CRISPR APPLICATIONS IN SUSTAINABLE AGRICULTURE AND FOOD SAFETY

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ABSTRACT
Today, factors such as the rapid increase in the world population, global warming, and climate change increase the importance of sustainable agriculture and food security. In addition, classical breeding techniques such as hybridization and mutation used in the agricultural field are insufficient due to these changing factors. For these reasons, it is important to use modern biotechnological methods in addition to agricultural and food applications. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), which is one of the important biotechnological methods at this point, eliminates some deficiencies in terms of sustainable agriculture and food safety. ZFN (Zinc Finger Nucleas) and TALEN (Transcription Activator-Like Effector Nuclease), which are CRISPR alternatives, are easy to use, yield results in a short time and are low-cost applications. In the field of agriculture, it is used against drought stress, one of the abiotic stress factors, and against pests, one of the biotic stress factors, in the cultivation of plants with high yield and nutritional value. In the field of food, it has a wide range of applications, such as controlling harmful microorganisms and managing beneficial microorganisms, ensuring food safety and extending shelf life. CRISPR is the modification or editing of some parts of the genome as an application. Designed to make targeted changes in specialized DNA sections in cell genomes, genome editing is a technique used for efficient and error-free editing of DNA. For example, in a study on tomatoes, the *SlAGO7 (Argonaute7)* gene responsible for the needle-leaf appearance was deleted with CRISPR technology and a needle leaf appearance was obtained. *It has been successfully applied to many crops including Arabidopsis thaliana, Oryza sativa, Nicotiana benthamiana, Zea mays, Triticum aestivum, Marchantia polymorpha, Sorghum bicolor, Solanum lycopersicum, Citrus sinensis, Cucumis sativus, Brassica oleracea, Gossypium hirsutum, Glycine max, Lotus japonicus, Hordeum vulgare, Papaver somniferum and Lactuca sativa.* It has also been used to destroy *E.coli* strains, which are important indicator bacteria for food safety. In addition, studies have shown that it has an eradicating effect on *Listeria monocytogenes*, *Salmonella spp*. and *Vibrio parahaemolyticus*, bacteria that pose a risk in the food field. Study results also showed that CRPISPR applications are hereditary. In addition to its advantages, the CRISPR method is still controversial because of its risks and legal regulations. The aim of this study is to highlight future studies by comparing the advantages and risks of CRISPR applications in sustainable agriculture and food security.
Keywords: CRISPR, sustainable agriculture, food security, stress factors, indicator microorganisms

SÜRDÜRÜLEBİLİR TARIMDA VE GIDA GÜVENLİĞİNDE CRISPR UYGULAMALARI

ÖZET

Günümüzde dünya nüfusunun hızla artması, küresel ısınma ve iklim değişikliği gibi faktörler sürdürülebilir tarım ve gıda güvenliğinin önemi artırmaktadır. Bunun yanı sıra tarımsal alanda kullanılan melezleme ve mutasyon gibi klasik ıslah teknikleri değişen bu faktörlerden dolayı yetersiz kalmaktadır. Bu nedenlerden tarım ve gıda alanındaki uygulamaların yanında modern biyoteknolojik yöntemlerin kullanılması önem arz etmektedir. Bu noktada önemli olan biyoteknolojik yöntemlerden biri olan CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) "kümelenmiş düzenli aralıklı kısa palindromik tekrarlar" uygulamaları sürdürülebilir tarım ve gıda güvenliği açısından bazı eksiklikleri gidermektedir. CRISPR alternatiflerinden ZFN (Zinc Finger Nucleas) ve TALEN (Transcription Activator-Like Effector Nuclease) göre kullanımı kolay, kısa sürede sonuç alınan ve düşük maliyetli uygulamalardandır. Tarım alanında abiyotik stres faktörlerinden kuraklık stresine karşı, biyotik stres faktörlerinden zararlılara karşı dirençli, verimi ve besin değeri yüksek bitkilerin yetiştirilmesinde kullanılmaktadır. Gıda alanında zararlı mikroorganizmaların kontrolü ve yararlı mikroorganizmaların yönetilmesi ile gıda güvenliğinin sağlanması ve raf ömrünün uzatılması gibi oldukça geniş kullanım alanına sahiptir. CRISPR uygulama olarak genom üzerindeki bazı kısımlarda değişiklik ya da düzenlemeler yapılmasıdır. Hücre genomlarında özelleşmiş DNA bölümlerinde hedeflenen değişiklikleri yapabilmek için tasarlanan genom düzenleme, DNA'nın verimli ve hatasız şekilde düzenlemesi için kullanılan bir tekniktir. Örneğin, domates üzerine yapılan bir çalışmada yassı yaprak görünümünden sorumlu *SlAGO7 (Argonaute7)* geni CRISPR teknolojisiyle silinmiş ve iğne yaprak görünümü elde edilmiştir. *Arabidopsis thaliana, Oryza sativa, Nicotiana benthamiana, Zea mays, Triticum aestivum, Marchantia polymorpha, Sorghum bicolor, Solanum lycopersicum, Citrus sinensis, Cucumis sativus, Brassica oleracea, Gossypium hirsutum, Glycine max, Lotus japonicus, Hordeum vulgare, Papaver somniferum ve Lactuca sativa* dahil birçok bitkide başarılı bir şekilde uygulanmıştır. Gıda güvenliği açısından da önemli indikatör bakterilerden *E.coli* strainlerini yok etmek için kullanılmıştır. Ayrıca gıda alanında risk oluşturan bakterilerden *Listeria monocytogenes*, *Salmonella spp*. ve *Vibrio parahaemolyticus* üzerine yok edici etkisi olduğu çalışmalarla ortaya koyulmuştur. Çalışma sonuçları, CRPISPR uygulamalarının kalıtsal olduğunu da göstermiştir. CRISPR yöntemi avantajlarının yanında riskleri ve yasal düzenlemeleri konusunda hala tartışmalıdır. Bu çalışmanın amacı, CRISPR uygulamalarının sürdürülebilir tarım ve gıda güvenliği alanındaki çalışmalarının avantajlarını ve risklerini kıyaslayarak gelecek çalışmalara ışık tutmaktır.

Anahtar kelimeler: CRISPR, sürdürülebilir tarım, gıda güvenliği, stres faktörleri, indikatör mikroorganizmalar

INTRODUCTION

Agriculture is very important and essential for humanity to survive and reproduce (Arora, 2018). The United Nations (UN) estimates that the world population will reach 10 billion by 2050. As available agricultural land and water diminish, global food demand will increase by 25%-70% above current production levels. Therefore, the problem of balanced and adequate nutrition will arise and the need for food will increase (Umesha et al., 2018; El-Mounadi et al., 2020). As a result of this increase in the world population, climate change has threatened agricultural production and food security (Akalın, 2014). Therefore, there is an urgent need to improve food production and accelerate sustainable agricultural development.

Agricultural sustainability is increasingly threatened by population growth, destruction of agricultural land, climate change and biotic-abiotic stressors (Umesha et al., 2018). One of these challenges is the failure to achieve food security. Food security covers the production and consumption chain of agricultural and food products from the field to the table. This scope includes the production, processing, storage, logistics and distribution of agricultural and food products under appropriate conditions (Taniş, 2022). Agricultural sustainability is of great importance in improving food security in the world (Arora, 2018).

One of the agricultural biotechnological methods in ensuring global food security is plant breeding technologies. Agricultural biotechnology is an indispensable field for plant breeding strategies that aim to develop tolerance to climate change and biotic and abiotic stresses that limit plant productivity (Anyshchenko, 2022; Tyczewska et al., 2023). Genome editing can be used for the benefit of societies struggling with malnutrition and the threat posed by climate change to agriculture. The use of CRISPR technology holds great promise as a powerful tool to improve plant nutrition, increase resistance to plant diseases and produce drought-resistant plants (Anyshchenko, 2022). Among the solutions to food safety issues, genome editing has been demonstrated that it can be used to eradicate pathogenic microorganisms, a worldwide public health problem (Mao et al., 2022). The aim of this research is to address the studies of CRISPR technology in the field of sustainable agriculture and food security. The benefits and applicability of CRISPR technology in addressing the adverse effects of global climate change will be discussed.

CRISPR TECHNOLOGY

The clustered regularly interspaced short palindromic repeats (CRISPR) system is a genome editing technique that enables efficient and error-free modification or editing of the relevant areas on DNA. Compared to the genome techniques ZFN and TALEN, it is easy to implement and low cost. In addition, it is advantageous in terms of ease of customization and application to multiple cell types (Bolukbas and Gucukoğlu, 2022; Mao et al., 2022; Gan and Ling; 2022).

The CRISPR system is an adaptive immune mechanism in bacteria that acts as a "gene weapon" against foreign genetic material such as bacteriophages (El-Mounadi et al., 2022; Mao et al., 2022). The working principle of the immune system is that CRISPR-associated proteins (Cas protein) use specialized sequences on guide RNA (gRNA) to target specific regions of exogenous nucleic acid under the influence of gRNA to interrupt and silence the external expression of the source gene (Mao et al., 2022).

In 1987, Ishino et al. first found a repetitive DNA sequence in the alkaline phosphatase isozyme gene (iap) in *Escherichia coli* (El-Mounadi et al., 2022). In 2012, Jennifer Doudna and Emmanuelle Charpentier co-discovered the CRISPR-Cas9 gene clip and revealed the potential of CRISPR-Cas technology in gene editing by cleaving DNA under the guidance of the Cas9 protein guide RNA. The use of CRISPR-Cas9 technology began in clinical medicine in 2013. Subsequently, RNA-targeted CRISPR effectors (Cas12, Cas13 and Cas14) were successively discovered (Mao et al., 2022).

In the CRISPR/Cas system, CRISPR sequences are transcribed into RNA (pre crRNA) and then processed into CRISPR RNA (crRNA) that serves the Cas effector. The Cas effector consists of a single protein or a multiprotein complex. There are combinations of different components in established CRISPR-Cas-based nucleic acid biosensors, but the main difference is based on the difference in Cas effectors. The currently reported CRISPR-Cas biosensor systems are divided into four categories according to the different effectors of Cas (Li et al., 2022; Mao et al., 2022).

Currently, in the CRISPR- Cas12 family, only CRISPR-Cas12a and CRISPR-Cas12b are used together for nucleic acid diagnostics and gene editing. The difference between Cas12 a and Cas12b is that Cas12b is a double RNA-guided endonuclease, while Cas12a is a single RNA-guided endonuclease (Mao et al., 2022).

APPLICATIONS IN THE FIELD OF FOOD SAFETY

Food science is the field of activity that investigates microbiological, chemical and physical parameters to ensure safe food production. Food security is the physical, social and economic access to sufficient, safe and nutritious food for people to have a healthy and balanced diet (Selle and Barrangou, 2015). With the increase in human population, food consumption, reduction in land area and climate change have emerged. Over the coming decades, annual crop yields are projected to increase by less than 1% and there is very limited room for expansion of arable land (Arulbalachandran et al., 2017).

If food safety is considered microbiologically, some microorganisms benefit the product process, while others need to be kept under control due to their negative effects. While harmful microorganisms cause many negative effects such as disease and spoilage in foods, beneficial microorganisms are used in the production of products such as traditional yogurt, probiotic yogurt, pickles and bread. The presence of valuable substrates such as simple carbohydrates, lipids, proteins, minerals and vitamins in the structure of foods provides a suitable environment for the growth and reproduction of beneficial and harmful microorganisms (Papadimitriou et al., 2015; Stout et al., 2017).

Cultures and probiotics have been utilized in food production for many years, but fermentation can be disrupted in some cases due to bacteriophage problems. The success achieved in the vaccination of *Streptococcus thermophilus* starter culture used in the fermentation of dairy products against viruses has paved the way for the use of CRISPR-Cas systems in the food industry. CRISPR/Cas9 loci were found in 62.9% of the genomes of lactic acid bacteria, which are important fermentation cultures, and 77% of the genomes of bifidobacteria, which are probiotic bacteria. The presence of CRISPR/Cas9 systems in these bacteria provides a perspective to overcome phage-induced fermentation failures. In addition, strain typing, plasmid inoculation, genome editing and antimicrobial activity can also be used in fermentation processes. Many of these applications are considered to be possible with the use of CRISPR/Cas9 system in the control of harmful microorganisms that cause food losses every year and in the management of beneficial microorganisms. (Bolukbas and Gucukoglu, 2022; Kılıç Tosun and Kesmen, 2022). It has also been reported that CRISPR-Cas system can be used in studies on the control of foodborne pathogenic microorganisms in order to increase food safety and extend shelf life. In a study by Gomaa et al. (2014), the CRISPR-Cas system designed using the Type I-E Cas3 enzyme was specifically used to destroy E. coli strains. This approach was tested shortly thereafter in E. coli and Staphylococcus aureus using Type II Cas9 and the antibacterial potential of CRISPR-Cas9 systems was evaluated.

According to statistics from the World Health Organization (WHO), approximately 600 million people worldwide become ill from food contaminated with pathogenic microorganisms, resulting in 420,000 deaths annually and huge economic costs associated with food recalls. Bacterial contamination in food and the environment is caused by *Escherichia coli (E. coli), Listeria monocytogens (L. monocytogens), Staphylococcus aureus (S. aureus)*, *Salmonella species (spp.)*, *Vibrio parahaemolyticus*. These pathogens pose a constant threat to food safety, a worldwide public health issue. Recently, fluorescence detection technology based on the CRISPR-Cas12 system has provided a new strategy for food safety testing, expanding from disease diagnosis to food safety monitoring. For example, *Vibrio parahaemolyticus*, common in seafood, is the main cause of seafood-related food poisoning (Mao et al., 2022).

One of the genotyping studies was carried out on *Lactobacillus buchneri*, which causes spoilage in pickles, especially in cucumber pickles, which adversely affects production by spoiling the flavor. After determining the occurrence and diversity of CRISPR-Cas systems in *L. buchneri* genomes, the use of the 36-nucleotide Type II-A CRISPR locus for identification yielded successful results. CRISPR loci were also used for genotyping *Enterococcus faecalis*, which is frequently used in fermented meats, and *Lactobacillus gasseri* and *Bifidobacterium*, known for their probiotic roles (Kılıç Tosun and Kesmen, 2022).

APPLICATIONS IN SUSTAINABLE AGRICULTURE

The CRISPR-Cas system can be used to achieve targeted genome modifications in different monocotyl and dicotyl plant cells (Ma et al., 2015). It has been successfully applied to many crops including *Arabidopsis thaliana, Oryza sativa, Nicotiana benthamiana, Zea mays, Triticum aestivum, Marchantia polymorpha, Sorghum bicolor, Solanum lycopersicum, Citrus sinensis, Cucumis sativus, Brassica oleracea, Gossypium hirsutum, Glycine max, Lotus japonicus, Hordeum vulgare, Papaver somniferum and Lactuca sativa* (Açar and Aka Kaçar, 2021). The aim of the application is to increase yield and quality such as herbicide tolerance, drought resistance, grain yield, plant size and weight, resistance to biotic and abiotic stresses, and improvement of sensory and nutritional properties (Baltacı and Arslan, 2020; Kılıç Tosun and Kesmen, 2022).

In classical breeding, it takes many years to eliminate undesirable characteristics in genotypes obtained by hybridization and mutation breeding. However, new-generation breeding techniques offer easy and effective techniques for the purpose. Genome editing techniques offer easy, effective and unique techniques for the mutation of new phenotypes or undesirable characters that are desired to be obtained through the breeding process. The CRSPR/Cas9 technique, which has grown in popularity in recent years, is a powerful system for the successful generation of targeted modifications. An RNA molecule complementary to the target DNA and an enzyme with nuclease activity that works in a complex with the RNA molecule allow the system to work quickly and effectively, making it desirable (Açar and Aka Kaçar, 2021).

Feng et al. (2013) demonstrated that CRISPR-Cas technology can be used for high-throughput targeted mutagenesis of multiple genes in *Arabidopsis* and rice. Li et al. (2013) studied 7 different target regions in *Arabidopsis* and *Nicotiana* plants and demonstrated the applicability of synthetic guide RNA:Cas9-based genome editing technology for multiple targets. In a study to increase yield in rice, multiple CRISPR sgRNAs targeting the *DEP1* (Dense and Erect Panicle 1) gene were designed and mutant plants with shorter, more erect and dense inflorescences were obtained by deletions in the target gene (Wang et al., 2017). Genome sequence-specific CRISPR/Cas9-based genomic modification of three genes, namely phytoene desaturase (*OsPDS*), betaine aldehyde dehydrogenase (*OsBADH2*), and mitogen-activated protein kinase (*OsMPK2*), which are involved against various abiotic stresses in rice (Shan et al., 2013).

In a study conducted in China using the CRISPR-Cas system, mutations were made in the *GmFT2a* gene that controls the photoperiod flowering time in soybeans. Thus, late flowering mutants with longer vegetative growth times were obtained under both long-day and short-day conditions and it was shown that this trait was stably transmitted to the next generations.

Wang et al. (2014) reported that CRISPR-Cas9 system can be used to create beneficial genetic traits in hexaploid bread wheat. In a study targeting four different genes in maize, CRISPR-Cas9 technology was compared with the TALEN method. The researchers found that both techniques can be used for genome editing in maize, but the CRISPR/Cas system is more advantageous in terms of cloning and multiplex genome editing.

Shan et al. (2014) demonstrated the successful application of CRISPR/Cas9 approach for *TaMLO* gene (Mildew resistance locus O) in wheat protoplasts. As a result of gene silencing by CRISPR application, the plant developed resistance to mildew.

In recent years, many genome editing studies have been carried out in cocoa production to obtain varieties that are more resistant to pests, high-yielding, drought-resistant, flavor and bean quality improved. In a study conducted for this purpose, the *TcNPR3* (Non-Expressor of Pathogenesis-Related 3) gene, which suppresses the defense response, was successfully deleted using CRISPR-Cas9 technology. Many studies have been conducted using CRISPR-Cas9 technology to increase the resistance of rice, which is highly sensitive to adverse environmental conditions, against various stress factors. In a study conducted to prevent bacterial blight disease caused by *Xanthomonas oryzae*, a common problem in rice, CRISPR-Cas9 technology was used to target the promoter of the disease susceptibility gene *OsSWEET13* and two disease-resistant knock-out mutants were produced.

Tomato, one of the crops to which CRISPR-Cas technology is most commonly applied, is a model crop that has been the subject of extensive research due to its simple diploid inheritance, high reproductive efficiency, short growth period and ease of genetic transformation. Studies on leaf shapes in tomatoes have shown that genetic mutations obtained with CRISPR-Cas technology are heritable (Wang et al., 2019). In a study conducted by Brooks et al. (2014), the *SlAGO7* gene, which is responsible for the typical compound and flat appearance of tomato leaves, was targeted and it was reported that deletion of this gene using CRISPR-Cas9 technology resulted in the appearance of needle or wiry-like leaves. The results showed that CRISPR-Cas technology could be successfully used to modify phenotypic traits of tomatoes and that genome editing with this technology is heritable.

In a study by Cermák et al. (2015), they used geminivirus replicons to create heritable modifications in the tomato genome at higher frequencies compared to traditional DNA transfer methods (e.g. *Agrobacterium*). A strong promoter was inserted into the head of the *ANT1* (Anthocyanin Mutant 1) gene, which controls anthocyanin biosynthesis, resulting in purple tomatoes with high production of anthocyanin in tomato tissues. Both TALEN and CRISPR-Cas9 technology were used in the study, and it was shown that both methods, in combination with geminivirus replicons, increase the efficiency of gene targeting. It was also reported that the modification in tomatoes was heritable and that there was no evidence of integration of replicon sequences outside the target region.

CRISPR-Cas technology has also been used to improve the nutritional content of plant products. For example, in a study by Andersson et al. (2017), starch quality was improved by deleting the gene encoding the *GBSS* (Granule-Bound Starch Synthase) enzyme responsible for amylose synthesis in potato protoplasts to increase the nutrient content of *Solanum tuberosum*, a tetraploid potato species (Kılıç Tosun and Kesmen, 2022).

For centuries, hybridization and mutation breeding techniques have been used to obtain new characters in classical breeding. However, global warming in recent years, world population increase and especially the decrease in agricultural lands have made classical breeding methods inadequate. There are many limitations in classical breeding. For example, heterozygotic conditions, self-incompatibility and polyploidy are factors that limit classical breeding and cause the breeding process to take a long time. Another limitation is that classical breeding requires large areas of land, while agricultural land is decreasing due to population growth. Labor-intensive processes also bring along factors that increase labor costs. Therefore, the necessity of using new-generation breeding techniques is increasing. New-generation technologies are easy, fast, and sustainable techniques that reduce costs, do not require large agricultural lands, and do not allow loss of time (Açar and Aka Kaçar, 2021).

CONCLUSION

The rapid increase in the world population has increased the importance of sustainable agriculture and food security. Agricultural production affects food production and yield. Global climate change, water scarcity, decrease in agricultural lands have caused classical breeding methods to be insufficient in agricultural practices. In addition, there is a need to improve food security in line with adequate and balanced nutrition in food production. In this context, biotechnological methods are promising. CRISPR technique is also important with successful studies in agricultural and food fields. CRISPR applications overcome many deficiencies such as the cultivation of plants resistant to abiotic and biotic stress factors in the agricultural field, improving yield and quality. In food safety, the detection of pathogenic microorganisms is important in terms of extending shelf life. With CRISPR applications, especially bacteriophage-induced spoilage is prevented. The studies conducted are very positive in the field of agricultural sustainability and food safety, but their long-term effects are not known. Therefore, it causes ethical and biosafety debates and moral concerns. There is still no legal legislation on CRISPR applications. Legal regulations should be organized to remedy this situation. Within this framework, scientific studies should continue. There is a need to increase studies, especially in the field of food safety.

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