392) as an internal control. The following primer pairs (Table 1) were designed to amplify a 115-bp *TaExtLP* fragment and a 102 bp *TaGLP* 9.1 fragment. All the primers used in qRT-PCR experiments were designed by Primer 3 program. Three technical replicates were carried out in order to quantify transcript level accurately. The ΔΔCq values for all the transcripts were averaged across all the treatments and experimental replicates. Student's t-test (GraphPad Prism 6) was applied to check for the statistical significance between drought-treated and –untreated control groups.

Table 1 QRT-PCR Primer list

Primer Name	**Sequence 5'—3'**	**Product Size**
β-Actin F	GACAA TGGAACCGGAATGGTC	110 bp
β-Actin R	GTGTGATGCCAGATTTTCTCCATg	
***TaExtLP* F**	AACCAGGGAAAACACAT CTT	115bp
***TaExtLP* R**	GGCAACAACAACAACAACTA	
***TaGLP* 9.1 F**	CACCAG GGATCACTAGACTA	102bp
***TaGLP* 9.1 R**	TGTCCGGAA ATCATGAAACT	

Results

From our RNAseq data, we found Germin-like protein 9-1 and Extensin-like protein gene expressions were differentially expressed in root and shoot tissues [6]. The abundance of *TaExtLP* and *TaGLP* 9-1 mRNA under 4 h and 8 h shock drought stress treatment was examined in root and leave tissues of drought tolerant and non-tolerant *T. aestivum* genotypes (Fig. 1).

Fig 1 Drought non-tolerant Atay85, Drought tolerant Müfitbey, and Gerek 79 *T. aestivum* cultivars

After total RNA extraction from root and leaf tissues, removal of genomic DNA was carried out. Semi-quantitative RT-PCR method was used to control the synthesized cDNAs (Fig 2).

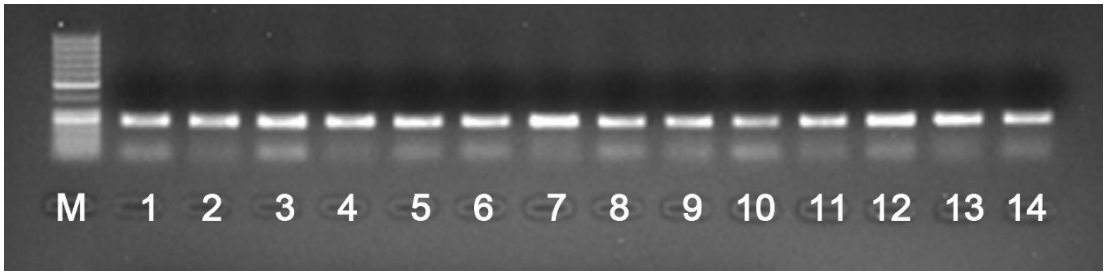

Fig 2 Agarose gel electrophoresis of amplified first-strand cDNAs by semi-qRT-PCR with housekeeping β-actin gene primers (M: GeneRuler 100bp DNA Ladder, MBI Fermentas)

Extensin-like protein (Arabidopsis, cell wall extensin) mRNA Expression: Extensin-like protein is the most abundant protein group present in the cell wall of higher plants

[7, 13]. Extensin expression in response to wounding, pathogen infection and ethylene treatment supports for the role of extensins in plant defense [14, 15, 16, 17, 8]. In our qRT-PCR experiments, it was observed that drought stress caused the up-regulation of this gene in root tissues. Maximum mRNA expression was observed in 4 h drought-stressed root tissues of tolerant and non-tolerant genotypes (Fig 3A, B, and C) .

In leaf tissues, *TaExtLP* mRNA was dramatically increased under 4 h drought-stressed Müfitbey.

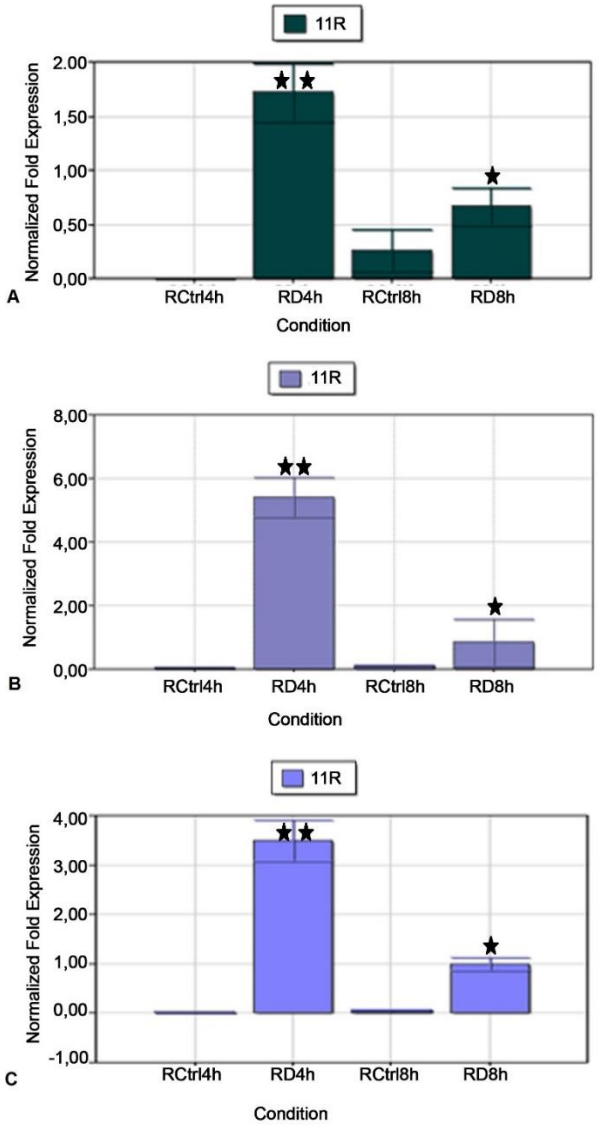

Fig 3 mRNA expression pattern of *TaExtLP* (cell wall) in 4 h and 8 h drought stressed root tissue of drought non-tolerant genotype Atay 85 (A), and Gerek (B), Müfitbey (C) cultivars. **RCtrl:** Root Control 4 h, **RD4h:** Root Drought 4 h. **RCtrl8h:** Root Control 8 h, **RD8h:** Root Drought 8 h. Error bars indicate the standard deviation of qRT-PCR each performed in triplicate. (*): $p \leq 0.05$, (**): $p \leq 0.01$.

On the other hand, Ext-like protein mRNA transcript was not much changed compared to the control tissue in 4 h drought stressed leaf tissues in non-tolerant genotype Atay. After 8 h of drought, mRNA expression level was not changed in leaf tissues (Fig 3).

Conversely, the different expression pattern was observed in leaf tissue of tolerant and non-tolerant genotypes. In non-tolerant genotype Atay 85, maximum mRNA was observed in 4 h drought stress treated leaf tissue and there was no significant difference in 8 h drought stress (Fig 4A). Whereas in the same tissue, down-regulation of this gene was shown in tolerant genotype Müfitbey (Fig 4B) and Gerek (data not shown).

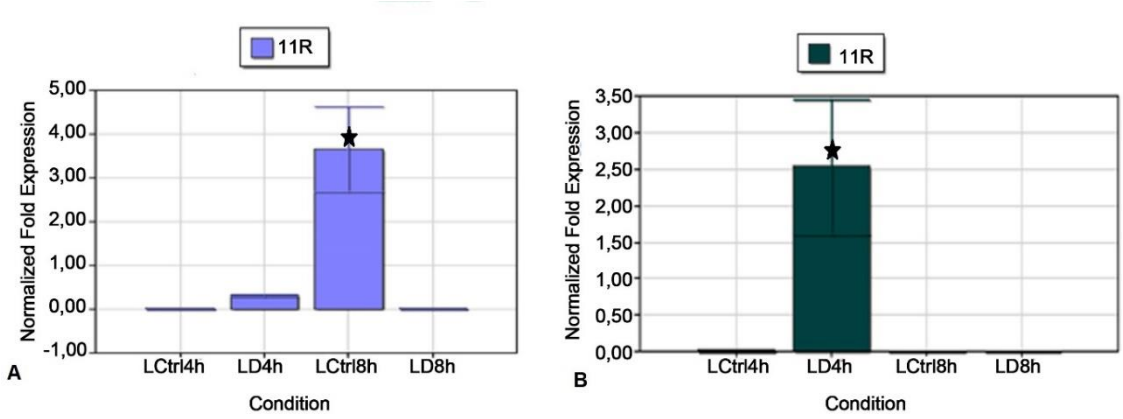

Fig 4 mRNA expression pattern of *TaExtLP* (cell wall) in 4 h and 8 h drought stressed leaf tissue of drought non-tolerant genotype Atay 85 (A), and non-tolerant Müfitbey (B) cultivars. **LCtrl4h:** Leaf Control 4 h, **LD4h:** Leaf Drought 4h, **LC8h:** Leaf Control 8h, **LD8h:** Leaf Drought 8h. Error bars indicate the standard deviation of qRT-PCR each performed in triplicate. (*): $p \leq 0.05$.

Germin-like proteins (GLPs) have been shown to implicate as plant cell defenders in many species to different conditions and diseases [19]. In root tissue, *GLP9-1* mRNA expression was induced by 4 h and 8 h drought stress (Fig 5A, B, and C). In the leaf tissue of non-tolerant genotype, there was no dramatic difference between control and drought stress. On the contrary, up-regulation was obtained in 4 h and 8 h drought stressed leaf tissues of tolerant genotype Müfitbey (Fig 6 B).

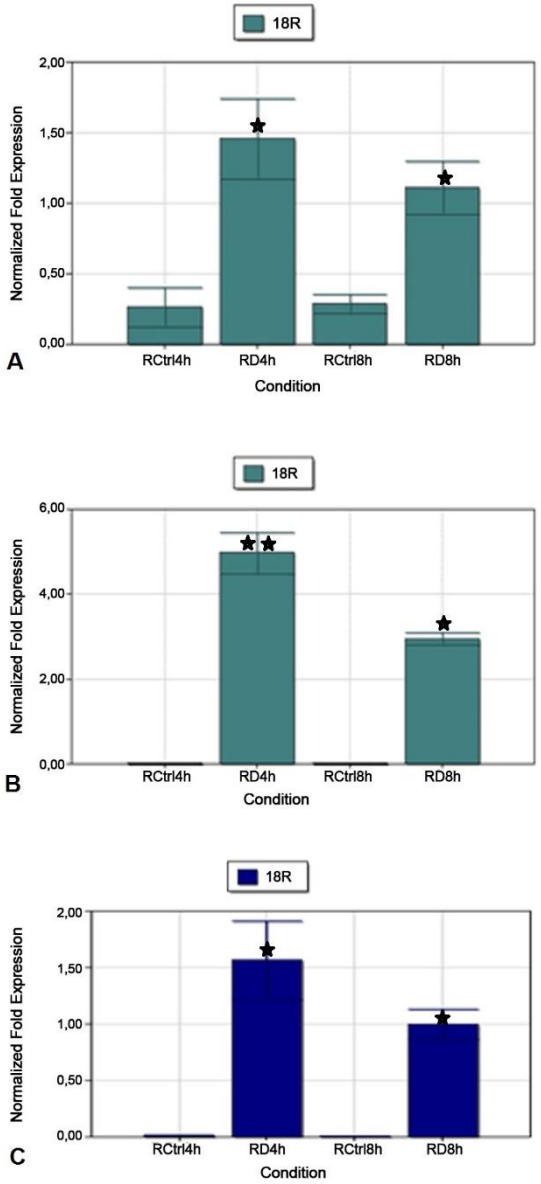

Fig 5 mRNA expression profile of *GLP9-1* in 4 h and 8 h drought stressed root tissue of drought non-tolerant genotype Atay 85 (A), and Gerek (B), Müfitbey (C) cultivars. **RCtrl:** Root Control 4h, **RD4h:** Root Drought 4h. **RCtrl8h:** Root Control 8h, **RD8h:** Root Drought 8h. Error bars indicate the standard deviation of qRT-PCR each performed in triplicate. (*): $p \leq 0.05$, (**): $p \leq 0.01$.

In root tissue, *GLP9-1* mRNA expression was induced by 4 h and 8 h drought stress (Fig 5A, B, and C). In the leaf tissue of non-tolerant genotype, there was no significant difference between control and drought stress. On the contrary, up-regulation was observed in 4 h and 8 h drought stressed leaf tissues of tolerant genotypes Müfitbey (Fig 6 B) and Gerek (data not shown).

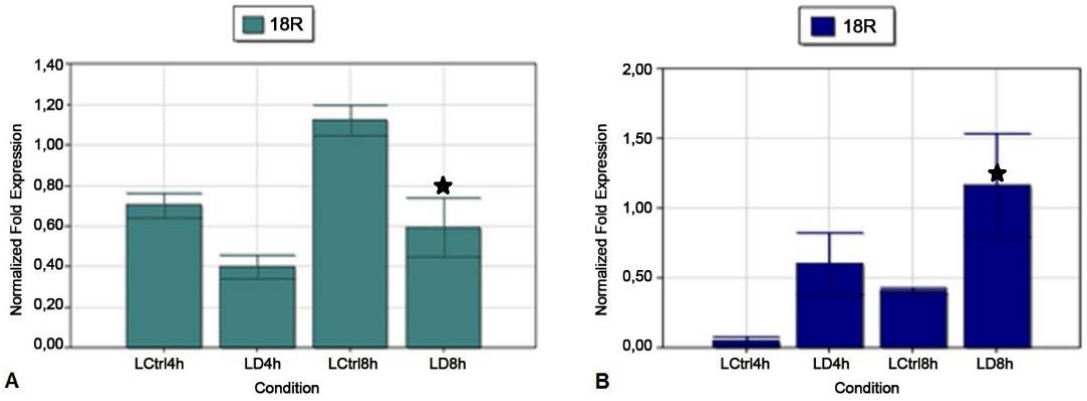

Fig 6 mRNA expression profile of *GLP9-1* in 4 h and 8 h drought stressed leaf tissue of drought non-tolerant genotype Atay 85 (A), and non-tolerant Müfitbey **(B)** cultivars. **LCtrl4h:** Leaf Control 4 h, **LD4h:** Leaf Drought 4 h, **LC8h:** Leaf Control 8h, **LD8h:** Leaf Drought 8h. Error bars indicate the standard deviation of qRT-PCR each performed in triplicate. (*): p ≤0.05.

Discussion

Extensin-like and Germin-like-genes are expressed in an organ-specific fashion and stress-related proteins in plants. Extensins are implicated in different biological processes such as embryonic development [20], root hair growth [21, 22], cell wall assembly and structure [23, 24], and biotic and abiotic stress responses [18, 25, 26]. Increased extensin accumulation and extensin cross-linking has been suggested to help in wound recovery and in the formation of a physical barrier against pathogens, thus avoiding the entry of pathogens into the vascular system [27]. In higher plants, the immune response can differ between leaf and root tissues [28, 29]. RNAseq data from *A. thaliana* showed that a significant number of genes (2424 genes) were differentially expressed between shoots and roots under normal conditions. Root-overexpression of at least nine encoded extensin proteins suggested specific roles of these glycoproteins in root tissues [30]. Glycine max extensin gene, *SbHRGP*3, expression has been shown in hypocotyl and the roots of seedlings [13]. In tomato, *LeExt*1, an Extensin-like protein expression was observed with tip growth, which proposes a role of the *LeExt*1 protein in root hair expansion. Comparative transcript analysis of *LeExt*1/GUS chimeric gene in four different transgenic plant species has proven its role in the regulation of apical/basal polarity in root tissues of transgenic tomato [31]. As a cell wall protein, extensins are also released into the root mucilage. Recently, Castilleux et al [32] reported a model about the influence of cell wall extensin on root secretions.

Up-regulation of cell wall modification related proteins lead to changes in the cell wall composition [33]. Different Expansin (*Exp*) and Extensin (*Xth*) mRNA gene expression profiles were reported at low temperatures in Arabidopsis and rice [34]. Extensin (*Xth*) down-regulation was observed to cold stress in Arabidopsis [35]. Freezing tolerance was improved by the overexpression of *AtXTH*21in transgenic Arabidopsis plants [35].
The Extensin-like gene identified from our RNAseq data was significantly up-regulated (about 4 fold) in response to 4 h and 8 h dehydration stress [6]. In this study, Extensin-like gene up-regulation was approximately 6 and 3.5 fold in 4 h stressed root tissues Gerek and Müfitbey cultivars. In leaf tissues in tolerant and non-tolerant cultivars, the different expression pattern was observed. Drought stress caused to up-regulation (4 fold) in 4 h stressed leaf tissues of Müfitbey. On the contrary, down-regulation (4 fold) was identified in Atay 4 h stressed leaf tissues. These results suggest that Extensin-like gene may have a role in drought tolerance.

Germin-like protein 9-1 (Oryza sativa subsp. japonica – apoplast, manganese ion binding, nutrient reservoir activity): Germins and GLPs were firstly reported in wheat as a specific marker for the start of germination [36]. Different enzymatic activities of six germin subfamilies (GER1-6) were identified with e.g. OXO activity in GER1 and SOD activity in GER2 [10]. The GER1 subfamily has also been reported to be involved in early plant development and germination [37, 38]. Dwarfism induction, cell morphology changes and disease increase (sheath blight and blast fungal) were observed in GLP1 down-regulated in transgenic rice plants [19]. GLPs have been studied in different plant species and implicated as plant cell defenders to biotic and abiotic stress conditions. GLPs have been reported to be resistant to proteases, extreme pH, heat, and sodium dodecyl sulphate [39].
GLP expression in *H. vulgarae* and a QTL on chromosome 8 of *O. sativa* have shown their involvement in disease resistance and complex trait of GLPs in cereal genomes [40, 41]. In our previous studies, we reported differential expression of GLP-like mRNA in ABA- treated wheat by DD mRNA experiments. In ABA-dependent pathway, GLP1 mRNA expression in ABA-treated plants rapidly increased in 1 h and maximal expression level was obtained in 8 h [40]. In the present study, we observed GLP transcript was significantly up-regulated (5 fold) in 4 h drought- stressed root tissues of tolerant cultivar

Gerek and then decreased in 8 h. On the other hand, there was no dramatic difference in leaf tissue of tolerant and non-tolerant cultivars. The qRTPCR results from root and leaf tissues from 3 different cultivars were in agreement with our RNAseq data.

Conclusion

The identification and elucidation of functional characteristics of the genes that play a role in the complex drought-response in wheat will be helpful for making the popular wheat varieties more productive with less amount of water. It is very significant to learn more about stress related genes for the elucidation of stress mechanism by transgenic plants. Although there are many studies about the Ext and GLP genes, the functions of these genes are still elusive. Downregulation or overexpression of Ext and GLP through gene editing methodology may shed more light on the functions of these genes in the future.

Acknowledgement
This work has been supported by the International Center for Genetic Engineering and Biotechnology (ICGEB) grand (#CRP/TUR09-03) to B. Cevher-Keskin. Bread wheat cultivars were provided from Agriculture and Forestry Translational Zone Agricultural Research Institute (Turkey) and the Ankara Agricultural Research Institute (Turkey).

Conflicts of Interest:
The author declare no conflict of interest.

Abbreviations
qRT-PCR: quantitative real time PCR, *TaGLP*: Germin-Like Protein of *T. aestivum, TaExtLP*: Extensin-like Protein of *T. aestivum*, RNAseq: RNA sequencing

References

1. Shao, H.B., et al., Water-deficit stress-induced anatomical changes in higher plants. C. R. Biology, 2008. 331: p.215–225.
2. Lisar, S.Y.S., et al., Water stress in plants: Causes, effects and responses. In Water Stress; Rahman, M., Hasegawa, H., Eds.; InTech: Rijeka, Croatia, 2012. p. 1–14.
3. Tardieu, F. Any trait or trait-related allele can confer drought tolerance: Just design the right
4. drought scenario. Journal Experimental Botany, 2011. 63: p.25–31.
5. Wolf, S., and S., Greiner, Growth control by cell wall pectins. Protoplasma, 2012. 249: p.169–175.
6. Tardieu, F., et al., Genetic and physiological controls of growth under water deficit. Plant Physiology, 2014. 164: p.1628–1635.
7. Cevher-Keskin, B., Microarray Analysis and Functional Characterization of Drought Stress Genes in Wheat, ICGEB CRP/TUR09-03, Final Project Report, 2015.
8. Ye, Z.H., and Varner, J.E. Tissue-specific expression of cell wall proteins in developing soybean tissues. Plant Cell, 1991. 3: p. 23–37.
9. Cassab, G. Plant cell wall proteins. Annu Rev Plant Physiol Plant Molecular Biology, 1998. 49: p. 281–309.

10. Dunwell, J., et al., Germin and germin-like proteins: evolution, structure, and function. Crit Review Plant Science, 2008. 27: p. 342–375.
11. Wu Y, et al., Expression and distribution of extensins and AGPs in susceptible and resistant banana cultivars in response to wounding and *Fusarium oxysporum*. Scientific Reports, 2017. 7: p. 42400.
12. Cevher-Keskin, B.C, et al., Quantitative expression analysis of *TaMPK4* and *TaTIP1* genes in drought tolerant and non-tolerant wheat (*Triticum aestivum* L.) cultivars. Plant Omics Journal, 2015. 8(3): p. 270-277.
13. Keskin B.C, et. al., Expression characteristics of ARF1 and SAR1 during development and the de-etiolation process. Plant Biology, 2012. 14: p. 24- 32.
14. Ahn, J.H., A novel extensin gene encoding a hydroxyproline-rich glycoprotein requires sucrose for its wound-inducible expression in transgenic plants. Plant Cell, 1996. 8: p. 1477–1490.
15. Tire, C., et al., Extensin gene expression is induced by mechanical stimuli leading to local cell wall strengthening in *Nicotiana plumbaginifolia*. Planta, 1994. 195: p. 175–181.
16. Hirsinger, C., et al., Characterization of a tobacco extensin gene and regulation of its gene family in healthy plants and under various stress conditions. Plant Molecular Biology, 1997. 33: p. 279–289.
17. Hirsinger, C., et al., The tobacco extensin gene Ext 1.4 is expressed in cells submitted to mechanical constraints and in cells proliferating under hormone control. Journal Experimental Botany, 1999. 50: p. 343–355.
18. Merkouropoulos, G., D.C. Barnett, and A.H. Shirsat, The Arabidopsis extensin gene is developmentally regulated, is induced by wounding, methyl jasmonate, abscisic and salicylic acid, and codes for a protein with unusual motifs. Planta, 1999. 208: p. 212–219.
19. Banerjee, J., M.K. Maiti, Functional role of rice germin-like protein1 in regulation of plant height and disease resistance. Biochem Biophys Res Communication, 2010. 394(1): p. 178–183.
20. Zhang, X., Y. Ren, J. Zhao, Roles of extensins in cotyledon primordium formation and shoot apical meristem activity in *Nicotiana tabacum*. Journal of Experimental Botany, 2008. 59: p. 4045–4058.
21. Velasquez, S.M., et al., O-glycosylated cell wall proteins are essential in root hair growth. Science, 2011. 332: p.1401–1403.
22. Velasquez, M., et al., Recent advances on the posttranslational modifications of EXTs and their roles in plant cell walls. Frontiers in Plant Science, 2012. 3: p. 93.
23. Pereira, C.S., et al., Extensin network formation in *Vitis vinifera* callus cells is an essential and causal event in rapid and H2O2-induced reduction in primary cell wall hydration. BMC Plant Biology, 2011.11: p.106.
24. Chormova, D., and S.C. Fry., Boron bridging of rhamnogalacturonan-II is promoted in vitro by cationic chaperones, including polyhistidine and wall glycoproteins. New Phytologist, 2016. 209: p. 241–251.
25. Sujkowska-Rybkowska M, and W. Borucki, Accumulation and localization of extensin protein in apoplast of pea root nodule under aluminum stress. Micron, 2014. 67: p. 10–19.
26. Zhang C., et al., Characterization of NtREL1, a novel root-specific gene from tobacco, and upstream promoter activity analysis in homologous and heterologous hosts. Plant Cell Reports, 2016. 35: p.757–769.
27. Showalter, A.M. Structure and function of plant cell wall proteins. Plant Cell, 1993. 5: p. 9–23.
28. Attard, A., et al., The immediate activation of defense responses in Arabidopsis roots is not sufficient to prevent *Phytophthora parasitica* infection. New Phytologist, 2010. 187: p. 449–460.
29. Balmer D, et al., Induced resistance in maize is based on organ-specific defense responses. The Plant Journal, 2013. 74: 213–225.
30. Widman, N., et al., Epigenetic differences between shoots and roots in Arabidopsis reveals tissue-specific regulation. Epigenetics, 2014. 9: p. 236–242.
31. Bucher, M., et al., The Expression of an Extensin-Like Protein Correlates with Cellular Tip Growth in Tomato. Plant Physiology, 2002. 128: p. 911-923

32. Castilleux, R., et al., Cell wall extensins in root–microbe interactions and root secretions Journal of Experimental Botany, 2018. 69(18): p. 4235–4247, https://doi.org/10.1093/jxb/ ery238
33. Zabotin, A.I., et al., Alterations in cell walls of winter wheat roots during low temperature acclimation. J. Plant Physiology, 1998. 152: p.473–479.
34. Imin, N., et al., Effect of early cold stress on the maturation of rice anthers. Proteomics, 2004. 4*:* p.1873–1882.
35. Seki, M., et al., Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant Journal, 2002. 31*:* p. 279–292.
36. Shi, H., AtHAP5A modulates freezing stress resistance in Arabidopsis through binding to CCAAT motif of AtXTH21. New Phytologist, 2014. 203*:* p. 554–567.
37. Membré, N., et al., *Arabidopsis thaliana* germin-like proteins: common and specific features point to a variety of functions. Planta, 2000. 211(3): p. 345–354.
38. Federico, M.L., et al., Spatial and temporal divergence of expression in duplicated barley germin-like protein-encoding genes. Genetics, 2006. 174(1): p.179–190.
39. Zimmermann, G., et al., The multigene family encoding germin-like proteins of barley. Regulation and function in basal host resistance. Plant Physiology, 2006, 142(1): p. 181–192
40. Manosalva, P., et al., A germin-like protein gene family functions as a complex qtl conferring broad-spectrum disease resistance in rice. Plant Physiology, 2008. 149(1): p. 286–296
41. Keskin, B.C, et al., Abscisic acid regulated gene expression in bread wheat (*Triticum aestivum* L.) Australian J Crop Science, 2010. 4: p. 617-625
42. Yi, H., and Richards, E.J., A cluster of disease resistance genes in Arabidopsis is coordinately regulated by transcriptional activation and RNA silencing. Plant Cell, 2007. 19(9): p. 2929–2939

Research Article

Samsulrizal, N.H. and N.Y. Yusof, In Silico Prediction of Cell Wall Remodeling Genes in Tomato, Banana, Melon and Grape International Journal of Life Sciences and Biotechnology, 2019. 2(2): p. 108-121.

In Silico Prediction of Cell Wall Remodeling Genes in Tomato, Banana, Melon and Grape

Nurul Hidayah Samsulrizal[1]*, Nik Yusnoraini Yusof [2]

ABSTRACT

Ripening is a complex developmental process and involves many events such as textural and constitutional changes. The texture of fleshy fruits is one of the major criteria for consumer choice. However, the molecular determinants of ripening- associated changes in texture or "softening" are relatively poorly understood and seem to involve a large number of cell wall remodelling factors. The recent completion of the tomato genome sequence has revealed more than 50 cell wall structure-related genes that are expressed during fruit development and ripening and may impact texture changes in this fruit. The aim of the project is to compare, on a genome-wide scale, ripening-related gene expression in a range of fleshy fruits and especially those linked with cell wall remodelling via computer simulation. Then by identifying orthologous genes in different fruit species to make predictions about those genes likely to important for the softening process in all fleshy fruits. Comparative genomics analysis of tomato (*Solanum lycopersicum*), banana (*Musa acuminate*), melon (*Cucumis melo*) and grape (*Vitis vinifera*), has been undertaken using Inparanoid, Multiparanoid and BLAST2GO software. This analysis showed that a total of 8,982 (25.86%) gene models could be identified in common between all four genomes based on comparison of amino acid sequences. Of these genes, 262 in tomato, 252 in grape, 261 in melon, and 198 in banana were identified as encoding cell wall structure-related proteins. However, comparison of the expression patterns of these genes revealed that most were expressed in tissues other than ripening fruits, and of the fruit expressed genes only a small number were common between different fruit species. This in silico analysis should provide additional clues as a target for manipulation of fruit softening in a range of fleshy fruit species. These also provide new opportunities to develop varieties of tomatoes that can survive the trip from the farm to the grocery store whilst maintaining excellent flavour and shelf-life.

ARTICLE HISTORY
Received
29 May 2019
Accepted
01 July 2019

KEY WORDS
Comparative genomics, inparanoid, cell wall

Introduction

The revolution in DNA sequencing technology in the last 10 years has enabled the sequencing and assembly of hundreds of genomes from organisms across the tree of life. These genomes include those from a wide range of fruit species. The objective of this

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project was to undertake a comparative genomics study to reveal similarities and differences in the types of cell wall-structure genes expressed in a range of fleshy fruit species. The fruit species chosen were tomato (*Solanum lycopersicum*), melon (*Cucumis melo*), grape (*Vitis vinifera*) and banana (*Musa acuminata*). Tomato is the model for investigations on the mechanistic basis of ripening and was chosen as our experimental platform and the genome assemblies for melon, grape and banana were of sufficient quality, annotated and with expression data to allow the bioinformatics analysis. The approach was made to identify related gene families and identify orthologous genes across these species.

Gene families can be defined as a set of genes inherited from a common ancestor that maintain their sequence and functional similarities [1]. This concept includes the gene paralogs (pairs of genes with similarities in sequence in the same species) and orthologs (genes that share similar sequences and have the same function in different species). Genes in the same family are expected to maintain their molecular structure and biochemical functions in different organisms [2] and sequence clustering helps to identify the gene family.

Studying relationships between genes within a family can provide important structure-function information and provides evidence for ancient genome duplications and *neofunctionalisation* as is apparent in tomato [3]. The changes in number of genes that are involved in certain biological processes could occur in several scenarios, for example, gene duplication that leads to gene families with multiple copies of genes which encode the same or related functions. The classic model in molecular biology assumes that duplication of a gene will generate several new genes that are free to be mutated, as long as one of the original genes retains its function [4]. The most likely outcome for these paralogues is that they degenerate into pseudogenes that are not transcribed (*nonfunctionalization*) [5, 6]. Alternatively, paralogs may still be functional and through mutation diverge from the original function (*neofunctionalization*) [4].

Here, the cell wall genes in melon, grape and banana were compared with those in tomato. The hypothesis to be tested was that cell wall structure-related genes that are common to all fleshy fruits and expressed during softening are likely to be key targets to allow the

manipulation of fruit firmness. These genes will be the targets of our next experiments in generating transgenic tomato. By identifying and manipulating these genes, it is hoped to improve tomato production in the future.

Materials and Methods

Similarity Search using BLAST

BLAST [7] programs used in this study were blastp (Protein-protein BLAST), blastn (Nucleotide-nucleotide BLAST) and tblastx (Nucleotide 6-frame translation-nucleotide 6-frame translation). The parameter used is the default parameter which is BLOSUM (BLOcks SUbstitution Matrix). BLOSUM62 with opening value space is 11 and the extension value space is 1. The BLAST program that is used in this study was downloaded from NCBI database and installed into a LINUX system on an external server. The similarity search analyses were then undertaken by batch collection (*batch blast*).

BLAST database format

In this study, formatDB was used for making a custom BLAST database. Putative cell wall structure-related genes were selected in each of the genome. This file was then used to construct the index for the BLAST database using a program from NCBI using a "formatdb" command.

Comparative analysis using in paranoid and multiparanoid

The genome sequences from four species of fleshy fruit, banana (*Musa acuminata*), melon (*Cucumis melo*) and grape (*Vitis vinifera*) were compared with that of tomato (*S. lycopersicum*). Only genomes that were assembled, annotated and had associated fruit-related expression data were used. All the pairwise proteome data from the Inparanoid [8] approach were then brought together by using Multiparanoid script. MultiParanoid is a powerful approach for searching for gene clusters among multiple strains so the pairwise orthologous clusters that had been generated from Inparanoid were directly transferred to it.

Gene classification by gene ontology (GO)

GO will provide a comparison of the classification of genes among genomes being studied [9]. It also overcomes issues in linking genes that have been annotated by different researchers when comparison of genes between species is required [10]. The results from

GO classification were used to identify all genes that had a putative cell wall base on cellular component distribution.

Manual curation with transcriptome database

Candidate genes will be manually selected and annotated based on GO classification and annotation from tomato genome (our reference genome). Then, the cell wall remodelling genes were selected out of all the proteins that were identified as orthologs in all four genomes. The curations also have been done using all transcriptome data from NCBI database, which focused on those genes expressed during ripening.

Results

Ortholog analysis

In this research, comparative analysis was done with the detection of orthologous groups using the Inparanoid program which applies *all-versus-all* sequence comparisons of two genomes with the special rules of cluster analysis. Although phylogenetic tree is a well-established method to distinguish orthologs and has been used to study the evolution of organisms, it is time-consuming and prone to errors [11]. Thus, the Inparanoid program was used as an alternative to the phylogenetic method. The *S. lycopersicum* gene models were compared to the list of genes from *C. melo*, *V. vinifera* and *M. acuminata*. The orthologs for each genome that were generated from Inparanoid program were sorted and viewed using Microsoft Excel program. The numbers of genes shared among all four species were obtained and calculated. Then, the cell wall remodelling genes in this group were identified. *V. vinifera* and *C. melo* were compared with *S. lycopersicum* because all of them were dicot genomes and the genomes have been completely sequenced [3, 12, 13, 14]. *M. acuminata* was used in a comparative analysis because it is a representative for monocot fleshy fruit bearing species [15]. From this analysis (Figure 1.1), a total of 3,013 of the predicted *S. lycopersicum* genes have orthologs in *C. melo*, whereas 2,763 of the gene models are shared in *V. vinifera* sequences and 1,607 of the gene models are represented by orthologous sequences in *M. acuminata*. The different trends of orthologous relationships between *S. lycopersicum* with the two dicots, *C. melo* and *V. vinifera*, and with the monocot *M. acuminata* likely reflect evolutionary processes that occurred in the ancestral genomes

of each group. Figure 1.1 also shows that all the dicot species (*S. lycopersicum*, *C. melo*, and *V. vinifera*) share a total of 1,340 orthologous sequences while 8,982 (26 %) orthologous genes were found to be common to all four species.

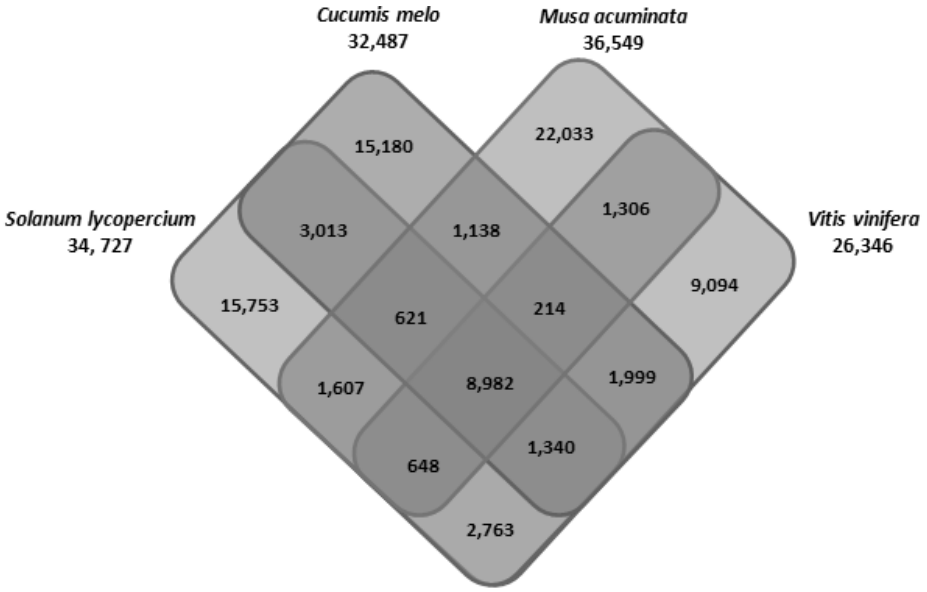

Fig 1-1 Venn diagram of the orthologous genes between *S. lycopersicum*, *M. acuminata*, -*C. melo* and *V. vinifera*. Numbers in the area of overlap indicate the number of orthologs predicted by reciprocal Inparanoid v 2.0 analysis (threshold E-value = $1x10^{-5}$)

Gene classification from GO

The Gene Ontology (GO) [http://www.geneontology.org] approach is probably the most widespread and the most extensive annotation scheme for the functional description of gene products [16]. A straight forward mapping of gene sequences was made using homology searches (blast hit) [17] to retrieve the GO terms associated with the hit obtained from the Blast results. The 8,982 sequences that had significant blast hits were loaded for GO mapping in Blast2GO suite (Table 1-1). GO terms of a total of 7,791 sequences were successfully assigned based on Gene Ontology Consortium in term of biological process, molecular function and cellular components which then were loaded through mapping to Gene Ontology database. From these, all sequences were assigned to biological processes, molecular functions and cellular components which GO terms could be assigned for more than one term for one sequence and each category was divided into other subcategories.

Table 1-1 Gene Ontology analysis generated using Blast2GO suite

	Quantity	Percentage (%)
Sequence has significant similarity (e-value $\leq 10^{-5}$)	8,982	100
Sequence has Gene Ontology assignment	7,791	86.74

Data mining for cell wall remodeling genes

Fruit softening is a complex process characterized by sequential disassembly and degradation of cell wall components mediated cooperatively by cell wall modifying enzymes [18]. There is also evidence for renewed cell wall biosynthesis during this process [19]. Five GO IDs were used in the identification of cell wall genes common between all orthologous groups, these were GO: 0005623, GO: 0005618, GO: 0044464, GO: 0030312, and GO: 0071944. Then, by using tomato as a model, comparison between the GO classification and tomato genes annotated as linked to cell wall structure, and remodelling [3] was undertaken manually using Microsoft Excel. The Tomato Genome Consortium (2012) reported there were 718 genes that were identified as cell wall structure-related genes in tomato genome. This information was then mapped to the orthologous cell wall sequences. The mapping resulted revealed that there is 262, 261, 252 and 198 cell wall structure-related genes where sequence were highly related in tomato, melon, grape and banana, respectively.

Discussion

Although there are numerous cell wall-related genes, not all are involved in the fruit softening process. For example, in tomato, out of more than 700 genes linked to cell wall metabolisms, only just over 50 were expressed in developing and ripening fruits [3]. Thus, peach and nectarine cultivars also reported only 14 cell wall related genes changed in expression in all cultivars tested [20]. Cell wall classification results using GO terms were mapped to transcriptome data from each of the fruits to identify the genes expressed during fruit development and ripening.

In tomato, 52 cell wall-related genes have been identified as being expressed during fruit development and ripening. However, only 12 genes showed large changes in expression during the ripening process and these included pectin methyl-esterases (PME), pectate lyases (PL), polygalacturonases (PG), and xyloglucan endotransglicosidases (XET) [3]. In melon and grape, approximately 100-200 cell wall genes were identified as expressed during fruit development and ripening which included those that encoded polygalacturonase, pectate lyase, cellulose, and xyloglucan endotransglucosylase. In banana fruits, around 90 genes were expressed during the developing and ripening stage [15], during ripening in bananas some of the most highly expressed cell wall genes were those encoding pectate lyase (2 genes), polygalacturonases (6 genes), pectinacetyl esterases (6 genes), xyloglucan endotransglucosylase/hydrolases (5 genes) and expansins (3 genes) [21].

Although the range of fleshy fruit species studied showed the expression of many of the same families of cell wall-related genes, the most surprising observation was that only a small number of truly orthologous genes were apparent that were expressed in all species (Supplementary Table 1). These include β-glucosidase, cellulose synthase, expansins, polygalactruonase and pectate lyases which were expressed in a wide range of fruits including others not studied in detail here such as apple [22] and strawberry [23].

In tomato, there are three *PL* genes that are expressed during fruit development and ripening (Solyc03g111690, Solyc05g014000 and Solyc06g083580) (Figure 1-2). The only one of these genes that has a close ortholog in melon, grape or banana was Solyc05g014000. However, this gene is expressed in tomato principally, during fruit development [3, 15]. Solyc06g083580, is expressed only in developing tomato fruits, but expression during ripening is very low [3] which fruit expressed orthologues in melon and grape [12, 13].

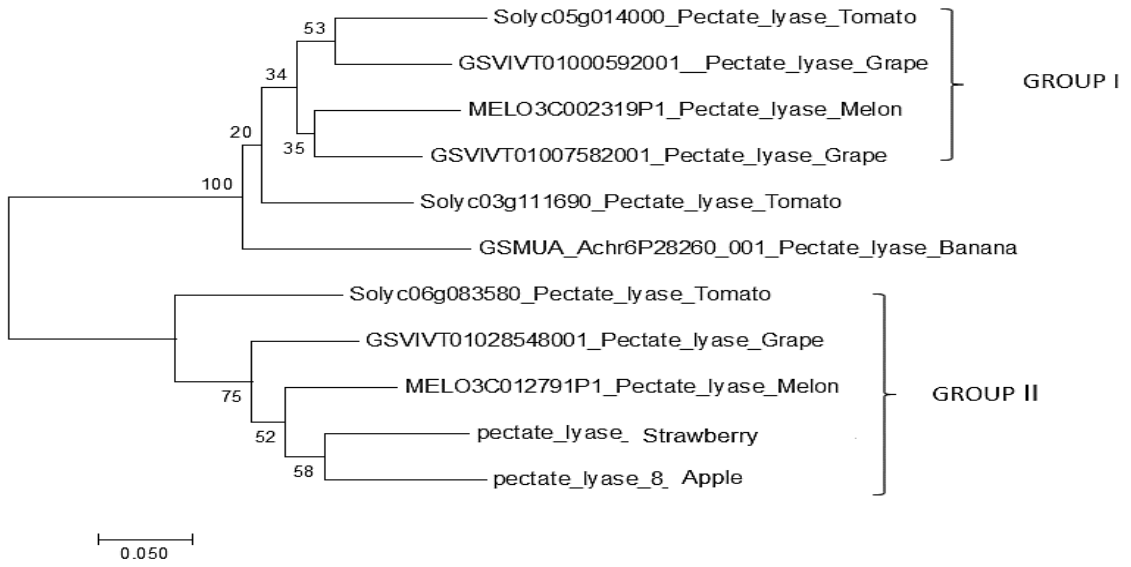

Fig 1-2 Molecular phylogenetic tree of pectate lyase (PL) amino acid sequences and the similar amino acid sequences in studied fruits. The dendrogram was generated by Mega 7.0 software using MUSCLE for the alignment and the maximum likelihood method for the construction of the phylogeny. Bootstrap tests were performed using 1,000 replicates and the percentage of the bootstrap value are shown in each branch where the value exceeds 50% is considered significant. The branch lengths are proportional to the phylogenetic distances

Interestingly, Solyc03g111690 is highly expressed in tomato during fruit ripening, but it does not have a close ortholog in melon, grape or banana. PL was shown to be important in fruit softening in banana [24], strawberry [25], apple [14] and very recently work in the Seymour lab has shown it is very important in tomato [26].

Tomato *PG* is perhaps the best know pectin degrading enzyme in tomato and is encoded by the gene Solyc10g080210. Orthologues of this gene are present in melon, grape and banana and fruit related expression of these orthologs occurs in tomato, melon and grape. In apple the *PG* most highly expressed during ripening [22] was more closely related to the tomato gene Solyc05g049980 (Figure 1-3) which was lowly expressed in ripening tomato fruits. In addition, Solyc06g009200, which is not expressed in developing or ripening tomato fruits, is orthologous to a gene that modulates softening in strawberry [27]. These data help highlight that species utilize a range of gene family members during cell wall disassembly.

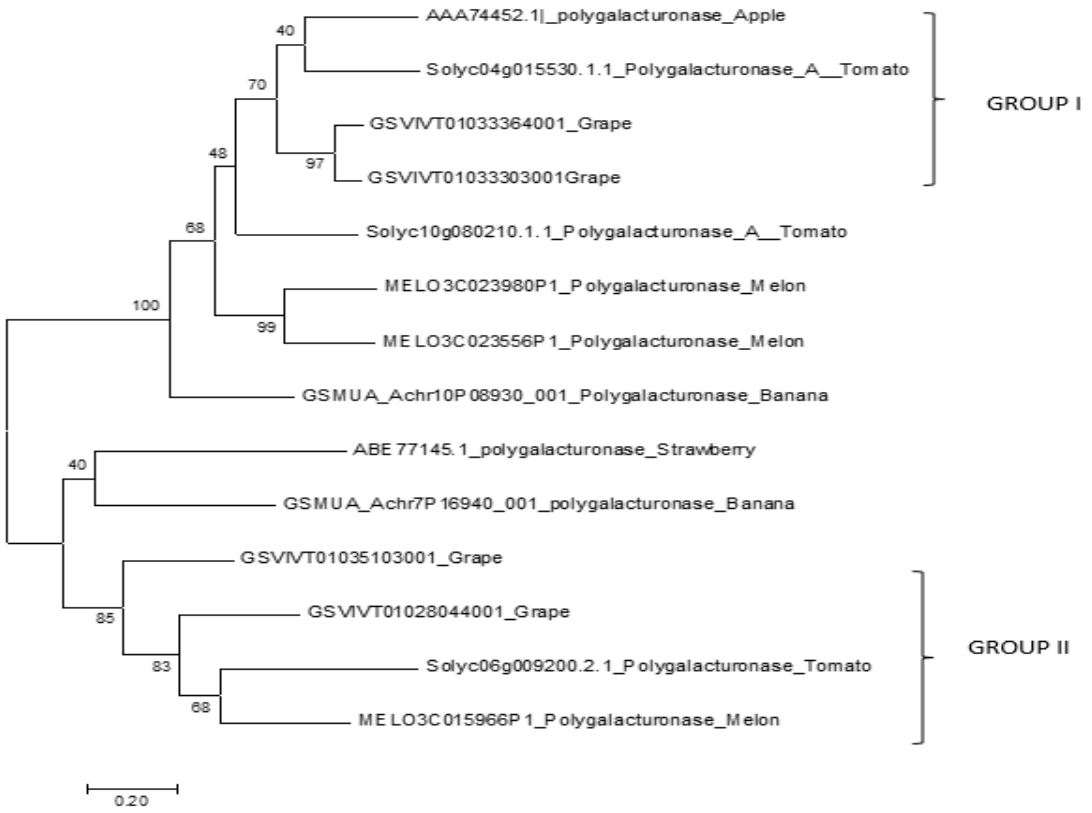

Fig 1-3 Molecular phylogenetic tree of polygalacturonase (PG) amino acid sequences and the similar amino acid sequences in studied fruits. The dendrogram was generated by Mega 7.0 software using MUSCLE for the alignment and the maximum likelihood method for the construction of the phylogeny. Bootstrap tests were performed using 1,000 replicates and the percentage of the bootstrap value are shown in each branch where the value exceeds 50% is considered significant. The branch lengths are proportional to the phylogenetic distances

One of the gene families where orthologues showed expression in all fruits were those encoding a CesA-like gene (Solyc08g061100) and also a glucan endo-beta glucosidase-like protein (Solyc03g115200) (Supplementary Table 1). During fruit development cellulose synthases are highly expressed in tomato and then their levels decrease at the breaker stage [3]. They are likely to be involved in the biosynthesis of cellulose [28], but the function of the CesA-like gene product from Solyc08g061100 has not been investigated in fruits. The role of the putative glucan endo-beta-glucosidase-like protein is even more obscure where in tomato its expression declines during fruit development and then increases during

breaker stage (Figure 1-4). Glucan endo-beta-glucosidase has a role in callose decomposition and others have reported that they are expressed during ripening [29].

Fig 1-4 Gene expression patterns based on Reads Per Kilobase of transcript per Million mapped reads (RPKM) value of *cesA*-like gene and β-glucosidase in tomato during fruit development and ripening (Tomato Genome Consortium, 2012)

Conclusion

In this project, the aim to select genes that were common to all the fleshy fruit species examined with respect to expression during fruit ripening. A comparison of the relationship between cell wall related genes in ripening tomato, melon, grape and banana revealed that there were only a small number of cell wall genes that were likely orthologues and expressed in all fruits that were surveyed. A limited number of these would then be targeted for further functional analysis.

Acknowledgments

I wish to thank International Islamic University Malaysia (IIUM) Research Initiative Grant Scheme (RIGS17-022-0597) for sponsored this work and The University of Nottingham, UK for providing the facilities.

References

1. Doolittle, R.F., Similar amino acid sequences: chance or common ancestry. Science, 1981. 214, pp.149-159.
2. Chothia, C., Lesk, A.M. The relation between the divergence of sequence and structure in proteins. The EMBO journal, 1986. 5(4):823-6.
3. Tomato Genome Consortium, The tomato genome sequence provides insights into fleshy fruit evolution. Nature, 2012. 485, pp.635-641.
4. Ohno, S. Evolution by gene duplication. 1970. New York: Springer-Verlag.
5. Nei, M. and Roychoudhury, A.K. Probability of fixation of nonfunctional genes at duplicate loci. American Naturalist, 1973. pp.362-372.
6. Petrov, D.A. and Hartl, D.L., Pseudogene evolution and natural selection for a compact genome. Journal of Heredity, 2000. 91, pp.221-227.
7. Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. Basic local alignment search tool. Journal of molecular biology, 1990. 215(3):403-10.
8. O'brien, K. P., Remm, M. and Sonnhammer, E. L. L. Inparanoid: a comprehensive database of eukaryotic orthologs. Nucleic Acids Research. 2005. 33, 476-480.
9. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T. and Harris, M.A., Gene ontology: tool for the unification of biology. *Nature genetics*, 2000. *25*(1), p.25.
10. Stein, L., Genome annotation: from sequence to biology. Nature Reviews Genetics, 2001. 2, pp.493-503.
11. Remm, M., Storm, C.E. and Sonnhammer, E.L., Automatic clustering of orthologs and in-paralogs from pairwise species comparisons. Journal of Molecular Biology, 2001. 314, pp.1041-1052.
12. Garcia-Mas, J., Benjak, A., Sanseverino, W., Bourgeois, M., Mir, G., González, V.M., Hénaff, E., Câmara, F., Cozzuto, L., Lowy, E. and Alioto, T., The genome of melon (Cucumis melo L.). Proceedings of the National Academy of Sciences, 2012. 109, pp.11872-11877.
13. Jaillon, O., Aury, J.M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., Choisne, N., Aubourg, S., Vitulo, N., Jubin, C. and Vezzi, A., The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature, 2007. 449, pp.463-467.
14. Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., Fontana, P., Bhatnagar, S.K., Troggio, M., Pruss, D. and Salvi, S., The genome of the domesticated apple (Malus domestica Borkh.). Nature genetics, 2010. 42, pp.833-839.
15. D'hont, A., Denoeud, F., Aury, J.M., Baurens, F.C., Carreel, F., Garsmeur, O., Noel, B., Bocs, S., Droc, G., Rouard, M. and Da Silva, C., The banana (Musa acuminata) genome and the evolution of monocotyledonous plants. Nature, 2012. 488(7410), p.213.
16. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A. Gene ontology: tool for the unification of biology. Nature genetics, 2000. 25(1):25.
17. Conesa, A., Götz, S., García-Gómez, J.M., Terol, J., Talón, M., Robles, M. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, 2005. 21(18):3674-6.
18. Seymour, G.B., Østergaard, L., Chapman, N.H., Knapp, S. and Martin, C., Fruit development and ripening. Annual Review of Plant Biology, 2013. 64, pp.219-241.

19. Vicente, A. R., Saladié, M., Rose, J. K. C. and Labavitch, J. M. The linkage between cell wall metabolism and fruit softening: looking to the future. Journal of the Science of Food and Agriculture, 87, 2007. 1435-1448.
20. Nilo, P. R., Campos-Vargas, R., Orellana, A. Assessment of Prunus persica fruit softening using a proteomics approach. Journal of Proteomics, 2012. 75, pp. 1618–1638.
21. Mbéguié-A-Mbéguié, D., Hubert, O., Baurens, F.C., Matsumoto, T., Chillet, M., Fils-Lycaon, B. and Sidibé-Bocs, S., Expression patterns of cell wall-modifying genes from banana during fruit ripening and in relationship with finger drop. Journal of Experimental Botany, 2009. p.erp079.
22. Atkinson, R.G., Sutherland, P.W., Johnston, S.L., Gunaseelan, K., Hallett, I.C., Mitra, D., Brummell, D.A., Schröder, R., Johnston, J.W., Schaffer, R.J. Down-regulation of POLYGALACTURONASE1 alters firmness, tensile strength and water loss in apple (Malus x domestica) fruit. BMC plant biology. 2012, 12(1):129.
23. Jiménez-Bermúdez, S., Redondo-Nevado, J., Muñoz-Blanco, J., Caballero, J. L., López-Aranda, J. M., Valpuesta, V., Pliego-Alfaro, F., Quesada, M. A. & Mercado, J. A. Manipulation of Strawberry Fruit Softening by Antisense Expression of a Pectate Lyase Gene. Plant Physiology, 2002. 128, 751-759.
24. Marín-Rodríguez, M.C., Orchard, J. and Seymour, G.B., Pectate lyases, cell wall degradation and fruit softening. Journal of Experimental Botany, 2002. 53, pp.2115-2119.
25. Shulaev, V., Sargent, D.J., Crowhurst, R.N., Mockler, T.C., Folkerts, O., Delcher, A.L., Jaiswal, P., Mockaitis, K., Liston, A., Mane, S.P. and Burns, P., The genome of woodland strawberry (Fragaria vesca). Nature Genetics, 2011. 43, pp.109-116.
26. Uluisik, S., Chapman, N.H., Smith, R., Poole, M., Adams, G., Gillis, R.B., Besong, T.M., Sheldon, J., Stiegelmeyer, S., Perez, L. and Samsulrizal, N., Genetic improvement of tomato by targeted control of fruit softening. Nature biotechnology, 2016. 34, pp.950-952.
27. García-Gago, J.A., Posé, S., Muñoz-Blanco, J., Quesada, M.A. and Mercado, J.A., The polygalacturonase FaPG1 gene plays a key role in strawberry fruit softening. Plant Signaling & Behavior, 2009. 4, p.766.
28. Delmer, D.P., Haigler, C.H. The regulation of metabolic flux to cellulose, a major sink for carbon in plants. Metabolic engineering, 2002. 4(1):22-8.
29. Goulao, L.F. and Oliveira, C.M., Cell wall modifications during fruit ripening: when a fruit is not the fruit. Trends in Food Science & Technology, 2008. *19*, pp.4-25.

Supplementary Table 1 Cell wall structure related genes expressed in tomato and likely orthologues in melon, grape and banana. These genes are expressed during fruit development and ripening

Gene Description	Short name	Tomato Gene ID	Melon Gene ID	Grape Gene ID	Banana Gene ID
Xyloglucan endotransglucosylase/hydrolase 9	SlXTH3d	Solyc03g093080	MELO3C017478P1	GSVIVT01029162001	GSMUA_Achr10P30870_001
Xyloglucan endotransglucosylase/hydrolase 14	SlXTH5	Solyc01g081060	MELO3C003441P1	GSVIVT01013055001	GSMUA_Achr1P23980_001
Xyloglucan endotransglucosylase/hydrolase 5	SlXTH16	Solyc07g052980	MELO3C018785P2	GSVIVT01000416001	GSMUA_Achr9P29980_001
Xyloglucan endotransglucosylase/hydrolase 2	SlXTH26	Solyc05g005680	MELO3C012004	GSVIVT01020228001	GSMUA_Achr3P10480_001
Xyloglucan endotransglucosylase/hydrolase		Solyc03g031800	MELO3C002480P1	GSVIVT01012635001	GSMUA_Achr1P08500_001
Beta-galactosidase	TBG6	Solyc02g084720	MELO3C007872P1	GSVIVT01018853001	GSMUA_Achr9P21610_001
Polygalacturonase A	PG-2a	Solyc10g080210	MELO3C023556	GSVIVT01033303001, GSVIVT01033364001	GSMUA_Achr10P08930_001
Polygalacturonase		Solyc05g049980	MELO3C006092P1	GSVIVT01019405001	GSMUA_Achr11P02870_001
Pectinesterase inhibitor		Solyc06g009190	MELO3C015963P1	GSVIVT01028041001	GSMUA_Achr11P05430_001
Pectinesterase inhibitor		Solyc07g017600	MELO3C013699P1	GSVIVT01023135001	GSMUA_Achr7P15620_001
Pectate lyase 1-27		Solyc06g083580	MELO3C012791	GSVIVT01028548001	GSMUA_Achr7P04580_001
Pectate lyase		Solyc05g014000	MELO3C002319P1	GSVIVT01000592001, GSVIVT01007582001	GSMUA_Achr6P28260_001
Mannan endo-1 4-beta-mannosidase		Solyc02g084990	MELO3C007842	GSVIVT01009746001, GSVIVT01018923001	GSMUA_Achr4P02940_001
Glucan endo-1 3-beta-glucosidase 1		Solyc03g115200	MELO3C017220	GSVIVT01007873001	GSMUA_Achr4P32790_001
Fasciclin-like arabinogalactan protein 19		Solyc07g045440	MELO3C024192P1	GSVIVT01014684001	GSMUA_AchrUn_randomP25500_001

Fasciclin-like arabinogalactan protein 10		Solyc10g005960	MELO3C024938P1	GSVIVT01030085001	GSMUA_AchrUn_randomP25500_001
Expansin	LeEXP1	Solyc06g051800	MELO3C025907P1, MELO3C003134P1	GSVIVT01024946001	GSMUA_Achr2P16370_001
Expansin (EXPA3)		Solyc03g031840	MELO3C015695P2	GSVIVT01023857001	GSMUA_Achr1P19730_001
Endoglucanase 1	Cel8	Solyc08g082250	MELO3C016287P1	GSVIVT01019523001	GSMUA_Achr4P08520_001
Endoglucanase 1		Solyc04g081300	MELO3C003760P1	GSVIVT01009881001	GSMUA_Achr4P19910_001
Cellulose synthase-like		Solyc11g066820	MELO3C017935P1	GSVIVT01028071001	GSMUA_Achr3P24160_001
Cellulose synthase		Solyc01g087210	MELO3C023114	GSVIVT01033297001	GSMUA_AchrUn_randomP09460_001
Cellulose synthase		Solyc08g061100	MELO3C003689	GSVIVT01035830001	GSMUA_Achr5P06050_001

Research article

Dogan Kubra, P.K. Akman, F. Tornuk, Improvement of Bioavailability of Sage and Mint by Ultrasonic Extraction. International Journal of Life Sciences and Biotechnology, 2019. 2(2): p.122- 135.

Improvement of Bioavailability of Sage and Mint by Ultrasonic Extraction

Kubra Dogan*[1] Perihan Kubra Akman[1], Fatih Tornuk[1]

ABSTRACT

Plant extracts are complex mixtures obtained from fruits, leaves, flowers, woods, resins and seeds of a fresh or dried plant by various methods. Because of the disadvantages of conventional extraction methods such as requirement of long periods and high amounts of chemicals, novel extraction methods such as ultrasound assisted systems have drawn higher attention in recent years. In this study, the ultrasound-assisted extraction (USE) conditions (temperature, time, ultrasonication power and solvent-solid ratio) were determined for the production of crude extracts from sage (*Salvia officinalis*) and mint (*Mentha piperita*). Bioavailability of the plant extracts were also compared to those obtained by classical hot-water extraction (HWE). USE parameters for the highest yield were 40 °C, 10 min, 400 W, as determined by preliminary experiments. Total phenolic contents of the mint and sage samples increased by ultrasound assisted liquid extraction method at the levels of 23.88 %and 14.97 %, respectively. The bioavailability of total phenolic contents in classic and ultrasound extraction of mint (*Mentha piperita*) extracts was 20.11 %, 32.45 % respectively. In conclusion, the results of the present study showed that ultrasound assisted extraction was more effective method for extraction of bioactive substances from sage and mint with shorter extraction time, increased bioactivity and bioavailability.

ARTICLE HISTORY
Received
25.06.2019
Accepted
08.07.2019

KEY WORDS
Medicinal herbs extract, ultrasound-assisted extraction, bioavailability

Introduction

Turkey is one of the most important gene centers of medicinal and aromatic plants in the World, and is located in a very fertile region which hosts thousands of endemic plant species. Aromatic plants have been used as remedy in traditional medicine for ancient times, as well as in food preservation due to their antimicrobial activity and in cosmetic and pharmaceutical industry in recent years [1]. The first records of treatment of people with aromatic plants belong to Mesopotamian civilization in BC. [2]. According to the World Health Organization (WHO), 25% of the pharmaceutical drugs used today are

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produced from medicinal plants. On the other hand, Food and Agriculture Organization (FAO) states that 30% of the drugs sold worldwide contain compounds derived from plant materials [3]. This is due to the abundance of bioactive compounds such as vitamins (E and C), glutathione, enzymes and phenolic compounds in medicinal and aromatic plants [4]. Phenolic compounds which are major bioactive constituents of aromatic plants are very important due to their effects on the organoleptic properties and nutritional quality of foods such as color, taste, odor, natural colorant in foods, and have alternative use as natural antioxidant and the positive effects on health in recent years [6]. The most common crops cultivated and used in the industry are sage, anise, juniper tar, nettle, thyme, rosehip, lemon balm, chamomile, cinnamon, vanilla, lion paw and mercury [7].

Although conventional extraction techniques such as Soxhlet extraction have been used for a number of decades in order to obtain bioactive extracts from plants, they have numerous disadvantages such as being time consuming and requirement of large amounts of chemicals, which cause high energy consumption and environmental pollution [8]. Therefore novel extraction techniques such as ultrasound-assisted, microwave-assisted, supercritical and accelerated extraction systems, alternative to conventional solvent extraction from plant sources have been investigated by different researchers to overcome these disadvantages [9, 10].

Ultrasonic assisted extraction, also called sound waves-assisted liquid extraction, is considered as one of the most efficient extract recovery techniques. In this technique, extraction is performed in gas or liquid environment by the effect of cavitation which is formed on liquid-liquid or gas-liquid interfaces [11]. In this method, acoustic vibrations are applied to the sample with frequencies above 20 kHz. [12]. In the use of ultrasound in extracting, the application is in the form of mass transfer by mechanical disintegration

of the cell wall. By disintegrating the cell wall in this way, it becomes easier for the liquid extract inside the cell to exit the cell. It is faster than other extraction methods and it is an effective non-thermal alternative method since the cell wall is destroyed by the application of ultrasound [13, 14]. Ultrasonic assisted extraction is commonly applied to obtain valuable compounds from a variety of matrices mainly food and plant materials [15]. Extraction of bioactive components by ultrasound application is one of the methods that provide high level of hand in a short time, reducing heat and energy consumption, reducing solvent consumption and easy to apply [16]. In this study, it was aimed to compare the bioavailability of sage and mint extracts obtained by conventional and ultrasonic assisted methods.

Material and Methods

Materials

Fresh sage (*Salvia officinalis*) and peppermint (*Mentha piperita*) were obtained from Zeytinburnu Medicine and Aromatic Plant Garden in Istanbul, Turkey. Ethanol, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and Folin&Ciocalteu's reagent purchased from Sigma Aldrich Chemical Co. (USA). Ethanol, dialysis membrane tube and bile salts were obtained from Merck (Germany).

Sample preparation for extraction process

After removing impurities, the plant materials were dried using a vacuum dryer (Daihan WOV-30, Gangwon-do, South Korea) at 40 °C for 8 h. The vacuum was adjusted by a vacuum pump (EVP 2XZ-2C, Zhejiang, China) with 6 kPa ultimate pressure and 2 L/s pump seed. The dried samples were grinded and then stored in desiccator at room temperature until extraction process.

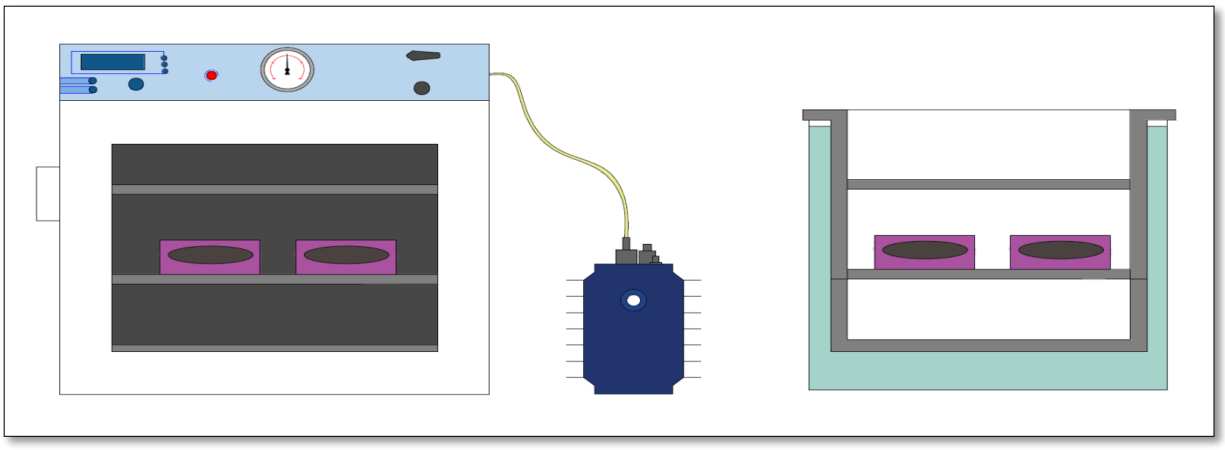

Fig 1 Vacuum drying system

Extraction Process

Classical hot water extraction (HWE)

For this purpose, 10g of the samples was incorporated with 100 mL of 80% ethanol (v/v) and mixed at 40 °C for 6 hours using a magnetic stirrer at 250 rpm. The ethanolic extracts were filtered and freeze-dried for the bioavailability assays.

Sonication-assisted liquid extraction (USE)

Ultrasound extraction process was performed using an ultrasonic processor (Hielscher UP400S, Germany) with 24 kHz frequency and 100µm amplitude values. The flow cell of the ultrasonic processor (Hielscher Flowcell D22-K) was combined with a 22 mm diameter probe (Hielscher sonotrode H22D) and the instant process volume was 15 mL. The samples were mixed with 80% ethanol (v/v) in a ratio of 1:10 (w/v) and then extracted at 40 °C for 10 min with ultrasound power of 400 W, which determined as optimum parameters with preliminary experiments to obtain maximum extraction yield. The temperature was kept at the constant value using a refrigerant during the process. After extraction, the extracts were filtered to remove impurities and freeze-dried to obtain crude powder for the bioavailability assays.

***In vitro* gastrointestinal digestion assay**

In order to simulate gastric and intestinal digestion of the extracts, the method described by McDougall et al. (2005) was performed with some modifications [17].

For stomach digestion; freeze-dried extracts were homogenously dissolved in 2.5 mL of water and completed to 20 mL of distilled water. Then 1.5 mL of pepsin solution (40 mg/ml) prepared with 0.1 M HCL was added to the mixture. The pH of the mixture was adjusted to 2.0 by adding 5 M HCl. The beaker was covered with parafilm and incubated for 2 h in a shaking incubator at 37ºC with stirring at 100 rpm. At the end of the incubation, 5 mL of PG fraction was separated and stored at -20ºC to use following analyses. For intestinal digestion, 5 mL of pancreatin (18 mg / mL), a mixture of bile (112.5 mg / mL bile salt) and 4.5 mL of $NaHCO_3$ (0.1 M) were added to the stomach digested beaker. The dialysis tube was completely immersed in the PG phase and the beaker was sealed with parafilm. It was incubated for 2 h in a shaking incubator with stirring at 100 rpm at 37ºC.

At the end of the period, the IN fraction in the dialysis tube was discharged into the falcon tube. The liquid outside the tube was discharged into a separate falcon tube as the OUT fraction. It was stored at -20ºC for the following analyses.

Bioavailability is determined by dividing the amount of substance present in the IN phase to the amount of substance present in the sample, as indicated in equation 2.1. This procedure is schematized in detail in Figure 2.

$$\%\ bioavailability\ = \frac{The\ amount\ of\ matter\ in\ IN\ phase}{The\ amount\ of\ the\ matter\ in\ the\ sample} \times 100 \qquad (2.1)$$

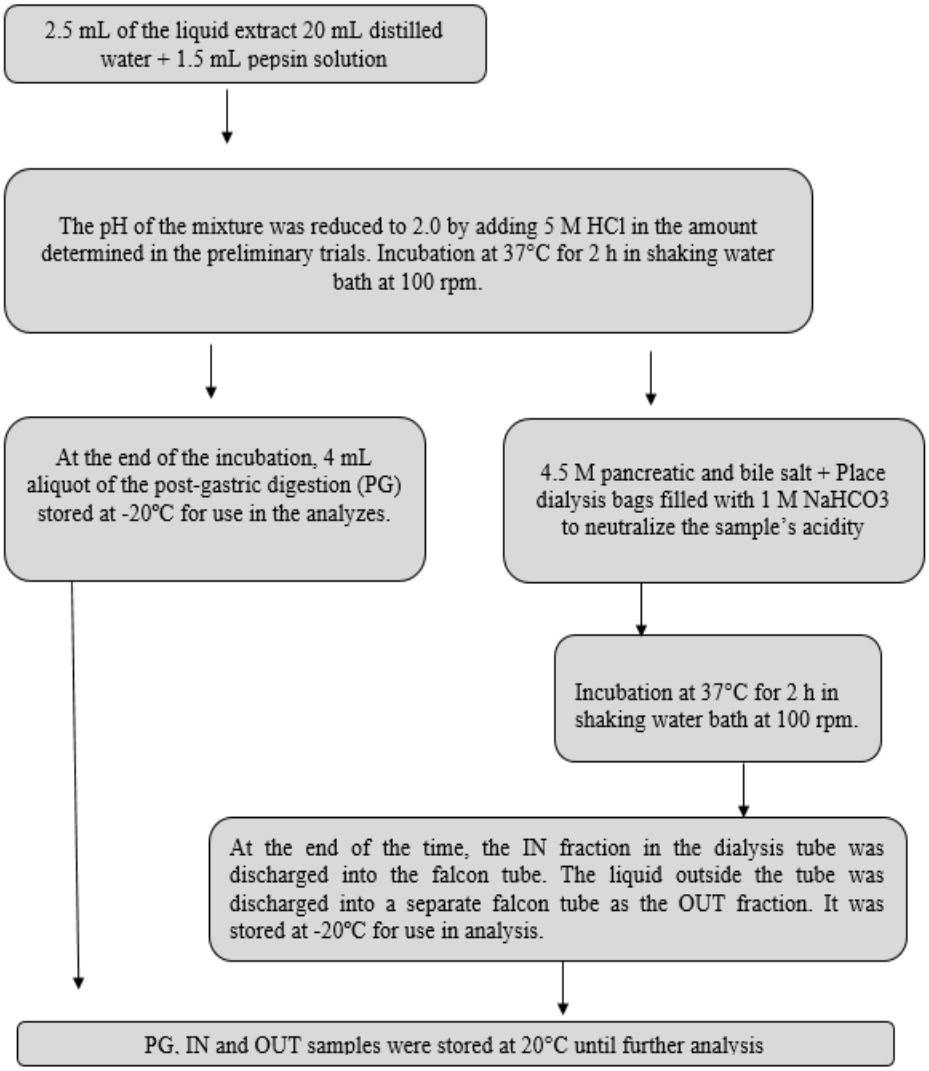

Fig 2 Flow chart of the in vitro gastrointestinal digestion method [31]

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of the samples was determined using Folin-Ciocalteu method as described by Singleton and Rossi (1965). Briefly, 0.5 ml of the ethanolic extract was mixed with 2.5 mL of 0.2 N Folin-Ciocalteau's reagent and after the incubation for 3 min, 2 mL of 7.5% Na_2CO_3 solution was added to the mixture. After keeping at the room temperature for 30 min, the absorbance of the samples was measured at 760 nm using a UV-vis spectrometer (Shimadzu, UV-1800, Japan). The results were

calculated using the following equation and were expressed as mg gallic acid equivalents in liters (mg GAE/L) [18].

$$TPC(mg\ GAE/L) = \frac{absorbance - 0.0791}{0.0103} \times dilution\ factor \quad (2.2)$$

Determination of total flavonoid content (TFC)

Determination of total flavonoid content (TFC) was performed according to the method described by Zhinsen et al. (1999). For this purpose, 1 mL of extract was mixed with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ solution (w/v). After 5 min, it was incorporated with 0.3 mL of 10% $AlCl_3$ solution (w/v) and mixed for 6 min. Following the addition of 2 mL of 1M NaOH, the volume was completed to 10 mL with distilled water. Then the absorbance values of the samples were measured at 510 nm with UV-VIS spectrophotometer (Shimadzu UV-1800, Japan). All the results were expressed as mg catechin equivalents (CAE)/100g of sample [19]. The formula used to calculate the total flavonoid content was given in Equation 2.3.

$$TFC\ \left(mg\frac{CAE}{L}\right) = [(151.6 \times absorbance) - 0.05454] \times dilution\ factor \quad (2.3)$$

Determination of antioxidant activity by DPPH radical scavenging assay

The antioxidant activity of the samples was determined according to the method described by Sanchez Moreno et al. (2002). For this purpose, 0.1 mL of the extract was mixed with 4.9 mL of 0.1 mM DPPH solution and the mixture was kept at 27 °C for 20 min. Then, the absorbance of the samples was measured at 517 nm using a UV-vis spectrometer (Shimadzu, UV-1800, Japan). The results were presented as mg Trolox equivalent (TEAC)/ 100 g of the sample [20].

Statistical analysis

Mean values and standard deviations of the data were evaluated using Excel software (Microsoft Office, 2017). Statistical analysis was performed by using a statistical software of SPSS 20.0 (SPSS, Inc., Chicago) with one way analysis of variance (ANOVA). Differences between the data were determined by Duncan's multiple comparison test with 95% confidence level.

Results and Discussion

Changes in bioactive components in extracts

Bioactive properties of sage and mint extracts are shown in Table 1. As seen in the table, ultrasonication process significantly increased the extraction yield of both sage and mint. Total phenolic contents (TPCs), total flavonoid contents (TFCs) and DPPH scavenging activities of the sage extracts were significantly higher than those of mint. Considering the effect of extraction technique on bioactive properties, ultrasonication enabled higher values than classical extraction, for instance, TPCs of mint and sage samples increased by 23.88 % and 14.97 %.by ultrasound assisted liquid extraction method, respectively.

Table 1 Bioactive properties and yields of sage and mint extracts

Sample	Extraction method	TPC (mg GAE/L)	TFC (mg CAE/L)	DPPH (mg TE/L)	Yield (%)
Sage	HWE	4066.24±4.01^{a}	356.53±0.92^{a}	66.41 ±0.96 ^{a}	3.36± 0.11^{a}
	USE	4491.14±2.11^{b}	378.45±1.83^{c}	101.6±4.34^{d}	5.28±0.23^{c}
Mint	HWE	198.35±3.67^{c}	65.67±1.23^{b}	72.09±1.04^{b}	3.82±0.19^{b}
	USE	245.73±5.45^{d}	87.35±1.56^{d}	83.67±1.62^{c}	5.79±0.13^{d}

*Different lowercase letters indicate the significance of the statistical difference between the data on the same row according to the ANOVA Duncan test ($p<0.05$)

Changes in post-digestive bioactive properties

Change in bioactive properties (TPCs, TFCs and DPPH scavenging activities) of sage and mint extracts obtained by classical or ultrasound assisted liquid extraction method after *in vitro* gastric and intestinal digestion (intestinal digestion, post-gastric (PG), IN (absorbed through the small intestine) and OUT (not absorbed from the small intestine)) are given in Figures 3, 4 and 5, respectively.

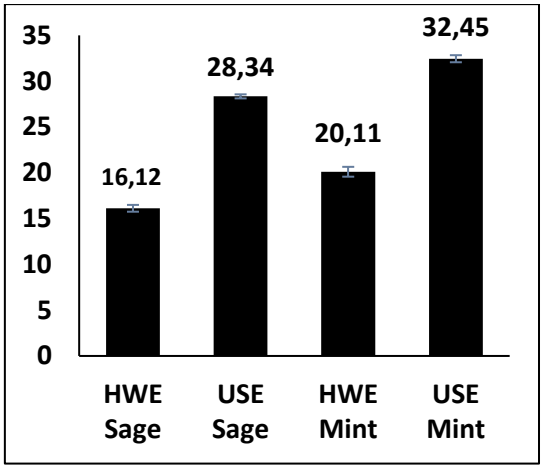

Figure 3 % Recovery of TPC

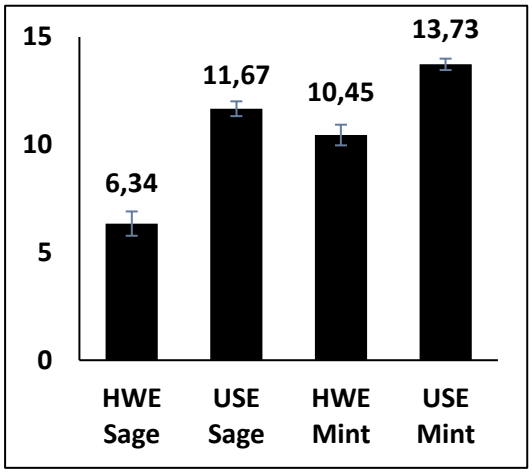

Figure 4 % Recovery of TFC

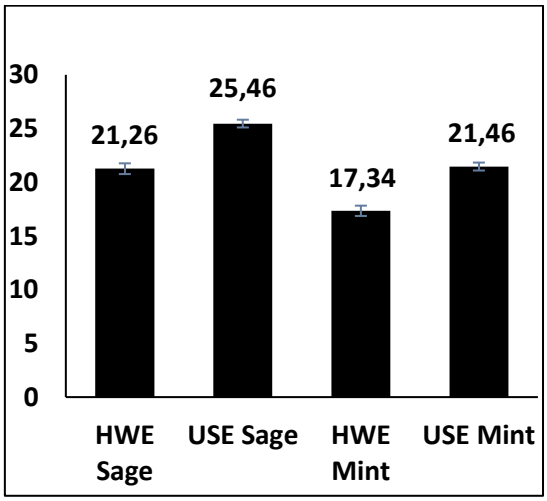

Fig 5 % Recovery of DPPH scavenging activity

Ultrasound was shown to improve the bioavailability of medicinal plants after gastrointestinal digestion. The bioavailability of TPCs in classic and ultrasound extraction

of sage extracts was 16.12% and 28.34% while it was 6.34% and 11.67% in the case of TFC, respectively. The bioavailability of DPPH scavenging activity was determined as 21.26 % and 25.46 % (Figure 6).

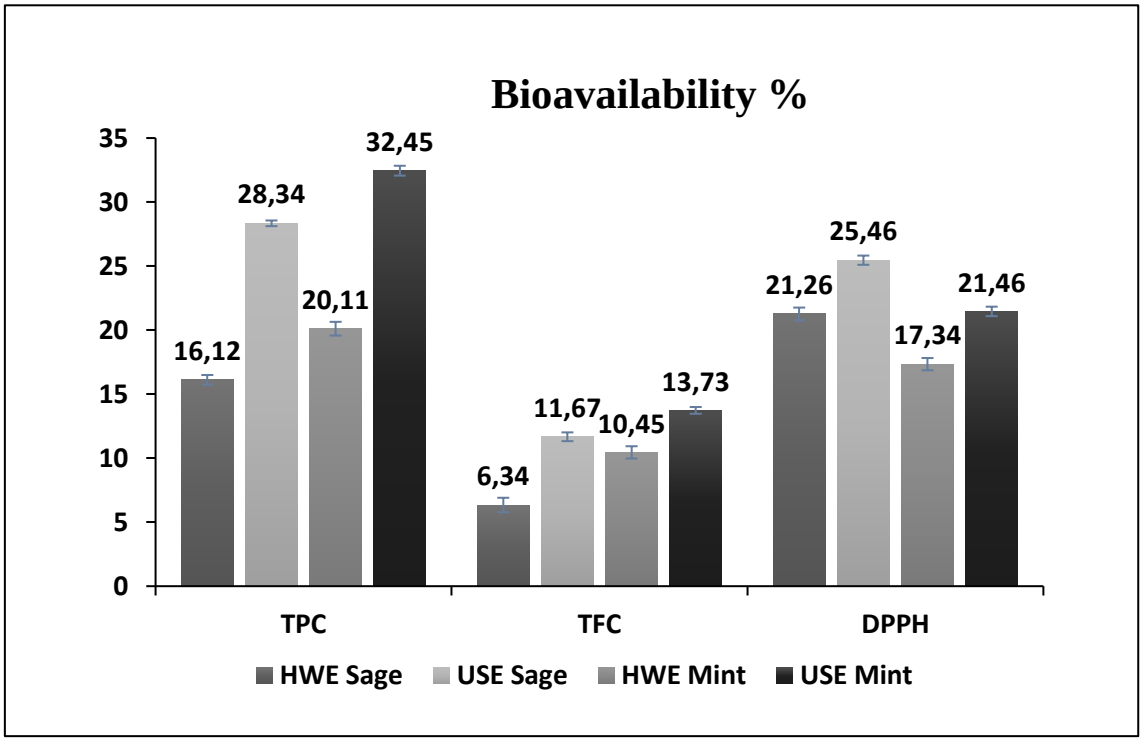

Fig 6 Recovery (%) of TPCs, TFCs and DPPH radical scavenging activities of sage and mint extracts after *in vitro* gastrointestinal digestion

The bioavailability of TPC of the mint extracts obtained by classical or ultrasound extraction was 20.11 % and 32.45 % while it was 10.45 % and 13.73 % for TFC while the bioavailability of the extract as measured by DPPH scavenging activity was determined as 17.34 % and 21.46 %, respectively.

Dahmoune et al. (2013) investigated effect of EU using solvents with different ethanol concentrations varying from 30% to 70% on bioactive properties of lemon peel and found that TPC values increased at higher ethanol ratios up to 62.93%. [21]. In another study, Wang et al. (2008) extracted the phenolic compounds from wheat bran by ultrasound-

assisted extraction technology at different processing conditions for extraction optimization. They found that extraction time was the most significant parameter for optimization and at the optimum extraction conditions (ethanol concentration, 64%; extraction temperature, 60 C; and extraction time, 25 min) TPC was 3.12 mg GAE/g of wheat bran [22].

Plant extracts with high antioxidant capacity, rich in phenolic acids, act as free radical terminators and reduce the effect of oxidative damage on dsDNA [23]. In one study, Skeva and Girousi investigated the antioxidant ability of *Camelia sinensis* (black and green tea) plant extracts *in vitro* by using a dsDNA biosensor to achieve oxidative damage on dsDNA. (Compared to Gallic acid, caffeic acid and trolox standard.)[23]. Putnik et al. (2018) used microwave assisted extraction (MAE) technique to obtain phenolic compounds from sage. They tested three different solvents (30% ethanol, 30% acetone and water), five different times (3, 5, 7, 9 and 10 min) and five different temperatures (30, 50, 60 and 80 °C) for extraction. The best result for the total polyphenols was obtained at 30% acetone as solvent and 80 °C for 10 minutes [24]. Bender et al., reported that *in vitro* oxygen radical absorbance capacity (ORAC) and antioxidant capacity (CAA) of peppermint extract were 1438 mmol trolox eq/g and s 27.9 mmol quercetin eq/g while Those values were 1351 mmol trolox eq/g and 35.3 mmol quercetin eq/g for sage, respectively [25]. Our results were in accordance with these findings. On the other hand, industrial microwaving of black pepper, sage and basil did not change their antioxidant properties [26].

Advantages of ultrasound assisted extraction are; inexpensive equipment, ease of use, environmentally friendly, maximization of the extract yield of the targeted component with minimum degradation and achieving high efficiency. Ultrasound-assisted extraction

accelerates mass transfer, thus providing more processing time and less solvent consumption compared to the classical extraction method [27, 28, 29] and increases penetration by breaking down cells [30].

Conclusion

In this study, separation of bioactive extracts from sage and mint was carried out by classical solvent extraction and ultrasound assisted extraction techniques. Although the classical extraction process was performed at higher temperatures than the ultrasound assisted extraction, TPC, TFC and DPPH scavenging ability of the extracts remained below the amount of phenolic material obtained by ultrasound assisted extraction.

When ultrasound assisted extraction method was examined, it was found that ultrasound assisted extraction was the most effective method for extracting of bioactive substances from sage and mint in a much shorter time compared to classical method. While increasing the amount of bioactive properties, it also increased the bioavailability of the extracts.

References

1. Cock, I.E. and Kukkonen, L., An examination of the medicinal potential of Scaevola spinescens: Toxicity, antibacterial, and antiviral activities. Pharmacognosy Research, 2011. 3 (2): p. 85-94. https://doi.org/10.4103/0974-8490.81955
2. Demirezer LO., et al., The structures of antioxidant and cytotoxic agents from natural source: anthraquinones and tannins from roots of *Rumex patientia*. Phytochemistry, 2001. 58(8): p. 1213–1217. https://doi.org/10.1016/S0031-9422(01)00337-5
3. Sağdıç, O., Özkan, G. and Göktürk Baydar, N., Yeni bitkisel doğal bir antimikrobiyal ve antioksidan: Üzüm posası. Gıda Teknolojisi, 2005. 9 (4): p. 78-80.
4. Raskovic, A., et al., Antioxidant activity of rosemary (Rosmarinus officinalis L.) essential oil and its hepatoprotective potential. BMC Complementary and Alternative Medicine, 2014. 14:225. https://doi.org/10.1186/1472-6882-14-225
5. Ignat, I., Volf I., and Popa, VI., A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. Food Chemistry, 2011. 126(4): p. 1821-1835. https://doi.org/10.1016/j.foodchem.2010.12.026.
6. Ozkan, G., et al., Phenolic compounds of Turkish endemic *Origanum sipyleum* L. extract, and its antioxidant and antibacterial activities. Journal of Food Lipids, 2007. 14(2): p. 157-169. https://doi.org/10.1111/j.1745-4522.2007.00077.x

7. Vesoul, J. and Cock, IE., An examination of the medicinal potential of *Pittosporum phylliraeoides*: toxicity, antibacterial and antifungal activities, 2011. Pharmacognosy Communications, 1(2): p. 8–17. https://doi.org/10.5530/pc.2011.2.3
8. De Castro, M. L., and Garcıa-Ayuso, L. E., Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Analytica Chimica Acta, 1998. *369*(1-2): p. 1-10. https://doi.org/10.1016/S0003-2670(98)00233-5
9. Perez-Serradilla, JA., Capote, PF. and Castro, LMD., Simultaneous utrasound-asisted emulsification-extraction of polar and nonpolar compounds from solid plant samples. Analytical Chemistry, 2007. 79: p. 6767-6774. https://doi.org/10.1021/ac0708801
10. Wang, L., and Weller, CL., Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology, 2006. 17: p. 300-312. https://doi.org/10.1016/j.tifs.2005.12.004
11. Adam, M., et. al, Extraction of antioxidants from plants using ultrasonic methods and their antioxidant capacity. Journal of Separation Science, 2009. *32*(2): p. 288-294. https://doi.org/10.1002/jssc.200800543
12. Ilbay, Z., Sahin, S., and Kirbaslar, S.I., Optimisation of ultrasound-assisted extraction of rosehip (*Rosa canina* L.) with response surface methodology. Journal of the Science of Food and Agriculture, 2013. 93: p. 2804–2809. https://doi.org/10.1002/jsfa.6104
13. Domeno, C., et al., A fast extraction technique for extracting polycyclic aromatic hydrocarbons (PAHs) from lichens samples used as biomonitors of air pollution: Dynamic sonication versus other methods. Analytica Chimica Acta, 2006. 569(1-2): p. 103-112. https://doi.org/10.1016/j.aca.2006.03.053
14. Hyotylainen, T. and Riekkola, M.L., Potential of effective extraction techniques and new analytical systems for profiling the marine environment. Trac-Trends in Analytical Chemistry, 2007. 26(8): p. 788-808. https://doi.org/10.1016/j.trac.2007.06.007
15. Khan, M. K., et al., Ultrasound-assisted extraction of polyphenols (flavanone glycosides) fromorange (*Citrus sinensis* L.) peel. Food Chemistry, 2010. 119: p. 851–858. 10.1016/j.foodchem.2009.08.046
16. Chemat, F., Tomao, V., and Virot, M., Ultrasound- assisted extraction in food analysis. In: Handbook of Food Analysis Instruments, 2008. Boca Raton, Florida, USA: CRC p. 85–103.
17. McDougall GJ., et al., Assessing potential bioavailability of raspberry anthocyanins using an in vitro digestion system. Journal of Agricultural and Food Chemistry, 2005. 53: p. 5896-5904. https://doi.org/10.1021/jf050131p
18. Lıe, Y., et al., Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chemistry, 2006. 96: p. 254-260. https://doi.org/10.1016/j.foodchem.2005.02.033
19. Zhinsen, J., Mengcheng, T. and Jianming, W., The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 1999. 64: p. 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2
20. Sanchez-Moreno C., Methods used to evaluate the free radical scavenging activity. Foods and Biological Systems, Food Science and Technology International., 2002. 8(3): p. 121-137. https://doi.org/10.1106/108201302026770
21. Dahmoune, F., et al., Valorization of citrus limon residues for the recovery of antioxidants: Evaluation and optimization of microwave and ultrasound application to solvent extraction. Industrial Crops and Products, 2013. 50: p. 77-87. https://doi.org/10.1016/j.indcrop.2013.07.013
22. Wang, J., et al., Optimization of ultrasound- assisted extraction of phenolic compounds from wheat bran. Food Chemistry, 2008. 106(2): p. 804-810. https://doi.org/10.1016/j.foodchem.2007.06.062
23. Skeva E., and Girousi S., A study of the antioxidative behavior of phenolic acids, in aqueous herb extracts, using a dsDNA biosensor. Central European Journal of Chemistry, 2012. 10(4): p. 1280-1289. https://doi.org/10.2478/s11532-012-0051-0

24. Putnik P., et al., Novel food processing and extraction technologies of high-added value compounds from plant materials. Foods, 2018. 7: p. 106. https://doi.org/10.3390/foods7070106
25. Bender C., et al., Antioxidant potential of aqueous plant extracts assessed by the cellular antioxidant activity assay. American Journal of Biology and Life Science, 2014. 2: p. 72-79.
26. Çoban, Ö. and Patır, B., Antioksidan etkili bazı bitki ve baharatların gıdalarda kullanımı. Gıda Teknolojileri Elektronik Dergisi, 2010. 2(5): 7-19.
27. Vieira, G.S., et al., Chemical and economic evaluation of natural antioxidant extracts obtained by ultrasound assisted and agitated bed extraction from jussara pulp (*Euterpe edulis*). Journal of Food Engineering, 2013. 119(2):196-204. https://doi.org/10.1016/j.jfoodeng.2013.05.030
28. Wu, H., et al., Ultrasound assisted enzymatic extraction and antioxidant activity of polysaccharides from pumpkin (*Cucurbita moschata*). Carbohydrate Polymers, 2014. 113: p. 314–324. https://doi.org/10.1016/j.carbpol.2014.07.025
29. Gonzalez, R., Effect of power ultrasound application on aqueous extraction of phenolic compunds and antioxidant activity from grape pomace (*Vitis vinifera* L.): Experimental kinetics and modeling. Ultrasonics Sonochemistry, 2015. 22: p. 506-514. https://doi.org/10.1016/j.ultsonch.2014.05.027
30. Vinatoru, M., An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrasonics Sonochemistry, 2001. 8(3): p. 303- 313. https://doi.org/10.1016/S1350-4177(01)00071-2
31. Kamiloğlu, S., et al., Kuru meyvelerin kuruyemişlerle birlikte tüketiminin flavonoidlerin *in vitro* biyoyararlılığına etkisinin incelenmesi. Gıda, 2014. 39(4): 227-233. https://doi.org/10.5505/gida.51523