TOKAT GAZİOSMANPAŞA ÜNİVERSİTESİ ZİRAAT FAKÜLTESİ

Tokat Gaziosmanpasa University, Faculty of Agriculture
TOKAT, TÜRKİYE

GAZİOSMANPAŞA ÜNİVERSİTESİ ZİRAAT FAKÜLTESİ DERGİSİ

Journal of Agricultural Faculty of Gaziosmanpasa

University
(JAFAG)

ISSN: 1300 - 2910

E-ISSN: 2147 - 8848

Cilt/*Volume:***39 Sayı/***Number:***2 Yıl/***Year:** **Ağustos 2022/***August 2022*

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Journal of Agricultural Faculty of Gaziosmanpasa University (JAFAG)

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https://dergipark.org.tr/tr/pub/gopzfd

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M. Furkan ÇITAK

***Basım**/Publisher:*

Tokat Gaziosmanpaşa Üniversitesi Matbaası, 60240, Taşlıçiftlik, Tokat/TÜRKİYE
Tokat Gaziosmanpasa University Press, 60240, Taşlıçiftlik, Tokat/TURKEY

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Journal of Agricultural Faculty of Gaziosmanpasa University (JAFAG)

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TR DİZİN

NINDEX COPERNICUS

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AMAÇ VE KAPSAM

Gaziosmanpaşa Üniversitesi Ziraat Fakültesinin 1985 yılından beri hakemli v bilimse süreli yayınıdır.Tokat Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi, Tarım bilimleri (tarım ekonomisi, zootekni, biyosistem mühendisliği, tarla bitkileri, su ürünleri mühendisliği, bahçe bitkileri, bitki koruma, toprak bilimi ve bitki besleme) alanındaki uluslararası bilimsel makaleleri Türkçe ve İngilizce olarak yayınlamayı amaçlamaktadır. Dergi yılda en az iki kez basılır. 2014 yılı itibariyle senede 3 baskı yapmıştır.

YAYIN POLİTİKASI

Dergide yayınlanacak makaleler İngilizce yayınlanır. Makaleler incelenmek üzere dergiye sorumlu yazar tarafından sunulur. Sunulan makalelerin başka bir yerde yayınlanmamış olması gerekir. Telif Hakkı Devir Sözleşmesi Formu tüm yazarlar tarafından imzalanmış olmalıdır.

Dergimizde yayınlanacak makaleler araştırma ve yayın etiğine uygun olmak zorundadır. Etik kurul kararı gerektiren klinik ve deneysel hayvan çalışmaları için ayrı ayrı etik kurul onayı alınmış olmalı ve belgelendirilmelidir. Dergimize gönderilecek bilimsel yazılarda, ICMJE (International Committee of Medcial Journal Editors) tavsiyeleri ile COPE (Committee on Publication Ethics)'un "Editör ve Yazarlar için Uluslararası Standartlar"ı dikkate alınmaktadır.

Dergiye sunulan makale, Dergi Sekreteryası tarafından yazım kuralları ve içerik açısından ön değerlendirmeye alınır. Dergide basılacak nitelikte bulunmayan makale yazara iade edilebilir. Uygun bulunanlar ise bilimsel açıdan değerlendirilmek üzere konusunda uzman hakemlere (maksimum 1 5 gün süre için) gönderilir. Hakem incelenmesinden sonra basıma uygun olmayan makaleler yazara bildirilir, makaleler iade edilmez. Hakem onayından geçenler içinde düzeltme yapılması istenen makaleler gerekli dokümanlarla yazara iletilir. Yazar gerekli düzeltmeleri en kısa sürede (maksimum 15 gün) tamamlayarak dergi e-posta adresine gönderir. Editörler kurulu nihai kararını vererek makaleyi uygun bulursa basım ünitesine gönderir. Basımına karar verilen ve düzeltme için yazarına gönderilen eserde, ekleme veya çıkartma yapılamaz.

Bir yazarın derginin aynı sayısında ilk isim olarak, en fazla iki eseri basılabilir.

Yayınlanan makalelerin tüm sorumluluğu yazar(lar)ına aittir

AIMS AND SCOPE

Journal of Agricultural Faculty is scientific, peer reviewed journal and belonged to the Tokat Gaziosmanpasa University Faculty of Agriculture since 1985. Journal of Agricultural Faculty of Tokat Gaziosmanpasa University aims to publish the international scientific paper on agriculture sciences (agricultural economics, animal science, biosystems engineering, field crops, fisheries engineering, horticulture, plant protection, soil science and plant nutrition). The journal is published at least twice in a year. The journal was published three issues in a year at 2014 year.

PUBLISHING POLICY

Manuscripts are published in English. The manuscripts are submitted to the journal from Turkey and the other countries for review by corresponding author. The manuscript submitted should not have been submitted and published in another journal

Manuscripts published in our journal must be appropriate to the research and publication ethics. Separate ethical board resolutions are needed for each clinical and experimental study on animals which requires ethical board decision. International Committee on Publication

Ethics' (ICMJE) recommendations and Committee on Publication Ethics' (COPE) "International Standards for Editors and Auditors" should be taken into consideration for the scientific manuscripts sent to our Journal.

Submitted manuscript to the journal is considered to preliminary assessment by the Editorial Board of journal. The Editorial Board has the right to decline the manuscript in event the manuscript does not meet the journal publishing rules. Manuscripts that meet the basic requirements are numbered and sent to three referees, experts in particular field of science, to peer review process (for max. 15 days period). Then, if the referees do not find the manuscript for publication, the related manuscript are not returned to the author, manuscript are archived. After peer reviewing, if the referees find the manuscript for publication with requires revision and corrections, author is informed, and the referee's suggestions and the related documents are sent to the corresponding author. The author is sent the corrected and revised manuscript to the Editorial Board as soon as possible (max. 30 days). Then, Editorial Board takes the final decision (positive or negative) for publication of manuscript. For the content of the accepted manuscripts, no editing, changes, including addition or deletion, can be made.

Only two manuscripts of each author, as the first author for one of the manuscript, can be published in same issue of the journal.

The author(s) are responsible for the content of the published manuscripts

ETİK İLKELER VE YAYIN POLİTİKASI

YAYIN ETİĞİ İLKELERİ

Gaziosmanpaşa ÜniversitesiZiraat Fakültesi Dergisi, yayın politikasında akademik ilke ve etik değerlere bağlıdır. Etik ilke ve değerlere ilişkin ulusal ve uluslararası standartlara uygun olarak yayın hayatını sürdürmektedir. Bu kapsamda, COPE (Committee on Publication Ethics) tarafından belirlenen standartlar ve YÖK "Bilimsel Araştırma ve Yayın Etiği Yönergesi"nde belirlenen esaslar dikkate alınmaktadır (https://publicationethics.org/, https://www.yok.gov.tr/Sayfalar/Kurumsal/mevzuat/bilimsel-arastirma-ve-…). Makale değerlendirme sürecinde kabul edilen araştırma ve yayın etiği standartlarına aykırılığı tespit edilen eserlerin yayın talebi reddedilir. Eserin yayınlanmasından sonra söz konusu aykırılığın tespit edilmesi halinde eser yayından kaldırılır.

Hakemli dergide yayın ilkeleri ile ilgili tüm taraflardan (yazar, dergi editörü, hakem ve yayımcı kuruluşlar) beklenen genel etik davranışlar ve sorumluluklara ilişkin tanımlamalar aşağıda belirtilmektedir.

Yazar(lar)ın Sorumlulukları

• Kaynakça listesi eksiksiz olmalıdır.
• İntihal ve sahte veriye yer verilmemelidir.
• Aynı araştırmanın birden fazla dergide yayımlanmasına teşebbüs edilmemeli,
• Bilim araştırma ve yayın etiğine uymalıdır.
• Tüm yazarların araştırmaya katkısı bulunmalıdır.
• Makalede geçen tüm veriler gerçek ve orijinal olmalıdır.
• Tüm yazarlar hatalı makalenin geri çekilmesini ve hataların düzeltilmesini sağlamak zorundadır.

Bilim araştırma ve yayın etiğine aykırı eylemler şunlardır:

• İntihal: Başkalarının fikirlerini, metotlarını, verilerini, uygulamalarını, yazılarını, şekillerini veya eserlerini sahiplerine bilimsel kurallara uygun biçimde atıf yapmadan kısmen veya tamamen kendi eseriymiş gibi sunmak,
• Sahtecilik: Araştırmaya dayanmayan veriler üretmek, sunulan veya yayınlanan eseri gerçek olmayan verilere dayandırarak düzenlemek veya değiştirmek, bunları rapor etmek veya yayımlamak, yapılmamış bir araştırmayı yapılmış gibi göstermek,
• Çarpıtma: Araştırma kayıtları ve elde edilen verileri tahrif etmek, araştırmada kullanılmayan yöntem, cihaz ve materyalleri kullanılmış gibi göstermek, ilgili teori veya varsayımlara uydurmak için veriler ve/veya sonuçlarla oynamak, destek alınan kişi ve kuruluşların çıkarları doğrultusunda araştırma sonuçlarını tahrif etmek veya şekillendirmek,
• Tekrar yayım: Bir araştırmanın aynı sonuçlarını içeren birden fazla eseri ayrı eserler olarak sunmak,
• Dilimleme: Bir araştırmanın sonuçlarını araştırmanın bütünlüğünü bozacak şekilde, uygun olmayan biçimde parçalara ayırarak ve birbirine atıf yapmadan çok sayıda yayın yaparak ayrı eserler olarak sunmak,
• Haksız yazarlık: Aktif katkısı olmayan kişileri yazarlar arasına dâhil etmek, aktif katkısı olan kişileri yazarlar arasına dâhil etmemek, yazar sıralamasını gerekçesiz ve uygun olmayan bir biçimde değiştirmek, aktif katkısı olanların isimlerini yayım sırasında veya sonraki baskılarda eserden çıkarmak, aktif katkısı olmadığı halde

ETHICAL PRINCIPLES AND PUBLICATION POLICY

PRINCIPLES OF PUBLICATION ETHICS

Journal of Agricultural Faculty of Gaziosmanpasa University is committed to academic principles and ethical values in its editorial policy. It continues its publication life in accordance with national and international standards regarding ethical principles and values. In this context, the standards set by COPE (Committee on Publication Ethics) and the principles set in the Council of Higher Education "Scientific Research and Publication Ethics Directive" are taken into account (https://publicationethics.org/, https://www.yok.gov.tr/Sayfalar/Kurumsal/mevzuat/bilimsel-arastirma-ve-…). The publication request of the works that are found to be in violation of the research and publication ethics standards accepted in the manuscript evaluation process is rejected. If the said contradiction is detected after the publication of the work, the work is removed from the publication.

Author’s responsibilities:

The references list should be complete;
No plagiarism, no fraudulent data is allowed;
It is forbidden to publish same research in more than one journal;
Authors obliged to participate in peer review process;
All authors have significantly contributed to the research;
Statement that all data in manuscript are real and authentic;
All authors are obliged to provide retractions or corrections of mistakes,
Authors should ensure that any studies involving human or animal subjects conform to national, local and institutional laws and requirements.

The actions against science research and publication ethics include;

Plagiarism: Presenting others' ideas, methods, data, applications, writings, figures or works as if they were their own works, partly or completely, without referring to the scientific rules.
Fraud: to produce data that is not based on research, to organize or modify the work submitted or published on the basis of unreal data, to report or to publish them, to make a research that has not been done.
Distorting: Dealing with the records of research and the data obtained, showing the unused methods, devices and materials used in the research, playing with data and / or results to fit the relevant theory or assumptions, or falsifying or shaping the results of the research in the interests of the people and organizations supported.
Slicing: Presenting the results of a research as separate works by disrupting the uniqueness of the research, by dissecting it inappropriately and making a large number of publications without reference to each other.
Unfair writ•er: To include people who do not have active contribution among the authors, not to include the people who have active contribution among the writers, to change the ranking of the authors without any justification and in an inappropriate way, to remove the names of those who have active contributions from the

nüfuzunu kullanarak ismini yazarlar arasına dâhil ettirmek.
• Diğer etik ihlali türleri: Destek alınarak yürütülen araştırmaların yayınlarında destek veren kişi, kurum veya kuruluşlar ile onların araştırmadaki katkılarını açık bir biçimde belirtmemek, insan ve hayvanlar üzerinde yapılan araştırmalarda etik kurallara uymamak, yayınlarında hasta haklarına saygı göstermemek, hakem olarak incelemek üzere görevlendirildiği bir eserde yer alan bilgileri yayınlanmadan önce başkalarıyla paylaşmak, bilimsel araştırma için sağlanan veya ayrılan kaynakları, mekânları, imkânları ve cihazları amaç dışı kullanmak, tamamen dayanaksız, yersiz ve kasıtlı etik ihlali suçlamasında bulunmak (YÖK Bilimsel Araştırma ve Yayın Etiği Yönergesi, Madde 8).

Hakemlerin Sorumlulukları
•Hakemlik süreci, bilimsel akademik yayıncılığın başarısında önemli bir konumda bulunmaktadır. Hakemler bu sürecin sağlıklı yürütülebilmesi ve iyileştirilmesine gayret göstermelidir.
•Hakemler araştırmayla, yazarlarla ve/veya araştırma fon sağlayıcılar ile çıkar çatışması/çakışması içerisinde olmamalıdır.
•Değerlendirmeleri tarafsız olmalıdır.
•Değerlendirilen makaleler hakem tarafından gizli tutulmalıdır.

Editörün Sorumlulukları
•Editörler bir makaleyi kabul etmek ya da reddetmek için tüm sorumluluğa ve yetkiye sahiptir.
•Editörler kabul ettiği ya da reddettiği makaleler ile ilgili çıkar çatışması/çakışması içerisinde olmamalıdır.
•Sadece alana katkı sağlayacak makaleler kabul edilmelidir.
•Hakemlerin ismini değerlendirme tamamlanana kadar saklı tutmalıdır.
•Makalenin yayımlanmasından sonra herhangi bir araştırmacı tarafından bilimsel hata tespit edildiğinde ilgili düzeltme/düzeltmelerin yayımlanmasını ya da geri çekilmesini desteklemelidir.

Yayımcının Sorumlulukları
•Yayıncılık etiğinin yayın kurulu tarafından izlenmesi/korunması,
•Akademik kaydın bütünlüğünü korumak,
•Etik standartlardan ödün vermemek,
•Gerektiğinde düzeltmeleri, açıklamaları ve özürleri yayımlamak,
•Okuyucunun dergide yayımlanan bir makalede önemli bir bilimsel hata ya da intihal, yinelenen makaleler gibi konularda herhangi bir uyarısı olduğu zaman ziraatderdi@gop.edu.tr adresine mail atarak editör kuruluna bildirebilir. Derginin bilimsel ve teknik yönden gelişmesi için bir fırsat olacağı bilinci ile, yapacağınız uyarılar/eleştiriler, editör kurulu tarafından memnuniyetle karşılanarak hızlı ve yapıcı bir şekilde iyileştirmelerimiz gerçekleştirilmektedir.

Değerlendirme Süreci
Yayımlanması için gönderilen eser, yayın ilkeleri doğrultusunda editör tarafından ön incelemeye alınır. Editör, dergide yayımlanabilecek nitelikte bulmadığı makaleleri hakemlere göndermeden yazara/yazarlara iade

work during publication or in later editions, and to use their influence even if there is no active contribution.

Other types of ethical violations: Not expressing the contributions of the persons, institutions or organizations that support them in the research, and their contributions in the research, Not to obey the ethical rules in human and animal research, to respect the rights of patients in their publications, To share the information contained in a work that he is commissioned to examine as an arbitrator with others, To use the sources, facilities and devices provided for scientific research out of their use purposes. To blame for a completely irrelevant, unwarranted and intentional violation of ethics (YÖK Scientific Research and Publication Ethics Directive, Article 8).

Peer review/responsibility for the reviewers:
To contribute to the decision-making process, and to assist in improving the quality of the published paper by reviewing the manuscript objectively.
Reviewers should have no conflict of interest with respect to the research, the authors and/or the research funders;
Judgments should be objective;
Reviewed manuscripts should be treated confidentially.

Editorial responsibilities:
Editors have complete responsibility and authority to reject/accept an manuscript;
Editors should have no conflict of interest with respect to manuscripts they reject/accept;
Only accept a paper when reasonably certain;
Preserve anonymity of reviewers.
No plagiarism, no fraudulent data.
When errors are found, promote publication of correction or retraction;
To act in a balanced, objective and fair way while carrying out their expected duties, without discrimination on grounds of gender, sexual orientation, religious or political beliefs, ethnic or geographical origin of the authors.

Duties of the Publisher
Monitoring/safeguarding publishing ethics by editorial board;
Guidelines for retracting manuscripts;
Maintain the integrity of the academic record;
Preclude business needs from compromising intellectual and ethical standards;
Always be willing to publish corrections, clarifications, retractions, and apologies when needed.
In an manuscript published in the journal, the reader can send an e-mail to ziraatdergi@gop.edu.tr when he has any warnings about important scientific error or plagiarism, recurring manuscripts. With the awareness that the journal will be an opportunity for the scientific and technical development of the journal, your warnings / criticisms are welcomed by the editorial board and our improvements are made quickly and constructively.

Evaluation Process
The submitted manuscript for publication is taken into consideration by the editor in accordance with the principles of publication. In case of finding not qualified to publish it in the journal, the editor has the right to make

kararı verme hakkına sahiptir. Ayrıca yazım kurallarına uymayan veya anlatım dili yetersiz olan makaleler, düzeltilmek üzere yazara/yazarlara iade edilir. Değerlendirmeye alınan makaleler, incelenmek üzere en az 2 hakeme gönderilir. Değerlendirmede çift yönlü kör hakemlik uygulaması esastır. Hakem değerlendirmesinden geçen makalelere ait düzeltmeler en kısa sürede sisteme yüklenmelidir.
Editör, hakem raporlarını ve/veya istenilen düzeltmelerin yeterli olup olmamasını dikkate alarak makalenin yayımlanıp yayımlanmamasına yönelik nihai karar vericidir. Makalenin yayımlanmasından önce makalede sayfa düzeni yapılarak son kontrol için yazarına gönderilir. Yazar makalenin son kontrolünü yaptıktan sonra ziraatdergi@gop.edu.tr mail adresine gönderir. Kontrolün düzgün yapılmaması sonucunda oluşabilecek baskı hataları yazarların sorumluluğundadır. İşlemi tamamlanan eserler kabul tarihi dikkate alınarak derginin yayınlanacak sayısında bulunması gereken makale limitleri dahilinde yayımlanır.

Alıntılanma Yüzdesi
Dergiye başvurusu yapılan makalelerin, hakemlik sürecine alınmadan önce intihal programı ile (iThenticate Plagiarism Detection Software) (http://www.ithenticate.com) taratılmış olması gerekmektedir. Tarama sonucunda Kaynaklar bölümü haricinde, benzerlik oranı %20 ve aşağı değeri taşıyan makaleler başvuruya kabul edilmektedir. Makale sisteme yüklendikten sonra iThenticate raporunun da ziraatdergi@gop.edu.tr mail adresine gönderilmesi süreç için gereklidir

Etik Kurul Onayı
Yazarlar yayımlatmak istedikleri makale ile ilgili olarak gerekli olan etik kurul onayını aldıkları kurumu ve onay numarasını Materyal ve Yöntem bölümünde mutlaka belirtmelidirler. Yayın kurulu gerekli gördüğünde "Etik Kurul Onay Belgesini" ayrıca isteyebilir. Makalenin etik kurul onayı gerektirip gerektirmediği aşağıda bildirilen kısımdan yazarlar ve alan editörleri tarafından mutlaka sorgulanması gerekmektedir.

Etik Kurul izni gerektiren araştırmalar aşağıdaki gibidir.
•Anket, mülakat, odak grup çalışması, gözlem, deney, görüşme teknikleri kullanılarak katılımcılardan veri toplanmasını gerektiren nitel ya da nicel yaklaşımlarla yürütülen her türlü araştırmalar
•İnsan ve hayvanların (materyal/veriler dahil) deneysel ya da diğer bilimsel amaçlarla kullanılması,
•İnsanlar üzerinde yapılan klinik araştırmalar,
•Hayvanlar üzerinde yapılan araştırmalar,
•Kişisel verilerin korunması kanunu gereğince retrospektif çalışmalar,
Ayrıca;
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Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
https://dergipark.org.tr/tr/pub/gopzfd

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2022) 39(2) 71-76
doi:10.55507/gopzfd.1105752

Scrutinization of Potential Concern in Estimating Hydrological Design Parameter in Changing Climate Conditions

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Alındığı tarih (Received): 19.04.2022 Kabul tarihi (Accepted): 09.06.2022

Abstract: Traditional approaches considered in estimating the design parameters of hydraulic structures assume that the data to be used in the analysis is stationary. However, the assumption of stationary has lost its validity, as the normal functioning of the hydrological cycle is deteriorated by climate change. Furthermore, the estimation of the required design parameter for hydraulic structures under non-stationary conditions would be evidence of failure for the structure in question. The aim of the study was to come up with what the regional frequency distribution characteristic of maximum rainfall with 2-h duration for the Euphrates-Tigris river basin would differ under non-stationary conditions. For this purpose, ITA and PITA approaches were used. Trends were detected in approximately 83% of the maximum rainfall series, with increasing variation in 80% of this amount. In order to reveal the variability in the regional frequency distribution of all rainfall series, the L-moments algorithm was applied to the data series of the first halve and second halve formed by the ITA method. It was found that remarkable differences were detected in the quantiles obtained for some risk levels.

Keywords: Euphrates-Tigris basin, frequency analysis, maximum rainfall

Değişen İklim Koşullarında Hidrolojik Tasarım Parametresinin Tahmininde Potansiyel Endişenin İncelenmesi

Öz: Hidrolik yapıların tasarım parametrelerinin tahmininde dikkate alınan geleneksel yaklaşımlar, analizde kullanılacak verilerin durağan olduğunu varsaymaktadır. Ancak, hidrolojik döngünün normal işleyişi iklim değişikliği nedeniyle bozulduğu için durağanlık varsayımı geçerliliğini yitirmiştir. Daha da ötesi, durağan olmayan koşullar altında hidrolik yapılar için gerekli tasarım parametresinin tahmini, söz konusu yapı için başarısızlığın kanıtı olacaktır. Çalışmanın amacı, Fırat-Dicle nehir havzası için 2 saat süreli maksimum yağışın bölgesel frekans dağılım özelliğinin durağan olmayan koşullarda ne gibi farklılık göstereceğini ortaya çıkarmaktır. Bu amaç için ITA ve PITA yaklaşımları kullanıldı. Maksimum yağmur serilerinin yaklaşık %83'ünde trend tespit edildi, bu miktarında %80'inde artan değişim bulundu. Tüm yağış serilerinin bölgesel frekans dağılımındaki değişkenliği ortaya çıkarmak için ITA yöntemiyle oluşturulan birinci yarı ve ikinci yarının veri serilerine L-momentler algoritması uygulanmıştır. Bazı risk seviyeleri için elde edilen niceliklerde dikkate değer farklılıklar tespit edildi.

Anahtar Kelimeler: Fırat-Dicle Havzası, Frekans Analizi, Maksimum Yağmur

1. Introduction

The IPCC (2013) highlighted that the variability in the global climate system was an indisputable fact. Under this reality of human intervention, the deterioration of the natural functioning of the hydrological cycle has allowed the experience of the changes in the amount and frequency of rainfall (Fotovatikhah et al. 2018; Li et al. 2018). The studies conducted on the climate of Turkey emphasized that there would be remarkable decreases in rainfalls and streamflows (Hemming et al. 2010; IPCC, 2007; Kitoh et al. 2008). Research on rainfall extremes, which have a considerable impact on the ecosystem, has increased recently (Cheng et al. 2015; Whang et al. 2012). In this context, a successful estimation of the design rainfall amount in the terms of planning, management, and cost of water-related structures, which are considered to be built in order to minimize the damage caused by maximum rainfalls, is also a necessity. On the other hand, it is assumed that the data to be used in the estimation of the design rainfall value based on the frequency analysis is provided under stationary climatic conditions. However, this assumption has lost its validity due to the reality of global climate change. The existence of trends in the rainfall datasets points out that its frequency distribution is inconstant over time. From this perspective, the stationarity of the relevant data should be satisfied to clear possible doubt while carrying out its frequency analysis. Hosking and Wallis (1997) underlined that performing a successful frequency analysis was based on reliable observations, also, that the assumption dealing with having analogous characteristics of frequency distributions belonging to the past and future observations would be ceased where

there was an upward or downward change in a given data over time.

Şen-Innovative Trend Analysis (ITA) has recently become a considerable tool to scrutinize variation in the meteorological data sequences (Şen, 2017). The distinctions of the approach from the others are that it does not expect to fit a certain distribution for the data to be analyzed, as well as it does not deals with the length of the data and serial dependence between observations. Şen (2020) proposed a new approach (PITA) to detect the change in frequency distribution behavior of past and future observations. In line with the above, the reliability of the data to be subjected to frequency analysis for the design rainfall value should be checked. Accordingly, the basic objectives of the study were a- to statistically evaluate variability in the maximum rainfalls with the ITA approach, b- to realize regional frequency analysis based on PITA (Probabilistic Innovative Trend Analysis) methodology and to reveal the difference in the frequency distributions of the past and future observations.

2. Materials and Methods

The materials and methods used in the study are given in the following subtitles.

2.1. Basin and Data

The Euphrates and Tigris rivers, which are the transboundary rivers of Turkey, merge within Iraq territory and flow into the Persian Gulf in Shatt al-Arab. The part of the basin within Turkey is 185.000 square kilometers. As of the geographical location of the basin belonging to these rivers, both rivers are vitally important water resources for Turkey and the Middle East. Therefore, the dispute regarding water allocation of the rivers among the riparian countries has been proceeding for a long time. Turkey has been claiming the demand for using more water due to the upstream position. Approximately 40 % of the mean annual streamflow to the Tigris River is supplied by the part of the basin in Turkey. The contribution to the Euphrates River by Turkey's territory is about 90 % (Daoudy, 2004; Kibaroglu and Unver, 2000). Approximately 28% of our country's water needs are supplied by the two rivers. The arid and semi-arid climate characteristics in the basin of these rivers are dominant. Precipitation falling in the basin of these rivers substantially occurs in the winter period (from October to April). A notable part of the precipitation during the period in question falls in the form of snow. Therefore, snowmelt from the highlands forms a considerable part of the river streamflows (Altınbilek, 2004; Ozdogan, 2011). Furthermore, the fact that the part in Turkey of the basin covers the GAP region, in which was planned 13 groups of irrigation and energy projects within the scope of the Southeastern Anatolian Project (GAP), has further increased its importance. The GAP has a critical role in the context of regional development and irrigation.

The annual average rainfall in the basin varied between 378 mm (Malatya Station) and 1234 mm (Bitlis station). This implies that the distribution of precipitation in the basin has a highly variable structure (Yurekli, 2015). In the northern and eastern parts of the basin, the altitude reaches up to 4,000 m. However, towards the south of the basin, the topography changes rapidly and the height decreases to 300 m. In the southern parts of the basin, evaporation is high as well as less precipitation.

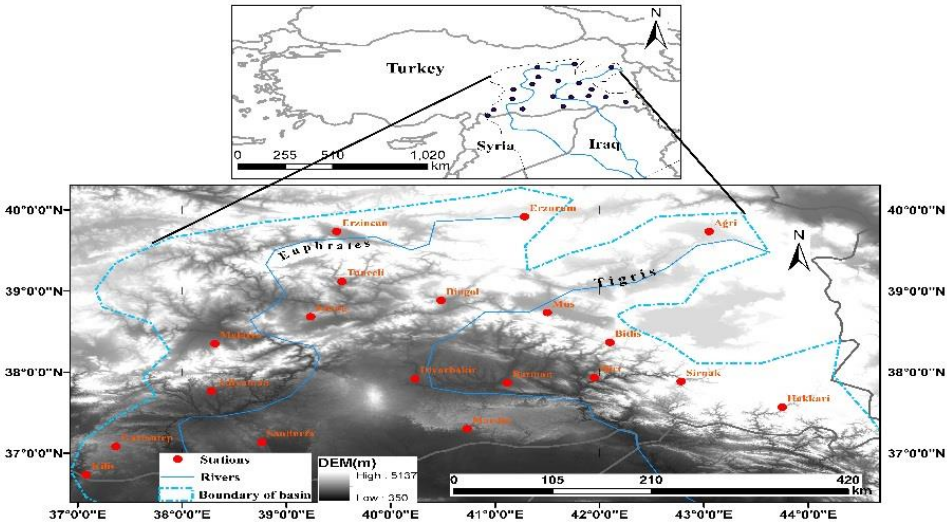

Figure 1. Geographic positions of the stations in the study
Şekil 1. *Çalışmadaki istasyonların coğrafik konumları*

The maximum rainfall data sequences with the duration of 2-h from 18 rainfall gauging stations in the upper Euphrates- Tigris basin were used as materials in the study to scrutinize variability in them. The missing maximum rainfall amounts with the duration of 2-h at any station in the study area were completed with the normal-ratio method. Some defining characteristics regarding the stations are presented in Table 1, and their geographic positions are available in Figure 1.

2.2. Şen-ITA Approach

The essence of this approach is based on splitting the original data into two equal parts and analyzing the scattering of two split data sets (first halve and second halve) against each other around the 1:1 line (with a slope of 45°). Based on this approach, the judgment on the trend is determined by the positioning of the data of the two halves according to the 1:1 line. The cases where the points corresponding to each pair of observations are above, below, or on the line with a slope of 45° point out an upward, downward trend or no change, respectively. In this state, a visual analysis is allowed, but it cannot be done statistically. But, the statistical significance test in the study conducted by Şen (2017) was brought to the literature. The statistical significance test is on the comparison of means averages belonging to the first (FH) and second (SH) halve series. The null hypothesis is accepted when the calculated slope (Scal) is smaller than the critical slope value, Scrit. Analysis details of this test are available in Yurekli (2021). The calculated test statistic and the confidence interval (CI) based on the critical test value were formulated in the following relationships.

$$S_{cal} = \frac{2(\bar{y}_2 - \bar{y}_1)}{n} \quad (1)$$

$$CI_{(1-\alpha)} = 0 \pm S_{crit}\sigma_S \quad (2)$$

$$\sigma_s = \frac{2\sqrt{2}}{n\sqrt{n}}\sigma\sqrt{1-\rho_{\bar{y}_1\bar{y}_2}} \quad (3)$$

Where $\bar{y}_1$ and $\bar{y}_2$ are the averages associated with the first and second halves; $\rho_{\bar{y}_1\bar{y}_2}$ is cross-correlation coefficient; σ is standard deviation of the full data; n is the number of observations in the full data.

The S_{crit} value (±1.960) is obtained based on the Standard Normal Distribution for the 5% (α) significance level.

2.3. Regional Frequency Analysis based on PITA Approach

The PITA approach is concerned with the probabilistic behavior of two equal datasets (FH and SH) split according to the ITA method. Pettitt (1979) underlined that the fact that parts of a time series had different frequency distributions indicated the existence of a change point in that series. The PITA approach was also based on this reference. Regional frequency analysis was performed with the L-moments algorithm using data sets of both halves for 18 stations in the basin. Thus, the regional variability in the probabilistic behavior of the data sequences belonging to both halves would be revealed. In the regionalization process, the L-moment algorithm is carried out in the stages described below (Hosking and Wallis, 1997).

First, the L-moments statistics, namely the measure of location, the coefficient of L-variation, the coefficient of L-skewness, and the coefficient of L-kurtosis (λ_1, L-CV, L-CS, and L-CK), of the data series of the two halves belonging to each station are calculated. In the next step, the discordancy (Di) between the stations in the tentatively formed region is tested by considering the L-moments statistics. With the detection of discordant station(s), it is decided to divide the relevant region into sub-regions as an alternative. This process continues until no discordant stations are found. Then, after it was found that none of the stations in the formed regions were discordant, the homogeneity of the region is tested with the H measure. If this measure is H<1 for a region, that region is judged to be acceptably homogeneous. In the last step, the regional distribution is determined among the candidate distributions depending on the goodness-of-fit measure (Z^{DIST}). The distributions satisfying the condition of the $\left|Z^{DIST}\right| \leq$ 1.64 are accepted as regional distributions. The details on how to perform these analyzes are available (Hosking and Wallis, 1997). The Generalized Logistic (GLOG), Generalized Extreme Values (GEV), Generalized Normal (GNO), Pearson Type III (PIII), and Generalized Pareto (GPA) distributions were considered as candidate distributions in the study. The implementation of the above-mentioned L-moments algorithm was achieved by the Fortran routines developed by Hosking (1996).

The quantile estimation at a given return period is fulfilled according to the index-storm approach of Dalrymple (1960). The mathematical relationship of the approach for site i is as follows;

$$Q_i(F) = \mu_i q(F) \quad (4)$$

Where Q(F) and μ_i are respectively the quantile and index rainfall value for the site i, and q(F) is the value dealing with the growth factor. F is the level of probability.

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3. Results and Discussion

The study was conducted to detect how the temporal variability in the maximum rainfall data sets with the duration of 2-h belonging to 18 rainfall stations of the basin would influence their probabilistic behavior. Firstly, the analysis of variability in the maximum rainfalls was scrutinized by the ITA approach. In line with this goal, after the full data set was divided into the first halve (FH) and second halve (SH), their calculated slope values (Scal) were compared with the critical slope value (Scrit). The ITA results for the stations in question are available in Table 1. Statistically, insignificant trend was detected in only three (Ağri, Erzurum, and Mus) of them out of all stations, while a statistically significant downward was also found in the three stations (Malatya, Mardin, Sanliurfa). On the other hand, the remaining stations had a statistically significant increasing change. The results highlighted that all rainfall series (excluding the three data) were subjected to statistically significant variability over time. In fact, this finding pointed out that it was imperative to perform frequency analyzes under the existing non-stationary conditions. The following findings are regionally in line with this basis, considering Şen (2020)'s proposal (PITA) for station-based.

Table 1. Geographic features and ITA results for the stations
Çizelge 1. *İstasyonların coğrafik özellikleri ve ITA sonuçları*

Rainfall Stations	Elevation (Meter)	Mean (mm)	ITA Results	Rainfall Stations	Elevation (Meter)	Mean (mm)	ITA Results
Adiyaman	669	713	0.034	Gaziantep	840	554	0.168
Agri	1640	526	**-0.037**	Hakkari	1720	783	0.098
Batman	550	492	0.118	Kilis	680	498	0.030
Bingol	1177	953	0.149	Malatya	977	377	-0.075
Bitlis	1545	1234	0.157	Mardin	1150	678	-0.108
Diyarbakir	677	487	0.063	Mus	1300	765	**0.022**
Elazig	1015	410	0.072	Siirt	895	718	0.042
Erzincan	1214	380	0.047	Sanliurfa	547	453	-0.078
Erzurum	1893	434	**-0.005**	Tunceli	914	884	0.093

*The bolded ones denote statistically insignificant trend.

In the study, the regional frequency analysis was applied to the FH and SH data sequences of all stations as the first attempt. It was found that not all of the regionalization steps described above were satisfied for the FH and SH data sets. Therefore, the possibility of evaluating all stations as a single region has been eliminated. Therefore, the process of forming sub-regions, in which all the regionalization phases would be achieved, was started. At first, efforts were made to divide the basin into two sub-regions. In the effort of two sub-regions for the FH, all stages of the regionalization process were able to be achieved when the Mus station was left out of action. On the other hand, the SH data of all stations were used for the aforementioned trial, and the regionalization process was completed. But, although two sub-regions were formed for the two halves, the stations assigned to their sub-regions showed no similarity in terms of numbers and names. The results of regionalization for both halve data sets are in Table 2. It was found that all of the candidate distributions considered in the study could be used as a regional distribution for the first sub-region (SR1) of the FH, while there were three candidate distributions (GLOG, GEV, GNO) for the first sub-region of the SH. But, for the second sub-regions (SR2) of the FH and SH, the same candidate distributions (GEV, GNO, PIII) were chosen as the regional distribution. Among the distributions found suitable based on the Z DIST value, the distribution having the smallest value for the sub-regions was chosen as the best fit (Table 2). These findings support the idea of Pettitt (1979). The first sub-regions of FH and SH indicate a different probabilistic structure. On the other hand, although the second sub-regions of these two halves have the same distribution characteristic, the differences in the stations in the region should not be overlooked.

Table 2. Heterogeneity and Goodness-of-Fit results for two halves
Çizelge 2*. İki yarı için Heterojenlik ve Uyum iyiliği testi*

Regionalization Tests	First Halve		Second Halve	
	$2FH\text{-}_{SR1}$	$2FH\text{-}_{SR2}$	$2SH\text{-}_{SR1}$	$2SH\text{-}_{SR2}$
H measure	0.41	-0.39	-0.96	0.69
Z^{DIST} measure	GEV (0.06)	PIII (-0.34)	GLOG (0.09)	PIII (-0.26)

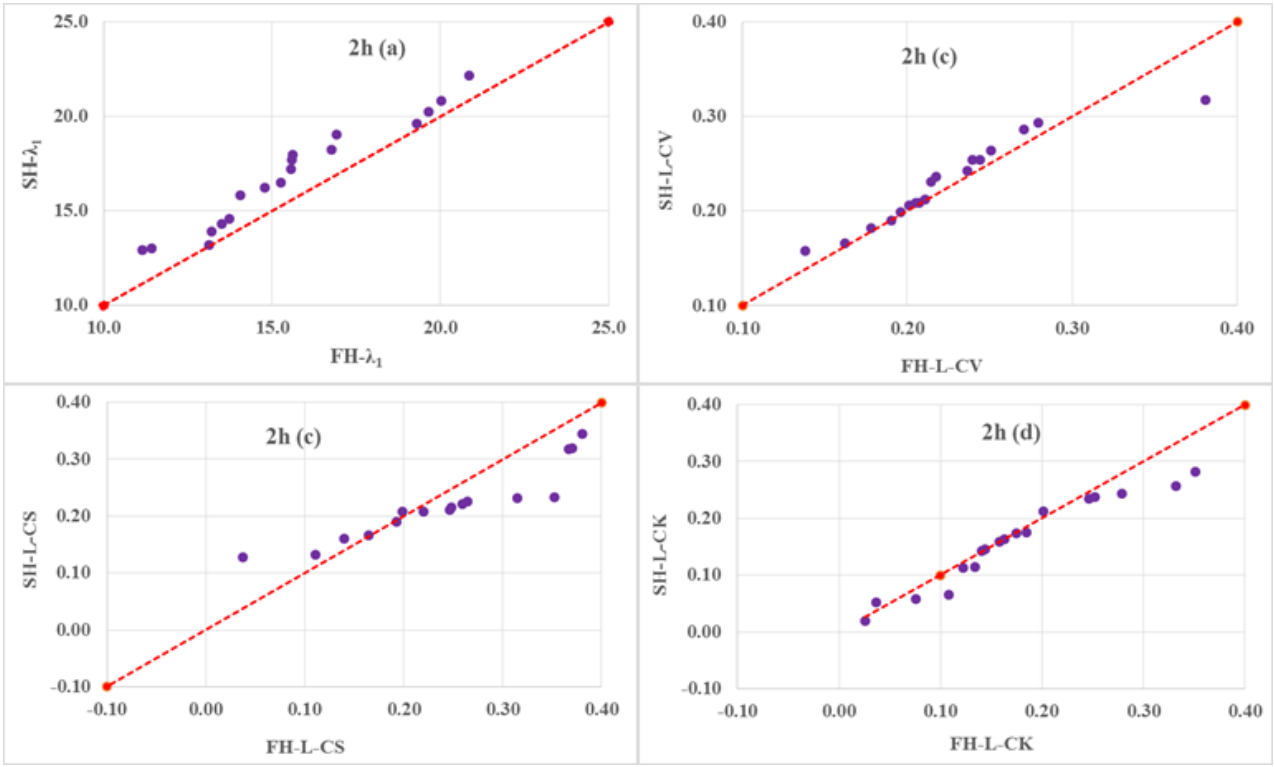

Figure 2. Variability in the L-moment statistics of the FH and SH rainfall series
Şekil 2*. FH ve SH yağmur serilerinin L-moment istatistiklerindeki değişim*

The first L-Moment and L-moment ratios of the rainfall data sequences with 2-h duration of the FH and SH were positioned according to the 1:1 line within the ITA mentality to visually reveal the change in these statistics (Figure 2). An increase was detected in the λ1 statistic of the Maximum rainfall data series with the 2-h duration. This detection determines that the maximum rainfall amounts of the FH are smaller than that of the SH. When analyzed in terms of L-CV statistics, it is seen in Figure 2b that there is a partial increase. In other words, this L-moment ratio showed no significant change in the region. In terms of the region, it is noteworthy that there is a significant decrease especially in large L-CS values (Figure 2c). On the other hand, the L-CK statistic is scattered around the 1:1 line in a structure similar to the L-CS. The change detected in these L-moment statistics was clear evidence that the data used in performing the regional frequency analysis was not obtained under stationary conditions. In the regionalization process, the variability in these statistical parameters, which have great importance in defining the regional distribution, would obstacle the decision of the distribution best fit to the region.

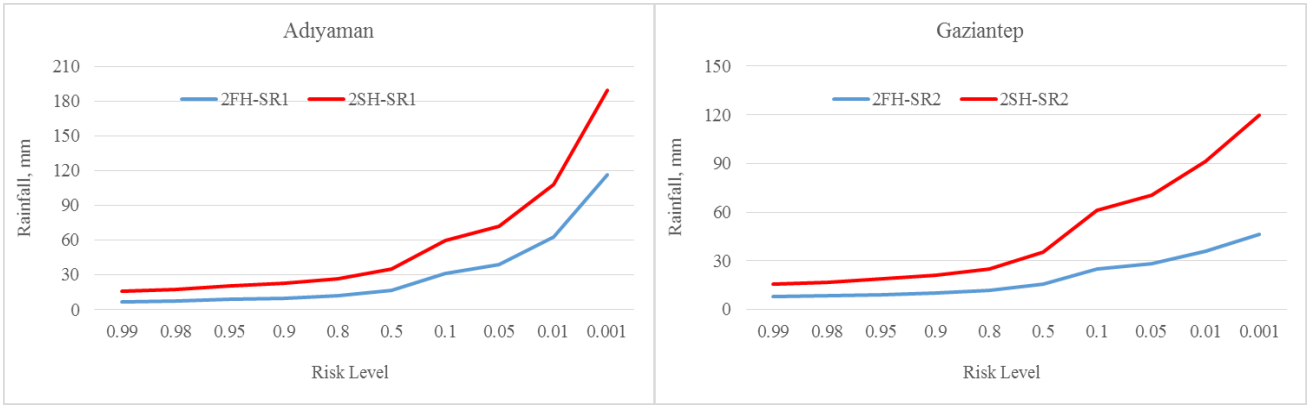

Figure 3. Variation of the quantiles estimates for risk levels
Şekil 3. *Risk seviyeleri için nicelik tahminlerinin değişimi*

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One of the main objectives in the current study was to come up with the change between the quantiles estimated regionally, but the assignments of the different stations to the sub-regions of the first and second halves was an obstacle to reaching the intended target. However, instead of this goal, the station-based quantile estimates for the FH and SH data sets regarding the stations selected, namely Adiyaman and Gaziantep, were performed based on the method of Dalrymple (1960). The relevant results are in Figure 3. The fact that there was an upward trend in the maximum rain series at these stations has caused an increase in the quantile estimates. The difference between the large quantile estimates for both halves also increased significantly.

4. Conclusion

The prevalent assumption in traditional practices to the frequency analysis of hydro-meteorological data sequences is that the hydrological variable has stationary over time. With the reality of global climate change, the fulfillment of the expectations from a hydraulic structure constructed based on executing frequency analysis under the assumption of stationarity would be insufficient. This study was conducted to detect how variability in climate conditions affects regional frequency analysis regarding rainfall series with the 2-h duration. The goal was achieved by the L-moments algorithm considering the PITA approach. With the ITA approach, statistically significant changes were detected in approximately 83% of the maximum rainfall series. This finding indicated that the frequency distributions of the rainfall series of two halves (FH and SH) split according to the ITA mentality would be different. The regionalization results based on the FH and SH rainfall series showed that the best fit regional distributions representing each halve series were different. Besides, the λ1, L-CV, L-CS, and L-CK statistics were compared to each other within the ITA procedure in order to come up with the variation in the regional frequency distribution behavior. In this respect, significant changes were detected. Additionally, the station-based quantile estimates at different risk levels for the first halve and second halve data sequences were carried out based on the procedure of Dalrymple (1960). In particular, the differences between large quantile estimates were greater.

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Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
https://dergipark.org.tr/tr/pub/gopzfd

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2022) 39(2) 77-84
doi:10.55507/gopzfd. 1131221

Bioinformatical Evaluation of PPARA and PPARG Candidate Genes for Milk Quality Characteristics in Turkish Saanen Goats

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Alındığı tarih (Received): 15.06.2022 Kabul tarihi (Accepted): 07.07.2022

Abstract: In this study, genetic information obtained in terms of milk yield and milk quality characteristics was used in order to increase the accuracy of breeding selection of Turkish Saanen goats. For this purpose, DNA sequencing of PPARG and PPARA genes from Turkish Saanen goats were compared with the sequences in gene databases and single nucleotide polymorphisms were examined. A relationship was found between some quality parameters (fat, protein and somatic cell count) of milk measurements of Turkish Saanen goats and PPARA and PPARG gene variants. Single nucleotide polymorphisms were detected in the exons and introns of the relevant gene variants, however, these polymorphisms did not have a statistically significant effect on quality parameters ($p>0.05$).

Key words: Bioinformatics, Candidate genes, PPARA, PPARG, Turkish Saanen goat

Türk Saanen Keçilerinde Süt Kalite Özellikleri ile İlgili PPARA ve PPARG Aday Genlerinin Biyoinformatik Değerlendirmesi

Öz: Bu çalışmada Türk Saanen'i keçilerinin damızlık seçiminde isabet derecesini artırmak amacıyla, süt verimi ve süt kalite özellikleri bakımından elde edilen genetik bilgilerinden yararlanılmıştır. Bu amaçla Türk Saanen keçilerinden alınan kanlardan izole edilen DNA örneklerinden PPARG ve PPARA genlerine ait DNA dizileme yapılarak elde edilen diziler, gen veri bankalarındaki dizilerle karşılaştırılarak tek nükleotid polimorfizmleri incelenmiştir. Türk Saanen keçilerinin süt ölçümlerine ait bazı kalite parametreleri (yağ, protein ve somatik hücre sayısı) ile PPARA ve PPARG gen varyantları arasında ilişki ortaya çıkarılmıştır. İlgili gen varyantlarının ekzon ve intronlarında tekli nükleotid polimorfizmleri tespit edilmiş, bununla beraber bu polimorfizmlerin kalite parametreleri üzerine istatistiksel olarak anlamlı bir etkisi bulunmamıştır ($p>0.05$).

Anahtar Kelimeler: Biyoinformatik, Aday genler, PPARA, PPARG, Türk Saanen keçisi

1. Introduction

The origin of Turkish Saanen goats is based on the Saanen goats brought to Turkey in 1959 for the purpose of improving goat breeding. The Turkish Saanen goat, which was created as a result of crossing Saanen goats and mostly Turkish Hair or Maltese goats in Western Anatolia, is bred as a dominant milk yielding goat in the region. Goat milk contains small fat globules and is easily digestible compared to other milks due to the structural differences of milk proteins. Due to its richness in short and medium chain, single and multi-fatty acids, it is an animal product recommended for infants and the elderly and those with stomach disorders (Haenlein, 2007; Ataç et al., 2018).

In this context, milk yield is one of the most economically important features in Turkish Saanen goat breeding. Along with the amount of milk, components of milk such as protein, fat and the number of somatic cells are among the most important quality criteria affecting the processing of milk (Najafi et al., 2009). Therefore, studies to increase the amount and quality of goat milk are important. There are limited studies conducted with Turkish Saanen goats and generally using morphological and physiological parameters. As a matter of fact, there are many candidate genes associated with growth, reproduction, milk and hair yield parameters in goats (Benjelloun et al., 2015; Işık, 2017; Yakan et al., 2018). Detection of these candidate genes, which can affect milk quality in goat breeding, will increase the accuracy of breeding selection.

Among the candidate genes affecting milk quality, the PPARA (Peroxisome Proliferator Activated Receptor Alpha) gene; it is a gene activated by many fatty acids and fatty acid-derived compounds. Discovered in the early 1990s, hepatic lipid metabolism;

It plays a key role by inhibiting the enzyme 3-hydroxy 3-methyl glutaryl coenzyme A reductase. Synthetic agonists of this gene reduce the plasma triglyceride level and increase the plasma HDL cholesterol level by stimulating the synthesis of apolipoprotein (Apo) AI and Apo-AII in the liver. It is also used in the treatment of dyslipidemia by inhibiting the production of Apo-CIII, a lipoprotein lipase inhibitor (Shipman et al., 2016). Ebrahimi et al., (2015) reported increasing PPARA gene expression in the liver tissue of goats fed diets rich in α-linolenic acid.

The PPARA gene is located on the 5th chromosome in goats and consists of 9 exons. PPARA activation regulates the uptake, utilization and degradation of fatty acids by regulation of genes involved in fatty acid transport, fatty acid binding and activation, and peroxisomal and mitochondrial fatty acid β-oxidation (Kersten 2014). PPARA helps to reduce pain and inflammation by inhibiting the release of various proinflammatory and pro-angiogenic enzymes (D'Agostino et al., 2007). In a study on goats; It was determined that PPARA gene expression increased in the liver tissue of goats fed α-linolenic acid-rich diets (Ebrahimi et al., 2015).

PPARG (peroxisome proliferator activated receptor gamma) is a ligand-dependent transcription factor. They act as sensors of hormones, vitamins, endogenous metabolites and xenobiotic compounds and are involved in the control of expression of many genes as members of the nuclear receptor superfamily. It is known that PPARG plays a role as a transcription factor in fat cell differentiation, fatty acid storage and glucose metabolism (Martin, 2010).

PPARG controls various sequences of biological processes by modulating the expression of specific target genes through a ligand-dependent mechanism (McKenna & O'Malley, 2002). PPARG is involved in the regulation of lipogenic pathways in the mammary gland. (Bionaz et al., 2013). PPARG gene polymorphisms or changes in the expression of this gene can lead to changes in the energy metabolism of the tissues and organs in which the gene is expressed, and may affect milk production and composition. A positive correlation was observed between the increase in milk production during lactation and the stearoyl-coenzyme A desaturase (SCD) enzyme in the mammary glands and PPARG, which regulates its expression in goats (Shi et al., 2013a). In addition, it has been stated that PPARG gene polymorphism affects milk, protein, dry extract and lactose yields and milk lactose content in goats (Ferreira et al., 2020), while in cattle meat tenderness (Fan et al., 2011), meat fatty acid profile and intramuscular It has been found to be associated with fat ratio (Lee et al., 2016).

This gene is involved in the synthesis and secretion of triacylglycerol from mammary gland epithelial cells in goats (Shi et al., 2013a). It has also been reported that PPARG stimulates the synthesis of monounsaturated fatty acids in goat mammary epithelial cells through the control of the enzyme stearoyl-coenzyme A desaturase (Shi et al., 2013b).

The information obtained according to the PPARG and PPARA genes can increase the success of selection studies, as well as save time and cost in breeding studies for the highlighted trait. However, understanding the genetic mechanism in the formation of components such as protein and fat, which also affect the nutritional value of milk, allows changes such as differentiation of nutritional value, reduction or increase of organoleptic properties for consumer demands (Pramod et al., 2018).

In this study, bioinformatics evaluation of PPARA and PPARG candidate genes related to milk yield and milk quality characteristics in Turkish Saanen goats was carried out.

2. Material and Methods

The study was carried out in a private dairy goat farm engaged in intensive breeding in Izmir-Turkey in 2019. The animal material was composed of 40 heads of Turkish Saanen goats, 3 years old and same lactation, with the same environmental conditions in terms of care, feeding and sheltering methods within the enterprise. Blood samples were taken from the vena jugular vein, once, from goats, into tubes with EDTA and without anticoagulant. Milk samples were taken from the milk obtained from the goats that were milked individually in the middle of the periods to represent the first three, 4-5 and 6-9 months of lactation. Goats were followed throughout lactation, milked with a milking machine and milk yields were recorded.

Goats were fed with goat milk feed containing 18 protein 2800 ME (kcal/kg). In the TMR program, a ration was applied according to 17% protein and 2600 ME (kcal/kg) dry matter and milk yield, the goats were fed in the same compartment in the barn and were not taken to the pasture. 1.5 kg of dried alfalfa grass and 1.0 kg of corn silage were used as roughage. According to the daily milk yield of the goats, additional milk feed needs were given individually in the feed vending machine with RFID system.

Dry matter (%), fat (%), true protein (%), total protein (%), lactose (%), non-fat dry matter (%) and

freezing point (°C) in goat milk samples, using Bentley150 device, somatic cell number (cells/mL-milk) was also determined using the Somacount150 instrument (Bentley Instruments, Inc., Chaska, Minnesota, USA). In the Bentley150 device, the composition of the milk is determined by the mid-infrared (MIR) spectrometry technique (Bentley150 Operator's Manual, 1999), in the Somacount150 device the somatic cell count (SHS) is determined by the flow cytometry method (Somacount150 Operator's Manual, 1998) has been detected.

RNA isolation from goat milk somatic cells was performed using "TriPure Isolation Reagent" (Sigma ALDRICH) according to the steps outlined in the instructions for use. Concentrations of isolated RNAs were measured by spectrophotometric methods. cDNA synthesis was performed using Thermo Scientific™ RevertAid RT Reverse Transcription Kit, RNA sample 12 µl for PCR, total 20 microliters, 5X Reaction Buffer 4 µl, RiboLock RNase Inhibitor (20 U/µL) 1 µl, 10 mM dNTP Mix 2 µl , RevertAid RT (200 U/µL) was added to 1 µl tubes.

Primers for PPARA, PPARG and ACTB gene regions taken as reference (control) genes were designed specifically for goat (*Capra hircus*) using the NCBI and ENSEMBLE gene banks. The specificity of the obtained primers was checked with the BLAST (Basic Local Alignment Search Tool, NCBI) program. Primers used for amplification of PPARA, PPARG, ACTB genes are given in Table 1.

Table 1. Primers used for amplification of PPARA, PPARG, ACTB genes
Çizelge 1. *PPARA, PPARG, ACTB genlerinin çoğaltılması için kullanılan primerler*

Gene	Primer	Primer Sequence (5'-3')
PPARA	Forward	GGATCAGATGGCTCCGTTATT
	Reverse	GCAGATCCTACACTCGATGTTC
PPARG	Forward	GGACATTCCGTTCCCAAGAG
	Reverse	GGATACAGGCTCCACTTTGATT
ACTB	Forward	CCCAGCACGATGAAGATCAA
	Reverse	GACAGCGAG GCCAGGAT

The synthesized cDNAs were studied with the Roche LightCycler 480 II instrument using the LightCycler® 480 SYBR Green I Master with primers designed for the indicated gene regions. Real Time PCR components total volume 10 µl Water, PCR-grade 1.9 µl, Forward Primer (F) (intermediate stock 10 µM) 0.3 µl, Reverse Primer(R) (intermediate stock 10 µM) 0.3 µl, Enzyme Mix (LightCycler® 480 SYBR Green I Master) 5 µl, cDNA 2.5 µl were placed in tubes.

In order to detect SNPs in PPARA and PPARG genes, primers used in PCR and Sanger sequence analyzes were designed specifically for Capra hircus using the NCBI and ENSEMBLE gene banks (Table 2). The specificity of the obtained primers was checked with the BLAST program.

Table 2. Primers and DNA sequences used for SNP analysis
Çizelge 2. *SNP analizi için kullanılan primerler ve DNA dizileri*

Primer		Primer Sequence (5'->3')
PPARA	Forward	CGATTCTGAGGCTGTCTAAGG
	Reverse	CGCTGCTGGGTTCTCAA
PPARG	Forward	GCAAAGCGAGTGTTTGTGAAG
	Reverse	TGTGTGGAAAGTGCGGTAAG

DNA samples were studied with PPARA and PPARG specific primers and FastStart High Fidelity PCR System, Thermal Cycler device using dNTPack (Roche) kit. PCR Grade Water 17.25 µl for a total of 25 µl of PCR components, FastStart High Fidelity Reaction Buffer (10x-with 18 mM MgCl2) 2.5 µl, PCR Grade Nucleotide Mix (10mM) 0.5 µl, DMSO 0.5 µl, Forward Primer (intermediate stock) 10 µM) 0.5 µl, Reverse Primer (intermediate stock 10 µM) 0.5 µl, FastStart High Fidelity Enzyme Blend (5U/µl) 0.25 µl, DNA sample was placed in 3 µl tubes. PCR temperature and cycles, initial denaturation 1 cycle, 94°C for 10 minutes, amplification 35 cycles for denaturation 94°C 2 minutes, attachment 57°C 30 seconds and elongation at 72°C 1 minute, final elongation 1 cycle After 7 minutes at 72 °C, it was cooled to 4 °C.

At the end of the PCR analysis, 1.4% agarose gel electrophoresis was performed to see the band formation in the PCR samples. The gel was visualized with a UV Transilluminator after running.

The obtained PCR products were purified using EXOSAP and then sequence PCR was established from the obtained purified PCR products. For an amplification tube (10 µl), the reaction mixture is BigDye Ready Reaction Mix 1 µl, Primer (3.2pmol/µl) 0.5 µl, Distilled water 4.0 µl, 10x Buffer 2 µl, Dye saving 2 µl and Purified PCR product 2.5 µl. has been placed. PCR amplifications were performed for 25 cycles at 96 °C for 10 seconds, at 50 °C for 5 seconds, at 60 °C for 4 minutes and cooled at 4 °C. The resulting sequence PCR products were cleaned with the Zymogen DNA Sequencing Clean-Up kit.

The data obtained at the end of Real Time PCR analysis were analyzed with Absolute Quantification

using Roche LightCycler480 software and normalized values were calculated for target genes for each sample.

Obtained nucleotide sequences were analyzed by multiple sequence alignment method with Molecular Evolutionary Genetics Analysis (Mega X) software and nucleotide polymorphisms were compared with DNA sequences in gene banks with BLAST. Possible single nucleotide polymorphisms (SNP) of gene variants were extracted with bioinformatics programs and their effects were determined.

One Way ANOVA was used in terms of milk components (dry matter, fat, true protein, total protein, lactose, nonfat dry matter, freezing point, somatic cell count, milk yield), target value (T/RPPARA and T/RPPARG) properties; According to the analysis assumptions in question, the normal distributio of the data was tested and transformations were applied for the features that did not show normal distribution.

T/RPPARA for PPARA and T/RPPARG for PPARG and logarithmic transformation for somatic cell number were applied. Since the freezing point properties for dry matter, true protein, total protein, lactose and non-fat dry matter and PPARG did not show normal distribution, SNP differences were analyzed with the nonparametric Mann-Whitney U test. IBM SPSS v25.0 program was used in all statistical analyzes of the study.

3. Results and Discussion

Descriptive statistics for milk components (dry matter, fat, true protein, total protein, lactose, nonfat dry matter, freezing point, somatic cell count), milk yield and target values (T/RPPARA and T/RPPARG) are given in Table 3.

Table 3. Descriptive statistics
Çizelge 3. *Tanımlayıcı istatistikler*

	Mean	Std. Deviation	Minimum	Maximum
T/RPPARA	.00048	.00029	.000000113	.0099
T/RPPARG	.00083	.00050	.000000100	.0165
Dry Matter (%)	11.67	0.25	8.18	16.10
Fat (%)	3.48	0.11	2.18	6.01
True protein (%)	3.49	0.13	2.26	6.79
Total protein (%)	3.72	0.11	2.43	7.37
Lactose (%)	4.67	0.19	3.40	8.86
Non-fat Dry Matter (%)	8.73	0.08	7.92	10.55
Freezing Point (°C)	-.578	0.002	-.550	-.594
Somatic Cell Count (x 1000) cell/mL-milk	899.01	98.62	111	2592
LMY milk (L)	520	77.45	480	650

When evaluated for the PPARA gene, 4 SNPs were determined in the samples examined, and two of these SNPs are located in the gene banks (PPARASNP1-rs640787651 and PPARASNP3-rs665742135). In addition, 2 detected SNPs are not included in the data banks (PPARASNP2 and PPARASNP4). As a result of the SNP analysis performed in the longest exons and intron regions of the PPARA gene, the gene variant registered in the gene banks with the number rs640787651 in the 6th and 7th introns of the PPARA gene was detected in 1 sample. In this gene variant, a single nucleotide polymorphism was seen in which Thymine was replaced by Cytosine. PPARASNP2 was detected in 6th and 7th introns in 1 sample, and there was a change in which Adenine nucleotide replaced Guanine. One SNP registered in the databases with the number rs665742135 has been detected (PPARASN3). It was found that Cytosine nucleotide replaced Thymine nucleotide in this SNP, which was detected in 8 samples and located in the seventh exon, but it was observed that the coded Serine amino acid did not change. In addition, a single nucleotide polymorphism was detected in the 7th and 8th introns of 2 samples, in which Adenine nucleotide replaced the Guanine nucleotide. This gene variant is also not included in the gene banks data (PPARSNP4).

In the analysis for the PPARG gene, 4 SNPs that were not registered in gene banks were found (PPARGSNP1, PPARGSNP2, PPARGSNP3, PPARGSNP4). While two of these SNPs are found in exons, two of them are determined in introns. The most common SNP in the samples for PPARA and PPARG genes is PPARGSNP1 found in 9 samples, and it is located in exon 6. In this single nucleotide polymorphism, it was determined that the Cytosine nucleotide was replaced by the Thymine nucleotide. However, it was determined that the coded Tyrosine amino acid did not change.

PPARGSNP2 and PPARGSNP3 were the only two SNPs detected in the same sample. These polymorphisms occurred in the 6th exon and 6th and 7th introns of the gene and are not available in data banks. In PPARGSNP2 detected in the sixth exon, it was determined that the Thymine nucleotide replaced the Cytosine nucleotide, but there was no change in the coded Phenylalanine amino acid. In the SNP detected in the introns, it was found that the Thymine nucleotide replaced the Adenine nucleotide. PPARGSNP4 was detected in 2 samples and was found in the 6th and 7th introns.

The following statistical models and terms were used for the one-way analysis of variance applied to reveal the SNP differences in the study.

$Y_{ijk}=\mu+a_i+e_{ij}$ (1)

In the equation, μ: the population mean in terms of the tested trait, a_i: i. define the group (SNP) effect and the e_{ij} random error effect.

Variance analysis results are given in Table 4 and Table 5. While the effects of SNP2 on the target value of T/RPPARA and SNP3 on T/RPPARG were significant ($p<0.05$), there was no significant difference between gene expression levels in terms of other characteristics ($p>0.05$).

According to the results of the analysis, the effect of PPARASNP2 was found to be significant in the change of the T/RPPARA ratio used in the analysis of the change in gene expression. Similarly, the effect of PPARGSNP3 change on T/RPPARG change was found to be significant.

The results of the nonparametric Mann-Whitney U test obtained in the study are given in Tables 6 and 7.

Table 4. Significancy levels for PPARA
Çizelge 4. *PPARA için önemlilik düzeyleri*

	PPARASNP1-rs665742135	**PPARASNP2**	**PPARASNP3-rs665742135**	**PPARASNP4**
T/RPPARA	0.755	0.006*	0.523	0.404
Fat (%)	0.188	0.069	0.068	0.109
Somatic Cell Count (x 1000) cell/mL- milk	0.179	0.454	0.792	0.848
Milk (gr)	0.877	0.305	0.100	0.517

*: P<0.05

Table 5. Significancy levels for PPARG
Çizelge 5. *PPARG için önemlilik düzeyleri*

	PPARGSNP1	**PPARGSNP2**	**PPARGSNP3**	**PPARGSNP4**
T/RPPARG	0.617	0.491	0.020*	0.491
Fat (%)	0.996	0.416	0.088	0.416
Somatic Cell Count (x 1000) cell/mL- milk	0.224	0.377	0.550	0.377
Milk (gr)	0.244	0.170	0.451	0.170

*: P<0.05

Table 6. Nonparametric statistics for PPARA
Çizelge 6. *PPARA için parametrik olmayan analiz sonuçları*

	PPARASNP1-rs665742135	**PPARASNP2**	**PPARASNP3-rs665742135**	**PPARSNP4**
Dry Matter (%)	0.567	0.118	0.143	0.286
True protein (%)	0.899	0.014*	0.026*	0.110
Total protein (%)	0.390	0.037*	0.339	0.328
Lactose (%)	0.567	0.044*	0.026*	0.091
Non-fat Dry Matter (%)	0.086	0.144	0.011*	0.374

*: P<0.05

Table7. Nonparametric statistics for PPARG
Çizelge 7. *PPARG için parametrik olmayan analiz sonuçları*

	PPARGSNP1	**PPARGSNP2**	**PPARGSNP3**	**PPARGSNP4**
Dry Matter (%)	0.972	0.155	0.075	0.155
True protein (%)	0.297	0.286	0.445	0.286
Total protein (%)	0.781	0.477	0.126	0.477
Lactose (%)	0.626	0.183	0.702	0.183
Non-fat Dry Matter (%)	0.972	0.534	0.098	0.534

In accordance with the nonparametric analysis results, the effect of PPARASNP2 on true protein, total protein and lactose was significant ($p<0.05$) for PPARA, while the effect of PPARASNP3-rs665742135 on true protein, lactose and dry matter was significant. There was no significant difference between gene expression levels and characteristics for PPARG ($p>0.05$).

On the other hand, target gene and reference gene expression ratio was used to examine the change of gene expression (T/RPPARA and T/RPPARG). Relationships between T/RPPARA, T/RPPARG, and traits were examined using the Pearson correlation test. As a result of the Pearson correlation test of T/RPPARA and T/RPPARG and milk yield values, a significant correlation of 0.05 was found between T/RPPARA and

True protein (%) and lactose (%). Although there was no significant correlation between T/RPPARG and milk yield values, it was determined that there was a negative correlation between dry matter (%), total protein (%) and somatic cell count (x1000 cells/mL- milk).

Relationships between T/RPPARA and rs665742135 gene variant and between T/RPPARG and SNP detected in 6th and 7th introns were investigated by regression analysis. The multiple regression model used in the study is given below:

$$Y= b_0+b_1X_1+ b_2X_2+b_3X_3+ b_4X_4+e_i \quad (2)$$

Where: Y= dependent (T/RPPARA and T/RPPARG) variable, X_i= (i = 1,2,3,4) independent (SNP) variables, b_i= (i: 1,2,3,4) denotes regression parameters and e_i: random error.

While the SNP numbered rs665742135 on T/RPPARA, which is the target value of PPARA, was found to be statistically significant ($p<0.05$), no significant contribution was found for other SNPs ($p>0.05$). While SNP3 was found to be statistically significant ($p<0.05$) on T/RPPARG, the target value of PPARG, other SNPs did not have a significant contribution ($p>0.05$).

Abousoliman et al. (2021) investigated the relationship between genome wide SNP and milk performance characteristics in Egyptian Barki Sheep and stated that PPARA has a critical role in the regulation of milk fat synthesis. While the effect of PPARASNP2 on protein and lactose was significant ($p<0.05$), the effect of PPARASNP3-rs665742135 on true protein, lactose and lean dry matter was significant, so further investigation of these SNPs as candidate genes would be beneficial.

The Peroxisome Proliferator-activated Receptor Gamma gene has a significant effect on the transcription of genes involved in lipid metabolism. Since the genes affected by the receptor are also highly expressed in the mammary glands of organisms, there may be a relationship between them and the characteristics related to milk production (Pramod et al., 2018). According to Shi et al. (2013b) found a significant relationship between the Peroxisome Proliferator-activated Receptor Gamma gene and the regulation of triacylglycerol synthesis in mammary cells in their study in goats. Ferreira et al. (2020) investigated the effects of polymorphisms in PPARG and UCP2 genes on milk yield and composition in goats; They reported that the PPARG-A allele they detected in the PPARG gene positively affected the total milk, fat, protein, dry matter, and lactose content even in heterozygous condition.

Yakan et al. (2018) examined the effects of some candidate genes on mastitis resistance, milk yield, and milk quality in goats, and reported that the expression of the Peroxisome Proliferator-activated Receptor Gamma gene was downregulated in the later stages of lactation. Although there was no significant correlation between T/RPPARG and milk yield values in our study, it was determined that there was a negative correlation between dry matter (%), total protein (%) and somatic cell count (x1000 cells/mL- milk), due to the downregulation of the receptor gene. At the same time, the significant effect of PPARGSNP3 on T/RPPARG change but not significant effect on milk characteristics may be related to sampling period in lactation and downregulation of the gene.

4. Conclusion

In the study, single nucleotide polymorphisms were detected in the exons and introns of the PPARA and PPARG genes, however, it was found that these polymorphisms did not cause any amino acid changes.

According to the results of nonparametric analysis, the effect of PPARASNP2 on true protein was found to be significant ($p<0.05$) for PPARA, but not for other features. There was no significant difference between gene expression levels and characteristics for PPARG ($p>0.05$).

As a result of analysis of variance, the effects of PPARASNP2 on target value T/RPPARA and PPARGSNP3 on T/RPPARG were found to be significant ($p<0.05$), but there was no significant difference between gene expression levels in terms of other characteristics ($p>0.05$). It was determined that the detected SNPs did not change the amino acid. Although the SNP2 effect was found to be significant on the traits examined in this study, it is thought to have no effect on the investigated traits since it was not found to be significant on other traits.

The multiple regression analysis examining the effect of SNPs on the target value of PPARA, T/RPPARA, while the SNP numbered rs665742135 was found to be statistically significant ($p<0.05$), no significant contribution of other SNPs was determined ($p>0.05$).

The Pearson correlation test results of T/RPPARA and T/RPPARG and milk yield values, a significant correlation of 0.05 was found between T/RPPARA and True protein (%) and lactose (%). Although no significant correlation was found between T/RPPARG and milk yield values, a negative correlation was found between dry matter (%), total protein (%) and somatic

cell count (x1000 cells/mL- milk). A positive correlation was found between PPARA gene expression and true protein and lactose values. Since a negative correlation was found between PPARG expressions and dry matter, total protein and somatic cell count, the effect of SNP rs665742135 on PPARA gene expression and PPARGSNP2 on PPARG gene expression. Since the effect on milk yield was found to be statistically significant ($p<0.05$), the relationship between the number of samples and milk yield parameters should be investigated in terms of these genes. In studies examining polymorphisms and gene expressions in PPARG Peroxisome proliferator receptor genes, there are significant differences in terms of goat breed, care and feeding styles of animals, and number of samples (Bionaz et al., 2013).

Introns can also affect mRNA metabolism, initiating transcription of the gene, editing and polyadenylation of the pre-mRNA, translation, and degradation of the mRNA product (Hou et al., 2013). In our study, it was found that PPARGSNP3's single nucleotide polymorphism in introns is important on T/RPPARG value. Although it occurs in introns, it may be useful to examine this SNP with more sample numbers, since its relationship with gene expression is important.

More detailed study of polymorphism in PPARA and PPARG genes and their expression levels in lactation by increasing the number of samples is necessary to identify candidate genes in terms of milk yield and milk yield characteristics.

Acknowledgment

The authors gratefully acknowledge the financial support of this research by Ege University Scientific Research Projects Coordination (BAP) (Project number: FGA- 2019-20593).

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Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
https://dergipark.org.tr/tr/pub/gopzfd

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2022) 39(2) 85-91
doi:10.55507/gopzfd.1073536

The Effect of Cold Marination on Some Physical Properties and Nutritional Composition of Sardine (*Sardina pilchardus*)

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Alındığı tarih (Received): 16.02.2022 Kabul tarihi (Accepted): 07.07.2022

Abstract: In the current study it was aimed to determine the effect of cold marination on the physical properties and nutritional quality of sardines. For this purpose, pH, colour, crude protein, crude oil, crude ash, moisture, fatty acid composition, amino acid, and mineral analyses were made in both raw and marinated sardine (marinated in 4% alcohol vinegar, 9% salt, and 0.3% citric acid at +4 °C for 36 hours). The *L** brightness value of sardine increased after marination. The crude ash content of the product was affected by marination, and the amount of crude protein and crude lipid increased ($p<0.05$). Glutamic acid, aspartic acid, alanine, and glycine contents of raw sardines increased after marination. In addition, there was an increase in the amounts of all essential amino acids except histidine. Palmitic acid was found to be the most abundant saturated fatty acid in sardines. The oleic acid content of the marinade increased with the marination process. It was determined that the Cd and Pb contents of raw and marinated sardines were below the limit values, and the Hg content of raw sardine was high. According to the results of the study, the marination process increased the brightness of the product, the amount of sweet and bitter amino acids, but it caused a decrease in omega 3 fatty acids.

Keywords: Sardine, marination, colour, fatty acids, amino acid, mineral

Soğuk Marinasyonun Sardalya'nin (*Sardina pilchardus*) Bazı Fiziksel Özellikleri ve Besin Bileşimi Üzerine Etkisi

Öz: Bu çalışmada soğuk marinasyonun sardalyanın fiziksel özellikleri ve besin bileşimi üzerine etkisinin belirlenmesi amaçlanmıştır. Bu amaçla hem çiğ hem de marine sardalyada (%4 alkol sirkesi, %9 tuz ve %0.3 sitrik asitte +4°C'de 36 saat marine edilmiştir) pH, renk, ham protein, ham yağ, ham kül, nem, yağ asiti kompozisyonu, amino asit ve mineral madde analizleri yapılmıştır. Marinasyondan sonra sardalyanın L* parlaklık değeri artmıştır. Ürünün kuru madde içeriği marinasyondan etkilenmiş, ham protein ve yağ miktarı artmıştır ($P<0.05$). Marinasyon sonrası çiğ sardalyanın glutamik asit, aspartik asit, alanin ve glisin içerikleri artmıştır. Ayrıca, histidin hariç tüm esansiyel amino asit miktarlarında artış olmuştur. Sardalyada en çok bulunan doymuş yağ asidi palmitik asit olarak tespit edilmiştir. Marinasyon işlemi ile marinatın oleik asit içeriği artış göstermiştir. Çiğ ve marine edilmiş sardalyaların Cd ve Pb içeriklerinin limit değerlerinin altında, çiğ sardalyanın Hg içeriğinin ise yüksek olduğu belirlenmiştir. Çalışma sonuçlarına göre marinasyon işleminin ürünün parlaklığını, tatlı ve acı aminoasit miktarını etkilediği buna karşın omega 3 yağ asitlerinde azalmaya neden olduğu tespit edilmiştir.

Anahtar Kelimeler: Sardalya, marinasyon, renk, yağ asitleri, amino asit, mineral

1. Introduction

Seafood is one of the most nutritious foods consumed around the world. It is recommended to consume seafood product with its high protein amounts, quality fatty acids, essential amino acids, minerals in the daily diet. However, when the population of Turkey is taken into consideration, the per capita consumption of seafood remains very low. Fish caught from the sea in a large part of Turkey is being consumed in the domestic market, the rest of the fish is sent to the fish meal and oil fabric and processing plants. For the last ten years, per capita consumption of seafood has not exceeded 7.1 kg in Turkey (TUIK 2021). While the average annual per capita consumption of seafood worldwide increased slightly from 19.9 kilograms to 20.5 kilograms between 2014 and 2019 (STATISTA 2021), these values could not be reached in Turkey. Considering the species obtained by caught, sardine is one of the first fish in Turkey. Considering the fish caught from Turkish seas in 2020, with 21265 tons, sardines are the 4th most caught fish after anchovy, sprat, and bonito (TUIK 2021).

Marination, which is one the seafood processing methods, is based on the interaction of acid and salt, giving flavour and aroma to the product, and extending its shelf life. With salting, which is one of the oldest processing methods, is tried to prevent spoilage by reducing the water content of the food. The pH of the

food is lowered by the acid added in marination and an edible product is obtained in a shorter time compared to salting. The method called marination has been tried in different ways in many kinds of seafood. The aim is not only to prevent microorganism growth but also it is used to tenderize or to change the taste, textural structural properties of raw materials (Gökoğlu et al. 2004). Studies on the use of marination technology in the processing of seafood are available in the literature (Gökoğlu et al. 2004; Kılınç & Çaklı 2004; Kılınç & Çaklı 2005; Kaba et al. 2014; Moreno et al.2017; Turan et al. 2017; Keskin et al. 2018; Kocatepe et al. 2019; Testa et al. 2019; Çorapcı et al. 2020; Szymczak et al. 2020; Çorapcı et al. 2021). The researchers studied the effects of marination under refrigerator conditions at different salt and acid concentrations in different fish species. 10% salt+4% (Turan et al. 2017; Keskin et al. 2018; Kocatepe et al. 2019) or 2% (Testa et al. 2019) acid for anchovy, 11% salt+ 4% acid (Çorapcı et al. 2020) or sea bream, 6% salt+ 5% acid (Szymczak et al. 2020) for Atlantic herring were used as a marination solution. In studies studied on sardines, 14% salt and 2%/4%/7% acid ratios were investigated by different researchers (Gökoğlu et al. 2004; Kılınç & Çaklı 2004; Kılınç & Çaklı 2005). In the present study, considering the previous studies, 4% alcohol vinegar+9% salt+0.3% citric acid was used for the sardine marination process. The study aims to determine the pH and colour changes observed in the product obtained by cold marination from frozen sardines and to determine the effect of marination on the proximate composition, fatty acids, amino acids, and mineral content of the product.

2. Material and Methods

2.1. Material

In the present study, 12 kg frozen sardines (*Sardina pilchardus*, Walbaum 1792) with average lengths of 13.25±0.20 cm and average weights of 19.64±0.93 were used as raw material for the marination process. Frozen sardines were purchased from a private company and brought to the laboratory within one hour under cold storage conditions. The study was carried out in November 2018.

Cold marination process

Frozen sardines were thawed under running water. The internal organs and heads of sardines were removed and cleaned. 2 kg of sardines were reserved for analysis before marination. After these processes, other sardines were marinated for 36 hours in marination solution (4% alcohol vinegar+9% salt+0.3% citric acid) with a fish: solution ratio of 1:1 at +4°C. After the marination, marinated sardines were removed from the solution and drained, and were make prepared for analysis.

2.2. Methods

Physical and proximate analysis were carried out in 2 replications and 3 parallels at Sinop University Fisheries Faculty, Fisheries Processing and Quality Control Laboratory. Fatty acids, amino acid, and mineral analyses were performed in 3 parallels at Sinop University Scientific and Technological Research Centre.

pH analysis

pH analysis was carried out using a pH meter. A 5 g of homogenized sample was transferred to the sample bottle. The measurement was carried out by immersing the pH meter probe into the sample.

Colour analysis

Konica Minolta (CRA-33a) colour measuring equipment was used for colour analyses. *L**, *a**, and *b** values were measured according to the International Commission on Illumination (CIE 1976).

Proximate analysis

The proximate composition (crude protein 984.13, crude lipid 960.39, crude ash 942.05), carbohydrates, and moisture contents were performed according to the AOAC official method (AOAC 1995; AOAC 2006). The energy value was calculated according to the method of Falch et al. (2010).

Amino acid analysis

Amino acid analyses were carried out using liquid chromatography (LC-MS/MS-Agilent Technology-6460 Triple Quad LC/MS) (Anonymous 1998). Ionization Modes: Electrospray ESI (- +), Atmospheric Pressure Chemical Ionization APCI (- +), Primary and Secondary Gases: Nitrogen (purity; 97%> and purity: 99.999%), Mass Filter and Detector: Triple Quadrupole, Dual mode (9 order), Liquid Chromatography Unit: Agilent Infinity 1260 HPLC, Nitrogen Gas Generator: Peak Scientific Instruments, NM32LA. JASEM quantitative amino acid LC-MS/MS analysis kit was used as a standard. Total sweet amino acids (Σ sweet AA) including glycine, alanine, and serine; and total bitter amino acids (Σ bitter AA) including isoleucine, arginine, phenylalanine, valine, tyrosine, leucine, methionine, and histidine, according to Dewi et al. (2016). The results were given in mg/100g protein.

Fatty acid analysis

Fatty acid compositions of raw and marinated sardine were analysed by GC/MS (Thermo Scientific/ Trace 1310 Gas Chromatography/ ISQ Single Quadrupole GC-MS) equipped with an auto sampler (AI-AS 1310 Series) (Gases Used: Nitrogen, Helium, Hydrogen, Dry Air. (99.999% purity), Available Detectors: GC-FID and GC-MS). A FAME mix (Supelco, 37 comp., Art No: CL40.13093, Bellefonte, PA, USA) was used to provide standards for comparison. The results were given in %.

Mineral analysis

Milestone (2018) method was used for the mineral analysis of samples. The acid (1 ml H_2O_2 30%, N 7 ml of HNO_3 65%) digestion of the sample in a closed vessel device using temperature control microwave (Ethos D, Milestone Inc. Sorisole, Italy) heating for the metal determination by spectroscopic methods. Analyses of elements were conducted using Agilent Technologies/7700X ICP-MS Systems. Analytical quality control was done using Agilent reference materials; std. 1: Agilent 8500-6940 2A (10 mg/kg-1 in 5% HNO3) was used for Li, Be, Na, Mg, K, Ca, Rb, Sr, Cs, Ba, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ag, Cd, Al, Ga, As, Se, Tl, Pb; std. 2 Agilent 8500 - 6940 Hg (10 mg/kg-1 in 5% HNO3) was used for Hg analysis. The results were given in mg kg^{-1} in the wet weight.

Statistical analysis

A one-way analysis of variance was used to determine the differences in data's using MINITAB 21.1.0. software program (Minitab Inc., State College, PA, USA). Differences between means was determined by Tukey's test (a level of $p<0.05$ was used to establish significant differences among means).

3. Results and Discussion

3.1. pH values and colour analyses results of raw and marinated sardines

The pH values of raw and marinated sardine are shown in Table 1. Ludorf and Meyer (1973) reported the pH value was between 6.00–6.50 in raw fish. Due to an increase in compounds such as ammonia in fish meat during the deterioration of the fish, the pH value also increases. An increase in pH value is an indicator, used in spoiling the raw fish. With the effect of the acids used in the marination process, the pH decreased and the desired ratio in the marinated products was achieved. In the present study, the pH value of raw sardines was found to be 6.28. Gökoğlu et al. (2004), Kocatepe et al. (2019), and Gökoğlu (2002) had been reported that the pH values of marinated fish should be ranged between 4.00-4.50. The pH of the marinated sardines obtained in the study (4.28) was similar to these studies.

Küçükgülmez (2012) reported the pH value of marinated sardine was 4.5. Besides, the pH value of sardines, marinated with a solution containing 7% acetic acid and 14% salt, was reported as 4.23 after the marination processing. Kılınç and Çaklı (2004) and Çorapcı et al. (2020) determined that the pH value of marine sea bream ranged between 4.04-4.28.

*L**, *a**, *b** values of raw sardine fillets were reported as 47.19, 4.44, and 2.86, respectively by Kılınç and Çaklı (2005). In our study, the *L** value was lower than Kılınç and Çaklı (2005), this may be due to the freshness of the sardine. Küçükgülmez (2012) reported *L**, *a** and, *b** values of marinated sardines (3% acetic acid+ 10% salt) to be 62.91, 1.79, and 12.54, respectively. The *L** value, which is an indicator of brightness, was higher in the present study, the reason for this may be the higher rate of vinegar and citric acid use in our study compared to the literature. Similarly, Szymczak et al. (2020) stated that the *L** and values of raw Baltic herring decreased after the marination process (5% acetic acid+ 6%salt).

Table 1. pH values and colour analyses result of raw and marinated Sardine

Çizelge 1. *Çiğ ve marine sardalyanın pH ve renk analizi sonuçları*

		Raw Sardine	Marinated Sardine
pH		*6.28±0.01*^{a}	*4.28±0.01*^{b}
Colour	***L****	39.39±0.19^{a}	71.26±0.33^{b}
	a*	4.13±1.24^{a}	-0.6±0.13^{b}
	b*	3.6±1.25^{a}	14.97±0.83^{b}

Mean (n=6) ± st. error

a, b,: The difference between groups with different letters is significant ($p\leq0.05$)

3.2. Proximate composition of raw and marinated sardines

Moisture content analysis showed that with the marination process, water emerged from the fish tissue ($p<0.05$). The crude ash and carbohydrate contents of the sardine did not change statistically with the marination process ($p>0.05$). With the marination process, the dry matter content of the product was affected, and the crude protein and the crude lipid amount increased ($p>0.05$) (Figure 1). Kılınç and Çaklı (2004) found the moisture, crude protein, crude lipid, and ash contents of frozen sardines as 73.70, 15.4, 4.44, and 6.17%, respectively. Similar to the present study, there was also an increase in crude protein, crude ash, and crude oil content with marination in the literature (Kılınç & Çaklı 2004; Czerner et al. 2015). The

diffusion of salt into fish meat caused an increase in crude ash. Similar results have been reported for marinated fish (Czerner et al. 2015; Cabrer et al. 2002; Özden 2005; Bilgin et al. 2011; Zhelyazkov et al. 2015). Marination also caused an increase in the energy content of the product ($p<0.05$). While the previous energy content of sardine was 98.56 kcal/100g, this value increased by about 62% in the marinated sardine (156.84 kcal/100g).

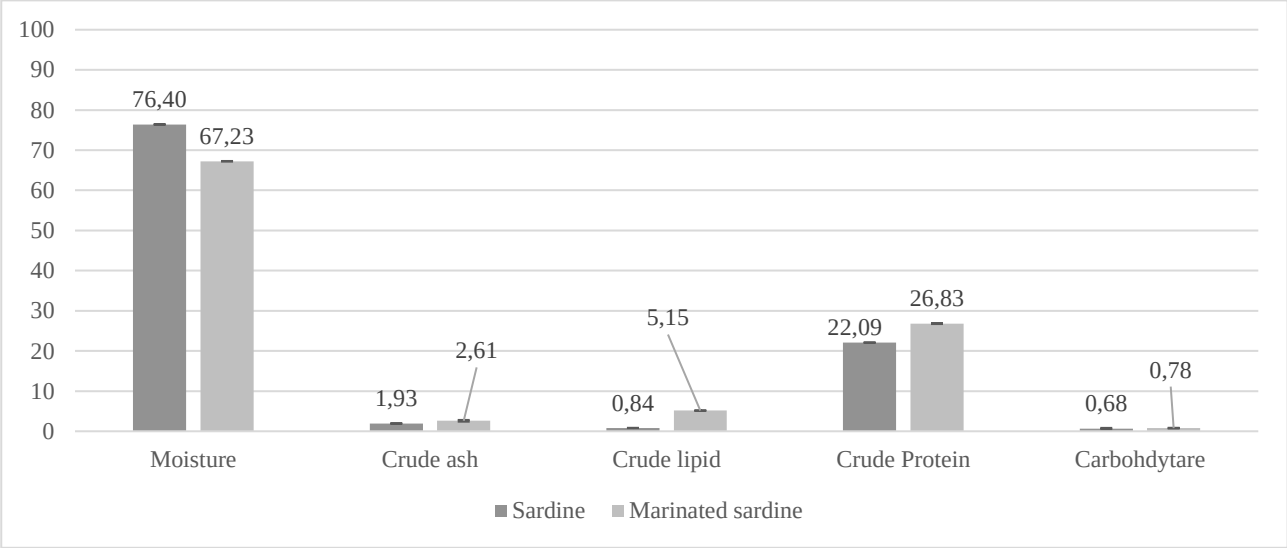

Figure 1. Proximate composition of raw and marinated sardine (%) (n=6)
Şekil 1. *Çiğ ve marine sardalyanın besin kompozisyonu*

3.3. Amino acid compositions of raw and marinated sardines

The amino acid compositions of raw and marinated sardines are shown in Table 2. Glutamic acid, aspartic acid, alanine, and glycine are responsible for flavour and taste. Özden (2005) reported that these amino acids are important by reason of they give marinated fish their characteristic taste and flavour. In the present study, glutamic acid, aspartic acid, alanine, and glycine contents of raw sardine increased after the cold marination process. The main essential amino acid in raw sardine was lysine. The lysine content of sardine increased with the marination process too ($p<0.05$). There was an increase in the amount of all essential amino acids except the histidine in the fish. ($p<0.05$). Similar results were reported by Kılınç and Çaklı (2004) and Özden (2005) about histidine contents of marinated sardine. The reason for this had been reported as the pH of marinades were not only total organic acid content but also the contents of basic and acidic amino acids such as histidine, glutamic acid, and aspartic acid: a decrease of histidine and an increase in glutamic and aspartic acid contents, presumably contribute to lowering pH of the marinade (Kılınç & Çaklı 2005).

The glutamic acid content, which plays an active role in the umami flavour, was high in raw sardine and marinated sardine.

Table 2. Amino acid compositions of raw and marinated Sardines (mg/100g protein)
Çizelge 2. *Çiğ ve marine sardalyanın amino asit kompozisyonu*

	Raw Sardine	Marinated Sardine
*Histidine**	0.62±0.02^a	0.45±0.03^b
Threonine	1.19±0.00^b	1.42±0.04^a
Valine	1.23±0.01^b	1.42±0.01^a
Methionine	0.72±0.01^b	0.90±0.00^a
Phenylalanine	1.10±0.01^b	1.22±0.01^a
Isoleucine	0.86±0.01^b	1.01±0.01^a
Leucine	1.70±0.06^b	2.14±0.00^a
Lysine	2.88±0.02^b	3.34±0.04^a
*Arginine**	1.44±0.00^b	1.71±0.06^a
Total Essential amino acid	11.74	13.61
Alanine	1.40±0.00^a	1.61±0.06^a
Aspartic Acid	2.26±0.05^a	2.73±0.14^a
Cysteine	0.27±0.00^a	0.32±0.02^a
Glutamic Acid	3.20±0.04^b	3.78±0.09^a
Glycine	0.92±0.04^b	1.13±0.02^a
Ornithine	0.06±0.01^a	0.06±0.00^a
Proline	0.94±0.00^b	1.03±0.01^a
Serine	1.15±0.01^b	1.33±0.02^a
Tyrosine	0.83±0.02^b	1.02±0.01^a
Taurine	0.39±0.00^a	0.21±0.01^b
Total Non-essential amino acids	11.42	13.22
Total amino acids	23.16	26.83
Sweet amino acids	3.47	4.07
Bitter amino acids	7.65	8.86

*: Conditionally essential amino acids.
Mean (n=3) ± st. error
a, b,: The difference between groups with different letters is significant ($p\leq0.05$)

It also had a high content of aspartic acid, one of the non-essential amino acids. Histidine and taurine content decreased with the marination process ($p<0.05$).

3.4. Fatty acid compositions of raw and marinated sardines

Fish is a high source of unsaturated fatty acids. The fatty acid compositions of raw sardine and cold marinated sardines are shown in Table 3. The most abundant saturated fatty acid in sardine was palmitic acid and present result was parallel with the literature (Karakoltsidis et al. 1995; Saglık and Imre 2001; Zlatanos & Laskaridis, 2007). Linoleic acid, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) were the highest percentages of fatty acids in raw sardines. Fish had low saturated fatty and high unsaturated fatty acid content. Fatty fishes such as mackerel, brook trout, herring, sardines, tuna, and salmon, etc. contain significant amounts of two important types of omega 3 fatty acids (DHA and EPA) (Turan et al. 2013). In present study, according to the results, sardine had high EPA and DHA contents. Also, the results show remarkable changes in the sardines with the marination process ($p<0.05$). Especially, butyric acid, palmitic acid, and DHA contents of sardines decreased after marination ($p<0.05$). The oleic acid content, which is an n-9 fatty acid, was increased by the marination process ($p<0.05$). As shown in Figure 1 and Table 3, raw sardines have a high protein, PUFAs (about 2.64 g/100g), and low-crude lipid content. This PUF content was higher than that of many protein foods listed by INFOODS (2021).

Sardine is the richest search for omega 3 fatty acids (Zlatanos & Laskaridis, 2007). Consumption of foods with high omega 3 content is important for the diet. Fast-food consumption habits have increased omega 6 intakes. The high levels of omega 3 fatty acids (above 30%) in *Sardine pilchardus* have also been reported by Zlatanos and Sagredos (1993). Similar results were found in present research. The recommended omega 3/omega 6 ratio to be taken daily is a minimum of 1. In present study, the omega 3/omega 6 ratio of sardines was above 1. After the marination process, this rate decreased but it did not fall below 1 value. ($p<0.05$). It is suggested that the omega 3/omega 6 ratio should be kept high, and this ratio should be 1:1 or 2:1 (Candela et al. 2011). Czerner et al. (2015) reported that the processing methods affected the omega 3/omega 6 ratios.

Table 3. Fatty acid compositions of raw and marinated sardines (%)

Çizelge 3. *Çiğ ve marine sardalyanın yağ asitleri kompozisyonu*

Fatty Acids%	Raw Sardine	Marinated Sardine
Butyric acid	7.32±0.13^a	4.30±0.25^b
Caproic acid	0.01±0.00^a	0.00±0.00^b
Caprylic acid	0.00±0.00^b	0.01±0.00^a
Capric acid	0.02±0.00^a	0.03±0.00^a
Undecanoic acid	0.01±0.00^a	0.01±0.00^a
Lauric acid	0.06±0.00^b	0.12±0.01^a
Tridecanoic acid	0.08±0.00^b	0.12±0.00^a
Myristic acid	4.25±0.04^b	5.42±0.29^a
Pentadecanoic acid	1.06±0.01^b	1.47±0.05^a
Palmitic acid	20.49±0.17^a	14.27±0.79^b
Heptadecanoic acid	1.30±0.01^b	1.49±0.05^a
Stearic acid	0.01±0.00^a	0.01±0.00^a
Arachidic acid	0.13±0.00^b	0.23±0.01^a
Heneicosanoic acid	0.06±0.01^a	0.08±0.00^a
Behenic acid	2.44±0.02^a	2.19±0.16^a
Tricosanoic acid	0.15±0.00^b	0.34±0.01^a
Lignoceric acid	0.37±0.01^b	0.55±0.00^a
Total SFA	37.76±0.07^a	30.63±1.65^b
Myristoleic acid	0.24±0.00^b	0.370±0.01^a
Pentadecanoic acid	0.14±0.00^b	0.19±0.01^a
Palmitoleic acid	0.33±0.01^b	0.68±0.02^a
Heptadecanoic acid	0.36±0.01^b	0.47±0.00^a
Oleic acid	3.65±0.00^b	21.12±1.29^a
Elaidic acid	0.21±0.00^b	5.33±0.40^a
Eicosenoic acid	1.71±0.01^b	2.21±0.13^a
Erucic acid	1.65±0.00^a	1.48±0.08^a
Nervonic acid	2.69±0.02^a	1.86±0.10^b
Total MUFA	10.99±0.05^b	33.72±2.02^a
Linolelaidic acid	0.16±0.00^a	0.01±0.00^b
Linoleic acid	8.22±0.04^a	9.25±5.36^a
Alpha-linolenic acid	0.64±0.00^b	1.67±0.09^a
Gamma-linolenic acid	1.24±0.01^b	1.99±0.12^a
Eicosadienoic acid	0.55±0.00^b	0.83±0.05^a
Arachidonic acid	2.44±0.022^a	2.19±0.16^a
Eicosatrienoic acid	0.30±0.02	0.35±0.02
Eicosapentaenoic acid (EPA)	8.82±0.02^a	7.29±0.45^b
Docosadienoic acid	0.15±0.00^b	0.34±0.01^a
Docosahexaenoic acid (DHA)	28.73±0.14^a	11.72±0.79^b
Total PUFA	51.25±0.10^a	35.63±7.06^b
omega 3	38.49±0.16^a	21.03±1.36^b
omega 6	12.76±0.07^a	14.61±5.02^a
omega 3/ omega 6	3.02	1.89
Total fatty acids	100.00±0.01^a	99.99±0.01^a

Mean (n=3) ± st. error

a, b,: The difference between groups with different letters is significant ($p\leq0.05$)

3.5. Mineral contents of raw and marinated sardines

Mineral contents of samples are shown in Table 4. The Be, Ag, Sb, Ga, and Tl contents of the samples are not shown in the table because they are <0.01 mg kg^{-1}. The major macro elements in sardine were K (4781.91 mg kg^{-1}) (Table 4). The recommended daily intake of K by the EGVM (2003) is 3500 mg K/day for adults. The macro element content of sardine had decreased after the marination process except for Na. The recommended

Na/K ratio for the prevention of heart diseases is less than 1 (Bu et al. 2012). In present study, the Na/K ratios of the raw sardine were determined as 0.38. The Na content of the sardine marinade was over the maximum measured value. The main reason for the increase in Na content of sardine marinade was the Na-containing salt used in marination process.

The determined dominant trace elements of sardine in present study were Zn, Fe, and As. The RDA for Zn 8-11mg/day for women and men (Dickinson 2000). The Zn content of sardine was quite high. Selenium is also a very valuable element for the immune system. The RDA of selenium is 55 µg/day for adults (Dickinson 2000). According to the Codex, the maximum Pb, Cd, and Hg levels permitted from the fish were 0.30, 0.10 (for sardines), and 0.50 mg/kg^{-1} (wet weight) (TGK 2021). Cd and Pb contents were lower than the limit values, but Hg level of sardine was high.

Table 4. Minerals contents of raw and marinated sardines (mg kg^{-1}) (wet weight)
Çizelge 4. *Çiğ ve marine sardalyanın mineral içeriği*

	Sardine	Marinated sardine
Ca	1199.77±26.94^{a}	639.68±3.36^{b}
Na	1839.52±43.00	*
Mg	595.47±15.39^{a}	250.75±1.71^{b}
K	4781.91±102.75^{a}	971.67±1.78^{b}
Li	0.21±0.00^{a}	0.17±0.01^{a}
Al	2.06±0.10^{b}	3.84±0.11^{a}
V	0.07±0.00^{a}	0.08±0.00^{a}
Cr	0.02±0.00^{b}	0.05±0.00^{a}
Mn	1.10±0.02^{a}	0.61±0.01^{b}
Fe	20.15±0.08^{a}	16.01±0.10^{b}
Co	0.03±0.00^{a}	0.02±0.00^{a}
Ni	0.11±0.00^{a}	0.09±0.00^{b}
Cu	1.12±0.00^{b}	1.83±0.02^{a}
Zn	27.52±0.11^{a}	12.18±0.19^{b}
As	13.11±0.05^{a}	2.79±0.00^{b}
Se	1.74±0.06^{a}	0.94±0.01^{b}
Rb	0.94±0.02^{a}	0.24±0.00^{b}
Sr	3.42±0.05^{a}	2.92±0.02^{b}
Cs	0.04±0.00^{b}	0.01±0.00^{a}
Ba	0.23±0.00^{a}	0.18±0.00^{b}
Pb	0.05±0.02^{a}	0.04±0.00^{b}
Cd	0.02±0.00^{a}	0.01±0.00^{b}
Hg	0.74±0.01^{a}	0.39±0.00^{b}

Mean (n=3) ± st. error, *over the maximum measured value.
a, b,: The difference between groups with different letters is significant ($p \leq 0.05$)

4. Conclusion

In the study, pH, colour values and nutritional composition of raw and marinated sardines were investigated. As an expected result of the marination process, the pH of the product decreased, the brightness (L^*) and yellowness (b^*) value increased, while the redness (a^*) decreased below zero. A product with high crude protein and crude lipid content was obtained due to water exit from the marinated tissue. Marinated sardines were high in essential amino acids. As with many processed products, a decrease in EPA, DHA, and total omega 3 fatty acids of marinated sardines was observed with the effect of processing. The mercury content of both raw sardines was higher than the limit values in the Turkish Food Codex. However, it is recommended to limit the consumption of sardines by children and pregnant.

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Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
https://dergipark.org.tr/tr/pub/gopzfd

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2022) 39(2) 92-96
doi:10.55507/gopzfd.1116199

Probabilistic Analysis of Variability in Reference Evapotranspiration

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Alındığı tarih (Received): 13.05.2022 Kabul tarihi (Accepted): 20.07.2022

Abstract: The amounts of hydro-meteorological data have varied over time due to human-induced global climate change. Obtaining the data to be used in the estimation of the crop water consumption parameter, which is of vital importance especially in the context of agricultural water management, under stationary conditions is a necessity in terms of reliability. The differentiation of data over time means that its frequency distribution behaviour also changes. The main goal of this study was on this change in question. For this purpose, annual reference evapotranspiration (ETo) values of Amasya and Samsun stations were used as material. The change in ETo data was analyzed with the ITA and PITA approaches. The ITA analysis regarding the Amasya station showed a statistically increasing change in the ETo data, whereas there was no statistically significant change for the Samsun station. Probability distributions fit most approximate to the data sequences of the first halve (FH) and second halve (SH) obtained by dividing the full data according to the PITA technique were Gama and Gumbel for the Amasya station, respectively. The normal distribution was found for two halves of the Samsun station. This finding confirmed that the data of the Amasya station has changed statistically over time. Remarkable differences were detected in the quantiles at especially higher risk levels for the Amasya station.

Keywords: Reference evapotranspiration, ITA approach, frequency analysis

Referans Evapotranspirasyondaki Değişkenliğin Olasılıksal Analizi

Öz: Hidro-meteorolojik verilerin miktarları, insan kaynaklı küresel iklim değişikliği nedeniyle zaman içinde değişmiştir. Özellikle tarımsal su yönetimi bağlamında hayati önem taşıyan bitki su tüketim parametresinin tahmininde kullanılacak verilerin durağan koşullarda elde edilmesi güvenilirlik açısından bir zorunluluktur. Verilerin zaman içinde farklılaşması, frekans dağılım davranışının da değiştiği anlamına gelir. Bu çalışmanın ana hedefi bahse konu bu değişim üzerine oldu. Bu amaçla Amasya ve Samsun istasyonlarının yıllık referans evapotranspirasyon (ETo) değerleri materyal olarak kullanılmıştır. ETo verilerindeki değişim ITA ve PITA yaklaşımları ile analiz edilmiştir. Amasya için ITA analizi, ETo verilerinde istatistiksel olarak artan bir değişim ortaya çıkarırken, Samsun istasyonu için istatistiksel olarak anlamlı bir değişiklik olmadı. Tam verilerin PITA tekniğine göre bölünmesiyle elde edilen birinci yarı (FH) ve ikinci yarının (SH) veri dizilerine en yakın olan olasılık dağılımları Amasya istasyonu için sırasıyla Gama ve Gumbel olmuştur. Samsun istasyonunun iki yarısı için normal dağılım bulunmuştur. Bu bulgu, Amasya istasyonuna ait verilerin zaman içinde istatistiksel olarak değiştiğini doğrulamıştır. Amasya istasyonu için özellikle daha yüksek risk düzeylerinde niceliklerde dikkate değer farklılıklar tespit edilmiştir.

Anahtar Kelimeler: Referans evapotranspırasyon, ITA yaklaşımı, frekans analizi

1. Introduction

Crop water consumption (ET), which is an important component of the hydrological cycle, has a key role in the successful assessments of water resources, especially in terms of drought and effective use of water (Yang et al. 2015). Therefore, the reliable prediction of this parameter in the optimal planning and operation of freshwater resources is of crucial importance. Although there are alternative estimation approaches for obtaining ET, the crop coefficient-based methodology is often accepted because it is easy and not expensive (Allen et al. 1998; Yee et al. 2015; Soubie et al. 2016). Allen et al. (1998) highlighted that crop water consumption was commonly calculated based on reference evapotranspiration (Eto) by using meteorological components. The FAO-56 Penman-Monteith relationship (FAO-PM) has been recommended as a reference approach in the literature in the calculation of the Eto. Global warming, which has been effective since the middle of the 20th century, has disrupted the natural functioning of meteorological events in the atmosphere (Akgül & Dino, 2020). Therefore, changes in meteorological parameters such as temperature, pressure, wind speed, airflow, precipitation, and relative humidity would impact reliable ET estimation. Allen et al. (2011) and Liu et al. (2021) highlighted that even small errors in the calculation of Eto based on climate components would cause remarkable negativities in the planning and operation of water resources.

Considering the explanations above, it is necessary to scrutinize the variability in the ET parameter which has critical importance for agricultural water management or the effective use of water resources under changing climatic conditions. Pettitt (1979) underlined the existence of a change-point when the separated parts of a given time series had different frequency distribution shapes. The innovative Trend Analysis (ITA) approach by Şen (2017) has recently been a favored tool to detect variability in the meteorological time series. Şen (2020) presented an approach (PITA) to the literature. The approach was based on the comparison of the frequency distributions of the split two halves considering the ITA technique. Under changing global climate conditions, it is necessary to examine whether the reference evapotranspiration time series is stationary over time in the context of effectively using water resources and agricultural water management of a region. The study was conducted on finding out variability in the Eto time series with the ITA technique and revealing probabilistically the difference in the frequency distribution shape of the Eto series over time.

2. Material and Method

The study was realized for two meteorology stations (Amasya and Samsun) in the Central Black Sea Region to analyze the variability of the Eto data sets. The geographical locations of the two stations are shown in Figure 1. The climatic parameters measured from 1984 to 2019 of these stations, which are wind speed, minimum and maximum relative humidity, minimum, maximum and average temperatures, and sunshine duration, were used as materials to form Eto datasets. The Eto data sequences on a monthly basis were obtained based on FAO-56 Penman-Monteith relationship, which is detailed In Allen et al. (1998). In the study, the annual Eto data series was formed by summing the Eto values of all months for each year, and then the annual Eto data sequences were used to be analyzed probabilistically with the PITA approach introduced by Şen (2020). For this purpose, first of all, the annual data was divided into two halves (First Halve and Second Halve) according to the ITA approach, the details of which were in Yurekli (2021). Thus, along with the application of the ITA technique, information was also obtained about the trend of annual Eto data sets. The probability distributions, namely Normal (N), Lognormal (LN), Three-parameter lognormal (LN3), Two-parameter gamma (G), Gumbel (EV), Generalized extreme value (GEV) and , Three-parameter gamma (Pearson III) was applied to determine the distribution that best fit the annual data of the first halve (FH) and second halve (SH) for the PITA analysis.

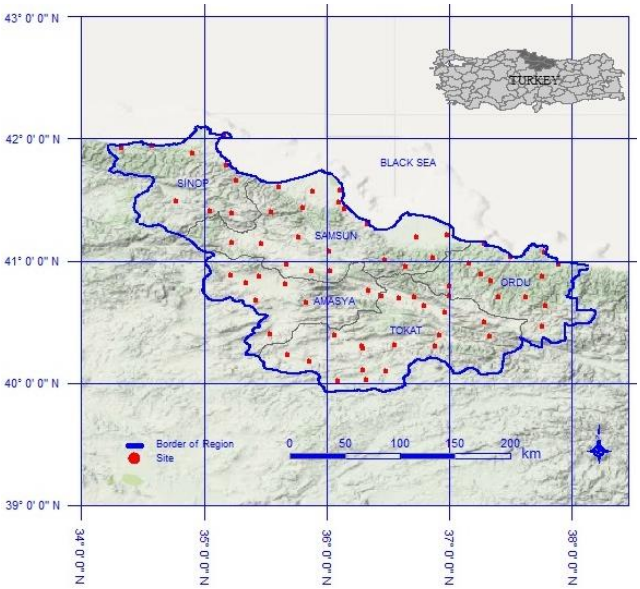

Figure 1. Geographical location of Amasya and Samsun Provinces selected for the study

Şekil 1. *Çalışma için seçilen Amasya ve Samsun İllerinin coğrafi konumu*

In the study, the Bayesian Information Criterion (BIC) suggested by Schwarz (1978) was used to identify the most adequate probability distribution model that fit the annual Eto data. According to the approach, among the candidate distributions, the distribution with the smallest BIC value was chosen as the most approximate model that best fit the data. The BIC is formulated as

$$BIC = -2\,logL(\hat{\Theta}) + klog(n)$$

In equation, where $L(\hat{\Theta})$ is maximum likelihood function; the “k” and “n” terms are the number of parameters and data points, respectively.

3. Results and Discussion

In the study, first of all, the homogeneity analysis of the annual Eto data was performed with the Mann-Whitney U test, details of which are given in Yurekli (2015). The null hypothesis for the Eto series of both stations was accepted. In other words, it was concluded that the datasets were statistically homogeneous. Then, the existence of the change in the Eto data sequences over time was determined by the ITA method. For this purpose, the annual Eto data sequences of the two stations were divided into two equal parts, the first and the second half. According to the ITA analysis, while the annual Eto data for Amasya station showed a statistically increasing change, a statistically insignificant decreasing trend was determined for

Samsun station. The calculated ITA test values of these stations were found to be 2.128 (± 1.885) and -1.550 (± 2.536), respectively. The values in parentheses indicate critical values. Visually scattering of the Eto data points of the two halves according to each other is shown in Figure 2. The scatter plot of the Amasya station shows that the stationary from small to large in the annual Eto data deteriorates. On the other hand, for Samsun station, it seems that stationarity is provided from small to large in Eto data. However, it should not be overlooked that there is a decreasing change in smaller data.

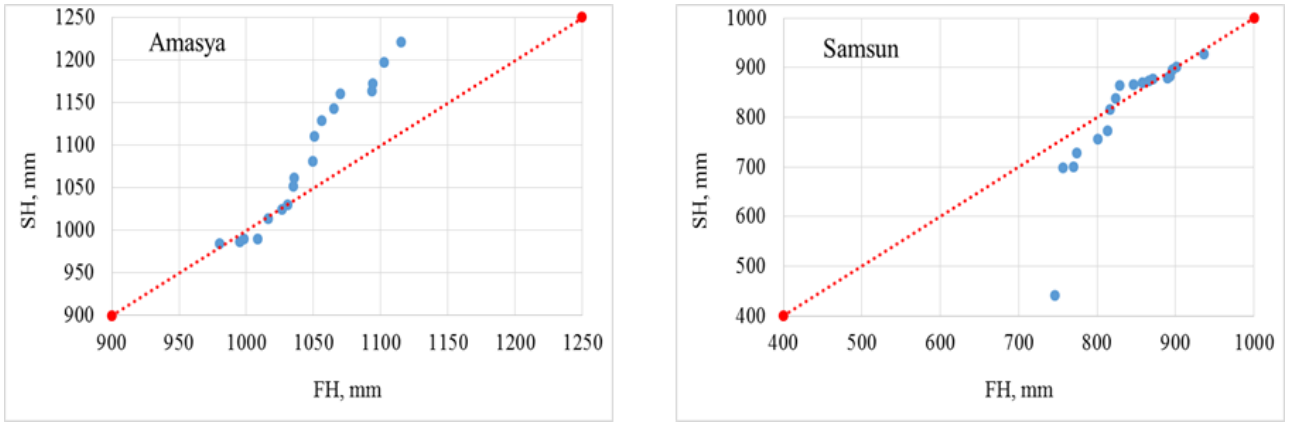

Figure 2. The scatter plots of data points in two halves according to each other
Şekil 2. *İki yarıdaki veri noktalarının birbirine göre dağılım grafikleri*

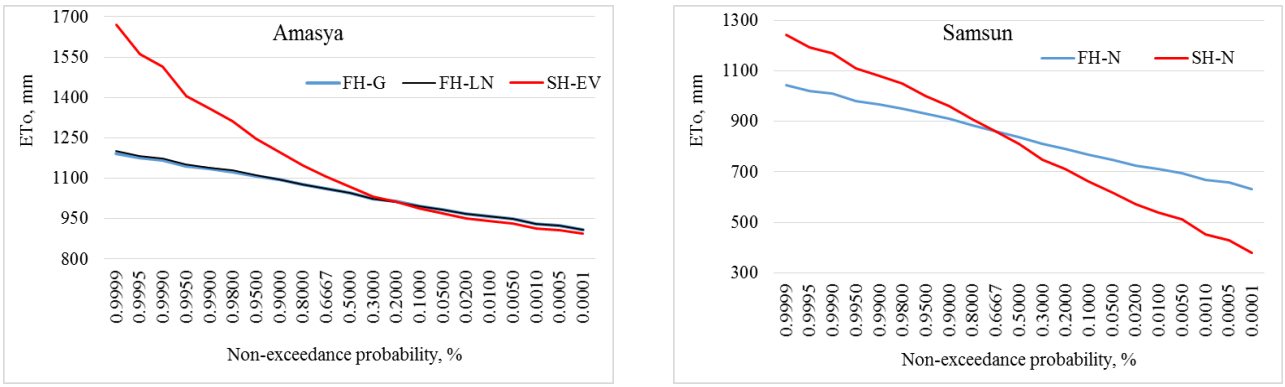

Figure 3. Variation of the quantiles obtained for the FH and SH (G, LN, EV, and N in the figure correspond to Gama, Lognormal, Gumbel, and Normal distributions)
Şekil 3. *FH ve SH için elde edilen niceliklerin değişimi (şekildeki G, LN, EV ve N Gama, Lognormal, Gumbel ve Normal dağılımlarına karşılık gelmektedir)*

For the Amasya station, the two-parameter Gamma distribution for the Eto data of the first halve (FH) and the Gumbel distribution for that of the second halve (SH) formed the smallest BIC values for the Eto data sequences. The BIC values of FH and SH for the aforementioned distributions are 187.75 and 213.0, respectively. However, the BIC value (187.76) of the lognormal distribution applied to the Eto data set belonging to the FH was very close to that of the gamma distribution. Although the data of the FH fit both gamma and lognormal distribution, the fact that the SH followed the Gumbel distribution was an indication that there was a change in the data, as stated by Pettitt (1979). Therefore, the stationarity of the Eto data series belonging to the Amasya station has deteriorated over time. Table 1 deals with the quantiles obtained from the distribution fitting most approximately to the Eto series associated with the FH and SH at different return periods. On the other hand, the Eto data sequences of both halves for the Samsun station followed the normal distribution as the most approximate distribution model. This finding is in line with the ITA results. The Normal distribution indicated the smallest value of the BIC when compared with those of the candidate distributions under consideration. The quantiles results associated with both halves for the normal distribution are available in Table 1. As can be seen in Table 1, the quantile results of the SH had a partial difference from those of the FH. The reason for this could be explained by the fact that the Eto data set of the SH showed a statistically

insignificant increase over time. Figure 3 shows the variation of the estimated quantiles for the non-exceedance probabilities between 0.9999 and 0.0001. For the Amasya station, the difference between the estimated quantiles became evident with the increase in the non-exceedance probability for the Eto data series of the FH and SH. The quantiles predicted toward smaller probabilities almost overlapped each other. On the other hand, although the FH and SH data sets of the Samsun station had the same probability distribution characteristics, the estimated quantiles for both halves showed an inverse difference variation structure at a 0.6667 probability level.

Table 1. Quantiles at some return periods (T) for non-exceedance probability
Çizelge 1. *Aşılmama olasılığı için bazı tekrarlanma periyotlarındaki (T) nicelikler*

T Year	Amasya			Samsun	
	FH(G)	FH(LN)	SH(EV)	FH(N)	SH(N)
100	1136.04	1139.87	1357.90	966.57	1080.81
50	1125.14	1128.33	1310.41	951.55	1049.13
20	1108.93	1111.24	1247.03	929.02	1001.62
10	1094.66	1096.26	1198.06	908.99	959.39
5	1077.53	1078.39	1147.01	884.74	908.23
2	1045.27	1045.05	1069.92	838.38	810.47
1.25	1013.69	1012.74	1012.61	792.02	712.70
1.11	997.47	996.23	988.25	767.76	661.55

4. Conclusion

Different methodologies have been developed for the estimation of crop water consumption parameters, which are of vital importance in the planning, management, and operation of water resources. Among them, crop coefficient-based ones are preferred because of being their easy and not expensive. However, one of the main problems in estimating in this way is the concern of making unreliable results by using climate data obtained under changing climate conditions, that is, under non-stationary conditions. Therefore, it is a necessity to analyze whether the assumption that the data to be used in the estimation of crop water consumption does not change over time overlaps with real conditions. This study was carried out to determine the change in annual reference evapotranspiration (ETo) values based on the FAO-56 Penman-Monteith relationship by using the climate data of the Amasya and Samsun stations. For this purpose, innovative trend analysis (ITA) and probabilistic innovative trend analysis (PITA) methods were used. According to ITA, a statistically increasing trend was detected in the annual ETo data for the Amasya, while a statistically insignificant increasing trend was found for the Samsun station. The PITA approach also confirmed this finding. The probability distributions most approximately followed for the first halve (FH) and second halve (SH) data sequences formed by dividing the full data based on the ITA approach were Gamma and Gumbel for the Amasya, respectively. Therefore, the different distribution behavior of the two halves is an indicator of the variability in the data. On the other hand, both halves of the Samsun station showed the best fit to the normal distribution. Significant differences became between the quantiles estimates from the Gamma and Gumbel distributions for the Amasya station, especially at higher risk levels. Although both halves of the Samsun station showed the same distribution characteristics, differences were found between the quantiles obtained according to the normal distribution at smaller and larger risk levels. Even if there is a statistically insignificant increase in the ETo data of the Samsun station, it should not be overlooked that this situation causes differentiation between the estimated quantiles.

Another important finding obtained with this study is that, based on the analysis made with the Mann-Whitney U test, although there was homogeneity in the ETo data of both stations, the method used in the present study revealed that there was variability at the Amasya station. This contradiction is probably related to the power of these methods to capture variability.

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Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
https://dergipark.org.tr/tr/pub/gopzfd

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2022) 39(2) 97-104
doi:10.55507/gopzfd.1119650

The Effect of Rye Translocation on Grain Yield and Agronomic Properties in the Recombinant Inbred Line Population Developed in Bread Wheat

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Alındığı tarih (Received): 25.05.2022 Kabul tarihi (Accepted): 02.08.2022

Abstract: Tolerance to biotic and abiotic stress factors is an important issue in wheat breeding studies. Rye translocation is an important genetic resource used in wheat breeding for tolerance to stress conditions and grain yield. In this study, the rye translocation and *Glu-B3b* allele, which is one of the Low Molecular Weight Gluten Subunits (LMW-GS), were identified by Polymerase Chain Reaction (PCR) method in the population of 145 recombinant inbreed lines (RILs) developed by crossing Tahirova-2000 and Tosunbey cultivars. It was determined that 85 out of 145 RILs carried the *Glu-B3b* allele. Statistical differences were analysed between the lines containing rye translocation or not in terms of the grain yield, thousand kernel weight and heading time. Physiologically, it was observed that the *1BL.1RS* rye translocation affects the heading time. An increase in root biomass was observed in genotypes carrying the rye translocation. It was determined that the thousand kernel weights of the lines were negatively affected by the rye translocation. These results indicated that the lines in the population can be used as gene resources for wheat breeding program and agronomic researches.

Keywords: Wheat, Rye translocations, *Glu-B3b, RILs*

Ekmeklik Buğdayda Geliştirilen Rekombinant Kendilenmiş Hat Popülasyonunda Çavdar Translokasyonunun Tane Verimi ve Tarımsal Özellikler Üzerine Etkisi

Öz: Buğday ıslah çalışmalarında biyotik ve abiyotik stres faktörlerine tolerans önemli bir konudur. Çavdar translokasyonu, buğday ıslahında stres koşullarına tolerans ve tane verimi için kullanılan önemli bir genetik kaynaktır. Bu çalışmada, Tahirova-2000 ve Tosunbey çeşitlerinin melezlenmesiyle geliştirilmiş 145 adet rekombinant kendilenmiş hat (*RIL*) popülasyonunda çavdar translokasyonu ve Düşük Moleküler Ağırlıklı Gluten Alt Birimlerinden (LMW-GS) biri olan *Glu-B3b* alleli Polimeraz Zincir Reaksiyonu (PCR) yöntemi ile tanımlanmıştır. 145 *RIL*'den 85'inin *Glu-B3b* alleli taşıdığı belirlenmiştir. Çavdar translokasyonu içeren ve içermeyen hatlar arasında tane verimi, bin tane ağırlığı ve başaklanma zamanı açısından istatistiksel farklılıklar analiz edilmiştir. Fizyolojik olarak *1BL.1RS* çavdar translokasyonunun başaklanma süresini etkilediği belirlenmiştir. Çavdar translokasyonunu taşıyan genotiplerde kök biyokütlesinde artış gözlenmiştir. Hatların bin tane ağırlıklarının çavdar translokasyonundan olumsuz etkilendiği belirlenmiştir. Bu sonuçlar, popülasyondaki hatların buğday ıslah programı ve agronomik araştırmalar için gen kaynağı olarak kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Buğday, Çavdar translokasyonu, *Glu-B3b, RILs*

1.Introduction

Wheat meets approximately 35% of the daily energy need, ranks first among the cereals cultivated in the World (Liu, 2007). While most of the wheat production in the world is consumed as a staple food, approximately 15% is used for animal feed (Shewry, 2009). Approximately 770 million tons of wheat was produced worldwide in 2020. According to the figures of 2020, approximately 20.5 million tons of wheat was produced on an area of 7.7 million hectares in Turkey (TUİK, 2020). According to these data, Turkey ranks 11th among wheat producing countries (TUİK, 2020).

While the increasing population in the world is rising the demand of grains, biotic and abiotic stress factors threaten wheat production and yield due to changing climatic conditions. The goal of many breeding studies in recent years is to develop varietiesa are tolerant to biotic and abiotic stress factors. In this context, rye translocations are important and widely used genetic resources in wheat breeding. The 1RS chromosome of rye, which can be located in different chromosomes of wheat, is frequently used by breeders (Graybosch, 2001). The 1RS rye chromosome carries genes that confer tolerance to diseases and insect pests. The 1RS chromosome arm of rye also contains genes

that provide resistance to diseases such as *Puccinia recondita f. sp. tritici, Puccinia graminis f. sp. tritici, Puccinia striiformis f. sp. tritici* and *Blumeria graminus f. sp. tritici* (Hsam et al., 2000). There are also studies reporting that rye translocation increases the adaptability and yield potential of bread wheat and provides tolerance to drought stress (Feldman & Levy, 2015; Yang et al., 2016; Leonardo et al., 2017; Tunca et al., 2018).

1BL.1RS translocation is widely used in wheat breeding programs. In the study published by Kim et al. in 2004, they determined that *1BL.1RS* translocation provides high yield potential by increasing the number of seeds in wheat. In the literature, it has been reported that wheat with rye translocation under arid conditions has higher root development and root biomass compared to wheat do not. Due to these characteristics of rye translocation, drought tolerance of the plant increases (Schlegel & Meinel, 1994; Rajaram et al., 2003). The effect of rye translocation on the regeneration capacity of the plant is positive, and it has been stated that the genotypes carrying the translocation have twice the regeneration capacity compared to the genotypes that do not (Agache et al., 1989). McKendry et al. (1996) reported in their study that genotypes carrying the *1BL.1RS* translocation had shorter plant height and a later heading period. In some studies, no advantages related to grain yield were observed in some wheats with different genetic bases and *1BL.1RS* translocation (Xue et al., 2014). This is thought to be due to gene suppressors in wheat (Carver &Rayburn, 1995; Leonardo et al., 2017).

Rye translocation, which has a positive effect on agronomic characteristics, generally affects the bread quality of genotypes negatively. It has been reported that the *1BL.1RS* rye translocation causes a decrease in the sedimentation volume in wheat, an increase in dough hardness and a decrease in gluten strength, and thus a decrease in the bread quality of wheat (Graybosch, 1990; Lelleya et al., 2004). It has been reported that the unfavorable effects on bread quality are due to the fact that *Sec-1* locus on the 1R chromosome of rye produces w-secaline protein and these monomeric proteins make the dough sticky (Lee et al., 1995). Another reason for the undesired effect is the loss of glutenin (*Glu-B3*) and gliadin (*Gli-B1*) loci from LMW-GS, which have the effect of improving the rheological quality properties of dough in translocation genotypes (Singh & Shepherd, 1988). Loss of these alleles causes a decrease in disulfide bonds in the protein scaffold that forms the gluten protein and decreases the dough quality (Wieser, 2007).

Rye translocations can be detected by different morphological, biochemical and molecular methods such as C-banding (Rayburn & Carver, 1988), Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) method (Zeller & Fuchs, 1983; Koebner & Shepherd, 1986) and Enzyme-Linked Immunoabsorbent Analysis (ELISA) (Andrews et al., 1996). Polymerase Chain Reaction (PCR) method is one of the fast and cost-effective methods that has been widely used in recent years. Genotypes carrying 1RS rye translocation can be detected easily with this method. Therefore, molecular screenings are widely used by breeders to determine the *1BL.1RS* rye translocation (Yamamoto & Mukai 2005; Landjeva et al., 2006; Tunca et al., 2018).

Mapping populations are used to identify genes controlling phenotypic characters in molecular level (Assanga et al., 2017). Backcross, F2, double haploid and recombinant inbred populations are the populations that are widely used for molecular detection of genes. Recombinant inbred line populations can be used to determine genotype x environment interactions of vegetative traits because they have homozygous pure lines (Hu et al., 2020).

In this study, 145 recombinant inbred lines obtained by crossing Tosunbey and Tahirova-2000 cultivars and five control cultivars were screened using PCR method for rye translocation and *Glu-B3b* allele. The effects of rye translocation on thousand kernel weight, plant height, grain yield, grain number per square meter, spike number per square meter, harvest index and NDVI measurements of recombinant inbred lines were investigated.

2. Materials and Methods

2.1. Plant materials, location and trials

In this study, 145 homozygous inbred Tosunbey x Tahirova-2000 cross lines obtained in another study and five control genotypes were used as material. Tosunbey, Tahirova-2000, Adana99, Nevzatbey and Altay2000 cultivars were used as control cultivars in the experiments. The lines and controls are white grained. Tahirova-2000 cultivar carries *1BL.1RS* rye translocation, while Tosunbey cultivar does not carry rye translocation.

Experiments were conducted in three crop years (2012-2015) on the experimental fields of Eskisehir Transitional Zone Agricultural Research Institute (300 31'N, 390 46 E). The seed rate was 450 seeds m^{-2}. Plot

dimensions were 1.2 m wide (6 rows with 20 cm row space) and 5 m long (Wintersteiger). Monthly precipitations for the experimental years of the research institute were provided in Table 1. The lowest precipitation was 254 mm in 2012-2013 and 319 mm in 2013-2014, below the long-term average. The highest precipitation was received in 2014-2015, and it was an extreme year with 125. 3 mm precipitation in June and total precipitation of 643 mm (Table 1). The experimental soils formed over deep alluvial sediment. The soils were high clay content, low organic matter (1.01-1.30%), medium in lime content and slightly alkaline character (pH=7.53-7.90) (Table 2). Depending on whether the growing season was rainy or not in Eskişehir conditions, 10 kg of pure nitrogen fertilizer (DAP and Amonyum Nitrat) was applied per decare. Fertilization was done as pure phosphorus at 6 kg/da in locations. Selective herbicide was used in weed control.

Table 1. Monthly precipitation of the experimental site at during 2013-2015growing seasons (mm).
Çizelge 1. *2013-2015 yetiştirme mevsimi boyunca deneme alanının aylık yağış miktarı (mm).*

Years	Sep.	Oct	Nov	Dec	Jan	Feb	Marc	April	May	June	July	Aug.	Annual Total
Long-Term	14.4	26.1	29.8	46.1	38.2	32.5	33.4	35.2	43.3	28.6	13.5	6.4	348
2012-13	0.0	16.1	14.5	78.2	18.5	36.5	33.2	37.8	9.5	14.0	0.8	0.0	254
2013-14	2.0	65.0	15	1.5	21.0	7	27.1	23.2	53.8	70.5	20.4	12.2	319
2014-15	41.4	66.1	26.2	72.1	39.0	60.9	46.0	41.3	61.2	125.3	0	63.5	643

Table 2. Soil chemical properties (0- to 30-cm soil layer).
Çizelge 2*. Toprak kimyasal özellikleri (0-30 cm toprak tabakası).*

Years	Texture	Potassium (K)	Phosphorus (P)	pH (1:2.5 Soil:Water)	Organic Matter	$CaCO_3$ (Lime)
		mg kg^{-1}	mg kg^{-1}		%	%
2013	C	107	3.58	7.90	1.10	9.27
2014	C	175	7.10	7,53	1,30	10,0
2015	C	142	7.40	7.80	1.01	10.28

C:Clay

2.2. DNA isolation and PCR analysis

DNA isolation was performed from 150 mg ground seeds of 145 recombinant inbred lines and five control lines using the ZR Plant/Seed DNA miniPrep™ Kit (Zymo, D6020). The *1BL.1RS* translocation (*Sec1Gene* primer*)* and the *Glu-B3b* allele were screened in 145 recombinant inbred lines and five control groups using the Biorad C1000 TouchThermalCycler device. PCR reaction conditions were described by Yamamoto and Mukai (2005) and Wang et al. (2010) was done as stated. PCR products were run on a 2% agarose gel for 1.5 hours and the gel was imaged using the BioradChemidoc MP gel imaging system.

2.3. Phenotypic measurements and observations

While determining the plant height, the distance from the tip of the last spikelet to the soil surface was measured and recorded in centimeters. During the measurements, attention was paid to ensure that the soil was not pits or bumps, and awn lengths were not included in the measurements (Torres & Pietragalla, 2012). As of January 1, the period in which approximately 50% of the plants in the plot were spiked has been determined as the number of days to heading (Tavella, 1978). From the seeds obtained from the wheat spikes in each line, 100 seeds were taken twice and counted with an automatic seed counting machine (Chopin-Numigral-I), and the weight of one thousand grains (g) was determined by taking the average and multiplying by 10, and this value was calculated according to the 14% moisture content. Harvest index was determined by Reynolds et al. (2001) according to the method used. According to this method; 50 spicate plants (Bb 50) were selected from among the plants cut from an area representing the plot and dried in paper bags at 75 °C for 48 hours (Ka 50). These plants were blended and their grains and stems were separated. In order to determine the grain yield, the parcel was harvested and the obtained product was weighed and converted to decare. 250 grains were counted and weighed to determine the grain weight.

NDVI values were determined using NTech, GreenSeeker Model 505 optical hand sensor in the first week of March, second week of April, last week of May and June to determine the early covering (NDVI) characteristics of the cultivars (Peňuelas et al., 1994). Measurements were taken when the light source (sensor) was 80 cm above the vegetation and walking at a constant speed along the five-meter plot. NVDI calculations were made according to the Equation 1.

$$NDVI = (R_{900} - R_{680})/(R_{900} + R_{680}) \quad (1)$$

R: indicates the reflection (Reflectance), while the subscript numbers indicate the wavelength (nm) of the rays.

2.4. Statistical analysis

The agronomic and physiological data obtained from the yield trials were analyzed in the JUMP statistical program, the effect of rye translocation according to the partial lattice trial design (Patterson & Hunter, 1983). Before the analysis of the data, the normal distribution was checked by performing the normality test. A very limited number of values (less than five) that did not fit the normal distribution were excluded. In the analyzes made according to the lattice trial design, the mean values of the genotypes were obtained as corrected values (Least Sq Mean). These values may differ from the arithmetic averages.

3. Results

3.1. Molecular scans of the *Glu-B3b* and *Sec-1* gene

The lines with and without the *Glu-B3b* allele as a result of molecular screenings for the *Glu-B3b* allele are given in Table 3 and Figure 1. As a result of molecular scanning by using *Glu-B3b* primers, bands with a length of 1570 bp (base pairs) were obtained in some lines which means *Glu-B3b* allele is included in these genotypes. Lines that did not carry the relevant allele did not produce bands. While 85 of the lines in its population carry the *Glu-B3b* allele, such as the Tosunbey variety, 60 lines do not carry the relevant allele (Table 3).

Table 3. Data on the *Glu-B3b* and *Sec-1* allele transport status of lines in the mapping population.
Çizelge 3. *Haritalama popülasyonundaki hatların Glu-B3b ve Sec-1 allel taşıma durumuna ilişkin veriler.*

Alleles	Lines Number
Genotypes carrying the *Glu-B3* allele	Tosunbey, 1, 2, 6, 11, 12, 13, 14, 15, 17, 18, 21, 23, 24, 25, 26, 27, 31, 33, 36, 38, 39, 40, 41, 45, 46, 47, 48, 49, 50, 51, 52, 56, 58, 60, 61, 63, 67, 69, 70, 71, 72, 73, 75, 76, 77, 79, 80, 81, 83, 84, 85, 86, 89, 93, 95, 96, 97, 99, 100, 108, 109, 112, 114, 116, 118, 119, 120, 126, 127, 129, 130, 131, 132, 134, 135, 136, 137, 138, 140, 144, 145, 146, 147, 148
Genotypes not carrying the *Glu-B3b* allele	Tahirova2000, 3, 4, 7, 8, 9, 10, 16, 19, 20, 22, 28, 29, 30, 32, 34, 35, 37, 42, 43, 44, 53, 54, 55, 57, 59, 62, 64, 65, 66, 68, 74, 78, 87, 88, 90, 91, 92, 94, 98, 101, 102, 103, 104, 105, 106, 107, 110, 111, 115, 117, 121, 122, 123, 124, 125, 128, 133, 139, 141, 141, 143, 149, 150
Genotypes carrying the *Sec1* allele	Tahirova2000, 3, 4, 7, 8, 9, 10, 16, 19, 20, 22, 28, 29, 30, 32, 34, 35, 37, 42, 43, 44, 53, 54, 55, 57, 59, 62, 64, 65, 66, 68, 74, 78, 87, 88, 90, 91, 92, 94, 98, 101, 102, 103, 104, 105, 106, 107, 110, 111, 115, 117, 121, 122, 123, 124, 125, 128, 133, 139, 141, 141, 143, 149, 150
Genotypes not carrying the *Sec1* allele	Tosunbey, 1, 2, 6, 11, 12, 13, 14, 15, 17, 18, 21, 23, 24, 25, 26, 27, 31, 33, 36, 38, 39, 40, 41, 45, 46, 47, 48, 49, 50, 51, 52, 56, 58, 60, 61, 63, 67, 69, 70, 71, 72, 73, 75, 76, 77, 79, 80, 81, 83, 84, 85, 86, 89, 93, 95, 96, 97, 99, 100, 108, 109, 112, 114, 116, 118, 119, 120, 126, 127, 129, 130, 131, 132, 134, 135, 136, 137, 138, 140, 144, 145, 146, 147, 148

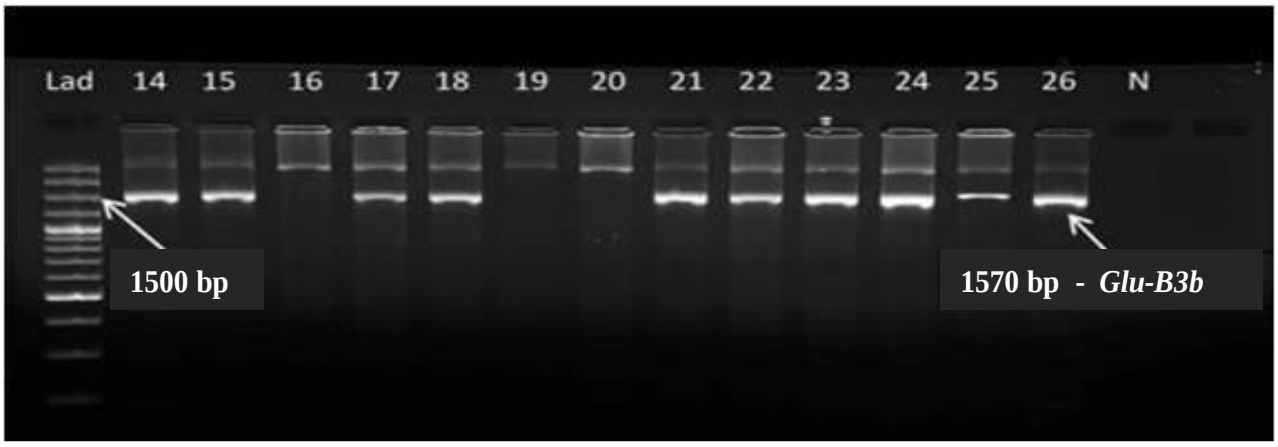

Figure 1. Results of PCR analysis using primers belonging to the *Glu-B3b* allele (Lad: Ladder, N: Negative control).
Şekil 1. *Glu-B3b alleli için kullanılan primerin PZR analiz sonuçları (Lad: Ladder, N: Negatif kontrol).*

The *Sec1Gene* primer was used to identify lines in the population carrying the *1BL.1RS* rye translocation. The agarose gel images of the lines in the mapping population are given in Figure 2, and the numbers of the lines with and without the rye translocation are given in Table 3. When the lines were screened using the *Sec1Gene* primer pair, 1216 base pairs (bp) long bands were obtained, but no bands were formed in the lines that did not carry the relevant allele. Among the lines in the recombinant inbred population, 60 lines

carry the *Sec1Gene* allele such as Tahirova2000 variety, while 85 lines do not carry the relevant allele such as the Tosunbey variety. As a result, it was confirmed that genotypes carrying the *Glu-B3b* allele did not carry the rye translocation (Table 3).

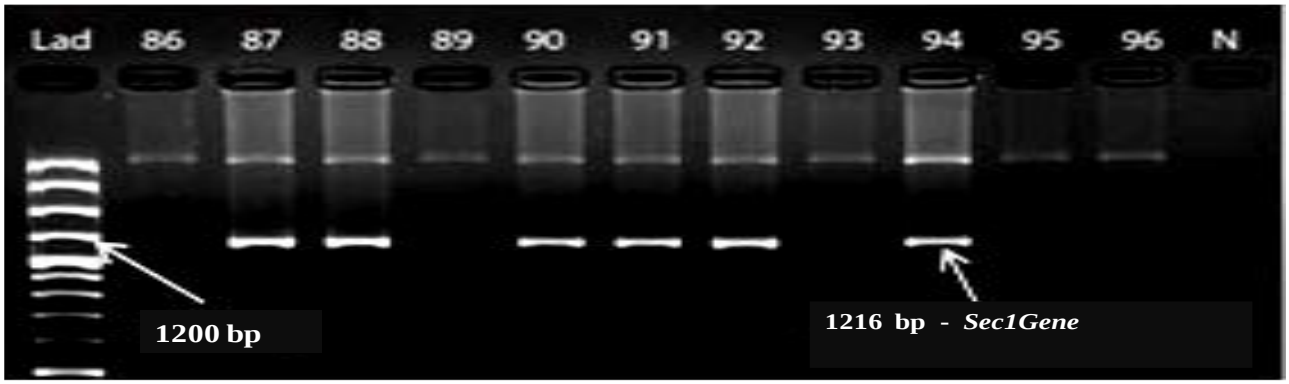

Figure. 2. Results of PCR analysis using primers of the *Sec-1* Gene (Lad: Ladder, N: Negative control).
Şekil 2. *Sec-1 Gene alleli için kullanılan primerin PZR analiz sonuçları (Lad: Ladder, N: Negatif kontrol).*

3.2. Effects of rye translocation on some agronomic traits

The average grain yield of the population used in the study was 473 kg/da (Table 5). The highest average grain yield was obtained in 2013 harvest year and the lowest grain yield was obtained in 2015 harvest year. The average grain yield in 2013, 2014 and 2015 was determined as 484, 486 and 443 kg/da, respectively (Table 4). The average thousand-grain weight of lines and control varieties in 2013, 2014 and 2015 was 45.3 g, 41.7 g and 38.3 g, respectively, and the average of these three years was 41.8 grams (Tables 4 and 5). The highest average in thousand kernel weight values was obtained in 2013, and the lowest average was obtained in 2015. As a result of the analyses, the average of the earliest heading was in 2014 and the average of the latest heading was in 2015. In the 2013, 2014 and 2015 harvest years in which the research was carried out, the average heading times were 135.8, 134.4 and 142.3 days, respectively, and the average of the three years was 137.4 days. (Tables 4 and 5). The average of plant heights were 89.9 cm in 2013, 87.1 cm in 2014 and 82.7 cm in 2015 (Table 4). When the average of three years is taken, the plant height is 86.6 cm (Table 5).

When the effects of lines with and without rye translocation on agronomic characteristics were examined, the difference between average grain yields was found to be 1% significant (Table 6). The average yields of lines carrying and not carrying rye translocation were determined as 459 and 482 kg/da, respectively. The average grain yield of lines carrying rye translocation was lower. It was determined that the thousand-grain weights of the lines were negatively affected by the rye translocation and the difference between the thousand kernel weights was significant at the 5% level (Table 6) The average thousand kernel weight of the lines with rye translocation was calculated 42 g, and the ones without rye translocation were 44 g. As a result of the analysis, it was determined that the difference between the average number of days of heading of genotypes and the interaction of genotype x environment were significant at the 1% level (Table 6). The mean heading times of the lines with and without the rye translocation were determined as 138 and 137.1 days, respectively (Table 5). It was determined that the heading occurred later in the lines carrying the rye translocation. No significant difference was observed between the mean plant heights of genotypes with and without rye translocation (Table 6). Similarly, it was determined that rye translocation did not cause a significant change on biomass (g/m^2), grain number per square meter, spike number and grain/head ratio, harvest index and NDVI analyzes (Tables 4, 5 and 6).

Table 4. Average values of agronomic traits by years.
Çizelge 4. *Agronomik özelliklerin yıllara göre ortalama değerleri.*

Sources of Variation	2013	2014	2015
Grain Yield (kg / da)	484^a	486^a	443^b
Biomass (g/ m^2)	1120.7^b	1164.2^a	1023.8^c
Thousand kernel weight (g)	45.4^a	41.5^b	38.2^c
Number of Grains per Square Meter	10709.4^b	11769.5^a	11623.1^a
Spike Per Square Meter	339.2^c	513.4^a	463.8^b
Grain / Spike Ratio	32.1^a	23.3^c	25.4^b
Harvest Index	0.4^a	0.4^b	0.4^a
Heading Time (Days)	135.8^b	134.4^c	142.3^a
Plant Height (cm)	89.8^a	87.0^b	82.6^c
NDVI 6 March	0.2^c	0.3^b	0.3^a
NDVI 18 April	0.5^a	0.4^c	0.5^b
NDVI 28 May	0.5^c	0.6^b	0.7^a
NDVI 21 June	0.1^c	0.3^a	0.3^b

Table 5. Three-year average data of the whole population, average data of grains with and without rye translocation, standard error and coefficients of variation.
Çizelge 5. *Tüm popülasyonun üç yıllık ortalama verileri, çavdar translokasyonu olan ve olmayan tahılların ortalama verileri, standart hata ve varyasyon katsayıları.*

Sources of Variation	Average	RT (-)	RT (+)	SE	CV
Grain Yield (kg / da)	473	482.6[a]	459.4[b]	91.8	19.4
Biomass (g/ m^2)	1104.3	1106.8	1099	220.6	20.0
Thousand kernel weight (g)	41.8	44.4b	42.1[a]	3.7	8.9
Number of Grains per Square Meter	11406.6	11559.3	11175.4	2346.30	20.6
Spike Per Square Meter	439.6	443.2	434.4	99.8	22.7
Grain / Spike Ratio	26.9	27.0	27.0	3.82	14.2
Harvest Index	0.43	0.44[a]	0.42[b]	0.03	7.2
Heading Time (Days)	137.4	137.1[b]	138.0[a]	2.6	1.89
Plant Height (cm)	86.6	86.8	86.3	7.6	8.8
NDVI 6 March	0.3	0.3	0.3	0.05	16.7
NDVI 18 April	0.4	0.5	0.5	0.09	18.4
NDVI 28 May	0.6	0.6	0.6	0.06	9.09
NDVI 21 June	0.2	0.2	0.2	0.05	17.2

RT: Rye Translocation. S.E.: Standard Error. C.V.: Coefficient of Variation

Table 6. Analysis of variance for agronomic properties.
Çizelge 6. *Agronomik özellikler için varyans analizi.*

Sources of Variation	Degree of Freedom (D.F.) Mean of squares (M.S.)	Year	Recurrence (Year)	Block (Year. Recurrence)	Rye Translocation	Error
Grain Yield (kg / da)	D.F.	2	3	30	1	828
	M.S.	8.88	6.9	12.91	**109.139.5****	8.335.60
Biomass (g / m^2)	D.F.	2	3	30	1	821
	M.S.	3.056.270	836.247	4.799.480	12.110	48.547
Thousand kernel weight	D.F.	2	3	30	1	827
	M.S.	7.663	757	1590	**80.12***	13.9
Grains per Square Meter	D.F.	2	3	30	1	822
	M.S.	197.607.058	40.506.979	522.302.398	29.581.114	5.480.723
Spike Per Square Meter	D.F.	2	3	30	1	822
	M.S.	4.829.728	52.371	442.333	15.573	9.971
Grain / Spike Ratio	D.F.	2	3	30	1	823
	M.S.	12.633	670	1.875	0.003	14.2
Harvest Index	D.F.	2	2	30	1	821
	M.S.	8.24	16.9	13.03	**0.065****	0.001
Heading Time	D.F.	2	3	30	1	828
	M.S.	5.344.30	159.69	14.56	**172.78****	6.64
Plant Height (cm)	D.F.	2	3	30	1	827
	M.S.	3.934.20	1.272.29	287.23	52.31	57.78
NDVI Mart (March)	D.F.	2	3	30	1	828
	M.S.	2.09	0.007	0.03	0.002	0.002
NDVI Nisan (Nisan)	D.F.	2	3	30	1	828
	M.S.	2.05	0.02	0.04	0.003	0.008
NDVI Mayıs (May)	D.F..	2	3	30	1	828
	M.S.	2.32	0.13	0.016	0.001	0.003
NDVI Haziran (June)	D.F.	2	3	30	1	828
	M.S.	3.14	0.02	0.02	0.006	0.003

** P <0.01 level. 0.01. * P <0.05 level of significance

4. Discussion and Conclusion

The *1BL.1RS* rye translocation is widely used in breeding programs because it provides resistance to biotic and abiotic stresses and drought tolerance. An increase in root biomass was observed in genotypes carrying the rye translocation. and the water use efficiency was higher than the genotypes carrying the rye translocation. The reason for including such important features in wheat is that the 1RS fragment of rye is translocated to wheat. In this study. RIL lines were screened with rye translocation *Sec1Gene* primers and 1216 bp long bands were observed in agarose gel images of PCR analysis results. Part of 1RS rye was translocated with part 1BL of Tahirova2000 rootstock.

Therefore. it has lost the *Glu-B3* locus. which is one of the low molecular weight glutenin subunits of gluten protein. which has an important quality enhancing feature in bread wheats. *Glu-B3b* allele was screened as a control in RIL lines using this information and the presence of genotypes without rye translocation was determined. In PCR scans were performed with *Glu-B3b* primer. bands with a length of 1570 bp were detected in the gel images of genotypes that did not have the rye translocation. Since this locus was absent in genotypes with rye translocation. no bands were observed. As a result of PCR analysis using *Sec1Gene* and *Glu-B3b* primers to determine the presence of rye translocation in genotypes. *1BL.1RS* rye translocation was detected in 60 of 145 RIL lines. while rye translocation was not observed in 85 genotypes. The Jump statistical program was employed for the reliability of the data and the analysis of the interaction of rye translocation and phenotypic characters to determine the effect of *1BL.1RS* translocation of the phenotypic characters of each line with the results obtained from molecular markers (Table 6).

The thousand kernel weight. which is an important quality component in bread wheat. increases in parallel with the increase in photosynthesis product. When environmentally suitable high yield conditions are provided. genotypes generally increase the thousand kernel weight and reflect this on yield. Studies conducted so far have also reported that the 1000 grains weight is an important feature affecting grain yield in cereals (Korkut et al.. 1993). Even Peterson et al. (1992) found that the effect of the environment on the thousand kernel weight was higher than the other quality criteria.

In this study. it was determined that the thousand-grain weights of the lines were negatively affected by the rye translocation. While the average thousand kernel weight of lines carrying rye translocation was 42.1 g. lines not carrying rye translocation were found to be 44.4 g (Table 5). The obtained result indicates that there may be decreases in thousand kernel weight of wheat with rye translocation in non-arid regions (Table 5). The *1BL.1RS* rye translocation in the lines decreased the 1000 grain yield. and in parallel. decreases in grain yield were observed. While the kilogram of wheat per decare was 459 kg/da on the lines carrying rye translocation. it was observed as 483 kg/da on the lines not carrying it. It is insufficient to argue that the result is only due to the rye translocation. Because grain yield is affected by both genetic structure and environmental factors. Peterson et al. (1992) reported that grain yield was affected by genotype x environment interaction. In this study. irrigation (35-40 mm) was applied to the trial material once in the summer of 2013 and 2014 in Eskişehir conditions. The relatively high yield potential in the studied location. as mentioned in the literature. did not show any improvement in the drought tolerance performance of the lines carrying rye translocation. and even had a negative effect (Xue et al.. 2014).

It was observed that the *1BL.1RS* rye translocation affects the heading time. The heading time is one of the most common features of transgressive expansion in wheat. The heading time is under the influence of many environmental factors besides the sensitivity to the photoperiod and vernalization genes (Shcherban et al.. 2015). The effect of rye translocation on *RIL* lines differed significantly at the 1% significance level. In addition to environmental factors affecting the heading time. translocation of rye also affects the duration of heading time and suggests that it is an important gene source that can be used in breeding later plants

The results obtained show that the recombinant inbred line population used in the research can be used in genetic resource development and in some physiological studies related to drought. especially in bread wheat.

Acknowledgement

This research was carried out within the scope of project number 12-YL-15 supported by Karamanoğlu Mehmetbey University BAP Coordinatorship and yield trials were established within the scope of project numbered 112O135 supported by TÜBİTAK.

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Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
https://dergipark.org.tr/tr/pub/gopzfd

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2022) 39(1) 105-112
doi:10.55507/gopzfd.1147100

Some Engineering Characteristics of Shelled and Unshelled Confectionery Sunflower Seeds Belonging to the Different Genotypes

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Alındığı tarih (Received): 30.06.2022 Kabul tarihi (Accepted): 13.08.2022

Abstract: In this study, the engineering characteristics of the shelled and unshelled sunflower seeds of the AYBAK-2013-12-DAÇ13099, AYBAK-2013-17-DAÇ130104, and 09 TRÇ 004 genotypes were determined. Moisture contents of the sunflower seeds were 7.22, 7.08, 7.34 (%d.b) of the shelled seeds, and 4.73, 4.19, 4.46 (%d.b) of the unshelled sunflower seeds, respectively. Dimensions, geometric mean diameter, sphericity, and surface area values of sunflower seeds showed statistically significant differences in terms of genotypes ($p<0.01$). The sphericity values were highest in 09 TRÇ 004 genotype (43.12%) in shelled seeds and in AYBAK-2013-12-DAÇ13099 genotype (40.51%) in unshelled seeds. The effect of genotypes on the investigated volumetric characteristics was found to be statistically significant ($p<0.01$). The highest L^* color characteristic gave higher values in the AYBAK-2013-12-DAÇ13099 genotype. The angle of repose values changed from 12.65° to 15.84° on the rubber surface of the shelled sunflower seeds. The static friction coefficient values were found in the range of 0.384-0.408 on the rubber surface of the shelled sunflower seeds. The engineering characteristics of shelled and unshelled seeds of different sunflower genotypes will contribute as important technical data in the design of machinery, facilities, and systems to be used for sowing, harvesting, and threshing mechanization and post-harvest technologies.

Keywords: Sphericity, hue angle, friction surfaces, sunflower genotypes

Farklı Genotiplere ait Kabuklu ve Kabuksuz Çerezlik Ayçiçeği Tohumlarının Bazı Mühendislik Karakteristikleri

Öz: Bu çalışmada, AYBAK-2013-12-DAÇ13099, AYBAK-2013-17-DAÇ130104 09 TRÇ 004 genotiplerine ait çerezlik ayçiçeği kabuklu ve kabuksuz tohumlarının mühendislik karakteristikleri belirlenmiştir. Çalışmada kullanılan ayçiçeği genotiplerinin kabuklu tohumlarının nem içerikleri sırasıyla %7.22, %7.08, %7.34 olarak kabuksuz ayçiçeği tohumlarının ise sırasıyla %4.73, %4.19 ve %4.46 (k.b) olarak belirlenmiştir. Kabuklu ve kabuksuz ayçiçeği tohumlarının geometrik özelliklerden boyutlar, geometrik ortalama çap, küresellik ve yüzey alanı değerleri, genotipler açısından istatistiki olarak önemli farklılık göstermiştir ($p<0.01$). Küresellik değerleri kabuklu tohumlarda 09 TRÇ 004 genotipinde (%43.12), kabuksuz tohumlarda AYBAK-2013-12-DAÇ13099 genotipinde (%40.51) en yüksek değerler vermiştir. Genotiplerin incelenen hacimsel özellikler üzerindeki etkisi istatistiki olarak ($p<0.01$) önemli bulunmuştur. En yüksek L^* renk karakteristiği, AYBAK-2013-12-DAÇ13099 genotipinde daha yüksek değer vermiştir. Doğal yığılma açısı değerleri, kabuklu ayçiçeği tohumlarında en yüksek lastik yüzeyde 12.65°-15.84° aralığında bulunmuştur. Statik sürtünme katsayısı değerleri, kabuklu ayçiçeği tohumlarında en yüksek lastik yüzeyde 0.384-0.408 aralığında bulunmuştur. Farklı ayçiçeği genotiplerine ait kabuklu ve kabuksuz tohumlarının mühendislik karakteristikleri, ekim, hasat ve harman mekanizasyonu ile hasat sonrası teknolojilerine ait kullanılacak makine, tesis ve sistemlerin tasarımında önemli teknik veriler olarak katkı sunacaktır.

Anahtar kelimeler: Küresellik, hue açısı, sürtünme yüzeyleri, ayçiçeği gentotipleri

1. Introduction

The importance of oilseed plants, which have a great place in human nutrition, is understood more and more every day. Sunflower, which is one of the oilseed plants, is one of the oil plants that has a very important place in the world and in Turkey (Dökülen, 2021). Sunflower (*Helianthus annuus* L.), being one of the most important oil crops grown, is also consumed as a confectionery. In addition, the inner part of the sunflower seed is used in the production of products such as cakes, cookies, bread, chocolate, and ice cream (Lofgren, 1997).

Confectionery sunflower varieties are black, white, black with white stripes, and are larger than oil sunflower seeds. It has a high percentage of bark and a thicker stem loosely attached to the core. The shell is easily separated from the seed and allows the shell of the seed as a whole to be removed (Fernandez-Martinez et al., 2009; Hladni et al., 2012). The most important production criteria that increase the market value of the products in Confectionery varieties; are seed yield, seed protein content, 1000 seed mass, husk/seed ratio, and seed separability from the husk.

The internal rate of sunflower seeds consumed as a confectionery should be at least 50%, and the thousand

seed mass should be greater than 80 grams (Lofgren, 1978). According to Lofgren (1978), confectionery sunflower seeds are divided into 3 groups according to their diameter (size). Grade 1 grains with a ratio of 15-25% on an 8.7 mm sieve are used as confectionerys. Grade 2 seeds on 8.7-7.1 mm sieves, which make up 40-60% of the whole product, are used in unshelled confectionerys or confectionery and bakery products. Grade 3 grains below 7.1 mm, which make up 15-20% of the whole product, are considered bird seeds.

According to TUIK data, sunflower for confectionerys was produced in Turkey in 2010 on a cultivation area of 900 000 decares of 150 000 tons and the yield was 167 kg da-1; For 2021, 200 000 tons of production is in question on 898 415 ha area and 223 kg da-1 yield has been reached (TUIK, 2021). Over a 12-year period, an increase of 33.33% in production values and a 33.53% increase in yield values was observed.

Meeting the food demand of the increasing world population is possible by increasing the quality and quantity of the product obtained from the unit area. Increasing the yield obtained from the unit area is realized by the use of high-quality seeds, as well as the use of many agricultural techniques. The engineering characteristics such as geometric, gravimetric characteristics, color characteristics, friction coefficient and angle of repose of sunflower seeds have great importance in terms of designing and projecting machinery and systems to be used in agricultural production from planting to harvest and post-harvest processes, classification, transportation and storage (Baryeh, 2001).

Size distribution data of sunflower seeds are essential for the design of cleaning, sorting, and separation equipment. At the same time, gravimetric properties are used for the design of equipment related to ventilation, drying, storage, and transportation (Kachru et al., 1994). Bulk density is used to determine the capacity of storage and handling systems. The angle of repose and friction characteristics are recognized by engineers as important features related to the rational design of silos and other storage structures affected by flow behavior (Kachru et al., 1994).

Studies on sunflower's engineering characteristics have been carried out by many researchers. Gupta & Das (1997), physical properties of Mordan sunflower variety seeds; Seifi & Alimardani (2010) determined the change of physical properties of sunflower seeds depending on their moisture content; Jafari et al., (2011) reported the mechanical properties of Shamshiri sunflower variety seeds, Gül et al. (2022), examined the physical, color and mechanical properties of seeds of oil sunflower varieties (Tunca, Reyna, Tarsan-1018).

The aim of this study is to determine the engineering characteristics (geometric, gravimetric characteristics, color characteristics, friction coefficient, and angle of repose) of shelled and unshelled sunflower seeds belonging to different confectionery genotypes (AYBAK-2013-12-DAÇ13099 and AYBAK-2013-17-DAÇ130104, and 09 TRÇ 004). Genotypes such as AYBAK-2013-12-DAÇ13099 and AYBAK-2013-17-DAÇ130104 are improvement lines and 09 TRÇ 004 is hybrid. With the results to be obtained from the study, it is thought that confectionery sunflower seeds will contribute to data acquisition in engineering applications for the design and development of machinery, facilities, and systems to be used in sowing, harvesting, and post-harvest processing technologies.

2. Materials and methods

Confectionery sunflower seeds, whose germination and viability were tested, were obtained from the Field Crops Department of the Faculty of Agriculture of Gaziosmanpaşa University. Seeds of sunflower genotypes were stored at 18.5ºC and 47% humidity before test. The engineering characteristic measurements of sunflower genotypes were carried out in the Biological Materials Laboratory of the Biosystem Engineering Department of Tokat Gaziosmanpaşa University, Faculty of Agriculture, between 15-30 September 2021. For the measurements, damaged and broken materials in sunflower seeds were removed from the samples. In this study, the sunflower genotypes such as [AYBAK-2013-12-DAÇ13099 (Improvement line) and AYBAK-2013-17-DAÇ130104 (Improvement line) and 09 TRÇ 004 (Hybrid)], were used (Figure 1).

The moisture content of the samples belonging to the sunflower genotypes was determined after drying the samples in a dry oven (oven) at a temperature of 105±1°C and for 24 hours on a dry basis (%d.b) (Suthar & Das, 1996). AYBAK-2013-12-DAÇ13099 and AYBAK-2013-17-DAÇ130104, and 09 TRÇ 004 genotypes have 7.22%, 7.08%, and 7.34% (d.b.) moisture content values for shelled seeds, respectively, and for unshelled seeds have 4.73%, 4.19% and 4.46% (%d.b), respectively.

Figure 1. Examples of the sunflower confectionery genotypes used in the study
Şekil 1. *Çalışmada kullanılan çerezlik ayçiçeği genotiplerine ait örnekler*

2.1. Geometric and gravimetric characteristics

In order to determine the geometric and volumetric characteristics of shelled and unshelled sunflower seeds were used 100 random samples. The length (*l*, mm), width (*w*, mm), and thickness (*t*, mm) were determined with a digital caliper with 0.01 mm precision (Figure 2). The geometric mean diameter (d_g), sphericity (S_p), and surface area (S_a) were determined from the following equations given by Mohsenin, 1980).

$$d_g = (l \,.w.\, t)^{1/3} \quad (1)$$

$$S_p = (d_g/l)100 \quad (2)$$

$$S_a = \pi (d_g)^2 \quad (3)$$

Figure 2. Size measurement of a sample confectionery sunflower seed
Şekil 2. *Örnek bir çerezlik ayçiçeği tohumunun boyut ölçümü*

Seed mass (M_a) was measured with a digital electronic balance with an accuracy of 0.001 g. For the measurement of thousand seed masses (T_m), 100 sample masses were taken in 4 replicates. The hectoliter method was used for the bulk density (B_d). The following equation was used for the seed volume (V_o).

$$V_o = \pi/6 \,(l \,.w.\, t) \quad (4)$$

2.2. Color characteristics

To determine the color characteristics of shelled and unshelled sunflower seeds, Minolta CR-400 Model (Tokyo, Japan) colorimeter device was used, considering CIE, *L**, *a**, and *b** color scales. The seeds were measured both shelled and unshelled seeds by taking the average value of all three measurements. According to the color scale changes, the redness-greenness (*a**) value, yellowness-blueness (b*) value as well as the hue angle ($H_a{}^o$) and chroma (C_r)value were determined by the following equations (McGuire, 1992). The chroma value (C_r) of seeds is close to 0 for pastel tones and 100 for vibrant tones as an indicator of vivid or pastel tone of seeds (Günaydın, 2020).

$$H_a{}^o = tan^{-1}\,(b^*/a^*) \quad (5)$$

$$C_r = (a^{*2} + b^{*2})^{1/2} \quad (6)$$

2.3. Mechanical characteristics

Friction surfaces such as PVC, laminated galvanized steel, rubber, and plywood were used to measure the friction coefficients of sunflower seeds, and the friction measurement device was given in Figure 4 (Yılmaz & Altuntas, 2020). For the friction coefficient measurement, the inclination angle (tanα) at the time when the seeds first started to move from different surfaces in the device that can be tilted with a screwed arm was taken into account.

Figure 3. Static friction coefficient measurement
Şekil 3. *Statik sürtünme katsayısı ölçümü*

For the determination of the angle of repose (A_r), a cylinder with 300 x 500 mm dimensions and an empty upper and the lower part was used, the seeds were filled to the top and raised to a cone formation on a flat plate surface. The angle of repose (A_r) of sunflower seeds was determined by the following equations (Kaleemullah & Gunasekar, 2002). The results were evaluated by using different surfaces (galvanized steel, plywood, rubber) for the angle of repose.

$$A_r = tan^{-1} (h / r) \quad (7)$$

Where;
r is the cone base radius (cm)
h is the cone height (cm).

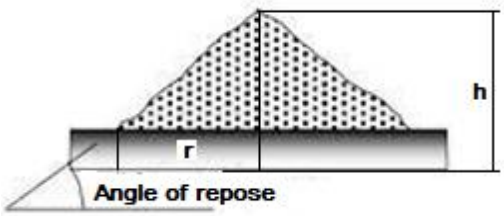

Figure 4. Measurement of the angle of repose
Şekil 4. *Doğal yığılma açısının ölçümü*

2.4. Statistical analysis

Kolmogorov-Smirnov test of normality was used to determine whether the data obtained in the study were suitable for normal distribution. It was determined that the normality tests before the analysis of the variance of the data were suitable for statistical analysis on the measurements of all studied engineering properties of the shelled and unshelled seeds of the sunflower genotypes. No transformation was applied for any of the measured properties. By using a one-way analysis of variance in the study, the effect of genotypes on the parameters examined with the DUNCAN multiple comparison tests was determined (SPSS, 2000).

3. Results and discussion

3.1. Geometric characteristics

The geometric and gravimetric characteristics values and variance analysis results are given in Tables 1 and 2 for AYBAK-2013-12-DAÇ13099 and AYBAK-2013-17-DAÇ130104, and 09 TRÇ 004 genotypes both shelled seeds and unshelled seeds, respectively. Statistically significant ($p<0.01$) differences were observed among the varieties in terms of geometric characteristics of the shelled and unshelled seeds. The length (23.95 mm) and width (7.96 mm) values gave high values for the shelled seeds of the AYBAK-2013-17-DAÇ130104 genotypes, and the length (14.56 mm) values of the width (5.25 mm) values for the unshelled seeds of the 09 TRÇ 004 genotype are higher than the other genotypes.

Table 1. The geometric characteristics of shelled sunflower seeds with different genotypes
Çizelge 1. *Farklı genotiplere ait kabuklu ayçiçeği tohumlarının geometrik özellikleri*

Genotypes	*l (mm)*	*w (mm)*	*t (mm)*	*d_g (mm)*	*S_p (%)*	*S_a (mm^2)*
AYBAK-2013-12-DAÇ13099	19.52±0.1975c**	7.18±0.1044b**	3.70±0.0463c**	8.00±0.0677c**	41.07±0.3234b**	202.35±3.3386c**
AYBAK-2013-17-DAÇ130104	23.95±0.1533a**	7.96±0.0852a**	4.16±0.0473b**	9.22±0.0691a**	38.58±0.2842c**	268.03±4.0156a**
09 TRÇ 004	20.99±0.0777b**	7.85±0.0634a**	4.53±0.0404a**	9.03±0.0493b**	43.12±0.2259a**	256.93±2.7799b**
F value	***222.68***	***24.18***	***85.15***	***109.69***	***65.59***	***105.95***

l: length, w: width, t: thickness, d_g: geometric mean diameter, S_p: sphericity, S_a: surface area, ±: standard error
***p<0.01. The difference between the same letters in the same column is insignificant.*

Table 2. The geometric characteristics of unshelled sunflower seeds with different genotypes
Çizelge 2. *Farklı genotiplere ait kabuksuz ayçiçeği tohumlarının geometrik özellikleri*

Genotypes	*l (mm)*	*w (mm)*	*t (mm)*	*d_g (mm)*	*S_p (%)*	*S_a (mm^2)*
AYBAK-2013-12-DAÇ13099	12.34±0.1412c**	4.45±0.0396c**	2.29±0.0182b**	4.99±0.0344b**	40.51±0.2991a**	78.86±1.0719b**
AYBAK-2013-17-DAÇ130104	14.56±0.0692a**	4.78±0.0389b**	2.50±0.346a**	5.56±0.0365a**	38.26±0.2011b**	97.49±1.3163a**
09 TRÇ 004	13.84±0.1231b**	5.25±0.0459a**	2.44±0.0319a**	5.60±0.0271a**	40.48±0.3185a**	98.48±0.9712a**
F value	***96.29***	***92.06***	***14.32***	***104.90***	***21.47***	***95.81***

l: length, w: width, t: thickness, d_g: geometric mean diameter, S_p: sphericity, S_a: surface area,
*±: standard error **p<0.01. The difference between the same letters in the same column is insignificant.*

The higher sphericity values were found in shelled seeds with a value of 43.12% in 09 TRÇ 004 genotype and 40.51% in unshelled seeds in AYBAK-2013-12-DAÇ13099 genotypeThe surface area values gave higher values as 268.03 mm^2 value for AYBAK-2013-17-DAÇ130104 genotype in shelled sunflower seeds and 98.48 mm^2 value in 09 TRÇ 004 genotype in unshelled sunflower seeds compared to other genotypes (Table 1, Table 2).

Seifi & Alimardani (2010) found that the length values of the SHF8190 sunflower variety seeds changed from 12.14 mm to 12.60 mm, the width values varied from 5.79 mm to 6.37 mm, the thickness values ranged from 3.85 mm to 4.09 mm, and the sphericity values between 52.82% and 55.42% for the different moisture contents (4%-22%). Gupta & Das (1997), found that the width values varied from 3.59 mm to 4.93 mm, and the thickness values changed from 2.09 mm to 2.72 mm for unshelled Mordan sunflower variety seeds at a moisture content of 6.2%. Jafari et al. (2011) found that the geometric mean diameter was between 5.74 and 10.63 mm and surface area values were changed from 103.5 to 354.9 mm^2 for seeds of shelled Shamshiri sunflower variety, while the geometric mean diameter was between 3.24 and 6.47 mm, and surface area values were varied from 5.74 mm and 10.63 mm for seeds of unshelled Shamshiri sunflower variety, respectively. According to the results of the literature, the geometric mean diameter and surface area of the shelled sunflower and unshelled sunflower seeds showed close values, while the sphericity values in the study found were lower than Seifi and Alimardani (2010).

3.2. Gravimetric Characteristics

The gravimetric characteristics of shelled and unshelled seeds of sunflower genotypes are given in Tables 3 and 4. While the higher values for seed masses were found as 0.164 g and 0.098 g in shelled and unshelled seeds of the 09 TRÇ 004 genotype respectively, the highest values in terms of seed volume were observed in the AYBAK 2013-17-DAÇ130104 genotype. The effect of genotypes was significant ($p<0.01$) and the differences between genotypes were remarkable in the statistical analyzes of all examined volumetric properties.

Table 3. The gravimetric characteristics of shelled sunflower seeds with different genotypes
Çizelge 3. *Farklı genotiplere ait kabuklu ayçiçeği tohumlarının gravimetrik özellikleri*

Genotypes	M_a *(g)*	T_m *(g)*	V_o *(mm³)*	B_d *(kg m⁻³)*
AYBAK-2013-12-DAÇ13099	0.120±0.0027c**	137.24±2.2861c**	274.90±6.6586c**	244.12±1.0820a**
AYBAK-2013-17-DAÇ130104	0.149±0.0028b**	173.37±0.0736b**	417.66±9.3918a**	227.02±2.9984b**
09 TRÇ 004	0.164±0.0024a**	188.85±2.3813a**	391.18±6.3049b**	248.84±4.4410a**
F value	***69.84***	***193.05***	***100.42***	***13.23***

*M_a: seed mass, T_m: thousand-grain weight, V_o: seed volume, B_d: bulk density. ±: standard error, **p<0.01. The difference between the same letters in the same column is insignificant.*

Table 4. The gravimetric characteristics of unshelled sunflower seeds with different genotypes
Çizelge 4. *Farklı genotiplere ait kabuklu ayçiçeği tohumlarının gravimetrik özellikleri*

Genotypes	M_a *(g)*	T_m *(g)*	V_o *(mm³)*	B_d *(kg m⁻³)*
AYBAK-2013-12-DAÇ13099	0.069±0.0023c**	61.90±2.7463c**	66.10±1.3031b**	473.91±2.8354b**
AYBAK-2013-17-DAÇ130104	0.087±0.0015b**	83.70±1.486b**	91.46±1.8653a**	461.98±4.3907c**
09 TRÇ 004	0.098±0.0033a**	95.91±0.7740a**	92.86±1.4306a**	517.17±3.8714a**
F value	***34.86***	***86.01***	***94.22***	***59.81***

*M_a: seed mass, T_m: thousand-grain weight, V_o: seed volume, B_d: bulk density. ±: standard error, **p<0.01.*
The difference between the same letters in the same column is insignificant.

Gupta & Das (1997) found that the seed masses were 0.049 g in shelled sunflower seed and 0.034 g in unshelled sunflower for the Mordan variety. According to the results of the literature, the seed masses values found were found to be higher than in this study. Polatlı (2013) found that the shelled sunflower seeds were classified into 4 different populations (populations 1, 2, and 3 in the F3 generation, whereas 4 populations in the F4 generation) determined the thousand seed masses as 100.26 g, 124.24 g, 101.89 g, and 116.80 g, respectively. Seifi and Alimardani (2010) found that the seed volumes of sunflower seeds were changed from 148.96 mm^3 to 177.81 mm^3 depending on the moisture content (4%, 12%, 16%, and 22%) for the Shamshiri variety. The shelled seeds gave higher values in terms of seed mass and seed volume than the unshelled seeds, in the study. Thousand seed mass and seed volume values of the shelled sunflower seeds gave higher values in this study than the varieties given in the literature. The reason for the change in these results is thought to be due to the differences in the varieties of genotypes used

in the studies, as well as the climate and soil factors in which the seeds were grown.

3.3. Color Characteristics

The results of the color characteristics of the shelled and unshelled seeds of the sunflower genotypes are given in Tables 5 and 6. The highest *L** characteristic was found in shelled and unshelled AYBAK-2013-12-DAÇ13099 genotype seed. Hue angle and chroma values calculated by using *L**, *a**, and *b** color characteristic values were also found to be higher in unshelled seeds in the AYBAK-2013-12-DAÇ13099 genotype. *L** and *a** color characteristics were statistically different at p<0.01 level for shelled sunflower seeds, while the difference between genotypes was statistically insignificant in unshelled sunflower seeds.

Gul et al. (2022), among the seeds of oil sunflower varieties, the color characteristics of Tunca variety *L**, *a**, and *b** values were determined as 23.44, 0.88, and 1.50, respectively; These values changed to 21.82, 0.81, and 1.24 in Reyna variety. While the difference between varieties on *L** and *a** color characteristics was insignificant, a p<0.01 difference was observed between varieties on *b** and chroma color characteristics. It was determined that *L** brightness values were higher in the Tunca variety.

Table 5. Color characteristics of shelled sunflower seeds with different genotypes
Çizelge 5. *Farklı genotiplere ait kabuklu ayçiçeği tohumlarının renk özellikleri*

Genoypes	*L**	*a**	*b**	C_r	H_a
AYBAK-2013-12-DAÇ13099	76.71±0.6907a**	4.72±0.1098a**	10.10±0.3609a*	11.16±0.3438a*	64.83±0.7859^{ns}
AYBAK-2013-17-DAÇ130104	72.27±0.6391b**	4.66±0.0779a**	9.68±0.3847ab*	10.75±0.3621a*	64.07±0.7978^{ns}
09 TRÇ 004	64.45±0.6621c**	4.14±0.0622b**	8.96±0.1222b*	9.87±01243b*	65.17±0.3409^{ns}
F value	***87.31***	***13.64***	***3.43***	***4.92***	***0.70***

±: standard error, ^{ns}: nonsignificant, * p<0.05, **p<0.01.
The difference between the same letters in the same column is insignificant.

Table 6. Color characteristics of unshelled sunflower seeds with different genotypes
Çizege 6. *Farklı genotiplere ait kabuksuz ayçiçeği tohumlarının renk özellikleri*

Genotypes	*L**	*a**	*b**	C_r	H_a
AYBAK-2013-12-DAÇ13099	54.37±0.9337^{ns}	3.94±0.0730^{ns}	8.12±0.2605a**	9.03±0.2571a**	64.00±0.5419a*
AYBAK-2013-17-DAÇ130104	52.96±1.0967^{ns}	3.79±0.0573^{ns}	7.27±0.2821b**	8.20±0.2540b**	62.25±0.9350ab*
09 TRÇ 004	54.27±0.7914^{ns}	3.83±0.686^{ns}	6.95±0.1137b**	7.94±0.0983b**	61.08±0.6472b*
F value	***0.69***	***1.44***	***6.85***	***6.90***	***4.05***

±: standard error, ^{ns}: nonsignificant, * p<0.05, **p<0.01.
The difference between the same letters in the same column is insignificant.

3.4. Mechanical characteristics

Angle of repose

The angle of repose values of shelled and unshelled sunflower seeds with different genotypes was examined on galvanized steel, plywood, and rubber surfaces, and the results are given in Tables 7 and 8. In sunflower genotypes, the highest value of angle of repose was found on the rubber surface for shelled sunflower seeds, while the highest value was observed on the plywood surface for unshelled sunflower seeds. According to the values of the angle of repose of the sunflower genotypes on different surfaces, statistically, significant differences were found between the shelled sunflower genotypes (p<0.01), while it was statistically insignificant in the unshelled sunflower seeds. While the angle of repose values was found to be between 12.65° and 15.84° on the rubber surface in shelled sunflower seeds, the values between 13.32° and 15.15° on the plywood surface in unshelled sunflower seeds were determined (Table 7, 8).

Table 7. The angle of repose values of shelled sunflower seeds with different genotypes
Çizelge 7. *Farklı genotiplere ait kabuklu ayçiçeği tohumlarının doğal yığılma açısı değerleri*

Genotypes	*Galvanized steel*	*Plywood*	*Rubber*
AYBAK-2013-12-DAÇ13099	8.60±0.3642b*	11.37±0.7652b**	12.65±0.5594b**
AYBAK-2013-17-DAÇ130104	8.37±1.0816b*	11.49±0.5539b**	13.26±0.6910b**
09 TRÇ 004	11.14±0.6812a*	14.47±0.5212a**	15.84±0.5911a**
F value	***4.01***	***7.95***	***7.52***

±: standard error *p<0.05, **p<0.01.
The difference between the same letters in the same column is insignificant.

Table 8. The angle of repose values of unshelled sunflower seeds with different genotypes
Çizelge 8. *Farklı genotiplere ait kabuksuz ayçiçeği tohumlarının doğal yığılma açısı değerleri*

Genotypes	*Galvanized steel*	*Plywood*	*Rubber*
AYBAK-2013-12-DAÇ13099	12.79±0.3719a**	15.15±0.7625^{ns}	12.94±0.2690^{ns}
AYBAK-2013-17-DAÇ130104	11.75±0.4187a**	13.59±0.4075^{ns}	11.76±0.4518^{ns}
09 TRÇ 004	9.54±0.5253b**	13.32±0.6222^{ns}	13.16±0.8684^{ns}
F value	***13.97***	***2.60***	***2.18***

±: standard error, ^{ns}: nonsignificant, **p<0.01.
The difference between the same letters in the same column is insignificant.

Gupta & Das (1997) found that the angle of repose values changed from 34° to 41° in shelled sunflower seeds of the Mordan variety, while they varied from 27° to 38° for unshelled sunflower seeds. Accordingly, the values observed in this study were lower than the literature values. The reason for this difference is thought to be due to the difference in the ecological conditions and soil characteristics in which the seeds are grown, together with the variety used.

Static friction coefficient

The results of the static friction coefficient values of shelled and unshelled sunflower seeds with different genotypes on galvanized steel, plywood, PVC, rubber, and laminate surfaces as different friction surfaces are given in Tables 9 and Table 10. According to the variance analysis results statistically p<0.01 differences were observed in terms of the static friction coefficients used on laminate, galvanized steel, and plywood friction surfaces in shelled sunflower seeds on the basis of genotypes (Table 9, Table 10). While the static friction coefficient values were found to be between 0.384 and 0.408 on the rubber surface in shelled sunflower seeds according to the genotypes, it was between 0.384 and 0.433 on the plywood surface in unshelled sunflower seeds. Altuntas et al. (2005) found that the static friction coefficients of fenugreek seeds ranged from 0.464 to 0.567 for the plywood surface when the moisture content rises from 8.9% to 20.1%. In the study, friction coefficient values gave higher results on rubber surfaces in shelled seeds and on plywood surfaces in unshelled seeds than on the other surfaces.

Khodabakhshian et al. (2010) found that the static friction coefficient values for galvanized steel, rubber, and plywood surfaces were as 0.38, 0.50 and 0.46. in Shahroodi variety sunflower seeds at 7% moisture content, respectively. Accordingly, the friction coefficient values found for the rubber surfaces for the shelled sunflower seeds in this study were found to be lower than the literature value.

Table 9. The static friction coefficient of shelled sunflower seeds with different genotypes
Çizelge 9. *Farklı genotiplere ait kabuklu ayçiçeği tohumlarının statik sürtünme katsayıları*

Genotypes	*PVC*	*Galvanized Steel*	*Laminate*	*Plywood*	*Rubber*
AYBAK-2013-12-DAÇ13099	0.236±0.0048^{ns}	*0.302±0.0047b***	*0.213±0.0055a***	*0.279±0.0058b***	*0.398±0.0052ab**
AYBAK-2013-17-DAÇ130104	0.231±0.0047^{ns}	*0.292±0.0040b***	*0.203±0.0041a***	*0.294±0.0058b***	*0.384±0.0067b**
09 TRÇ 004	0.242±0.0056^{ns}	*0.319±0.0058a***	*0.184±0.0040b***	*0.317±0.0086a***	*0.408±0.0051a**
F value	***0.32***	***7.68***	***10.64***	***7.81***	***4.50***

±: standard error, ^{ns}: nonsignificant, *p<0.05, **p<0.01.
The difference between the same letters in the same column is insignificant.

Table 10. The static friction coefficient of unshelled sunflower seeds with different genotypes.
Çizelge 10. *Farklı genotiplere ait kabuksuz ayçiçeği tohumlarının statik sürtünme katsayıları*

Genotypes	*PVC*	*Galvanized Steel*	*Laminate*	*Plywood*	*Rubber*
AYBAK-2013-12-DAÇ13099	0.337±0.0099a^{ns}	*0.340±0.0039a**	*0.246±0.0037^{ns}*	*0.433±0.0082a***	*0.388±0.0040**
AYBAK-2013-17-DAÇ130104	0.313±0.0076ab^{ns}	*0.317±0.047b**	*0.238±0.0045^{ns}*	*0.412±0.0081a***	*0.367±0.0040b**
09 TRÇ 004	0.306±0.0085b^{ns}	*0.317±0.0077b**	*0.231±0.0058^{ns}*	*0.384±0.0063b***	*0.396±0.0081a**
F value	***3.38***	***5.56***	***2.40***	***10.39***	***6.39***

±: standard error, ^{ns}: nonsignificant, *p<0.05, **p<0.01.
The difference between the same letters in the same column is insignificant.

4. Conclusion

The engineering characteristics such as geometric, gravimetric, color, and mechanical properties of the confectionery sunflower genotypes were determined. It was observed that the *L** characteristic value of both the shelled and shellless seeds of the AYBAK-2013-12-DAÇ13099 genotype was higher, and the chroma value was higher for the shellless seed than for the other

genotypes. Geometric sizes of the seeds belonging to the confectionery sunflower genotypes differ according to the genotypes, which will create differences in the design of the equipment to be used in post-harvest cleaning, classification, and separation, and it can be said that the differences in the volumetric properties may cause differences in the design of the equipment related to ventilation, drying, storage and transportation of the genotypes. In terms of mechanical properties, angle of repose and friction properties gave different results for different surfaces, and the use of both shelled and unshelled seeds in the study. In general, different sunflower genotypes gave different results for the parameters examined in the study. The reason for this is that the genotypes used in the experiment have different geometric, gravimetric, colour and mechanicial characteristics as well as genetic and cultivating characteristics. These results will cause significant differences in terms of designs in post-harvest production technologies. As a result, it is thought that the findings on sunflower seeds will create important engineering data in the design of the equipment related to sowing, harvest, cleaning, classification, and post-harvest technologies.

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Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
https://dergipark.org.tr/tr/pub/gopzfd

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2022) 39(2) 113-119
doi:10.55507/gopzfd.1138189

Optimization of Bioactive Components in Fresh Red Watermelon Juice of Ultrasound Assisted Extraction Conditions With Response Surface Methodology

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Alındığı tarih (Received): 30.06.2022 Kabul tarihi (Accepted): 13.08.2022

Abstract: In this study, optimization of bioactive components in fresh red watermelon juice which was applied ultrasound for different amplitude and time with response surface methodology (RSM) was performed. As a result of the optimization, lycopene, ascorbic acid, total phenolic content and DPPH were determined as 28.74 mg/100 mL, 4.34 mg/100 mL, 122.2 mg GAE/L and 54.26%, respectively. When compared to the fresh red watermelon juice samples applied ultrasound with control samples, it was found that lycopene, total phenolic content and DPPH values increase and ascorbic acid content decreased.

Keywords: Bioactive components, Fresh Red Watermelon Juice, Response Surface Methodology, Ultrasound

Taze Kırmızı Karpuz Suyundaki Biyoaktif Bileşiklerin Ultrases Destekli Ekstraksiyon Koşullarının Tepki Yüzeyi Metodolojisi ile Optimizasyonu

Öz: Bu çalışmada, farklı genlik ve sürelerde ultrases uygulanmış kırmızı karpuz suyundaki biyoaktif bileşiklerin tepki yüzeyi metodolojisi (TYM) kullanılarak optimizasyonu gerçekleştirilmiştir. Optimizasyon sonucunda ultrases uygulanmış örneklerin likopen, askorbik asit, toplam fenolik madde ve DPPH değerleri sırası ile 28.74 mg/100 mL, 4.34 mg/100 mL, 122.2 mg GAE/L ve %54.26 olarak tespit edilmiştir. Kontrol örneği ile kıyaslandığında ultrases uygulanmış kırmızı karpuz suyu örneklerinin likopen, toplam fenolik madde ve DPPH değerlerinde artış görülürken, askorbik asit içeriğinde azalma meydana gelmiştir.

Anahtar kelimeler: Biyoaktif bileşikler, taze kırmızı karpuz suyu, tepki yüzeyi metodu, Ultrases

1.Introduction

Watermelon [*Citrullus lanatus* (Thunb.)] is generally considered to be of Citrullus species which belongs to the *Cucurbitaceae* family of flowering plants (Seyed & Elnaz, 2006). Although the Middle East, Europe, the USA, Africa, Japan, and India are the most important watermelon producing areas, watermelon is cultivated in most parts of the world (Fehér, 1993). It is one of the most economically and a pleasant-tasting fruit (Olayinka & Etejere, 2018). Watermelon contains several bioactive compounds such as carotenoids, unsaturated fatty acids, xanthophylls, phenolic compounds, and citrulline (Zamuz et al., 2021). Bioactive compounds has properties; inhibition and induction of gene expression, enzymes, receptor activities (Santos et al., 2019). Carotenoids in waterlemon that give to the varios flesh colors; orange, red, yellow (canary and salmon). Flesh color is show of the potential health benefits of watermelon (Bang et al., 2007; Bang et al., 2010).

Food processing objective is producing more usable, compact, appealing, value-added, and shelf-stable product. The most traditional food processing method is heating for retard growth of foodborne pathogens and reduce microbial growth. These process can cause changes at color, taste, and textural modifications in heat-sensitive food (Singla & Sit, 2021). One of the green and innovative techniques is ultrasound-assisted processing (Bindes et al., 2019). Ultrasonication, as a novel non-thermal treatment, is applied in the food technology especially in biotechnology (Alijan et al., 2021). Many researchers have found that non-thermal treatment (ultrasound technology) of mango peel and juice (Santhirasegaram et al., 2013; Mercado-Mercado et al., 2018), grapefruit juice (Aadil et al., 2015), tomato juice (Zhang et al., 2019), rosehip nectar (Atalar et al.,2020), amazon fruits juice (De Souza Carvalho et al., 2020) had minimal losses to nutritional value and quality.

RSM is an statistical technique, less time-consuming and laborious. It has successfully and constantly been showed that it can be used in optimizing ingredients (Lee et al., 2006).

When the literature was searched, no study was found

on the optimization of bioactive compounds in fresh red watermelon juice using the RSM. The objective of this study was to investigate the effects of various ultrasound treatment of fresh red watermelon to its antioxidant capacity, ascorbic acid, lycopene, and total phenolic contents as a result of RSM optimization.

2. Material and method

2.1. Watermelon juice samples preparation

Fresh red watermelons were carefully collected from local producers (Tekirdağ, Turkey) and kept at 4°C. The seeds, and ripe parts of the watermelons were removed from the outer pods then the remaining edible parts were crushed with the help of a blender (Waring Commercial Blender Model HGB2WTS3, USA) to prepare its juice. Watermelon juice after filtered through a sterilized double-layer muslin cloth, it was sterilized and filled into 100 mL airtight bottles. Untreated watermelon juice was chosen as the control (MJ-C). Watermelon juice was pasteurized in a water bath (90 °C) for 30 seconds, then quickly cooled to 20 °C (MJ-P). The UP200St- ultrasound device of Hielscher Ultrasonics (UP200St, Germany) was used in the study to treated with ultrasound. The device was operated at 26 kHz, 200 w. Samples were stored at -20 °C until analysis.

2.2. Experimental design

RSM was used to determine the effect of ultrasound technology on the lycopene, total phenolic content, ascorbic acid, and DPPH of the fresh red watermelon juice, Central Composite Design was chosen and a two-factor, five-level experiment design was created (Table 1). For the study, 13 trial designs were created. The lack-of-fit tests, R2 and corrected -R2 coefficients, and ANOVA results were model adequacy values. Independent variables were determined as duration within the range of m (time) and n (amplitude).

The following equation models was created with quadratic-polynomial equation formula;

$$y = b_o + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2 \quad (1)$$

Table 1. Dependent and independent variables of RSM analysis and results of bioactive compounds
Çizelge 1. *RSM analizinin bağımlı ve bağımsız değişkenleri ve biyoaktif bileşenleri üzerine etkisi*

Run no.	Independent variables		Dependent variables							
	Time (m)	Amplitude (m)	Lycopene (mg/100 mL)		AA (mg/100 mL)		TPC (mg GAE/L)		DPPH (% inhibition)	
			Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted
1	8 (+1)	50(-1)	27.80	27.63	3.83	3.76	122.60	123.87	54.90	54.88
2	8 (+1)	70 (+1)	25.32	25.09	2.55	2.55	108.24	109.90	53.40	53.35
3	6 (0)	60 (0)	26.38	26.45	3.16	3.16	125.87	125.23	55.32	55.32
4	2 (-1.41)	60 (0)	28.64	28.72	5.07	5.04	107.41	106.68	51.55	51.54
5	6 (0)	60 (0)	26.38	26.45	3.16	3.16	125.87	125.23	55.32	55.32
6	6 (0)	60 (0)	26.38	26.45	3.16	3.16	125.87	125.23	55.32	55.32
7	6 (0)	60 (0)	26.38	26.45	3.16	3.16	125.87	125.23	55.32	55.32
8	10 (+1.41)	60 (0)	25.73	25.84	3.53	3.56	109.81	108.97	53.05	53.07
9	4 (-1)	70 (+1)	28.72	28.50	4.09	4.15	119.88	121.47	53.76	53.74
10	6 (0)	80 (+1.41)	27.44	27.56	3.16	3.13	106.71	105.71	51.57	51.59
11	4 (-1)	50 (-1)	27.25	27.09	3.67	3.65	108.80	110.00	52.94	52.96
12	6 (0)	60 (0)	26.38	26.45	3.16	3.16	125.87	125.23	55.32	55.32
13	6 (0)	40 (-1.41)	28.62	28.69	3.80	3.84	108.79	108.21	52.36	52.35
MJ-C			25.16		5.42		112.55		50.86	
MJ-P			21.45		2.70		104.86		46.64	

RSM: Response surface methodology TPC: Total phenolic content; AA: ascorbic acid; DDPH: radical scavenging activity; GAE: gallic acid equivalent; MJ-C: watermelon juice; MJ-P: thermal pasteurized watermelon juice

The coefficients of the polynomial were indicated by b_{12} (interaction effects), b_{11} and b_{22} (quadratic effects), b_1 and b_2 (linear effects), and b_0 constant term).

2.3. Determination of lycopene content

For the determination of lycopene concentration method was used as reported previously by Oms-Oliu et al., (2009) with some changes. The lycopene concentration (mg/L) of fresh red watermelon sample was estimated using the eguation below:

$$\text{Lycopene concentration} = \text{Abs}503 \times MW \times DF \times 1000\varepsilon \times L \quad (2)$$

Molecular weight (MW) of lycopene; 536.9 g/mol, dilution factor (DF), path length (L) in cm, and molar extinction coefficient (ε) for lycopene;172.000 L/mol/cm.

2.4. Determination of total phenolic contents

Total phenolic content assay as described by Singleton & Rossi, (1965) was used. The dilution was carried out by taking 1.5 ml from the fresh red watermelon juice sample. Folin-Ciocalteau reagent (0.2 N, 2.5 mL) was added. After 2 mL of 7.5% Na_2CO was

added, the prepared solution was incubated for 3 minutes. The red watermelon juice samples were left for 30 minutes in the dark at 24 °C (normal room temperature). Spectrophotometer (SP-UV / VIS-300SRB, Spectrum Instruments, Australia) was used to measurements absorbance (760 nm). Standard curve for gallic acid was constructed, results were given as mg GAE/L.

2.5. Determination of ascorbic acid contents

The Vitamin C (AA) content of the red watermelon samples was determined by Association of Official Analytical Chemists (AOAC) (Association of Official Analytical Chemists. [AOAC], 1990). Briefly, watermelon juice sample (five mL) was diluted with distilled water and two ml of the diluted solution was taken and glacial acetic acid (20%, 25 ml) was added. Titration was done using 0.05 g/100 mL DCIP (6-dichlorophenol indophenol) solution. Results were given as milligram ascorbic acid equivalents mg/100 with using standard curve for ascorbic acid.

2.6. DPPH free-radical scavenging activity

For the determination of DPPH radical scavenging activity of the fresh red watermelon samples, method, as described by Grajeda-Iglesias et al. (2016) was used with some changes. Shortly, two ml of 0.1 mM DPPH (Sigma-Aldrich, USA) was added to 100 µL of red watermelon juice sample as blank control and standard, or to tubes containing distilled water. The prepared solutions were incubated during 30 minutes under normal room conditions. Measurements were performed on tubes with a wavelength of 517 nm in a spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Australia). The percentage of inhibition of the DPPH value was calculated with the following formula:

$$\text{DPPH (\% inhibition)} = (T_0\text{-}T_1)/T_0)*100 \quad (3)$$

The T_0= absorbance value of control, T_1= absorbance value of the examined sample

2.7. Statistical analysis

Results were expressed as mean ± standard deviation (SD). All analyses were carried out in triplicate for each samples. Analysis of variance (one-way-ANOVA) was used for determination of the differences between the data with a significance level of $p<0.05$. SPSS 22.0 package software (SPSS Inc., Chicago, USA) was used for statistical analysis calculations.

3. Results and Discussion

3.1. Optimization of bioactive compounds

An innovative, non-toxic, environmentally friendly non-thermal pasteurization method that has emerged as an alternative to traditional thermal technologies to minimize changes in the organoleptic and nutritional properties of food is; ultrasound method (Bhargava et al., 2021; Fan et al., 2021; Valiati et al., 2022). Watermelon juice has attracted great attention from consumers in recent years due to its richness in lycopene, phenolic compounds, and high antioxidant activity. Since bioactive substances are sensitive to high-temperature processing, they hurt their nutritional and sensory properties. Therefore, non-thermal treatments are needed to enrich or further preserve the bioactive compounds of watermelon juice. One of the aims of this study is to increase the bioactive components with ultrasound. Optimization results of lycopene, ascorbic acid, total phenolic substance, and DPPH content of watermelon juice are shown in Table 1. The result of the optimization, the quadratic modeling equation of the lycopene value of watermelon juice is given below.

$$Lycopene\ (\frac{mg}{100\ mL)=} 29{,}44 + 1{,}980m - 0{,}233n + 0{,}05151m^2 + 0{,}004175n^2 - 0{,}04931mn \quad (4)$$

R^2 values, analysis of variance (ANOVA), discordance, and regression coefficients are shown in Table 2. Watermelon juice is a fruit juice included lycopene, an important beverage used in food industry such as nutraceutical supplements and energy drinks (Vani et al., 2021). In our study, significant increases were detected in lycopene amounts as a result of ultrasound treatments. When we examined the effects of time and amplitude, an increase in proportion to the amount of lycopene was found in general (Figure 1A). R^2 level of RSM modeling showed high agreement with 98.60% (Table 2). Two-way and one-way effects of modeling were not found to be statistically significant ($p<0.05$). As a result of the optimization, lycopene was determined as 28.74 mg/100 mL (Table 3). Compared to the MJ-C sample, a 14.23% increase in the amount of lycopene was detected. Similar increases were found in the amount of lycopene in ultrasound treatment applied to red watermelon juice (Yıkmış, 2020). However, another study found reductions in ultrasound treatment applied to guava juice. Significant reductions in the amount of lycopene were detected in thermal pasteurization (Campoli et al., 2018). The reason for this decrease in lycopene is that it undergoes oxidation and isomerization with thermal heat (Shi & Le Maguer, 2000).

Table 2. ANOVA results of bioactive compounds (Lycopene, ascorbic acid, TPC, and DPPH)
Çizelge 2. *Biyoaktif bileşiklerin (Likopen, askorbik asit, TPC ve DPPH) ANOVA sonuçları*

Source	DF	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
		Lycopene (mg/100 mL)		AA (mg/100 mL)		TPC (mg GAE/L)		DPPH (% inhibition)	
Model	5	98.5000	0.0000	541.1900	0.0000	95.1900	0.0000	7201.7800	0.0000
Linear	2	116.0900	0.0000	597.4100	0.0000	2.3400	0.1670	1498.5000	0.0000
m	1	201.6700	0.0000	973.4100	0.0000	2.1800	0.1830	2414.3900	0.0000
n	1	30.5200	0.0010	221.4100	0.0000	2.4900	0.1580	582.6000	0.0000
Square	2	67.4500	0.0000	544.0200	0.0000	191.9300	0.0000	15585.6600	0.0000
m^2	1	31.3500	0.0010	1088.0300	0.0000	234.4000	0.0000	17859.1300	0.0000
n^2	1	128.7600	0.0000	86.1700	0.0000	258.2400	0.0000	22074.8200	0.0000
2-Way Interaction	1	125.4100	0.0000	423.1100	0.0000	87.4000	0.0000	1840.6100	0.0000
m*n	1	125.4100	0.0000	423.1100	0.0000	87.4000	0.0000	1840.6100	0.0000
Error	7								
Lack-of-Fit	3								
Pure Error	4								
Total	12								
R^2		98.60%		99.74%		98.55%		98.98%	
Adj R^2		97.60%		99.56%		97.52%		99.97%	
Pred. R^2		90.34%		97.39%		90.03%		99.82%	

m: time; n: amplitude; DF: degrees of freedom; TPC: Total phenolic content; AA: ascorbic acid; DDPH: radical scavenging activity; GAE: gallic acid equivalent;

Table 3. Maximum optimization values according to RSM.
Çizelge 3. *RSM'ye göre maksimum optimizasyon değerleri.*

Variable	Setting			
Time (m) (min.)	8.8			
Amplitude (n) (%)	46.9			
Response (MJ-UT)	**Fit**	**SE Fit**	**95% CI**	**95% PI**
Lycopene (mg/100 mL)	28.74	0.202	(28.267; 29.222)	(28.111; 29.378)
AA (mg/100 mL)	4.346	0.047	(4.2339; 4.4579)	(4.1972; 4.4945)
TPC (mg GAE/L)	122.2	1.56	(118.52; 125.90)	(117.32; 127.10)
DPPH (% inhibition)	54.26	0.157	(53.884; 54.628)	(53.753; 54.758)

TPC: Total phenolic content; AA: ascorbic acid; DDPH: radical scavenging activity; GAE: gallic acid equivalent; MJ-UT: ultrasound-treated watermelon juice.

As a result of RSM, the ascorbic acid R2 value showed a high correlation with 99.74% (Table 2). As a result of the optimization, the quadratic polynomial equation of the ascorbic acid value of fresh red watermelon juice is given below.

$$AA\ (\frac{mg}{100\ mL)=}3{,}141 + 0{,}2347m + 0{,}0136n - 0{,}07119m^2 + 0{,}000801n^2 - 0{,}02125mn \quad (5)$$

No statistically significant differences were found in the two-way interaction of m and n values in ultrasound treatment ($p<0.001$). The amount of AA in the MJ-C sample was determined as 5.42 mg/100 mL. It was determined that AA amount is decreased after ultrasound treatment. These decreases are observed when the three-dimensional graph of the modeling is examined (Figure 1B). After the optimization, AA was determined as 4.35 mg/100 mL in this study. However, ultrasound treatment preserved the ascorbic acid content more than thermal pasteurization. Similar effects were observed in ultrasound treatments applied to kiwi juice (Wang et al., 2019) and red grape juice (Margean et al., 2020). The decrease in the amount of ascorbic acid may be due to the sensitivity of ascorbic acid to the amount of heat generated as a result of cavitation.

Polyphenols are one of the non-nutritive bioactive components responsible for the taste, aroma, and color of vegetables and fruits (Dall'Asta et al., 2022). Today, there has been increasing interest to polyphenol-rich ingredients into foods. The effect of food processing technologies on the stability and biological activity of polyphenols is an important factor (Debelo et al., 2020). The effects of ultrasound parameters on total phenolic substance and DPPH are shown in Table 1. RSM optimization, the quadratic polynomial equations of TPC and DPPH values of fresh red watermelon juice are given below.

$$TPC\ \left(mg\ \frac{GAE}{L}\right) = -190{,}8 + 32{,}42m + 7{,}326n - 1{,}0878m^2 - 0{,}04567n^2 - 0{,}3180mn \quad (6)$$

$$DPPH\ (inhibition) = 7{,}903 + 4{,}1925m + 1{,}16118n - 0{,}18854m^2\_0{,}008385n^2 - 0{,}028974mn \quad (7)$$

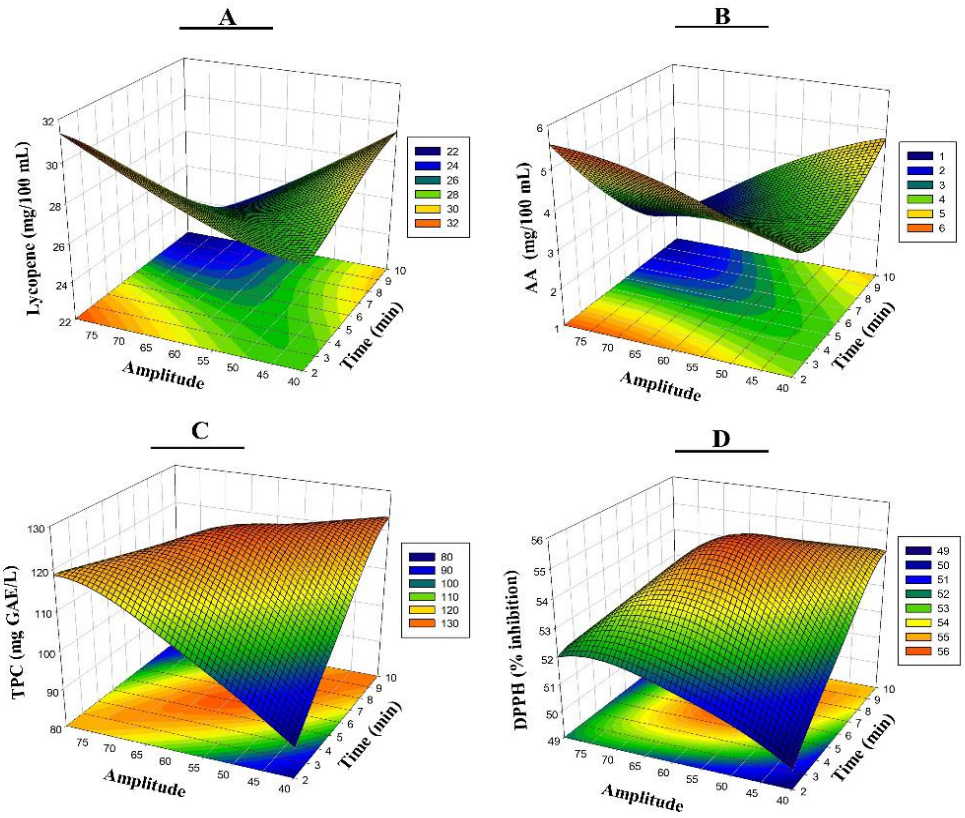

Figure 1. Response surface plots (3D) of lycopene (A), ascorbic acid (B), TPC (C), and DPPH (D), as functions of significant interaction factors.
Şekil 1. *Önemli etkileşim faktörlerinin fonksiyonları olarak likopen (A), askorbik asit (B), TPC (C) ve DPPH'nin (D) tepki yüzey grafikleri (3D).*

The results of the changes in DPPH antioxidant activity values during fermentation of watermelon juice samples applied with ultrasound at different times and amplitudes are expressed in Table 1. As a result of RSM, TPC and DPPH R2 values showed a high correlation with 98.55% and 98.98%, respectively (Table 2). Increases in the amounts of TPC and DPPH were detected with m and n. These increases are observed when the three-dimensional graph of the modeling is examined (Figure 1CD). The linear effects of the variables (m and n) on TPC were found to be statistically significant ($p>0.05$). However, linear effects on DPPH are insignificant ($p<0.001$). No significant effects on TPC and DPPH were detected in the two-way interaction of the variables ($p<0.001$). After the optimization, TPC and DPPH were determined as 122.20 mg GAE/L and 54.26%, respectively. Similar increases in TPC and antioxidant values with ultrasound were reported in functional beverage of Turkish (Doguer et al., 2021), chokanan mango juice (Santhirasegaram et al., 2013), strawberry juice (Wang, et al., 2019), lactic acid fermented mulberry juice (Kwaw et al., 2018), purple cactus pear (Zafra-Rojas et al., 2013), quince juice (Yıkmış et al., 2019) and gilaburu vinegar (Erdal et al., 2022) samples. Significant reductions were detected with thermal pasteurization. The effect originating from ultrasound is associated with the cavitation mechanism that occurs when bubbles form and burst during the propagation of sound waves in a liquid medium. When high-powered ultrasound is applied to food products or ingredients, these bubbles burst, producing free radicals across and intense shock waves the cell membrane (Aadil et al., 2013; Ordóñez-Santos et al., 2017; Wang et al., 2019; Yıkmış et al., 2021).

4. Conclusion

In this study, the optimization of bioactive components in fresh red watermelon juice using ultrasound with RSM was determined. Compared to the MJ-C sample, lycopene, total phenolic content, and DPPH values increased by 14.23%, 8.57%, and 6.69%, respectively. With the ultrasound treatment, a decrease of 19.81% in ascorbic acid content was detected. The results of this research have shown that ultrasound technology had a important effect on bioactive compounds in fresh red watermelon water.

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Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
https://dergipark.org.tr/tr/pub/gopzfd

Araştırma Makalesi/*Research Article*

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2022) 39(2) 120-128
doi:10.55507/gopzfd.1088185

Determination of Grapevine Rootstocks Resistancy to Cadmium (Cd) Toxicity

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Alındığı tarih (Received): 15.03.2022 Kabul tarihi (Accepted): 18.08.2022

Abstract: In this study, response of 12 grapevine rootstock genotypes to cadmium (Cd) toxicity were investigated. The Cd application to the soil was made at the beginning of the experiment at 4 different (0, 5, 10 ve 20 mg Cd kg^{-1}) doses. Shoot, leaf and root dry matter yields, leaf Cd, N, P and Zn contents were determined to assess genotype tolerance of Cd toxicity. Present findings revealed that based on shoot, leaf and root dry weights, leaf Cd, N, P and Zn contents, there were Cd-sensitive and resistant genotypes among the present ones. At the greatest Cd dose (Cd20), the greatest Cd contents (µg $plant^{-1}$) were observed in 8B (6.13), 420A (5.35) and 1103P (4.69) rootstocks and the lowest Cd contents were observed in 99R (1.27) and SO4 (1.58) rootstocks. Among the grapevine rootstocks, SO4 with quite lower leaf Cd accumulation than the other genotypes and increasing shoot and leaf dry weights and leaf N, P and Zn content was identified as resistant against toxic Cd conditions. On the other hand, 8B, 420A, 1103P, 5BB, Harmony genotypes with decreasing shoot, leaf and root dry weights under Cd toxicity conditions, higher leaf Cd accumulations and significantly decreasing leaf N, P and Zn contents were considered as sensitive to Cd toxicity.

Key words: Grapevine rootstocks, cadmium, toxicity, heavy metal, nutrient uptake

Asma Anaçlarının Kadmiyum (Cd) Toksisitesine Karşı Dayanıklılıklarının Belirlenmesi

Öz: Bu çalışmada 12 asma anaçgenotipinin kadmiyum (Cd) toksisitesine tepkisi incelenmiştir. Toprağa Cd uygulaması deneme başlangıcında 4 farklı dozda (0, 5, 10 ve 20 mg Cd kg^{-1}) yapılmıştır. Sürgün, yaprak ve kök kuru madde verimi ile yaprak Cd, N, P ve Zn içerikleri belirlenmiştir. Elde edilen sonuçlar değerlendirildiğinde, asma anaçları arasında sürgün, yaprak ve kök kuru madde verimi ile yaprak Cd, N, P ve Zn içerikleri bakımından Cd toksisitesine karşı hassas ve dayanıklı anaçların olduğu ortaya çıkmıştır. Kadmiyum uygulamasının en yüksek olduğu Cd20 dozunda Cd içeriği en fazla en fazla 8B (6.13 µg $plant^{-1}$), 420A anacında (5.35 µg $biki^{-1}$) ve 1103P (4.69 µg $plant^{-1}$) anaçlarında olduğu buna karşın en düşük Cd içeriği ise 99R (1.27 µg $plant^{-1}$) ile SO4 (1.58 µg $plant^{-1}$) anaçlarında olduğu ortaya çıkmıştır. Asma anaçları arasında SO4 genotipinin diğer anaçlara göre yapraklarında çok daha az Cd birikimi yapması, sürgün ve yaprak kuru madde verimi ile N, P ve Zn içeriklerinde artışların olması bu anacın toksik Cd koşullarına karşı dayanıklı olabileceğini göstermiştir. Buna karşın toksik Cd koşullarında yapraklarında yüksek düzeyde Cd biriktiren ve kuru madde (gövde, yaprak, kök) verimlerinde önemli düzeyde azalma meydana gelen 8B, 420A, 1103P, 5BB, Harmony asma anaçlarının ise Cd toksisitesine karşı hassas olduğu ortaya çıkmıştır.

Anahtar Kelimeler: Asma anaçları, kadmiyum, toksisite, ağır metal, besin alımı

1. Introduction

Cadmium (Cd) is among the most dangerous heavy metals and pollutants in ecosystem and it has toxic impacts on living organisms (Alengebawy et al., 2021). Soils are contaminated by cadmium through parent material or industrial activities and phosphorus fertilizers-like anthropogenic activities. Of the Cd reached to soils through human activities, 56% comes from use of phosphorus-containing fertilizers, 40% comes from atmospheric and 2-5% comes from manure treatments (sludge-livestock) (Cheng et al., 2014; Suhani et al., 2021). Since cadmium has a more water solubility and the mobility than the other metals, it is more up taken by the plants. Therefore, it is the most dangerous metal accumulated in soils. Cadmium is not an essential element for plants, it is a heavy metal that makes it toxic to plants. Cadmium disrupts the root activity of plants, negatively affects plant nutrient uptake and use. At the same time, it negatively affects photosynthesis in plants, impairs carbohydrate metabolism and adversely affects plant yield and quality (Stachowiak et al., 2015; Yang et al., 2021).

Climatic conditions, soil properties and agricultural practices significantly affect the uptake of Cd by plants (Hart et al., 1998; Hussain et al., 2021). Cadmium causes in decelerated plant growth, low biomass, browning in root tips and ultimately plant die outs (Anjum et al., 2008; Erdem, 2021). Cadmium ions reduce root and shoot lengths. Inhibition of root growth is the most significant indicator of Cd^{+2} toxicity (Sgherri et al., 2001;2002; Xiao et al., 2022). Cadmium also negatively affects chlorophyll synthesis (Haider et al.,

2021; Song et al., 2019). Cadmium ion especially influence photosynthesis, respiration and N metabolism of the plants (Cheng et al., 2014; Nascimento et al., 2021). Cadmium restrict uptake, transport and use of water and plant nutrients (Ca, Mg, P, K and Fe) (Haider et al., 2021). Cadmium uptake and transport largely vary with the plant species, thus plants have different tolerance levels to Cd toxicity (El Rasafi et al., 2021; Grant et al., 1998). Plant tolerance to Cd toxicity largely depend on Cd accumulation capacity and localization of the plants (Obata & Umebayashi, 1993; Shrivastava et al., 2019). Plant genetics also play a great role in Cd uptake and accumulation by the plants. Researchers reported that there are significant genetic variations in Cd tolerance of tobacco (Erdem et al., 2017; Lugon-Moulin et al., 2004), soybean (Zhi et al., 2020), maize (Rasool et al., 2020), wheat (Greger & Löfstedt, 2004; Zhou et al., 2020), and lettuce (Loi et al., 2018).

Grafting in grapevines could be increase the yield and quality of grapes and increase the resistance of plants to various stress factors such as extreme heat, cold stress, drought and heavy metals (He et al., 2020; Savvas et al., 2010; Yuan et al., 2019). Some vine rootstocks prevent Cd uptake and transport in xylem by plant roots by ion exclusion or retention mechanism. Plants are protected from heavy metal toxicity through these mechanisms. It has been reported that Cd accumulation in the leaves of the eggplant and tomato grafted to *Solanum torvum* rootstocks decreased (Yuan et al., 2019). In a study investigating the responses of grafted Malus plants to Cd toxicity, it was reported that applications varied according to rootstock, scion and rootstock-scion combinations (He et al., 2020).

In modern viticulture, grafted vine saplings must be used due to the phylloxera. Several different genotypes are used as rootstock in vine saplings. Grapevine rootstocks different macro and micronutrient uptake and grafted plants have different nutrient uptakes from the seed-propagated plants. Grapevine rootstocks may also differ in response to biotic and abiotic stress conditions (Bavaresco et al., 2003; Ibacache & Sierra, 2009; Lecourt et al., 2015; Zamboni et al., 2016).

In regions with industrial pollution, significant relationships were reported between grapevine roots and soil Cd concentrations of the vineyards located in regions with industrial pollution (Angelova et al., 1999). Al- Obeed et al., (2011) reported that fruit Cd contents of the different grape cultivars irrigated with treated domestic wastewater varied with the cultivars. Miklos & Erdei (1997) reported that high Cd concentrations adversely affected the development and nutrition of grape genotypes (Leányka and Ezerjó,), and plant roots had higher Cd concentrations than leaves.

In this study, the effects of increasing doses of toxic Cd on plant growth and leaf Cd, N, P and Zn content twelve different grapevine rootstocks on were investigated.

2. Material and Methods

2.1. Material

Plant Material: The research was carried out in the greenhouses of Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Horticulture. Twelve different rootstocks (1103P, 1045P, 5BB, 8B, SO4, 420A, 99R, 110R, 140 Ru, Dogridge, Harmony, 41B) were used in the study. The rootstock saplings used in the study were grown in the greenhouse in 1-liter plastic bag with a 1:1 substrate mixture (peat-perlite).

Soil Material: The soil used for the pot experiment had the following chemical and physical properties: texture was clay, $CaCO_3$ content was %15.8, pH was 7.85, organic matter was 1.69 %, available P_2O_5 concentration was 5.60 kg da^{-1}, K_2O concentration was 16.4 kg da^{-1} and the DTPA extractable concentrations (ppm) of Zn, Fe and Cd were 0.52, 2.11, 0.005, respectively.

2.2 Method

The study was carried out in 5-liter plastic pots with 4 replications according to the randomized plots experimental design under open sections with 35% shading net conditions. Air-dried 4 mm sieved 4 kg soil, prior to filling the pots, was homogeneously mixed with a fertilizer solution containing 250 mg N kg^{-1} soil as $Ca(NO_3)_2.4H_2O$, 100 mg P kg^{-1} soil as KH_2PO_4, 2.0 mg Fe kg^{-1} soil as Fe-EDTA and 2.0 mg Zn kg^{-1} soil as $ZnSO_4.7H_2O$. The treatments were control (Cd0), 5.0 (Cd5), 10 (Cd10) and 20 (Cd20) mg Cd kg^{-1}, and Cd were applied to soil in the form of $(CdSO_4)_3.8H_2O$. Cd doses were given to the soil at the beginning of the experiment together with basic fertilizers and mixed with the soil in a homogeneously. After the fertilizer and Cd applications, one sapling was planted in each pot. The shoots on the seedlings were left 4-5 cm tall and only one shoot was allowed to grow according to the state of the dormant buds. In order to prevent water loss by evaporation in the soil, the top of the pot were dressed with 2-3 cm thick perlite and the plants were watered daily with distilled water.

Destructive harvest of the plant parts begun on the 134^{th} day according to the decrease in growth in the plants and the severity of Cd toxicity in the leaves. The

plants were destructively harvested separately as roots, shoots and leaves. After the destructive harvest of the plant parts, they were washed with tap water, and dried at 65 ºC for 48 hours to obtain their dry weight. Dried leaf samples were then ground in agate mill, subjected to wet-digestion in $HNO_3 – H_2O_2$ in a microwave oven and Cd, P and Zn concentrations of the samples were determined in an ICP-OES (Varian Vista) device (Kacar and ? İnal, 2008). Total nitrogen analysis in leaf samples was made according to the Kjeldahl distillation method (Bremner, 1965). The Cd, N, P and Zn contents of the leaves were calculated by multiplying the leaf weights with Cd, N, P and Zn concentrations. It has been recommended to examine the effect of Cd on physiologically important micronutrients, particularly P (Wang, 1987) and Zn (Kim et al., 1988).

Statistical Analysis: The research was carried out in 4 replications, with one plant in each pot. Experimental data were subjected to variance analysis in accordance with randomized plots–factorial design. Duncan multiple range test was used to compare significant means.

Cluster analysis was performed to determine the relation between varieties. Cluster analysis was carried out using the Cd, N, P, Zn, shoot, leaf and root dry weight **v**alues of the control treatment. Since the units of the variables used in the analysis are not the homogeneous, the variables are standardized and then the Euclidean distance is calculated. The dendrogram showing the similarities and differences of rootstocks was obtained by cluster analysis according to Ward's method.

3. Result and Discussion

Toxic Cd negatively affects the nutrient uptake and transport of plants and causes physiological disorders in plants, resulting in reduced growth. Roots absorb Cd from the soil and transport it through xylem to upper section of the plants with the aid of transpiration stream (Salt et al., 1995; Zhang et al., 2022). Heavy metals absorbed by the roots initially combine with proteins, polysaccharides, and nucleic acids, then transported to shoots (Goyal et al., 2020). Therefore, generally a positive relationship is encountered between increasing Cd doses applied to soils and shoot Cd concentrations. Significant increases were observed in Cd contents of grapevine rootstocks with increasing Cd treatment doses. Rootstocks, Cd doses and rootstock x dose interactions were found to be significant at 5% level (Table 1). Average Cd content of grapevine rootstocks at Cd0 dose (0.66 µg plant^{-1}) increased to 1.96 µg plant^{-1} at Cd5 dose, to 2.34 µg plant^{-1} at Cd10 dose and to 3.15 µg plant^{-1} at Cd20 dose. Significant differences were observed in Cd contents of rootstock genotypes with increasing Cd doses. At the greatest Cd dose (Cd20), the greatest Cd contents were observed in 8B (6.13 µg plant^{-1}), 420A (5.35 µg plant^{-1}) and 1103P (4.69 µg plant^{-1}) rootstocks and the lowest Cd contents were observed in 99R (1.27 µg plant^{-1}) and SO4 (1.58 µg plant^{-1}) rootstocks (Table 1). Such findings on Cd contents indicated that rootstock response to Cd toxicity was different. Some rootstocks may prevent Cd uptake or transport through ion exclusion or retention, thus may mitigate heavy metal phytotoxicity. He et al. (2020) reported that Cd uptake of apple cultivars varied according to both rootstock and scion. Various plant species may have quite different Cd tolerance, uptake and transport (Chun et al., 2020; He et al., 2020; Yang et al., 2021; Zhou et al., 2017). Foliar Cd accumulations were reduced through grafting eggplant and tomato scions onto Solanum torvum rootstocks (Yuan et al., 2019).

Table 1. Effects of increasing Cd doses on leaf Cd and N contents of grapevine rootstocks
Çizelge 1. *Asma anaçlarında artan Cd dozlarının yaprak Cd ve N içerikleri üzerine etkileri*

Rootstock	Cd Content (µg plant^{-1})				N Content (mg plant^{-1})			
	0	5	10	20	0	5	10	20
5BB	0.90^{abC}	2.58^{abcAB}	1.89^{bB}	3.09^{cA}	83.8^{abA}	88.0^{aA}	52.6^{b-eB}	39.5^{cdB}
8 B	0.74^{abcC}	2.51^{abcB}	1.74^{bcBC}	6.13^{aA}	82.2^{abcA}	80.1^{abcA}	60.4^{b-eA}	65.5^{abcA}
SO4	0.60^{cdB}	A 2.75^{abA}	2.74^{abA}	1.58^{deAB}	58.2^{cdAB}	77.9^{abcA}	2.5^{deB}	71.4^{abB}
420A	0.85^{abC}	3.14^{aC}	2.47^{abB}	5.35^{abA}	82.5^{abcA}	59. 3^{abcAB}	71.5^{bcdAB}	53.1^{bcdB}
1613 C	0.65^{bcdB}	2.05^{a-dA}	2.04^{cbA}	3.14^{cA}	78.7^{abcA}	76.0^{abcA}	50.3^{cdeA}	731.7^{abA}
99 R	0.75^{abcC}	0.87^{eC}	2.55^{abA}	1.27^{eB}	91.3^{aA}	75.8^{bcAB}	67.7^{bcdBA}	47.0^{bcdC}
110 R	0.58^{cdC}	1.48^{cdeB}	2.36^{abA}	2.29^{cdeA}	64.5^{a-dB}	83.7^{abcA}	74.5^{abcAB}	60.4^{bcdB}
140 Ru	0.58^{cdB}	2.17 $^{a-dA}$	2.27^{bA}	2.25^{cdeA}	50.4^{dA}	65.7^{abcA}	52.5^{bcdeA}	40.9^{cdA}
1103P	0.57^{cdC}	1.93^{b-eB}	3.62^{aA}	4.69^{bA}	59.6^{bcdA}	62.2^{abcA}	82.1^{abA}	70.2^{abA}
1045 P	0.61^{cdB}	1.02^{eB}	2.27^{bA}	2.12^{cdeA}	63.0^{bcdAB}	50.9 ^{abcB}	67.8^{bcdAB}	90.9^{aA}
Dogridge	0.54^{cdB}	1.51^{cdAB}	0.82^{bcB}	2.21^{cdeA}	50.0^{dA}	65.9abA	29.8^{eA}	59.7^{bcdA}
Harmony	0.48^{dB}	0.89^{eB}	2.47^{abA}	2.27^{cdeA}	47.9^{dB}	43.9^{cBC}	102.4^{aA}	34.4^{dC}
41B	0.77^{abcA}	2.86^{abA}	3.06^{abA}	3.43^{cA}	70.5^{a-dA}	65.7^{abA}	67.6^{bcdA}	58.3^{bcdA}
Average	**0.66^{D}**	**1.96^{C}**	**2.34^{B}**	**3.15^{A}**	**68.2^{A}**	**67.4^{A}**	**62.1^{AB}**	**58.1^{B}**

Means shown with similar capital letters in the same row are not significant ($p<0.05$). Means shown with similar lowercase letters in the same column are not significant ($p<0.05$).

Considering the effects of increasing Cd doses on N contents of grapevine rootstocks, , Cd doses and rootstock x dose interactions were found to be significant at 5% level. The average N content of grapevine rootstocks in control treatment (68.2 mg plant^{-1}) decreased to 62.1 mg plant^{-1} in Cd10 and to 58.1 mg plant^{-1} in Cd20 treatments (Table 1). N content of the rootstocks either increased or decreased with increasing Cd doses, but generally a decrease was observed in majority of the rootstocks. For instance, N content of 420A genotype with a high Cd content in leaves with increasing Cd doses was measured as 82.5 mg plant^{-1} in Cd0 treatment and the value decreased to 53.1 mg plant^{-1} in Cd20 treatment. On the other hand, N content of SO4 genotype with a low Cd content in rootstocks was measured as 58.2 mg plant^{-1} in Cd0 treatment and the value increased to 71.4 mg plant^{-1} in Cd20 treatment (Table 1). Nitrogen deficiency in plants grown in soils with Cd toxicity was attributed to inhibition of nitrate reductase activity within the rootzone and about 70% reduction in nitrate absorption by Cd (Borchard et al., 2014; Genchi et al., 2020; Nascimento et al., 2021) reported that Cd toxicity induced metal bindings onto Sulphur hydride groups of proteins, resulted in replacement of macro nutrients, structural destructions or activity inhibition, thus ended up with nutrient deficiency. In previous studies conducted on tomato plants, significant decreases were reported in green herbage N concentrations with Cd treatments (Chaffei et al., 2004; Gouia et al., 2000).

In terms of the effects of increasing Cd doses on P contents of grapevine rootstocks, Cd doses, rootstocks and rootstock x dose interactions were found to be significant at 5% level (Table 2). While the average P content of grapevine rootstock was 4.80 mg plant^{-1} in Cd0 treatment, the value decreased to 2.68 mg plant^{-1} in Cd10 and to 2.74 mg plant^{-1} in Cd20 treatments (Table 2). P contents of the rootstocks either increased or decreased with increasing Cd doses, but generally a decrease was observed in majority of the rootstocks. For instance, P content of 420A genotype was 5.74 mg plant^{-1} in Cd0 treatment and the value decreased to 4.08 mg plant^{-1} in Cd20 treatment. On the hand, P content of SO4 genotype was 4.56 mg plant^{-1} in Cd0 treatment and the value increased to 5.90 mg plant^{-1} in Cd20 treatment (Table 2).

Rizwan et al. (2018) indicated that plants grown in contaminated sites might have different nutrient quantities since Cd accumulation largely depended on plant species and varieties. High Cd concentrations resulted in decreased P contents in tomato (Borges et al., 2019), strawberry (Muradoglu et al., 2015), maize (Anwar et al., 2017) and lettuce (Rizwan et al., 2017).

Table 2. Effects of increasing Cd doses on leaf P and Zn contents of grapevine rootstocks
Çizelge 2. *Asma anaçlarında artan Cd dozlarının yaprak P ve Zn içerikleri üzerine etkileri*

Rootstock	P Content (mg leaf^{-1})				Zn Content (µg leaf^{-1})			
	0	5	10	20	0	5	10	20
5BB	7.62^{aA}	5.99^{aA}	3.03^{cdeB}	2.65^{cB}	114.2^{aA}	75.6^{aB}	36.7^{cdC}	32.1^{gC}
8 B	6.35^{abA}	5.02^{abA}	4.33^{bcdA}	4.99^{abA}	64.8^{b-eA}	64.1^{abA}	56.8^{abcA}	54.9^{b-eA}
SO4	4.56^{b-eAB}	4.87^{abAB}	3.34^{cdeB}	5.90^{aA}	51.2^{deAB}	59.7^{abAB}	39.9^{cdB}	70.0^{abcA}
420A	5.74^{abcA}	4.32^{abA}	5.27^{abcA}	4.09^{bcA}	74.6^{bcA}	43.9^{bB}	70.9^{abA}	50.2^{c-fB}
1613 C	5.86^{abcA}	5.40^{abA}	4.16^{bcdeA}	5.05^{abA}	73.7^{bcdA}	59.0^{abA}	48.5^{bcA}	62.5^{a-dA}
99 R	5.83^{abcA}	5.93^{aA}	4.62^{bcdAB}	3.25^{bcB}	77.2^{bA}	57.7^{abB}	52.7^{bcB}	54.2^{b-eB}
110 R	5.73^{abcA}	5.68^{abA}	5.26^{abcA}	4.69^{abA}	57.4^{b-eB}	56.3^{abB}	54.7^{bcB}	77.0^{aA}
140 Ru	4.23^{bcA}	4.88^{abA}	3.81^{bcdeA}	3.28^{bcA}	56.3^{b-eA}	56.8^{abA}	43.3^{cdA}	39.6^{efgA}
1103P	5.03^{bcdAB}	4.89^{abAB}	6.05^{abA}	3.67^{bcB}	52.5^{cdeAB}	50.6^{abAB}	68.9^{abA}	39.4^{efgB}
1045 P	4.57^{b-eAB}	3.32^{bB}	5.09^{abcAB}	6.11^{aA}	64.8^{b-eAB}	40.3^{bB}	53.2^{bcAB}	70.9^{abA}
Dogridge	3.60^{deA}	5.02^{abA}	2.07^{eA}	3.53^{bcA}	49.1^{eA}	59.3^{abA}	22.7^{dA}	44.2^{d-gA}
Harmony	2.74^{eB}	3.25^{bB}	7.26^{aA}	2.82^{cB}	46.5^{eB}	38.1^{bC}	80.1^{aA}	28.0^{gD}
41B	5.26^{bcdA}	4.99^{abA}	4.37^{bcdA}	3.94^{bcA}	59.4^{b-eA}	49.6^{abA}	50.56^{bcA}	43.9^{defA}
Average	**4.80^{bcdA}**	**3.89^{abAB}**	**2.68^{deB}**	**2.74^{cB}**	**56.8^{b-eA}**	**3829^{bB}**	**37.3^{cdB}**	**37.7^{efgB}**

Means shown with similar capital letters in the same row are not significant ($p<0.05$). Means shown with similar lowercase letters in the same column are not significant ($p<0.05$).

Considering the Zn contents of the rootstocks under increasing Cd doses, rootstocks, Cd doses and rootstock x dose interactions were found to be significant at 5% level (Table 2). While the average Zn content of grapevine rootstocks was 56.8 µg plant^{-1} in Cd0 treatment, the value decreased to 37.3 µg plant^{-1} in Cd10 and 37.7 µg plant^{-1} in Cd20 treatments (Table 2). As it was in N and P contents, either decreasing or increasing Zn contents of the rootstocks were observed with increasing Cd doses, but generally a decrease was observed in majority of the rootstocks. For instance, while the Zn content of 5BB genotype was 114.2 µg plant^{-1} in Cd0 treatment, the value decreased to 32.1 µg plant^{-1} in Cd20 treatment; Zinc content of 420A

genotype was 74.6 µg plant^{-1} in Cd0 treatment and the value decreased to 50.2 µg plant^{-1} in Cd20 treatment. On the other hand, Zn content of SO4 genotype was 51.2 µg plant^{-1} Cd0 treatment, the value increased to 70.0 µg plant^{-1} in Cd20 treatment (Table 2). Such decreases in Zn concentrations under increasing Cd conditions were attributed to antagonistic relationships between Cd – Zn. There are several other studies reporting decreased Zn uptakes with increasing Cd treatments (Erdem et al., 2012; Kinay et al., 2021; Tiecher et al., 2017). More Cd uptake of plants grown under Zn deficiency was attributed to competition of Zn and Cd with similar chemical characteristics for absorption points on membranes (Cakmak et al., 2000; Zhou et al., 2019) and increased membrane permeability under Zn deficiency (Cakmak & Marschner, 1988). Wu et al. (2003) applied increasing Cd doses (0, 0.1, 1.0 and 5.0 µM) to barley plants and determined green herbage Cd and Zn concentrations. It was observed that with Cd treatments to growth environment, not only the Zn concentration of plant tissues decreased, but also Zn transport from the roots to green herbage was inhibited.

Wu et al. (2004) applied increasing Cd doses (1 and 10 mM Cd) to cotton plants and reported decreasing Zn, Cu and Fe concentrations with increasing Cd doses. Hussain et al. (2016) also reported decreasing Zn contents of spinach with increasing Cd treatments. Such decreases were mainly attributed to similar transport and distribution mechanisms of Cd and Zn (Lin & Aarts, 2012). Cadmium toxicity symptoms are encountered in plants when the Cd accumulation reached to a certain threshold and such symptoms includes recessed growth and development, small plant size and browning (Genchi et al., 2020).

Reductions in photosynthesis, growth, and photosynthetic pigments are responses of higher plants to Cd toxicity (Kaya et al., 2019; Luo et al., 2016). For such decreases, rootstocks, Cd doses and rootstock x dose interactions were all found to be significant at 55 level (Table 3, 4 and 5). While the average shoot dry weight was 5.12 g plant^{-1} in Cd0 treatment, the value decreased to 4.27 g plant^{-1} in Cd20 treatment. The greatest decrease in shoot dry weights with Cd treatments were observed in Harmony, 110R and 5BB rootstocks and an increase was observed only in shoot dry weights of SO4 genotype (Table 3). In Harmony genotype with the greatest decrease in shoot dry weight, the value was measured as 5.00 g plant^{-1} in Cd0 treatment and 57% decrease was observed with Cd20 treatment and the value decreased to 2.13 g plant^{-1}. On the other hand, in SO4 genotype, shoot dry weight was measured as 4.44 g plant^{-1} in Cd0 treatment and the value increased to 4.92 g plant^{-1} with about 11% increase in Cd20 treatment (Table 3).

Table 3. Effects of increasing Cd doses on shoot dry weights of grapevine rootstocks

Çizelge 3. *Asma anaçlarında artan Cd dozlarının sürgün kuru ağırlıklarına etkisi*

Rootstock	Shoot Dry Weight (g plant^{-1})			
	0	5	10	20
5BB	5.25^{bcA}	4.78^{cA}	4.34^{bcAB}	2.91^{cdB}
8 B	5.49^{bcAB}	6.51^{abA}	5.43^{abAB}	4.41^{abcC}
SO4	4.44^{cB}	6.89^{aA}	3.43^{cdC}	4.92^{aB}
420A	6.88^{abA}	4.38^{cdAB}	6.27^{aB}	5.37^{aAB}
16163 C	4.32^{cA}	2.13^{efB}	1.94^{eB}	2.73 ^{cdAB}
99 R	5.14^{cA}	4.98^{cA}	4.49^{bcAB}	2.95^{cdB}
110 R	7.47^{aA}	5.00^{bcB}	4.39^{bcBC}	4.13^{abcC}
140 Ru	5.33^{bcA}	3.80^{cdeA}	3.46^{cdA}	3.45^{bcdA}
1103P	3.74^{cA}	2.82^{defB}	2.70^{deB}	3.05^{cdAB}
1045 P	3.96^{cA}	4.05^{cdA}	5.16^{abA}	3.45^{bcdA}
Dogridge	4.36^{cA}	2.95^{defA}	2.84^{deA}	3.79^{a-dA}
Harmony	5.00^{cA}	2.44^{egB}	5.75^{abA}	2.13^{dB}
41B	4.43^{cA}	4.31^{cdA}	3.58^{cdA}	4.15^{abcA}
Average	**5.12^{A}**	**4.42^{B}**	**4.31^{B}**	**4,27^{B}**

Means shown with similar capital letters in the same row are not significant ($p<0.05$). Means shown with similar lowercase letters in the same column are not significant ($p<0.05$).

Table 4. Effects of increasing Cd doses on leaf dry weights of grapevine rootstocks

Çizelge 4*. Asma anaçlarında artan Cd dozlarının yaprak kuru ağırlıklarına etkisi*

Rootstock	Leaf Dry Weight (g plant^{-1})			
	0	5	10	20
5BB	3.31^{a-dA}	3.73^{aA}	2.12^{b-eB}	1.71^{bcdB}
8 B	3.36^{abcA}	3.14^{abA}	2.50^{a-dA}	2.78^{abA}
SO4	2.39^{cdeAB}	3.05^{abA}	1.57^{deB}	2.95^{aA}
420A	3.79^{aA}	2.41^{abB}	3.11^{abcAB}	2.56^{abcB}
1613 C	3.27$^{abc\ A}$	2.37$^{ab\ B}$	1.88$^{d\ C}$	2.67 ^{abcB}
99 R	3.40^{abA}	2.97^{abAB}	2.55^{a-dB}	1.63^{cdC}
110 R	3.35^{abcA}	2.92^{abAB}	2.62^{a-dB}	2.59^{abcB}
140 Ru	2.37^{deA}	2.58^{abA}	2.11^{b-eA}	2.06^{a-dA}
1103P	2.52^{b-eA}	2.45^{abA}	3.18^{abA}	2.16^{a-dA}
1045 P	2.69^{b-eA}	2.09^{bA}	2.85^{abcA}	3.06^{aA}
Dogridge	2.15^{eA}	2.79^{abA}	1.22^{eA}	2.50^{abcA}
Harmony	2,13^{eB}	1,86^{bB}	3,62^{aA}	1,27^{dC}
41B	3.11^{a-dA}	2.62^{abaA}	2.40^{a-eA}	2.47^{abcA}
Average	**2.90^{A}**	**2.73^{AB}**	**2.49^{B}**	**2.31^{C}**

Means shown with similar capital letters in the same row are not significant ($p<0.05$). Means shown with similar lowercase letters in the same column are not significant ($p<0.05$).

Average leaf dry weight of the rootstocks was measured as 2.90 g plant^{-1} in Cd0 treatment and the value decreased to 2.31 g plant^{-1} in Cd20 treatment. The greatest decreases in leaf dry weights with increasing Cd treatments were observed in 99R, 5BB and Harmony genotypes and an increase was observed only in leaf dry weight of SO4 genotype (Table 4). In 99R genotype with the greatest decrease in leaf dry weight, the value was measured as 3.40 g plant^{-1} in Cd0 treatment and a

52% decrease was observed in Cd20 treatment and the value decreased to 1.63 g plant^{-1}. On the other hand, in SO4 genotype, leaf dry weight was measured as 2.39 g plant^{-1} in Cd0 treatment and the value increased to 2.95 g plant^{-1} with about 23% increase in Cd20 treatment (Table 4).

Significant changes were not observed in root dry weight of the rootstocks with increasing Cd doses. The average root dry weight of the rootstocks was measured as 5.97 g plant^{-1} in Cd0 treatment and the value increased to 6.00 g plant^{-1} in Cd20 treatment. The greatest decreases in root dry weights were observed in Harmony and 41B genotypes and an increase was observed in root dry weight of 420A genotype (Table 5). In Harmony genotype with the greatest decrease in root dry weight, the value was measured as 4.06 g plant^{-1} in Cd0 treatment and the value decreased to 2.63 g plant^{-1} with about 35% reduction in Cd20 treatment. On the other hand, in 420A genotype, root dry weight was measured as 5.37 g plant^{-1} in Cd0 treatment and the value increased to 8.18 g plant^{-1} with 52% increase in Cd20 treatment (Table 4).

Table 5. Effects of increasing Cd doses on root dry weights of grapevine rootstocks
Çizelge 5. *Asma anaçlarında artan Cd dozlarının kök kuru ağırlıklarına etkisi*

Rootstock	Root Dry Weight (g plant^{-1})			
	0	5	10	20
5BB	4.26^{dA}	5.49^{deA}	6.18^{abcA}	4.78^{c-fA}
8 B	8.45^{abA}	7.16^{cdA}	7.15^{abcA}	8.38^{aA}
SO4	8.81^{aA}	8.21^{bcA}	7.86^{abA}	8.58^{aA}
420A	5.37^{cdB}	3.98^{efB}	5.62^{cdeB}	8.18^{aA}
1613 C	7.26 ^{abcA}	7.08$^{cd\ A}$	5.48 ^{cdeB}	6.43 $^{a-dAB}$
99 R	5.90^{bcdA}	5.79^{cdeA}	4.92^{cdA}	5.46^{b-eA}
110 R	3.71^{dB}	5.68^{deA}	3.53^{deB}	3.41^{efB}
140 Ru	4.92^{cdA}	3.29^{efA}	4.98^{cdA}	4.44^{defA}
1103P	5.16^{cdA}	4.83^{defA}	5.29^{cdA}	6.65^{a-dA}
1045 P	7.74^{abcA}	10.08^{abA}	8.69^{aA}	7.04^{abcA}
Dogridge	4.10^{dA}	5.62^{deA}	4.76^{cdA}	4.95^{c-fA}
Harmony	4.06^{dA}	2.78^{fAB}	1.97^{eB}	2.63^{fAB}
41B	9.17^{aAB}	11.12^{aA}	7.97^{abAB}	7.51^{abB}
Average	**5,97^{A}**	**6.17^{A}**	**5.74^{A}**	**6.00^{A}**

Means shown with similar capital letters in the same row are not significant ($p<0.05$). Means shown with similar lowercase letters in the same column are not significant ($p<0.05$).

Chun et al. (2020) applied different Cd doses (0, 2.5, 10, 20, 50 and 100 mg Cd L^{-1}) to 9 different citrus rootstocks under aquaculture conditions and reported significant decreases in relative growth rates of the rootstocks with increasing Cd doses. As compared to the control, 12.2, 24.1, 57.8 and 94.4% decrease was observed respectively with Cd2.5, Cd10, Cd20 and Cd50 doses. Significant decreases were reported in dry matter content of maize plants with increasing Cd treatments to soils. About 11.9% yield reduction was reported with 10 mg kg^{-1} Cd treatment and 23.5% with 20 mg kg^{-1} Cd treatment (Khurana & Jhanji, 2014). Such decreases in dry matter yields were basically attributed to phytotoxic effect of Cd (El Rasafi et al., 2021; Pereira et al., 2011). Cd stress significantly inhibited plant growth in grafted apple combinations (He et al., 2020). It was reported in previous studies that Cd toxicity inhibited the efficiency of photosynthetic enzymes participating into Calvin cycle and chlorophyl biosynthesis, thus had negative impacts on photosynthesis and ultimately reduced plant yields (Zulfiqar et al., 2021).

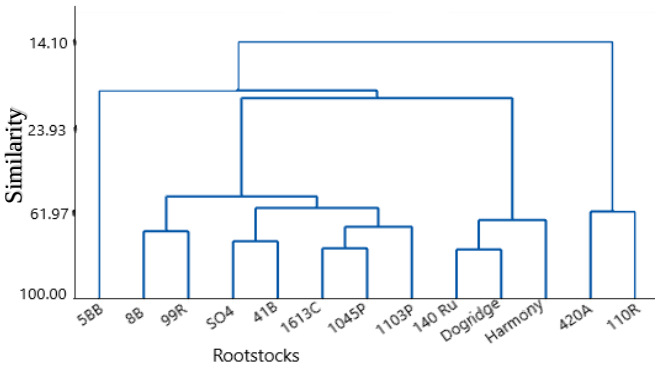

Figure 1. Genetic groupings of grapevine rootstock according to Cluster analysis
Şekil 1. *Kümeleme analizine göre asma anaçlarının genetik gruplamaları*

The dendogram obtained as a result of cluster analysis to determine the similarities between rootstocks in terms, shoot dry weight, leaf dry weight and root dry weight values, and N, P, Zn and Cd contents is given in Figure1. As a result of the analysis, rootstocks were divided into 6 groups according to their similarity. 140 Ru, Dogridge and Harmony have made up the first group. In this group, the similarity rate between 140Ru and Dogridge was 78%, and Harmony joined this group with a 64.9% similarity rate. This group can be characterized by its low nutrient content, and lower root and leaf dry weight. The second group has consisted of 1613, 1045P and 1103P rootstocks. The similarity rate between 1613C and 1045p was 77.4%. 1103P was 68% similar to these two rootstocks. The rootstocks in this group had lower shoot dry weight compared to the others. SO4 and 41B formed the third group with a similarity rate of 74.28%. The most distinctive feature of these two rootstocks was their high dry root weight. The rate of similarity between 8B and 99R, which make up the fourth group, was 69.8%. These two rootstocks were noted for their relatively high N content and high leaf dry weight. 420A and 110R have made up the fifth

group with a 61.2% similarity rate between them. In addition to high shoot and leaf dry weight, low dry root weight emerged as the common feature of this group. The 5BB rootstock alone formed the sixth group. The most distinctive features of 5BB that made it different from other rootstocks were its high Cd, P and Zn content.

4. Conclusions

Cadmium pollution encountered in soils poses serious threats on sustainable agriculture and human health (Genchi et al., 2020). Present research revealed new information about the effects of toxic Cd doses on growth, leaf Cd, N, P and Zn contents of grapevine rootstocks. High soil Cd concentrations negatively influence photosynthesis, respiration and nitrogen processes, then recess plant growth and development and reduce plant yields. Recess in plant growth (shoot, leaf, root) under increasing Cd doses was attributed to Cd-induced disorders in plant metabolism and insufficient uptake of plant nutrients. Present findings revealed that based on shoot, leaf and root dry weights, leaf Cd, N, P and Zn contents, there were Cd-sensitive and resistant genotypes among the present ones. Quite lower leaf Cd accumulation than the other genotypes and increasing shoot and leaf dry weights and leaf N, P and Zn content revealed that SO4 genotype was more resistant against toxic Cd conditions. On the other hand, 8B, 420A, 1103P, 5BB, Harmony genotypes with decreasing shoot, leaf and root dry weights under Cd toxicity conditions, higher leaf Cd accumulations and significantly decreasing leaf N, P and Zn contents were considered as sensitive to Cd toxicity. It was determined that the responses of grafted vines to Cd toxicity were changed by rootstock-scion interaction. However, it should be evaluated for Cd accumulation and tolerance in different rootstock/scion combinations.

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