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Virus diseases limiting greenhouses and open field production of cucurbits in Antalya province

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ABSTRACT

The Mediterranean region in Türkiye is noted for the production of cucurbits and other vegetables. As such, the aim of this study was to sample symptomatic cucurbits crops from open fields and greenhouses where there is active cultivation of these crops. Young shoot, leaves, and fruits exhibiting virus-like symptoms (yellowing, mosaic, necrotic) were collected. Out of 968 plant samples collected and tested through RT-PCR and PCR, 949 were discovered to be infected with several viruses. The identified virus diseases included ZYMV, WMV, PRSV, SqMV, CGMV, CYSDV, BPYV, CABYV, ToLCNDV and CMV. In terms of hosts exhibiting a high incidence of virus infections, cucumber (363 samples), squash (277 samples), melon (201 samples), and watermelon (108 samples) emerged as the top four hosts. Additionally, viruses with notable high incidences in the collected samples, as recorded through molecular testing in decreasing order included ZYMV at 28.1%, CYSDV at 15.5%, and WMV at 14.4%. Also, 90% of samples collected from open fields had single or multiple infection. In contrast, 26.4% of samples from greenhouses exhibited mosaic symptoms and 74.6% showed yellowing symptoms. Notably, MNSV and BPYV, were detected in these samples. The samples also exhibiting mixed infections predominantly displayed mosaic symptoms, including mixed infections such as ZYMV with WMV, ZYMV with CMV, CMV with WMV, and CMV with PRSV. In contrast, samples obtained from open fields showed a higher prevalence of yellowing symptoms, such as ToLCNDV with CMV, ToLCNDV with ZYMV, ZYMV with CMV with CYSDV, and ZYMV with CMV with CVYV.

1. Introduction

The family Cucurbitaceae, referred to as the gourd family within the order Cucurbitales, predominantly inhabits tropical and subtropical regions. Cucurbitaceae consist of approximately 975 species distributed across roughly 98 genera of flowering plants (Xu and Chang 2017). Commercially cultivated members of the Cucurbitaceae family in Türkiye include pumpkin (*Cucurbita pepo*), watermelon (*Citrullus lanatus*), cantaloupe (*Cucumis melo*), cucumber (*Cucumis sativus*), Armenian cucumber (*Cucumis melo* var. *flexuosus*), butternut squash (*Cucurbita moschata*), and ornamental gourd (*Lagenaria siceraria*).

Pumpkin, an annual vegetable, is cultivated in all parts of the world, however, its production is more dominant in tropical and subtropical regions (Whitaker and Bemis 1975). Fruits of various varieties exhibit diverse shapes, sizes, and colors. The global pumpkin production is estimated at 2.8 million tons over an area of 1.5 million hectares with China leading production with 7.4 million tons, followed by Ukraine with 1.3 million tons, Russia with 1.2 million tons, and the USA with 1.1 million tons. Türkiye's significant economic income earned from pumpkin cultivation amounts to 771.651 tons (FAO 2021).

Watermelon, an herbaceous annual plant within the Cucurbitaceae family, is believed to have been initially cultivated in Africa (Decoteau 2000). Its distribution and cultivation in Anatolia and Europe are estimated to have occurred in the late 14th to early 15th centuries (Kütevin and Türkeş 1987). This crop is reportedly the most extensively cultivated among several cucurbit worldwide, with a total production volume of 101.6 million tons over an area of 3million hectares. China dominates global production with 60.9 million tons, while Türkiye ranks second with 3.5 million tons (FAO 2021).

Melon, a traditional crop of Asian origin, has been cultivated across a wide geographic range since around 2000 BCE (Robinson and Walters 1997). According to FAO data, melon is cultivated across 1.1million hectares globally, yielding a total production of 28.6 million tons. Unsurprisingly, China leads its production with 14 million tons, followed by Türkiye with 1.9 million tons (FAO 2021).

Cucumber, an annual herbaceous plant, is reportedly one of the oldest cultivated vegetables, dating back to 3000 years ago (Yawalkar 1985). It ranks second only to watermelon in terms of global production quantity, with a total of 93.5 million tons produced across 2.2 million hectares. China, Türkiye, Russia,

Ukraine, and Mexico are the top cucumber producing countries in the world (FAO 2021). However, yield of these crops are severely threatened by biotic stresses including, bacteria, viruses, fungi, insects, nematodes and other pathogens.

Viruses which cause yield and quality losses in cultivated plant species are notably distinct from other plant pathogens. The absence of chemical control measures for viruses and the insufficient implementation of existing controls by growers contribute to more devastating symptoms and greater yield losses compared to those caused by other plant pathogens.

Cucurbits serve as hosts to various viral pathogens, resulting in economic losses due to disease. Nearly 60 viral pathogens have been reported on cucurbits with the notable ones being Zucchini yellow mosaic virus (ZYMV), Cucumber mosaic virus (CMV), Watermelon mosaic virus (WMV), Papaya ringspot virus (PRSV), Melon necrotic spot virus (MNSW), Cucurbit chlorotic yellows virus (CCYV), Beet pseudo-yellows virus (BPYV), Squash mosaic virus (SqMV), Cucurbit yellow stunting disorder virus (CYSDV), and Cucumber vein yellowing virus (CVYV) (Lecoq and Desbiez 2012).

The presence of more than 60 viruses, with varying modes of transmission (seed-borne and insect vectors, mechanical) often leads to confusion with symptoms that are due to environmental stresses. In order to mitigate this confusion and assess the status of viral diseases in cucurbits in both open field and greenhouse settings, surveys were conducted over a span of two years in the Antalya province. This study aimed to elucidate the current situation in both open field and greenhouse environments, as well as to identify any new potential harmful viral diseases. Based on the symptoms exhibited by viral diseases, the study proceeded to categorize them into three classes, as shown in Table 1a.

As observed in Table 1a viruses transmitted by whiteflies are characterized by yellowing symptoms whereas those transmitted by seeds and aphids, or leafhoppers, typically result in mosaic-like symptoms, and the others, transmitted by fungal pathogens, are majorly distinguished by necrotic lesions on infected plants. For this purpose, samples in surveys were examined in three broad symptom groups: yellowing, mosaic, and necrotic.

2. Materials and Methods

2.1. Sample collection

Survey studies were conducted by visiting open fields and greenhouses where cucurbit cultivation took place from the easternmost (Gazipaşa, Alanya, Serik Aksu) to the westernmost (Demre, Kumluca Finike, Elmali) parts of Antalya province. Cucurbit samples collected during these studies were observed based on symptoms (yellowing, mosaic, and necrotic) and collected using a symptom-driven sampling method. Plant samples showing symptoms typical of viral infections were obtained from young shoot leaves and fruits.

The collected plant samples were packaged in plastic bags and placed in a cold chain and then taken to the laboratory with each sample bearing the information of location of collection, date of collection, as well as the species or variety of the plant. They were then stored in a deep freezer at -20°C.

2.2. Nucleic acid extraction, PCR (RT-PCR) and gel electrophoresis

Total nucleic acid (TNA) isolation was carried out on all collected leaf samples. The CTAB method as previously reported

by Doyle and Doyle (1990) was slightly modified and used for isolation. Isolated nucleic acids were adjusted to a concentration of 80-100 ng using the nanodrop spectrophotometer.

For RNA-based viruses, RT-PCR protocols were applied using the One-step RT-PCR kit purchased from Thermo Scientific. In addition, specific primer pairs listed in Table 1b were used for the virus identification of viral pathogens. In the study, only Tomato yellow leaf curl virus (TYLCV) was identified as a DNA-based pathogen among the viral pathogens. To detect the presence of TYLCV, PCR studies were conducted using Dream Taq Green Buffer Master mix and its protocol from Thermo Scientific with specific primer pairs listed in Table 1b.

The total volume of the study was prepared to be 25 µl. For the detection of each virus, 1 µl of TNA containing an average of 80-100 ng RNA/DNA was used. After, PCR products were run on 1.5% agarose gel for an hour. The gel was stained with a solution containing ethidium bromide at a concentration of 0.5 µg ml⁻¹, and resulting bands were visualized under UV light.

3. Results

In the survey conducted in Antalya province, a total of 968 plant specimens were collected, with 949 testing positive for various viral infections through molecular analyses (Table 2a). Molecular testing identified 11 distinct viral pathogens, including ZYMV, WMV, PRSV, SqMV, CGMV, CVYV, CYSDV, BPYV, CABYV, ToLCNDV, and CMV (Figure 1). Separate studies in Korea and Europe identified seven viruses - WMV, ZYMV, PRSV, CFMMV, CGMMV, KGMMV, and ZGMMV in cucurbit crops (Lee et al. 1981; Kim et al. 1995; Lee et al. 1990; Ko et al. 2007; Ryu et al. 2000; Rhee et al. 2010; Kwon et al. 2014).

Host-specific virus infections were investigated, revealing cucumber as the crop with the highest number of positive samples (363). Second to cucumber was pumpkin which exhibited 277 positive samples for the examined viruses, while melon had 201 samples, and watermelon displayed the lowest infection rate with 108 testing positive for the viruses.

Upon analysis of the data presented in Tables 2a and 2b it becomes evident that ZYMV predominates among the viral pathogens tested. Specifically, out of 267 leaf and fruit samples, ZYMV was detected in 28.1% of samples collected. This finding is coherent with the report by Kamberoglu et al. (2016), where ZYMV was determined as a prevalent virus in the Çukurova region (Adana and Mersin). Secondly, 147 samples tested positive for CYSDV (15.5%). Following closely, in third place, 137 samples showed presence of WMV (14.4%). Research conducted in China has consistently reported the widespread prevalence of WMV across nearly all regions of cucurbit production (Zhang et al. 2007; Tian et al. 2016; Wang et al. 2017; Zhao et al. 2022).

Upon examination, based on the cultivation environment, it was observed that out of 502 samples collected from open fields, 452 were found to be infected with mosaic-type symptomatic viruses (90.03%). Among the samples exhibiting yellowing symptoms in open fields (Figure 2a, b) (Figure 3), 50 samples showed a 10% infection rate. Among the samples collected from greenhouses, 118 (26.4%) out of the total (447) were discovered to be infected with mosaic-causing viruses (Figure 4) (Figure 5a and b). Moreover, 329 samples collected from greenhouses exhibited yellowing symptoms, with a 74.6% infection rate.

Upon examination of symptom types as delineated in Tables 2a and 2b, it becomes evident that among mosaic-type viral pathogens Zucchini yellow mosaic virus (ZYMV) prevail as the most common. Specifically, ZYMV was predominantly detected in open-field pumpkin species, with 89 positive samples, followed by 65 positive samples in cucumbers, 35 positive samples in melons, and 27 positive samples in watermelons.

No viral pathogens showing necrotic symptoms were observed. From all of the samples collected 24 randomly selected samples were subjected to RT-PCR using specific primers for MNSV, all of which tested negative. In Turkey, MNSV was first detected in cucumber species in 2010 by Fidan et al. (2010). A subsequent study by, Koç et al. (2014) confirmed the presence of the agent in pumpkins, indicating an expanded inter-species host range. Kwak et al. (2015) reported the detection of four different strains of MNSV in watermelon plants in different regions of Korea.

Among viral pathogens showing yellowing symptoms, CYSDV was the most predominant. 58 of the samples which were collected from covered cucumber fields were confirmed positive for CYSDV (Figure 2b). This was followed by 41 positive samples collected from covered melon fields and 24 positive samples in covered pumpkin fields. Additionally, Brown et al. (2007) reported that economic losses attributed to CYSDV could be as high as 80% in highly susceptible melon varieties (Arslan et al. 2020a)

ToLCNDV was detected in greenhouse production as follows :31 melon samples, 24 cucumber samples 18 pumpkin samples and 4 watermelon samples, In open field production ToLCNDV was detected in13 cucumber samples, 9 melon samples, 6 pumpkin and 3 watermelon samples. (Figure 3). Panno et al. (2016) identified ToLCNDV in pumpkin species cultivated in the open fields of Sicily, Italy, while Juárez et al. (2014) were the first to report ToLCNDV detection in pumpkin plants.

Table 1(a). Modes of Transmission and Symptom Types Induced by Viral Pathogens

Type of symptoms	Plant virus	Mode of transmission
Yellowing and chlorosis	Cucumber vein-yellowing virus (CVYV)	Silverleaf whitefly (<i>Bemisia tabaci</i>)
	Cucurbit yellow stunting disorder virus (CYSDV)	Silverleaf whitefly (<i>Bemisia tabaci</i>)
	Beet pseudo-yellows virus (BPYV)	Greenhouse whitefly (<i>Trialeurodes vaporariorum</i>)
	Cucurbit aphid-borne yellows virus (CABYV)	Several aphid species
	Tomato Leaf Curl New Delhi virus (ToLCNDV)	Silverleaf whitefly (<i>Bemisia tabaci</i>)
	Cucurbit chlorotic yellows virus (CCYV)	Silverleaf whitefly (<i>Bemisia tabaci</i>)
Necrosis	Melon necrotic spot virus (MNSV)	<i>Olpidium bornovanus</i> (Fungi)
Mosaic	Zucchini yellow mosaic virus (ZYMV)	Aphis spp.
	Watermelon mosaic virus (WMV)	Aphis spp.
	Papaya ringspot virus (PRSV)	Aphis spp.
	Squash mosaic virus (SqMV)	Striped cucumber beetle (<i>Acalymma spp.</i>) Spotted cucumber beetle (<i>Diabrotica spp.</i>)
	Cucumber mosaic virus (CMV)	Aphis spp.
	Cucumber green mottle mosaic virus (CGMMV)	Mechanical, Seed

Table 1(b). The list of PCR primer pairs used for virus diseases in this study

Virus diseases	Primer Pairs	Annealing temperature (°C)	Amplicon size (bp)	References
CVYV	CVYVF: AGCTAGCGCGTATGGGGTGAC CVYVR: GCGCCGCAAGTGCAAATAAAT	55	450	Papayiannis et al. 2005
CCYV	CCYVF: TCCCGGTGCCAAGTGAAGACA CCYVR: TACGCGCGGCAGAGGAATTT	55	375	Sarikaya et al. 2023
CYSDV	CYSDVF: AGTGACATGCCTAACTGTTACTT CYSDVR: ATAGCTGCTGCAGATGGTTC	54	364	Papayiannis et al. 2005
BPYV	BPYVF: TCGAAAGTCCAACAAGACGT BPYVR: CTGATGGTGC GCGAGTG	55	251	Papayiannis et al. 2005
CABYV	CABYVF: GAATACGGTCGCGCTAGAAATC CABYVR: CTATTTTCGGGTTCTGGACCTGGC	62	600	Kassem et al. 2007
ToLCNDV	To-A1F: GGGTTGTGAAGGCCCTTGTAAGGTGC To-A1R: AGTACAGGCCATATACAACATTAATGC	55	504	Fidan et al. 2023a
MNSV	MNSVF: CTCCATAAGCGCCAAGCAACC MNSVR: AGCGGGGGAAAACAGAAGAA	50	485	Koç et al. 2014
ZYMV	ZYMVF: TCACCACACATGGAGTTTC ZYMVR: ATGCAACCTTGTGAGCA	61	550	Zhao et al. 2015
WMV	WMVF: GGCTTCTGAGCAAAGATG WMVR: CCCAYCAACTGTGGAAG	53	408	Desbiez et al. 2009
PRSV	PRSVF: ATCACAATGATTACGCGCTGCG PRSVR: CTCTATTCTAAGAGGCTCGAATAG	59	1200	Usharani et al. 2013
SqMV	SqMVF: TTACAGACTTGCTCTAGTG SqMVR: AAATAACAGCATCTGGCATAT	61	550	Zhao et al. 2015
CMV	CMVF: TAACCTCCCAGTTCTCACCGT CMVR: CCATCACCTTAGCTCCATGT	52	513	Fidan et al. 2023b
CGMMV	CGMMVF: CTAATTATTCTGTGCTGGCTGCGGATGC CGMMVR: CTTGCAGAATTACTGCCATA	56	976	Tian and Posis 2014

Table 2(a). Viral pathogens identified through PCR/RT-PCR tests conducted on collected cucurbit samples

Symptom types	Plant virus	Cucumber		Pumpkin		Melon		Watermelon		Total
		F*	GH*	F*	GH*	F*	GH*	F*	GH*	
Mosaic	ZYMV	65	12	89	27	35	8	27	4	267
	CMV	42	13	11	12	4	5	6	4	97
	WMV	43	4	27	13	21	-	25	4	137
	PRSV	23	4	14	12	-	-	12	-	65
	SqMV	1	-	3	-	-	-	-	-	4
	CGMV	-	-	-	-	-	-	4	-	4
Necrosis	MNSV	-	-	-	-	-	-	-	-	0
	CVYV	3	42	-	6	-	13	-	4	68
Yellowing	CCYV	-	-	-	8	-	-	-	-	-
	CYSDV	4	58	3	24	6	41	3	8	147
	BPYV	-	-	-	-	-	-	-	-	0
	CABYV	-	12	-	4	-	28	-	-	44
	ToLCNDV	13	24	6	18	9	31	3	4	108
	TOTAL	194	169	153	124	75	126	80	28	949

*F: Open Field * GH: Greenhouse

Table 2(b). Percentages of Viral Pathogens Observed in Cucurbit Species

Plant virus	Cucumber (363)	Pumpkin (277)	Melon (201)	Watermelon (108)	Total	%
CVYV	45	6	13	4	68	7.1
CYSDV	62	27	47	11	147	15.5
BPYV	0	0	0	0	0	0
CABYV	12	4	28	0	44	4.6
ToLCNDV	37	24	40	7	108	11.4
CCYV	0	8	0	0	8	0.84
MNSV	0	0	0	0	0	0
ZYMV	77	116	43	31	267	28.1
WMV	47	40	21	29	137	14.4
PRSV	27	26	0	12	65	6.8
SqMV	1	3	0	0	4	0.42
CMV	55	23	9	10	97	10.22
CGMMV	0	0	0	4	4	0.42

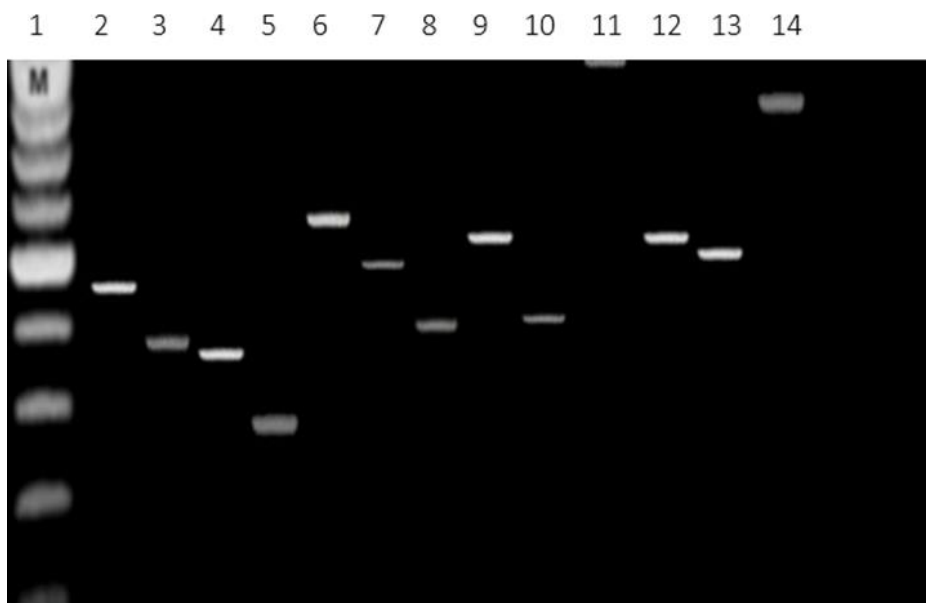
**Figure 1.** Agarose gel electrophoresis results of PCR and RT-PCR, 1) 100bp DNA marker; Lane 2) MNSV: 485 bp; Lane 3) CCYV: 375 bp; Lane 4) CYSDV: 364 bp; Lane 5) BPYV: 251 bp; Lane 6) CABYV: 600 bp; Lane 7) ToLCNDV: 504 bp; Lane 8) WMV: 408 bp; Lane 9) ZYMV: 550 bp; Lane 10) CVYV: 450 bp; Lane 11) PRSV: 1200 bp; Lane 12) SqMV: 550 bp; Lane 13) CMV: 513 bp; Lane 14) CGMMV: 976 bp.



Figure 2. (a) The yellowing type symptoms of CVYV observed in cucumber plants and melon plants, (b)The symptoms of CYSDV observed in cucumber plants (A, B) and melon plants, as well as the symptoms of CABYV observed in melon plants (C).



Figure 3. Symptoms of ToLCNDV observed in cucumber plants (A) and pumpkin plants (B, C).



Figure 4. Symptoms of PRSV observed in pumpkin fruit (A) and symptoms of WMV observed in watermelon fruit (B, C).

The least recorded viral pathogens were Squash Mosaic Virus (SqMV) and Cucumber Green Mottle Mosaic virus (CGMMV) (Figure 5b), with infection rates of 0.42%. No detections of Beet pseudo-yellows virus (BPYV) and Melon necrotic spot virus (MNSV) were recorded. According to Bi et al. (2019), the rapid spread of CGMMV in watermelon cultivation has led to massive yield reductions and substantial economic losses in regions such as Israel (Reingold et al. 2013) and Australia (Tesoriero et al. 2016). Noteworthy, CGMMV was exclusively identified as a viral pathogen in watermelon in this study.

CCYV, a newly reported Crinivirus with limited geographical distribution, has been reported only in Japan (Gyoutoku et al. 2009), Taiwan (Huang et al. 2010), and China (Gu et al. 2011; Kavalappara et al. 2021). In this study, CCYV was detected in pumpkin plants at a rate of only 0.84%.

PRSV was found in cucumber, pumpkin, and watermelon but not in melons. CMV, ToLCNDV, CYSDV, and ZYMV were observed in all species examined (cucumber, melon, watermelon,

and pumpkin) and in both greenhouse and open field environments. A decade ago, the presence of WMV, ZYMV, CMV, CABYV, and PRSV agents in cucumber, melon, watermelon, and pumpkin plants grown intensively in Diyarbakır and Mardin provinces was determined by Kızmaz et al. (2016). In Kastamonu province and its surroundings, where cucurbits are grown, the presence of WMV, CMV, ZYMV, and PRSV was confirmed, while SqMV infection was not reported in the study (Topkaya 2020).

In total, the top five pathogens observed in cucurbits, in order of frequency, were ZYMV (28.1%), CYSDV (15.5%), WMV (14.4%), ToLCNDV (11.4%), and CMV (10.22%) (Figure 6). China accounts for approximately 64.02% of cucumber production, making it the largest producer of cucurbits (FAO 2021). Among the most significant RNA viruses affecting the cucurbit group in China are Tobacco mosaic virus (TMV), ZYMV, WMV, CGMMV, and CMV (Liu et al. 2019; Desbiez et al. 2009 Zhao et al. 2022).



Figure 5. (a) Symptoms of ZYMV observed on watermelon plant (A), cucumber plant (B), pumpkin plant (C), and cucumber fruit (D). (b) Symptoms of CGMMV observed on watermelon leaves (A, B) and watermelon fruit (C).



Figure 6. Symptoms of CMV (1), PRSV (2), WMV (3), and ZYMV (4) observed in squash plants.

Mixed infections have been identified in both greenhouse and open field cultivation. These are typically observed as mixed infections of viruses causing mosaic symptoms, while in some cases, they appear as mixed infections of viruses causing both mosaic and yellowing symptoms. When mixed infections are examined according to the cultivation environment, mosaic-type symptoms ZYMV+WMV with 18 positive samples, ZYMV+CMV with 21 positive samples, CMV+WMV with 14 positive samples, CMV+PRSV with 5 positive samples were more commonly observed in greenhouse cultivation conditions, whereas yellowing-type symptoms ToLCNDV+CMV with 9 positive samples, ToLCNDV+ZYMV with 3 positive samples, ZYMV+CMV+CYSDV with 3 positive samples, ZYMV+CMV+CVYV with 4 positive samples, were more prevalent in open fields, as confirmed in this study.

Mixed viral infections manifest as mosaic+mosaic symptoms (ZYMV+WMV, ZYMV+CMV, CMV+PRSV) and mosaic+yellowing symptoms (ToLCNDV+CMV). Upon examination of triple infections, mosaic+yellowing symptoms (ZYMV+CMV+CVYS, ZYMV+CMV+CYSDV) have been observed. In 2020, mixed infections of WMV+ZYMV and WMV+PRSV were found in cucurbit fields in Kastamonu (Topkaya 2020).

4. Discussion

When examining viral diseases that limit cucurbit production based on symptoms, it was observed that they could be categorized into three groups. Among samples displaying symptoms of yellowing, 375 out of 384 were found to be infected with one or more viruses causing yellowing. Similarly, out of 584 samples showing mosaic-like symptoms, 574 were found to be infected with viruses. No samples showing necrotic symptoms were found. However, 24 randomly selected samples displaying necrotic symptoms were tested and found to be negative for viral infection. These results indicate the effectiveness of symptomology in distinguishing virus-infected areas within cucurbit crops.

Observations based on symptoms revealed that whitefly-transmitted viruses predominantly caused symptoms of yellowing. Specifically, only CABYV was identified as a virus transmitted by aphids and causing yellowing. Mosaic-like symptoms were generally attributed to viruses transmitted by aphids, seed, and mechanical means. It was observed that viruses causing yellowing symptoms were prevalent in greenhouses, while those causing mosaic-like symptoms were common in open fields (Arslan et al. 2020b). Factors contributing to the prevalence of viruses causing yellowing symptoms in greenhouses include the conducive environment created by unsuitable climate conditions for protected cultivation, allowing whiteflies to thrive. Additionally, the development of resistance of whiteflies against effective pesticides hinders their population control. The widespread of monoculture through the large cultivation of a single economic crop on a large area of land quickens the adaptability of the virus to a host and thereby contributing to their easy spread.

In open fields, the prevalence of mosaic-like symptoms is attributed to factors such as dense planting, larger cultivation areas compared to protected cultivation, and less frequent monitoring and maintenance, leading to increased mechanical transmission. Furthermore, the inability to conduct timely spraying during periods of heavy rainfall and overcast weather in

open fields, unlike in protected cultivation, contributes to the highest incidence of mosaic-like symptoms.

Observations indicated that viruses causing symptoms of yellowing were intense in greenhouses, while mosaic-like symptoms were prevalent in open fields, initially transmitted by aphids and later dispersed mechanically during harvesting. Symptoms observed in cucurbit viruses provide a general idea of which viruses are present. However, leaf symptoms caused by ZYMV, PRSV, and WMV are very similar, making it impossible to distinguish these viruses based solely on leaf symptoms. Therefore, in areas where symptoms are observed, testing must be conducted using DAS-ELISA, RT-PCR, and qRT-PCR techniques to distinguish among the three viruses in cases of mixed infections. This study demonstrated the reliable use of RT-PCR/PCR techniques in viral disease diagnostics.

In areas where viral diseases are prevalent in cucurbits, effective vector control, including the use of biological control agents against vectors, and proper weed management are essential. Moreover, given that ZYMV is the most widespread virus in this study and globally, preference should be given to varieties resistant or tolerant to viruses. Otherwise, efforts should focus on breeding for resistance or employing molecular techniques such as CRISPR-Cas9. With the continuous emergence of new virus species or entirely new viruses, overcoming them economically is becoming increasingly challenging. Viruses possess significant potential to adapt to natural selection pressures due to factors such as large population sizes and the absence of repair mechanisms in their genomes, enabling rapid replication.

In summary, research is needed to understand the distribution of reservoir plants for viruses and their transmission to cultivated plants, the detection and characterization of local isolates from different locations in Türkiye, including both wild and cultivated forms, and the collection of isolates from common host pools at specific times, which may lead to the formation of new sub-isolates capable of breaking resistance in plants. The impact on agricultural ecology and economic losses in agricultural fields due to both mixed infections and the development of new strains through mutation cannot be predicted.

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Assembly and annotation of the first complete mitochondrial genome of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

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ABSTRACT

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), is a significant economic pest with a global distribution. Despite the increasing volume of literature on mitochondrial genome sequencing, complete mitochondrial genome sequences have not been reported for *P. solenopsis* yet. Here, we assembled the complete mitochondrial genome of *P. solenopsis* using high throughput DNA sequencing technology. The genome is 14,831 bp in length and is comprised of 37 genes: 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, and two ribosomal RNA (rRNA) genes. The total length of all PCGs is 9,678 bp, accounting for 65.2% of the overall mitogenome. Among the PCGs, *atp8* is the smallest gene (99 bp) and *nad5* is the largest (1,593 bp). Of the 13 PCGs, seven (*nad3*, *cox3*, *atp6*, *atp8*, *cox2*, *cox1*, *nad2*) are encoded on the majority strand (J-strand), while six (*nad1*, *nad6*, *cob*, *nad4L*, *nad4*, *nad5*) are on the minority strand (N-strand). The analysis revealed a predominant A+T base composition, making up 90.3% of the total genome. However, the non-coding regulatory control region (CR) is missing due to the non-overlapping endpoints of the linear assembly. This assembly provides comprehensive information for investigating the evolutionary relationships between scale insects and for the precise identification of insects.

1. Introduction

The superfamily Coccoidea, known as scale insects, resides in the suborder Sternorrhyncha (Hemiptera) and contains around 8500 species. Pseudococcidae (mealybug), the second largest family in Coccoidea, includes 2143 species broadly dispersed around the globe (García et al. 2016). Most mealybug species are considered economically important pests since they are phloem feeders on several plant parts. Furthermore, they are known to play an important vector role for several plant viruses. One such pest, *Phenacoccus solenopsis* Tinsley 1898, is a highly invasive example of the family Pseudococcidae reported in more than 70 countries (EPPO 2024) causing detrimental damage to cotton plants (Figure 1).

Despite the existence of vast amount of literature on pest status, little is known about the genetics and phylogeny of the Pseudococcidae. The current taxonomy of the family is mainly focused on the morphological characters measured on female adults (Gullan and Cook 2007). However, precise identification of mealybugs may become difficult and cumbersome using only morphological characters. On the other hand, mitochondrial genome information can present an opportunity to rapidly resolve genetic structure, evolutionary relationships and precise identification of insects (Cameron 2014). However, complete mitochondrial genome information in the family Pseudococcidae is still scarce, and absent for *P. solenopsis*. Therefore, we utilized high throughput DNA sequencing technology to assem

mitogenome of the *P. solenopsis* collected from Denizli province in Türkiye and annotated it in detail.

2. Materials and Methods

2.1. Sample collection and DNA extraction

Cotton mealybug samples were collected from a *Gossypium hirsutum* L. cultivated field in Adaköy (37,95887°N, 29,00747°E) in the Sarayköy district in Denizli province, Türkiye in August 2023. The samples were immediately stored in absolute ethanol and brought to the laboratory for long term storage at -20°C until DNA extraction. The samples were initially identified morphologically, and more precise identification was carried out based on BLAST comparison of mtCOI sequences in NCBI database. For total DNA, a single female specimen was subjected to the CTAB extraction protocol (Doyle and Doyle 1987). The resulting total DNA quality was checked by running it on 1% agarose gel and then, stored at -20°C until library preparation.

A whole genome shotgun sequencing approach was utilized with 1 µg of DNA as input material. Enzymatic digestion was carried out using fragmentase enzyme (New England Biolabs, Inc) producing 300-1200 bp DNA fragments. DNA fragments were then cleaned using AMPure size selection kits (Beckman Coulter, Brea, CA). The remaining protocol followed NEBNext® V Ultra II DNA Library Prep Kit (New England

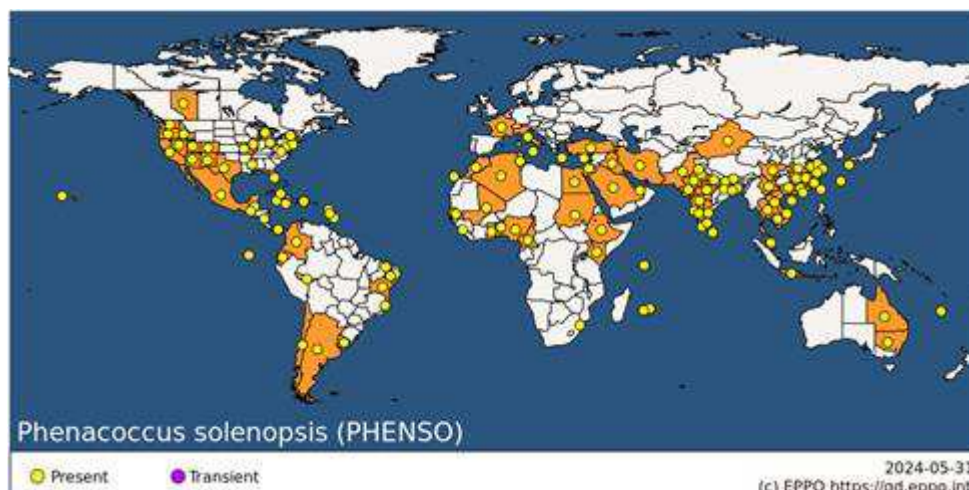


Figure 1. Global distribution of *Phenacoccus solenopsis*.

Biolabs, Inc.) according to the manufacturer's recommendations and index sequences were added to the genomic libraries. The resulting library, with an average insert size of 400 bp, was sequenced using the paired-end 150 sequencing method on the Illumina NovaSeq 6000 platform by MacroGen Ltd.

2.2. Mitochondrial assembly

The raw reads were at first subjected to demultiplexing and debarcoding protocol using "je demultiplex" software (Girardot et al. 2016) with default values and then, cleaned from low quality reads using "trim galore" (Martin 2011) and finally analyzed for quality metrics using "FastQC" (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>).

The clean reads were assembled with "MEGAHIT" (Li et al. 2015) and "metaSPAdes" (Nurk et al. 2017) with default values resulting in several nuclear and mitochondrial contigs. Among these contigs, mitochondria associated ones were baited using complete mitogenomes from closely related taxa on "bbduk" (Bushnell 2020) of BBTools Packages (<https://jgi.doe.gov/data-and-tools/bbtools/>). The baited contigs were then used as a second round bait file for the selection of reads from the original cleaned fastq file followed by final assembly construction by "MEGAHIT" and "metasSPAdes". The resulting two route assemblies were checked against each other for consistency and further BLAST checked for similarities to mitogenome of closely related taxa.

2.3. Mitochondrial genome annotation and analyses

The assembled mitogenome was initially annotated for PCGs, rRNA, and tRNA genes using MITOS v2 web server (Bernt et al. 2013). Since initial annotation by MITOS2 was lacking for some PCGs (*atp8*, *nad4L*, *nad6*), the ORF Finder server (<https://www.ncbi.nlm.nih.gov/orffinder/>) was used for capturing missing PCGs. Furthermore, the ORF finder was used for verification of boundaries of PCGs, *rrnS* and *rrnL* reported by MITOS2. In parallel to PCGs, several tRNA's were not captured by MITOS2, hence a BLAST search of closely related taxa was utilized to find missing tRNA's, leading to improved annotation quality. The graphic representation of *P. solenopsis* mitogenome were visualized by Proksee (<https://proksee.ca/>), an updated version of the CGView web server (Grant and Stothard 2008).

3. Results and Discussion

3.1. General Features

The mitogenome sequence of *Phenacoccus solenopsis* was assembled into a single contig of 14831 bp in length (Figure 2). However, the non-coding regulatory control region (CR) was apparently missing from the final mitogenome assembly as the end point of linear assembly was not overlapping on each other. Furthermore, CR is known to have a repetitive AT-rich composition in many insect species and close taxa, and yet was not apparent in the finished mitogenome. The ambiguity about CR most probably stemmed from an underrepresentation of AT-rich sequences in Illumina read data (Shen et al. 2015) leading to a downsized final assembly of mitogenome. Furthermore, in order to avoid mis-assemblies in the final data, assembly programs are prone to skip repetitive regions from the final assembly. There are only three full mitogenomes available for mealybugs in the GenBank database (OY390719; NC_066716; OX465514) for comparison and none of them clearly indicated the presence of CR region in their annotation. However, close examination of two accessions revealed the presence of a probable CR region of approximately 500 bp and 2100 bp for *Planococcus citri* Risso and *Balanococcus diminitus* Leonardi, respectively. On the other hand, there was no apparent CR region for *Phenacoccus manihoti* Matile-Ferrero mitogenome sequence. For other *Coccoidea* species, the annotation for the CR region was either completely missing or variable between 310 bp and 403 bp (NC_085772; NC_070232; NC_067791; NC_063660; NC_057479). Despite not having certain length information in the current study, the putative CR *P. solenopsis* was likely to lie between *rrnL* and *trnM* genes.

3.2. Protein-coding genes (PCGs)

The annotation of the newly assembled mitogenome revealed a total of 37 genes, including 13 protein-coding genes (PCG), two ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes (Figure 2). Overall nucleotide composition of *P. solenopsis* mitochondria had a significant A+T bias (90.3 %) even in the absence of AT-rich CR in the final assembly (Table 1). The length of all PCG's totaled 9678 bp and accounted for 65.2% of the overall mitogenome (Table 1). Among the PCG's, *atp8* was the smallest gene (99 bp) whereas *nad5* was the largest gene (1593 bp) which is similar in other known *Coccoidea*

on the N-strand with varying lengths between 45 bp and 71 bp (Figure 2).

In a typical animal mitochondrial genome, all tRNA genes, except *trnS* (GCT), are folded into clover leaf-like secondary structures where four arms (AA-arm, D-arm, AC-arm and T-arm) are concurrently forming (Wolstenholme 1992). In the current research, only 10 of the predicted tRNA genes (*trnM*, *trnI*, *trnW*, *trnL2*, *trnD*, *trnN*, *trnS1*, *trnE*, *trnF*, *trnH*, *trnP*) had the typical clover leaf secondary structure and the remaining tRNAs (*trnG*, *trnR*, *trnT*, *trnY*, *trnQ*, *trnV*, *trnK*, *trnA*, *trnS2*, *trnL1*, *trnC*) had irregular secondary structures where the D-arm/T-arm, or both were absent. Heavy tRNA truncation was prevalent in nematodes where 20 of the 22 tRNAs lacking T-arm or D-arm were reported in the literature (Wolstenholme et al. 1987). Furthermore, arm reduction in tRNA was exemplified in other arthropod species, such as arachnids and mites (Domes et al. 2008). In fact, loss or reduction of D-arm or T-arm in tRNA genes were a common feature in closely related scale insect species such as *Ceroplastes japonicus* Green and *Saissetia coffeae* Walker (Deng et al. 2019; Lu et al. 2020). Therefore, the arm reduction in tRNA genes reported here is not a specific event for *P. solenopsis*.

Furthermore, rather than MITOS2 auto-annotation, the positions of 8 tRNA were only located by multiple sequence alignment in the current mitogenome assembly. Whether the results of unusual secondary structures in tRNA genes or limited identification ability of tRNA search programs, the overall tRNA results indicate a heavy truncation of tRNA genes for the *P. solenopsis* mitogenome.

Rather than nuclear machinery, mitochondria orchestrate its own transcription and translation systems. Hence, dysfunctional tRNAs encoded by the mitochondrial genome could be detrimental to organisms unless a replacement from the nuclear genome could be transported to the mitochondria to assist a deficient tRNA function. Furthermore, the post-transcriptional tRNA editing scheme is proposed as a likely mechanism for some species (Lavrov et al. 2000; Segovia et al. 2011). The current results add up another case for truncated/deficient tRNA genes in scale insect's mitochondria even these species function normally. Since the limited mitochondrial genome is available, it cannot be assured tRNA truncation is a common feature for all scale insects. Hence, more research is needed to explore mitogenomes in this taxon.

Table 1. Nucleotide composition and size of *P. solenopsis* genes

Genes	A	T	G	C	AT	Size (bp)
<i>rrnS</i>	0.44	0.46	0.06	0.04	0.90	618
<i>rrnL</i>	0.44	0.47	0.06	0.03	0.91	1142
<i>nad1</i>	0.34	0.54	0.08	0.03	0.88	903
<i>nad6</i>	0.37	0.55	0.04	0.03	0.92	465
<i>cob</i>	0.34	0.52	0.06	0.07	0.86	1059
<i>nad4L</i>	0.43	0.51	0.05	0.01	0.93	267
<i>nad4</i>	0.35	0.56	0.06	0.03	0.91	1332
<i>nad5</i>	0.35	0.55	0.07	0.03	0.91	1593
<i>nad3</i>	0.40	0.51	0.03	0.06	0.92	354
<i>cox3</i>	0.40	0.52	0.03	0.05	0.92	753
<i>atp6</i>	0.41	0.49	0.03	0.06	0.91	618
<i>atp8</i>	0.40	0.48	0.02	0.09	0.89	99
<i>cox2</i>	0.43	0.45	0.04	0.07	0.89	696
<i>cox1</i>	0.37	0.46	0.07	0.10	0.83	1539
<i>nad2</i>	0.42	0.53	0.01	0.04	0.94	981
All genes	0.38	0.51	0.06	0.05	0.89	
PCG	0.37	0.52	0.06	0.05	0.89	
All Genome	0.43	0.48	0.06	0.03	0.90	

Table 2. Mitogenome structure of *P. solenopsis*, direction, position and start/stop codons

Gene	Direction	Position	Start codon	Stop codon
<i>nad1</i>	N	2154-3056	ATG	TAA
<i>nad6</i>	N	3122-3586	ATT	TAA
<i>cob</i>	N	3588-4646	ATG	TAA
<i>nad4L</i>	N	4742-5008	ATT	TAA
<i>nad4</i>	N	4969-6300	ATA	TAA
<i>nad5</i>	N	6522-8114	ATT	TAA
<i>nad3</i>	J	8454-8807	ATT	TAA
<i>cox3</i>	J	8871-9623	ATG	TAA
<i>atp6</i>	J	9624-10241	ATA	TAA
<i>atp8</i>	J	10249-10347	ATC	TAA
<i>cox2</i>	J	10472-11167	ATT	TAA
<i>cox1</i>	J	11212-12750	ATA	TAA
<i>nad2</i>	J	12800-13780	ATA	TAA

4. Conclusion

In this study, we sequenced and assembled the first mitogenome of *P. solenopsis* and described its mitochondrial features. The results indicated the presence of gene arrangement and reduction of tRNA genes. Nucleotide bias was aberrant toward A+T bases, a common feature in other scale insect mitogenomes (Cameron 2014; Deng et al. 2019; Han et al. 2021). The ability to use high throughput sequencing technology provides the opportunity to reach fast and accurate insect mitogenomes. However, they are not without drawbacks. Despite high coverage rates presented by Illumina data, current assembly was missing in the CR region in the final *P. solenopsis* mitogenome due to, most probably, the existence of high repetitive and AT content in the region. Hence, non-coding CR regions rich in repetitive sequences should be PCR amplified with the primers developed from *trnY* and *rrnS* site and, then Sanger sequencing could be utilized to reveal the CR region details in *P. solenopsis*. Overall, in its current form, *P. solenopsis* mitogenome can provide useful data for the investigation of phylogenetic relationships between different populations and across different scale insect lineages.

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Dynamics and competitiveness of Nigeria's sesame production in international trade: Vector error correction method

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ABSTRACT

This research study evaluated the dynamics and competitiveness of Nigeria's sesame (*Sesamum indicum*) production in international trade. Secondary data were used, in the data period of 1982 to 2022 (41 years). The data used were sourced from NBS, CBN, FAO, World Bank publication, and General Household Survey-Panel (GHS-P) in conjunction with Federal Ministry of Agriculture and Rural Development. The econometric tools used were Augmented Dickey-Fuller (ADF) unit root test, Johansen co-integration test, Zivot – Andrews (Z-A), Lee Strazicich (LM) structural break unit root tests, and vector error correction model (VECM). The result of the ADF unit root test shows that all the test variables were stationary at first difference I (1). The results of the Johansen co-integration test for the time series data shows that the trace test statistics indicate 2 co-integrating equations, the Max-Eigen values also indicates 2 co-integrating equations. The estimated long run effect using VECM shows that area, yield, and world trade in oilseed, were positively and significantly related to the dynamics and competitiveness of Nigeria's sesame seed in international trade. The real exchange rate had a negative coefficient and was non-significantly related to the dynamics and competitiveness of Nigeria's sesame seed in international trade. The coefficient of the error correction term (ECM) is with the expected negative sign and statistically significant at 1% level of probability, this is an indication of a move back towards equilibrium with a magnitude of -0.95. Policies that would encourage exportation of sesame should be pursued and enhancing research activities on improving quality of sesame produced.

1. Introduction

Sesame (*Sesamum indicum*) is one of the world's most ancient oilseed crops cultivated in tropical and subtropical regions (Bedigian 2015). Nigeria's sesame production and export in 2022 was recorded at 450000 tonnes and 297022 tonnes respectively (FAO 2024). Nigeria's sesame area and yield in 2022 was reported at 365080 ha and 1232.9 Kg ha⁻¹ respectively (FAO 2024). Nigeria's sesame export value and agricultural products export value in 2022 was recorded at 330.9 million USD and 1.561 billion USD respectively (FAO 2024). According to FAO (2019), the world production of sesame exceeded 5.5 million tonnes in 2017, out of which 57% was produced in Africa and about 40% was produced in Asia. Sesame ranks third among the oilseed crops in the world (Umar 2020). The world trade in oilseed export in 2022 was reported at 197679390 tonnes (FAO 2024). Sesame seeds are very nutritious and are used for edible oil and food (Wacal et al. 2021). Sesame is a good source of healthy fat, plant protein, and can be used as an alternative to

animal fats and animal protein in human diets. Sesame is also a good source of minerals, vitamins, and fibre (Zebib et al. 2015). Sesame, in terms of health benefits, is good as an antioxidant and as an ingredient for the pharmaceutical industry. Sesame seeds bring a lot of foreign exchange when exported (FAO 2011). By 2025, it is expected that the global sesame seed international market will be valued at US \$17.77 billion (Cision PR Newswire 2019).

Today, competitiveness is one of the most used terms in global trade. In the context of this study, competitiveness on a macroeconomic level is based on market shares for export (Fagerberg 1988). Competitiveness is defined as the ability to produce goods and services that meet the test of international markets and simultaneously expand and maintain real income that raises the welfare of a country's citizens (Haque 1995). According to Umar (2020), competitiveness is substantially

related to the productivity growth of the countries, both at the microeconomics and at macroeconomics level. The real exchange rate (RER) is the key determinants of agricultural export for many countries. It is expected that as domestic currencies depreciate, the agricultural export will increase and vice versa, and this is a measure of competitiveness. The yield (YED) is included in the model to measure the contribution of the agricultural production capacity and technology in use in Nigeria to sesame export earnings. The area (ARE) was used as an indirect measure to capture the effect of rainfall variation. [Abbott and Bredahl \(1992\)](#), reported that the progress and long term competitiveness of agriculture is not associated with short term factors such as price and input costs. For this reason, price and interest rates are not considered in this model. The research gap is that Nigeria's agricultural produce is not competitive enough in the global market. Enough documents on the competitiveness of Nigeria's sesame is needed for policy decisions makers. The specific objectives are to: examine the stationarity and co-integration involving factors of the time series data, and evaluate the factors influencing export competitiveness of sesame production in Nigeria.

2. Materials and Methods

This research study was conducted in Nigeria. Nigeria is located between longitudes 3° and 14° east and latitudes 4° and 14° north ([NBS 2016](#)). The country has a land mass of 923 768000 km² and is bordered by the Republics of Benin to the west, Niger to the north, Chad to the north-east, and Cameroon to the east, and the Atlantic Ocean to the south. The estimated population of Nigeria stands at 229152217 persons growing at a rate of 2.39% per annum ([NPC 2024](#)). Agriculture remains the base of the Nigerian economy, supplying food for most Nigerians and also exporting primary seeds/crops and fruits to other countries. Agriculture employs two-thirds of Nigeria's labour force, contributes over 40% to the Gross Domestic Product (GDP) and provides about 88% of non-oil earnings ([NBS 2016](#)). Secondary data were used, the data covered the period 1982 to 2022 (41 years). The data were sourced from World Bank (WB) publications, Central Bank of Nigeria (CBN), Food and Agriculture Organization (FAO), National Bureau of Statistics (NBS), and general household survey-panel (GHS-P) sourced from the National Bureau of Statistics in conjunction with the Federal Ministry of Agriculture and Rural Development. The data were analyzed using the following descriptive and econometric tools:

2.1. Augmented Dickey-Fuller (ADF) model for unit root test

The unit root test is conducted to determine primarily the level of integration among factors under consideration. The unit root test is evaluated through the application of Augmented Dickey-Fuller (ADF). The Augmented Dickey-Fuller model as developed by [Dickey and Fuller \(1981\)](#), [Dickey and Wayne \(1979\)](#) is stated thus:

$$\Delta Y_t = \pi Y_{t-1} + \sum_{j=1}^p \gamma_j \Delta Y_{t-1} + \varepsilon_t \quad (1)$$

Where, Δ = Notation for the First Difference Operator, π and γ_j = Estimated Parameters, ε_t = Disturbance Error Term, Y_t = Time Series to be Tested, P = Proxy for the Maximum Lag Length for the Variables.

2.2. Johansen co-integration test

Co-integrating is the statistical analysis of the existence of long run equilibrium relationships between the factors. The Johansen co-integrating test is a superior test that relies on asymptotic properties. The Johansen co-integrating test was applied because the factors included in the model are non-stationary at their level form but become stationary after difference. The Johansen co-integrating method gives two (2) test statistics. Firstly, the value of the Likelihood ratio (LR) test which is based on the minimum Eigen-value. Secondly, the value is based on the trace statistics of the stochastic matrix. In other words, the Johansen co-integrating test is analyzed via the Trace statistics and Maximum Eigen-value. The decision rule is that if either is greater than the 5% critical value, we reject the null hypothesis of no co-integration among the variables. If the LR is greater than the critical value, the hypothesis of co-integration is accepted.

The null hypothesis for LR test based on Eigenvalues is as follows:

$$LR_\lambda = T \sum_{i=r+1}^n \{(\ln(1 - \lambda_i^*)) - (\ln(1 - \hat{\lambda}_i))\} \quad (2)$$

Where, $\hat{\lambda}_i$ = Eigen-value of the Unrestricted Model, λ_i^* = Eigen-value of the Restricted Model

T = Total Number of Observation, n = Number of Endogenous Factors, If $LR >$ Critical Value, then the null hypothesis is rejected (the critical value is at $n - r$ degree of freedom), r = The number of co-integration relations of unrestricted model.

2.3. Vector Auto Regressive (VAR)

When the data used are stationary at the same level of differencing, and there is co-integration, then VAR can be combined with error correction and become the vector error correction model (VECM). In general, the model can be defined as:

$$y_{t,i} = c + \sum_{i=1}^p \phi_i y_{t-1} + \varepsilon_t \quad (3)$$

Where,

y_t = the element vector of y at time t , ϕ_i = Matrix order $n \times n$, element are the coefficient of vector y_{t-1} for $i = 1, 2 \dots, p$, p = The lag length, c = Vector intercept, ε_t = Random vector of shock

2.4. The Vector Error Correction model (VECM)

The Vector Error Correction Model (VECM) can be defined as a multiple time series model that evaluates the speed at which a dependent variable returns to equilibrium relationship after a change in an independent variable ([Alabi et al. 2022](#)). VECM is interested in both long term and short term relationships. Also, a negative error correction coefficient from VECM gives sufficient evidence of the presence of a short run equilibrium relationship. The size of the error correction coefficient gives the speed of adjustment towards equilibrium. If two (2) factors are co-integrated at the first (1st) difference order, their relationship can be expressed as Vector Error Correction Model (VECM) by taking past disequilibrium as an explanatory factor for the

dynamic behavior of the current factor. The VECM corrects the equilibrium error in one period by the next period.

2.5. The Model Specification

The implicit form of the dynamics and competitiveness of Nigeria’s sesame (*Sesamum indicum*) production in international trade using vector error correction model (VECM) is given as:

$$SES = f(ARE, YED, WOT, RER) \tag{4}$$

Where, SES = The Share of Sesame Export Value as a Percentage of Nigeria’s Agricultural Export Value (%), ARE= Area (Ha), YED= Yield (Kg^{ha}⁻¹), WOT= World Trade in Oil Seed (Metric Tonnes), RER= Real Exchange Rate (Naira per Dollar). The Vector Error Correction Model (VECM) with co-integration rank $r \leq k$ is presented in its original form as follows:

$$\Delta y_t = c + \Pi Y_{t-1} \sum_{i=1}^{p-1} \Gamma_i \Delta Y_{t-1} + \varepsilon_t \tag{5}$$

Where, y_{t-1} = Vector variable endogenous with 1st lag, Δ = first difference operator, ε_t = noise term, c = vector intercept, Π = matrix coefficient of co-integration, $\Pi = \alpha\beta$, α = vector adjustment, with matrix order $(k \times r)$, β = vector cointegration, long term parameter matrix $(k \times r)$, Γ_i = matrix with order $k \times k$ of endogenous coefficient of the i^{th} variable. The model specification of dynamics and competitiveness of Nigeria’s sesame (*Sesamum indicum*) production in international trade is stated thus:

$$\begin{aligned} Ln SES = \alpha_0 + \alpha_1 LnARE_{1t} + \alpha_2 LnYED_{1t} + \alpha_3 LnWOT_{1t} \\ + \alpha_4 LnRER_{1t} + \alpha_5 \mu_{t-1} + \varepsilon_{it} \end{aligned} \tag{6}$$

Where,

SES = The Share of Nigeria’s Sesame Export Value as a Percentage of Nigerian Agricultural Export

Value (%), ARE_{1t} = Area (Ha), YED_{1t} = Yield (Kg^{Ha}⁻¹), WOT_{1t} = World Trade in Oil Seed (Metric Tonnes), RER_{1t} = Real Exchange Rate (Naira per Dollar), μ_{t-1} = Lag of the Residual Term Representing Short Run Disequilibrium Adjustment of the Estimates of the Long Run Equilibrium Error, ε_{it} = Stochastic Error Term, α_5 = Coefficient of the Error Correction Term, α_0 = Constant Term, $\alpha_1 - \alpha_4$ = Estimated Parameters. The Real Exchange Rate (RER) following Kingu (2014) is calculated by using the following equation:

$$RER = \frac{CPI_{Nigeria}}{CPI_{USA}} \times NER \tag{7}$$

Where, $CPI_{Nigeria}$ = Consumer Price Index of Nigeria, CPI_{USA} = Consumer Price Index of United States of America (US), NER = The Nominal Exchange Rate in Local Currency (₦\$⁻¹).

3. Results and Discussion

3.1. Descriptive statistics of variables in dynamics and competitiveness of Nigeria’s sesame production in international trade

The data covering 41 years on dynamics and competitiveness of Nigeria’s sesame production in international trade were subjected to descriptive statistics to examine their means, minimum, and maximum values over the period as well as to evaluate the skewness, kurtosis and the spread and are presented in Table 1. Also, the trends of sesame area and yields within the period of 1982 to 2022 are displayed in Figures 1 and 2. The mean values of ARE, YED and WOT were 366 873.51 ha, 230487 100g ha⁻¹ and 90994416.75 tons respectively. The mean values of SEV and APEV were 201.5551 million USD and 21, 582, 877.875 thousand USD respectively. The variables ARE, YED and WOT have maximum values of 4070100 ha, 19932 100g ha⁻¹ and 215460396 tons in 2015, 2012, and 2020 respectively (Table 1, Figures 1 and 2), while the maximum values of SEV and APEV were 352 million USD and 1871.2 million USD and this was recorded in the years 2019, and 2021 respectively. The minimum values of ARE, YED, WOT, were 85500 ha, 2999 100g ha⁻¹, and 31907094 tons, and this was recorded in the years 1987, 1985, and 1989 respectively (Table 1, Figures 1 and 2). The kurtosis, skewness, and Jarque - Bera statistic tests were conducted for the normality of data, the kurtosis of 3 and skewness of 0 implies that the series are normally distributed, while the probability of the Jarque – Bera statistics of greater than 0.05 implies the acceptance of the null hypothesis that the series are normally distributed. All the factors are negatively skewed since their skewness values are less than 0. The factors have kurtosis less than 3 implying that they are not thick tailed, as kurtosis measures the symmetry of the distribution. The probability of estimates greater than the stipulated 0.05 indicate that all the factors are normally distributed. This result is in line with the findings of Umar (2020).

Table 1. Descriptive statistics of variables in dynamics and competitiveness of sesame (*Sesamum indicum*) production in international trade in Nigeria

Variables	ARE (Ha)	YED(100g Ha ⁻¹)	WOT(tons)	SEV(1000 USD)	APEV(1000 USD)
Mean	366873.51	230487	90994416.75	201551.25	21582877.875
Maximum	4070100	19932	215460396.9	352008	1871202
Minimum	85500	2999	31907094	68000	601069
Std Deviation	634561.72	3122.03	59353571.31	101065.068	651061.7504
Skewness	-0.03571	-0.476825	-0.03224	-0.03563	-0.03748
Kurtosis	2.29824	2.385362	2.36482	2.367624	2.36521
Jarque- Bera	0.360297	0.3254656	0.28006926	0.28858673	0.27050938
Sum	15041814	230487	3730771087	363224820	122126046
Probability	0.0678956	0.068923	0.072466	0.081254	0.09732
Observation	41	41	41	41	41

Source: FAOSTAT (2024), SEV- Sesame Export Value, APEV-Agricultural Product Export Value, USD-United States Dollar.

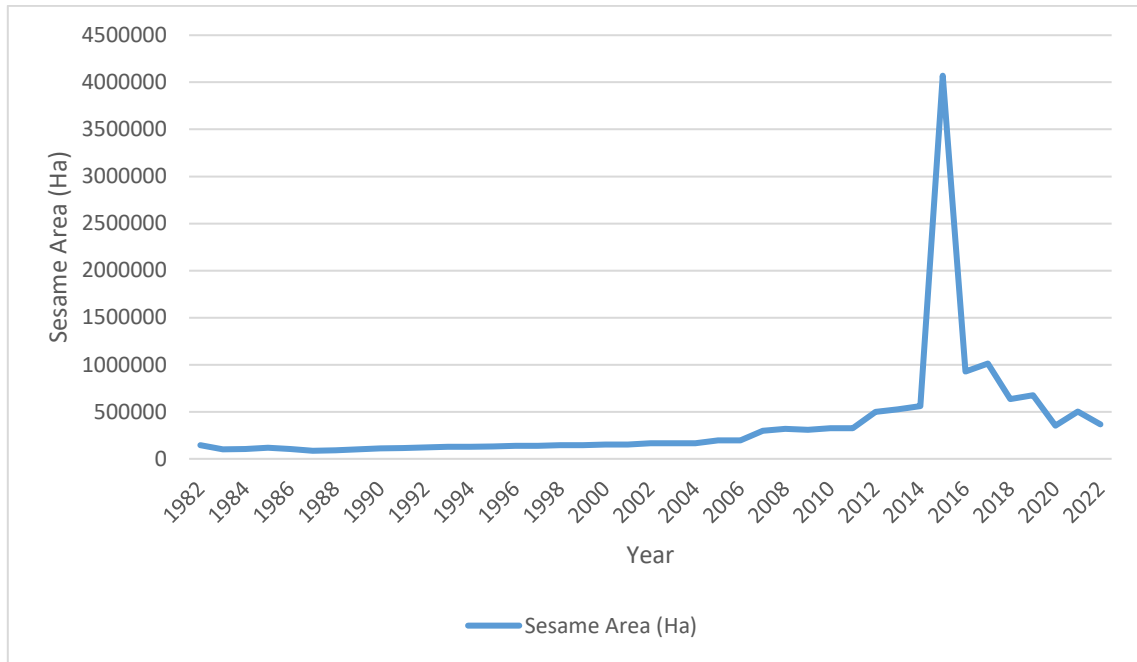


Figure 1. Trends of sesame area in hectare from 1982 to 2022.

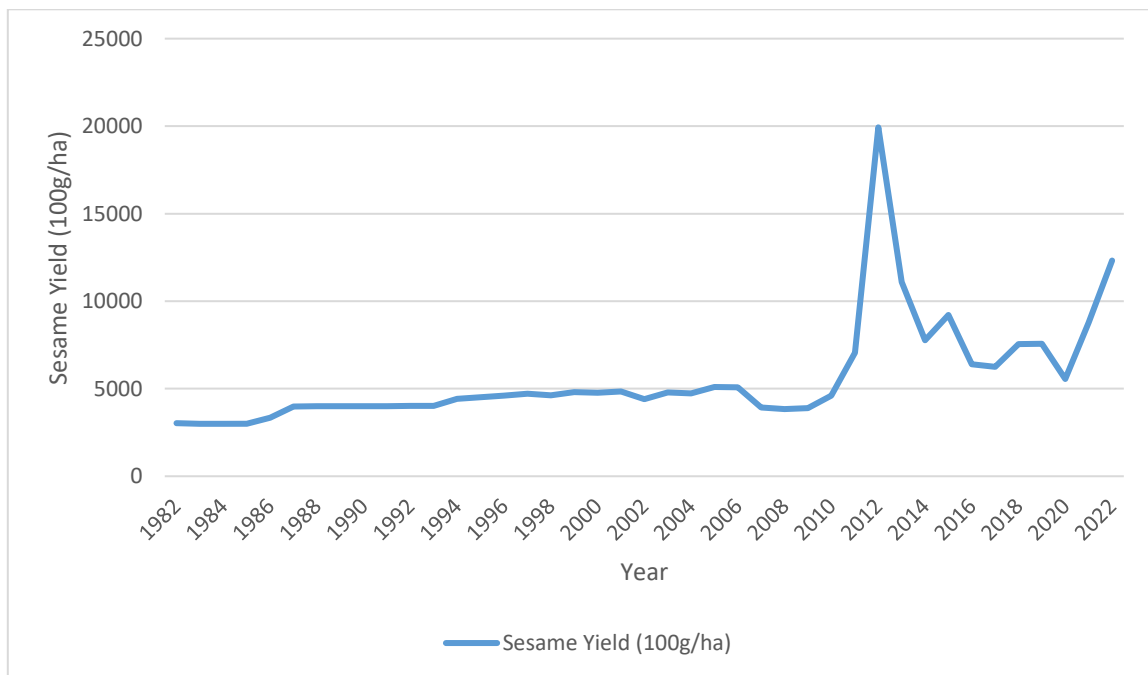


Figure 2. Trends of sesame yield (100 g ha⁻¹) from 1982 to 2022.

3.2. The Augmented Dickey-Fuller (ADF) stationarity

The unit root test is used to examine the stationarity of the time series data. The Augmented Dickey-Fuller (ADF) Statistic was used to test for the stationarity of the series, the results are presented in Table 2. The critical value at 5% is reported in columns 3. The ADF test statistics are reported in columns 2 and 5. The unit root test was conducted to give information on the characteristics of the time series data used in explaining the dynamics and competitiveness of sesame production in Nigeria. As observed in Table 2, the variables SES, ARE, YED, WOT, and RER attained stationarity after first difference I (1). The

result after the unit root tests are displayed in column 6 and the decision is that the time series data were stationary at first difference order one I (1). This gives the justification for using the vector error correction model (VECM) test to test for cointegration and examining short and long run relationships between the variables influencing the competitiveness of sesame production in international trade. This result is in conformity with the findings of Alabi et al. (2022), who obtained similar results while evaluating the export performance of ginger in Nigeria and Okuduwor et al. (2022), who obtained similar result while assessing agricultural export evidence on economic growth in Nigeria.

3.3. Johansen co-integration test

The co-integration test was carried out using the Johansen co-integration test, which is a superior test that relies on asymptotic property and it also robust. The results of the Johansen co-integration test for the time series data are presented in Table 3. The trace test statistics indicate 2 co-integrating equations. The Max-Eigen statistics also indicates 2 co-integrating equations. The trace statistics and the Max-Eigen statistics are greater than their respective critical values for the 2 co-integrating equations at these points. Thus, the null hypothesis of no co-integrating equations among the time series data is rejected. This signifies that even though the time series of the variables are stationary at 1st difference, their linear combinations are co-integrated. This further means that there exists a long run relationship among the variables at 5% level of significance. This result supports the findings of Alabi et al. (2022) and Aro-Gordon (2017).

3.4. Unit root test with structural break using Zivot-Andrews (Z-A Tests) and Lee Strazicich (LS) Lagrange Multiplier (LM) test

Table 4 shows the Zivot- Andrews (1992) unit root test with a single structural break. From the outcome, the data are not stationary after and at first difference. Therefore, we relied on another, stronger test, the Lee and Strazicich (2004). Table 5 shows the Lee and Strazicich (2004) unit root test indicating two

structural breaks. The outcome confirmed that all variables are non-stationary in the level, and are not stationary at first difference.

3.5. The dynamics and factors influencing the competitiveness of Nigeria's sesame seed in international trade

Table 6 shows the long run effect of ARE, YED, WOT and RER on the dynamics and competitiveness of Nigeria's sesame seed in international trade. The signs and the magnitude of the estimated coefficients indicate that the area of land (ARE) measured in hectares used to cultivate sesame seed has a positive and significant effect on sesame seed production and export at 1% level of significance. This is in line with both apriori expectation and also economic criteria. The result indicates that a unit increase or decrease in ARE will lead to a 0.70 increase or decrease in sesame (SES) export. This shows that the policy of promoting SES production by the government has and can significantly increase the production and export of Nigeria's sesame seed. The yield of sesame production (YED) measured in Kg/ha¹ shows a positive coefficient and significant effect on SES production and export at 1% level of significant. This is also in agreement with apriori and economic criteria. The result indicates that a unit increase in YED will lead to a 0.53 increase in Nigeria's sesame seed export. This has resulted from the

Table 2. Augmented Dickey-Fuller (ADF) stationarity test

Variables	ADF Test Statistics at Levels	5% Critical Value	Order of Integration	ADF Test Statistics at First (1 st) Difference	Order of Integration
SES	- 0.39	-2.96	NS	-5.89	I (1)
ARE	- 2.06	-2.96	NS	-7.94	I (1)
YED	- 0.84	-2.96	NS	-7.58	I (1)
WOT	-0.67	-2.96	NS	-7.95	I (1)
RER	- 0.27	-2.96	NS	-5.81	I (1)

NS- Not Stationary.

Table 3. Johansen co-integration test

Null- Hypothesis	Trace Statistics	5% Critical Value	Null-Hypothesis	Max-Eigen Statistics	5% Critical Values
$r = 0^*$	149.34	68.71	$r = 0^*$	74.97	34.87
$r \leq 1^*$	69.86	46.74	$r \leq 1^*$	41.58	28.69
$r \leq 2$	27.60	28.68	$r \leq 2$	14.53	22.24
$r \leq 3$	8.48	16.38	$r \leq 3$	5.86	15.37
$r \leq 4$	2.50	3.92	$r \leq 4$	2.75	3.95

Table 4. Unit root test with structural break using Zivot-Andrews (Z-A Tests)

Variables	Levels			First Difference		
	A	B	C	A	B	C
SES_t	-3.51 (4)	-2.16 (4)	- 2.46 (4)	- 3.41 (4)	-3.76 (4)	-4.52 (4)
TB	1987	2004	1995	2016	2015	2013
$AREA_t$	0.30(4)	-1.17 (4)	- 1.39 (4)	-4.16 (3)	-4.89 (4)	- 4.92 (4)
TB	2016	2016	1994	2008	2016	2016
YED_t	-4.89 (5)	- 4.20(4)	-3.92 (4)	-4.02 (4)	-4.61 (5)	-5.56 (5)
TB	2002	2004	1992	2004	2001	2005
WOT_t	-2.74 (5)	-2.68 (4)	-3.24 (4)	-4.50 (4)	- 3.87 (4)	- 4.69 (4)
TB	2002	2003	1998	2011	2018	2011
RER_t	-4.81 (5)	- 4.39 (4)	- 3.78 (4)	- 4.36 (4)	- 4.53 (4)	- 4.63 (4)
TB	2000	2004	2011	2009	2006	2002

*-Significant at 10% Probability Level, **-Significant at 5% Probability Level, ***Significant at 1% Probability Level, TB-Time of Break, () – Optima Lag Length.

Table 5. Unit root test with structural break using Lee Strazicich (LS) Lagrange Multiplier (LM) Test

Variables	Level	First Difference	I (...)
	A	A	
SES_t	-5.78 (6)	- 6.45 (7)	
TB_1	2003	1998	I (1)
TB_2	2012	2017	
$AREA_t$	- 4.37 (5)	-6.26(7)	
TB_1	2000	2001	I (1)
TB_2	2017	1987	
YED_t	- 5.45 (6)	-5.49 (7)	
TB_1	2004	2001	I (1)
TB_2	2015	1996	
WOT_t	-5.81 (7)	-6.91 (7)	
TB_1	2002	2009	I (1)
TB_2	2012	1994	
RER_t	- 5.92(7)	-6.93 (7)	
TB_1	2004	2011	I (1)
TB_2	1996	2016	

*-Significant at 10% Probability Level, **-Significant at 5% Probability Level, ***Significant at 1% Probability Level, TB-Time of Break, () – Optima Lag Length.

Table 6. Estimated long run result

Variable	Coefficient	Standard Error	t- Value
ARE	0.70***	0.096	7.24
YED	0.63***	0.043	14.75
WOT	0.96**	0.102	9.45
RER	-0.05	0.034	1.48
$R^2 = 0.692$			
$DW = 1.91$			
$F = 69.9**$			

DW-Durbin Watson Statistics, **- Significant at 5% Probability Level, ***- Significant at 1% Probability Level.

government's export promotion program, that encourages the development of Micro small and Medium enterprises and which has expanded products and markets for domestic products under various international trade agreements (such as the African Growth and Opportunity Act of the United States of America (AGOA), ECOWAS trade liberalization scheme, and the growth of the oil and gas industry in Nigeria). This export growth is mainly of a primary nature that commands low prices in the international market due to poor quality, worse still in the environment of volatile exchange rate. The world trade in oil seed (WOT) has a positive and significant effect on Nigeria's SES export at a 5% level of significance, this is in agreement with a priori and economic criteria. The result indicates that a unit increase in WOT will lead to 0.96 increase in Nigeria's SES export, similarly, a unit decrease in WOT will lead to a 0.96 decrease in Nigeria's SES export trade in international market. The growth in Nigeria's SES when world trade in SES export increases will need investment in the production of SES to sustain it, but agricultural credit is difficult to access in Nigeria, this limits the productivity of the farmers. The farmers are resource poor farmers who may not be exposed to improved techniques. The real exchange rate (RER) indicated a negative coefficient and effect on SES export. The result shows that if Naira depreciates by a unit, SES export will increase by 0.05, if Naira appreciates by a unit, SES export will decrease by 0.05. The Nigerian Naira exchange rate has been volatile over the years, and this has implications on the export and import of goods and services including sesame. When the Naira depreciates it makes Nigeria's sesame export cheaper to others from other countries, making it the preferred product. When the Naira appreciates, Nigeria's SES export will be more expensive to the rest of the world, so it will not be preferred. The RER exhibits a negative effect on sesame

seed export (SES), but the effect of this on its share of export market growth is low (0.05), this potentially large source of export trade is limited by low human and capital investment in the production of sesame seeds, this is worsened by the primary nature of the seeds, given that this commands low prices, and can be labelled low quality by the market leaders. This results agrees with Umar (2020). The summary statistics in Table 6 shows that the model's estimates are generally robust. The R^2 of 0.692 implies that about 69.2% of total variation in SES is explained by the regressors with the 30.8% accounted for by exogenous factors in the model covered by the error term. The overall model is statistically significant at 5% level of significance as shown by the F-statistics estimated at 69.9. The Durbin Watson statistics was estimated at 1.91 which is very close to 2, this depicts the presence of minimal positive serial correlation. These observations necessitate the test for long run relationships.

The Estimated Long Run Equation is stated thus:

$$SES = 0.70ARE + 0.63YED + 0.96WOT - 0.05 RER \quad (8)$$

(0.096) (0.043) (0.102) (0.034)

3.6. VAR lag length selection criteria

The stationarity of the residuals is potent evidence that there is convergence to long run equilibrium among the integrated variables. To be able to ascertain whether there is co-integration among the variables of interest, it is important to first determine the optimal lag of the variables to be used (Table 7). The result shows that all the selection criteria indicate that 1 lag length is selected for application of this study.

3.7. Error correction method

The error correction model (ECM) is designed for use with non-stationary time series that are known to be co-integrated. The ECM has co-integration relations built into the specification so that it restricts the long run behavior of the endogenous variables to converge to their co-integrating relationships, while allowing for short run adjustment dynamics. The co-integration term is known as the error correction term since the deviation from long run equilibrium is corrected gradually through a series of partial short run adjustments. The summary of the error correction model is given in Table 8. The coefficients SES, ARE, YED, and WOT display signs of conformity to a priori expectation, while the coefficient RER does not. The model shows five (5) significant variables, they are SES ($P<0.05$), ARE ($P<0.05$), YED ($P<0.01$), ECM ($P<0.01$) and Intercept ($P<0.05$). The parameter estimates of WOT and RER are statistically not significant. The coefficient of the error correction term is with the expected negative sign and statistically significant at 1% level of probability, this is an indication of a move back towards equilibrium with a magnitude of -0.94. The magnitude shows that if there is any deviation, the long run equilibrium is adjusted speedily where about 94% of the disequilibrium may be removed in each period. The coefficient of error correction model was negative at -0.94. This measures the speed of adjustment towards the long run equilibrium. The coefficient of multiple determinations (R^2) of 0.92 signifies that 92% of dynamics and competitiveness of Nigeria's sesame (*Sesamum indicum*) production in international trade were explained by the test variables included in the model. This signifies goodness of fit, the F-statistics of 32.97 was statistically significant at 1% probability level. This confirmed the good explanatory power of the entire model. This result is in agreement with the findings of Alabi et al. (2022) and Umar (2020).

4. Conclusion and Recommendations

This study has established that Nigeria has exported sesame seeds in tonnes to the international market within the stipulated period of 1982 to 2022 (41 years). The test variables included in the model were area (ARE), yield (YED), world trade in oil seed (WOT), and real exchange rate (RER). All the test variables were significant and stationarity at first difference I(1) using the Augmented Dickey-Fuller unit root test. The Johansen Co-Integration test conducted shows that co-integration equation

exists among the variables. The Trace statistics have 2 co-integrating equations, the Max-Eigen statistics test also indicates 2 co-integrating equations. This further means that there exists a long run relationship among the variables at a 5% level of significance. The Zivot- Andrews (1992) unit root test, with a single structural break, shows that the data are not stationary after first difference, and are not stationary at first difference. The Lee and Strazicich (2004) unit root test showing two structural breaks confirmed that all variables are non-stationary in the level, and are not stationary at first difference. The vector error correction model was employed because of the existence of co-integration among the test variables. The estimated long run equation shows that the signs and the magnitude of area of land (ARE), cultivated for sesame seed, yield (YED), world trade in oil seeds (WOT) have positive and significant effect on the competitiveness of Nigeria's sesame seed in international trade. While, the real exchange rate (RER) has a negative effect on the competitiveness of Nigeria's sesame seed in international trade. The short run equation shows that the signs and the magnitude of area of land (ARE), cultivated for sesame seed, yield (YED), world trade in oil seeds (WOT) have positive coefficient but only area of land (ARE), cultivated for sesame seed, and yield (YED) have significant effects on the competitiveness of Nigeria's sesame seed in international trade. The coefficient of error correction model was negative at -0.95. Based on these findings the following recommendations were made:

- (i) Macroeconomic policies that will stabilize the real exchange rate should be formulated and implemented,
- (ii) The policies that would encourage exportation of sesame should be pursued. Such policies should be directed towards the provision of storage facilities, granting of tax holidays and long term export credit at concessionary interest rates to exporters of sesame,
- (iii) Enhancing the productive capacities of farmers of sesame such as provision of basic farm inputs, fertilizer input, chemical input, extension services, and access to land input. Feeder roads infrastructures should be constructed for easy movement of sesame produce to nearby market centres,
- (iv) Research activities should be financed on improving the quality of agricultural produce devoid of contaminations, pesticides, mycotoxins, and aflatoxins,
- (v) Government should be consistent on favourable policies that affect international trade such as real exchange rate, and enhancing capacity buildings on proper packaging.

Table 7. VAR lag length selection criteria

Lag	LogL	LR	FPE	AIC	SIC	HQ
0	-165.0	NA	5.83	6.94	8.27	8.04
1	68.7*	379.6*	1.26*	-0.67*	1.67*	0.24*
2	109.4	48.03	2.70	-0.13	4.16	1.48

*-Indicates Lag Order Selected by the Criterion, LR-Likelihood Ratio Test, FPE-Final Prediction Error, HQ-Hannan-Quin Information Criterion, AIC-Akaike Information Criterion, SIC-Schwartz Information Criterion, Log L-Log Likelihood.

Table 8. Error correction model

Variable	Coefficient	Standard Error	t-Statistics
ECM	-0.95***	0.2398	-3.96
D(SES (-1))	0.69**	0.2685	2.57
D(ARE (-1))	0.08**	0.0323	2.48
D(YED(-1))	0.04***	0.0107	3.74
D(WOT (-1))	0.19	0.1376	1.38
D(RER (-1))	0.09	0.0638	1.41
Intercept	10.15**	3.406	-2.98
R^2	0.92		
Adjusted R^2	0.90		
F- Statistics	32.97***		

*- Significant at 10% Probability Level, **- Significant at 5% Probability Level, ***- Significant at 1% Probability Level.

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Expression profiling of BSD domain-containing genes in apricot during different developmental stages

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ABSTRACT

Plant transcription factors are evolutionarily conserved proteins that play an important role in the transcriptional regulation of gene expression by binding to their specific DNA sequences. BSD (mammalian BTF2-like transcription factors, synapse-associated proteins, and DOS2-like proteins) transcription factors are conserved in various species, from protozoa to humans, and are characterized by a typical BSD domain. However, little information is available about their possible roles in plant growth and development, and to date, members of this transcription factor family have not been systematically identified and analyzed in apricot. In this study, two BSD domain-encoding genes were identified in the apricot genome. Expression profile analysis by RT-qPCR revealed that both genes participate in different developmental stages of three different organs in apricot. *PaBSD1* was expressed higher than *PaBSD2* only in the stamen. Moreover, *PaBSD2* was higher expressed than *PaBSD1* in four different fruit stages, young leaf, leaf bud, sepal and petal. This study reveals the critical roles of BSD transcription factors in apricot development, with *PaBSD1* showing higher expression in stamen and *PaBSD2* in various fruit stages and leaf tissues. These findings provide a foundation for future functional studies and apricot breeding programs.

1. Introduction

Transcription factors (TFs) are genomic constituents that play important roles in controlling plant growth and development and response to stress factors by activating or repressing genes. The identification and characterization of these TFs, which play a critical role in the rearrangement of gene expression, are very important for elucidating extremely complex plant growth processes. *Arabidopsis thaliana* is an important model plant of plant molecular biology, and approximately 2000 TFs have been discovered in its genome. Nearly two decades ago, Doerks et al. (2002) discovered BSD (named after BTF2-like transcription factors, synapse-associated proteins, and DOS2-like proteins) TFs with a structurally conserved domain in various species from primitive protozoa to humans. The BSD domain is approximately 60 amino acids long, has two highly conserved adjacent tryptophan and phenylalanine residues at the C terminus, and three α -helix that are likely involved in DNA binding. Interestingly, the BSD domain also participates in the structures of different protein families. For example, the BSD domain is also found in members of the U-box family of proteins known to be involved in ubiquitination, indicating that BSD participates in different cellular regulations (Doerks et al. 2002).

Since it is a relatively newly discovered TF family, there are limited studies on BSD domain-encoding genes, which have a conserved domain in all living groups. For example, in yeast, BSD domain-containing DOS2 (DELOCALIZATION OF SWI6 2) has been shown to be involved in RNA interference and heterochromatic histone modification (Li et al. 2005). Reichmuth et al. (1995) showed that SAP47 (SYNAPSE-ASSOCIATED

PROTEIN OF 47 kDa) is required for the short-term plasticity and association functions of synapses in *Drosophila melanogaster*. In mammals, BTF2 (BASIC TRANSCRIPTION FACTOR 2) is a component of the general transcription and DNA repair factor IIIH core complex and is involved in the nucleotide excision repair of damaged DNA (Wang et al. 1995). To date, BSD domain-containing transcription factors have been identified in *Arabidopsis thaliana* (Park et al. 2009) and *Musa acuminata* (banana) (Ba et al. 2014). In *Arabidopsis*, ten genes encoding the BSD domain have been identified and of these, *AtBSD1* has been shown to be expressed in all tissues (Park et al. 2009). In banana, *MaBSD1*, a homolog of *AtBSD1*, has been shown to play a role in cell proliferation during somatic embryogenesis and its expression increases in parallel with ethylene accumulation and ripening (Ba et al. 2014; Shivani et al. 2017). Moreover, in tomato, *SIBSD1* positively regulates growth and fruit quality, but opposite pleiotropic effects on leaf senescence were detected in transgenic phenotypes obtained with knockdown or overexpression (Fan et al. 2020).

Apricot is a diploid species with eight pairs of chromosomes belonging to the genus *Prunus*, subgenus *Prunophora* Focke, and section *Armeniaca* (Lam.) Koch of the family Rosaceae (Olmsted 1941; Raji et al. 2014). Apricot (*Prunus armeniaca* L) cultivars are categorized into four eco-geographic groups: Central Asian, Iranian-Caucasian, European, and Dzhungar-Zailing. This fruit is commercially grown in 65 countries, highlighting its global agricultural importance (Kostina 1969). Although the roles of BSD TFs in plant growth and development have been described

in Arabidopsis, banana and tomato, no information is available about their possible functions in apricot. In this study, BSD TFs were identified for the first time in apricot and their expression patterns at different developmental stages were revealed by RT-qPCR. The results obtained from this study will not only contribute to the possible roles of BSD TFs in the plant kingdom but will also form the basis of functional characterization studies to be carried out in apricot in the coming years.

2. Materials and Methods

2.1. Plant materials and tissue sampling

Fifteen-year-old apricot trees from the Burdur Mehmet Akif Ersoy University Garden (37°, 01', 18" N; 30°, 17', 49" E) were selected for analysis. These trees represent a typical apricot growing environment in Burdur, Turkey. To analyze the expression pattern of *PaBSD* genes, 12 different tissues, including flower bud, leaf bud, young leaf (2 cm diameter), mature leaf (5 cm diameter), flower organs such as sepals, petals, stamens, carpels, young fruit (30 DAB), large green fruit (45 DAB), breaker fruit (60 DAB), and mature fruit (75 DAB), were sampled from the three different apricot trees. These samples were collected, separated and immediately frozen in liquid nitrogen and stored at -80°C.

2.2. Identification of BSD genes in apricot

To identify the BSD gene family in apricot, the BSD domain (IPR005607) was first obtained from InterPro (<https://www.ebi.ac.uk/interpro/>) and then used as a BLASTP query in The Genome Database for Rosaceae (GDR, <https://www.rosaceae.org/>), *Prunus armeniaca* Genome v1.0 (apricot) with an e-value of 10^{-5} (Jung et al. 2019). The existence of BSD domain in candidate proteins obtained above were further confirmed by SMART (<http://smart.embl-heidelberg.de/>) (Letunic et al. 2021) and Conserved Domain Database (CDD) (<https://www.ncbi.nlm.nih.gov/cdd/>). Finally, sequences that did not contain BSD domains were eliminated, two genes encoding BSD domains were detected in apricot and named *PaBSD1* (PARG09221m01) and *PaBSD2* (PARG09221m02), respectively.

2.3. RNA extraction and RT-qPCR analysis of BSD genes in apricot

Total RNA extraction was conducted using the Plant/Fungi Total RNA Purification Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturers' instructions. Removal of DNA contamination was performed using the RNase-Free DNase I (Norgen Biotek Corp., Thorold, ON, Canada). The RNA concentration was measured with a microplate spectrophotometer (Epoch Microplate Spectrophotometer, Biotek Instruments, Inc.), and the quality of RNA was checked by agarose gel electrophoresis. The first strand of cDNA was synthesized using a VitaScript™ FirstStrand cDNA Synthesis Kit (Procomcure Biotech) according to the manufacturer's protocol. Gene-specific primers were designed Primer-BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). The apricot *TRANSLATION ELONGATION FACTOR 2 (TEF2)* (Tong et al. 2009) and *ACTIN (ACT)* (Niu et al. 2014) genes served as the internal reference gene. The primer sequence details are given in Table 1.

The quantitative real-time PCR (qRT-PCR) was run using the iTaq Universal SYBR Green Super Mix (Bio-Rad Laboratories,

Hercules, CA, USA). The reaction system contained 5 µl of iTaq Universal SYBR Green Super Mix, 1 µl of cDNA template, 0.5 µl of each forward and reverse primer, and 3.5 µl of Nuclease-free water. The reaction procedure's setting was as follows: 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s, annealing and extension at 60°C for 40 s. Each sample had three biological and three technical replicates.

The relative expression levels of *PaBSDs* were calculated by applying the $2^{-\Delta C_t}$ method (Livak and Schmittgen 2001).

Table 1. Primer sequences specific to the *PaBSD* genes used in this study.

Primer name	Primer sequence (5'-3')
<i>PaBSD1-F1</i>	TGTCGTGTAGGCAAGTGGTGA
<i>PaBSD1-R1</i>	CGAACTTCGCAGCAGACGAG
<i>PaBSD2-F2</i>	TGTCGTGTAGGCAAGTGGTGA
<i>PaBSD2-R2</i>	GAGGGGTCGTTTGGCCTGAA
<i>TRANSLATION ELONGATION FACTOR 2-F</i>	GGTGTGACGATGAAGAGTGATG
<i>TRANSLATION ELONGATION FACTOR 2-R</i>	TGAAGGAGAGGGAAGGTGAAAG
<i>ACTIN-F</i>	GTTATTCTTCATCGGCGTCTTCG
<i>ACTIN-R</i>	CTTACCATTCCAGTCCATTGTC

2.4. Statistical analyses

In this study, samples were collected from three different apricot trees in triplicate, and RT-qPCR data were statistically analyzed with SPSS software, version 17 (SPSS Inc., Chicago, IL, USA). The overall statistical significance of the data was revealed by Student's t-test at the $P < 0.05$ level.

3. Results and Discussion

RT-qPCR is the most widely used analysis method to measure the expression of low-level expressed genes with high sensitivity and accuracy, combining traditional PCR and fluorescence techniques (Bustin 2000). In this study, to reveal the possible roles of *BSD* genes in apricot development, the expression patterns of their genes in 12 different stages of leaf, flower and fruit were analyzed by RT-qPCR (Figure 1). There is no statistical difference observed for the expression of both genes in the leaf bud and mature leaf stages. *PaBSD2* was expressed higher than *PaBSD1* in young leaf. *PaBSD2* was expressed higher than *PaBSD1* at the flower bud stage. In other flower organs, except carpel, *PaBSD2* was found to have higher expression, consistent with the flower bud. Apricot fruit development is a complex process in which many genes cooperate to alter numerous biochemical and physiological processes. According to the analysis results, expression of both *PaBSD* genes was detected, with *PaBSD2* being higher, at four different stages of apricot fruit. The differential expression of *PaBSD1* and *PaBSD2* across various tissues and developmental stages indicates their involvement in apricot leaf, flower, and fruit development. Specifically, the higher expression of *PaBSD2* in fruit stages suggests its pivotal role in fruit maturation and ripening, akin to its homologs in banana and tomato, which are known to influence the growth and ripening processes. These insights pave the way for future functional analyses and potential biotechnological applications in apricot cultivation.

Banana, which has a typical climacteric fruit, is an herbaceous perennial plant belonging to the *Musa* family (Liu et al. 2021). *MaBSD1* expression was investigated by RT-qPCR in

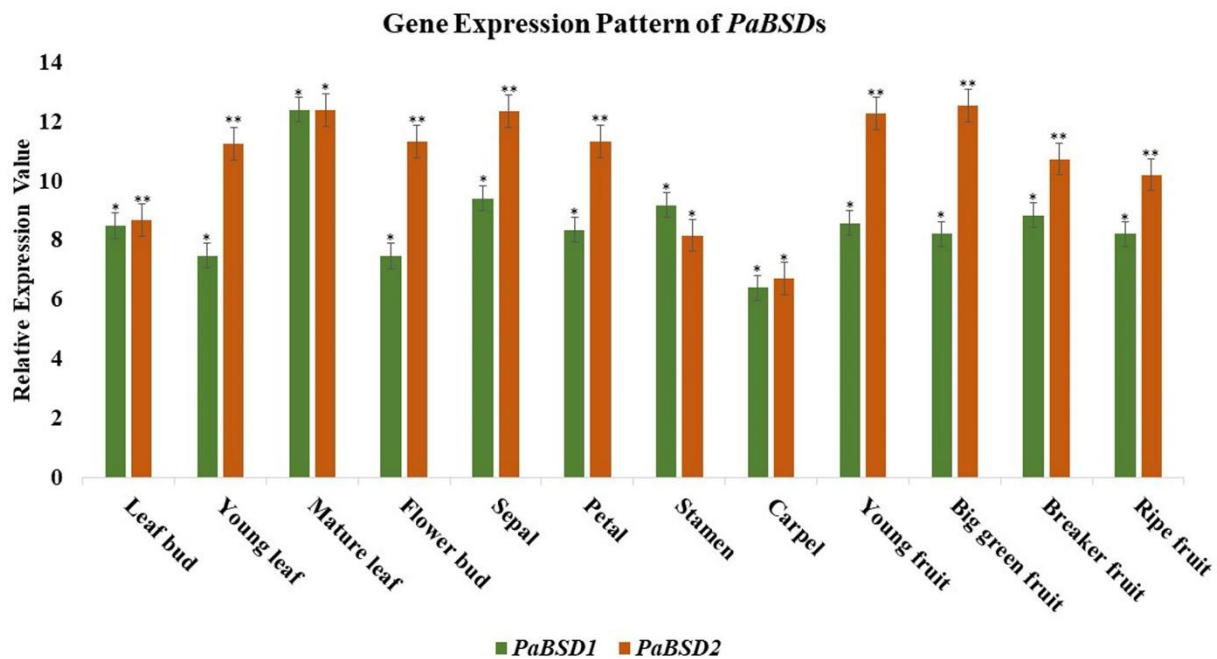


Figure 1. The expression profiles of *PaBSD* genes at the different developmental stages. Bars represent the mean of replicates \pm standard deviation. * and ** indicate a significant difference at $P < 0.05$ as determined by the Student's t-test.

banana under three different ripening conditions, including natural ripening, ethylene-induced and 1-MCP delayed ripening (Ba et al. 2014). *MaBSD1* expression was constant between days 0 and 7 of natural ripening, but started to increase on day 12 in accordance with the amount of ethylene, and on day 18, it was expressed 40 times more than on day 0. In contrast to natural ripening, there was a delay in *MaBSD1* expression in 1-MCP-treated fruits, and accumulation increased on days 30-36. Finally, *MaBSD1* expression increased rapidly 3-7 days after ethylene treatment. Fan et al. (2020) screened the expression of *SIBSD1*, the *AtBSD1* homolog in tomato, by RT-qPCR in six different tissues. The analysis showed that *SIBSD1* was expressed in all tissues examined, with the highest expression in the root and the lowest in the leaf.

A transcriptome is a snapshot of gene expression at a specific time and place in a tissue or cell, provided by capturing the total RNA within that tissue. This technique reveals, not only the expression of target genes, but also the combination of entire isoform sequences across cells and tissues. In this part of the study, to reveal the divergent roles of *BSD* genes in plant growth and development, digital expression profiles were examined based on transcriptome data from different model plants. In *Arabidopsis thaliana*, it was determined that both *BSD* genes expressed highest in the dry seed and lowest in the mature pollen stage among 48 developmental stages (Winter et al. 2007). In *Oryza sativa*, it was determined that the *AtBSD1* homolog was expressed highest in the shoot apical meristem and lowest in the seedling root at six different developmental stages (Jain et al. 2007). It has been shown that *SIBSD1* (Solyc04g077600), which is the *AtBSD1* homolog in *Solanum lycopersicum*, is expressed highest in the root and lowest in the leaf. In addition, *SIBSD1* showed the highest expression in the 3 cm fruit of *S. lycopersicum*, it also showed high expression in the immature green fruit stage in *Solanum pimpinellifolium*, suggesting that *BSD* genes have different functions in different species of the

same genus (Sato and Orozco López 2012). *Prunus persica* is the taxonomically closest species to *Prunus armeniaca* and has two *BSD* genes. Based on the transcriptome data, it was determined that the first of these (Prupe.1G583500) was expressed highest in the shoot meristem and lowest in the bud, the second (Prupe.5G213500) was expressed highest in the fully opened flower and lowest in the bud and stem (Verde et al. 2013). Taken together with the RT-qPCR and transcriptome results obtained from these taxonomically distant species, it can be said that *BSD* domain-encoding homologues are firmly associated with plant growth and development.

4. Conclusion

In this study, two homologous genes encoding the *BSD* domain in apricot were identified for the first time, and their expression patterns in 12 different developmental stages of three different organs were revealed. The expression analysis results of *PaBSD* genes showed that these genes may play important roles in apricot growth and development, consistent with studies in other plants. As a result, this study will facilitate the understanding of the roles of *BSD* genes in growth and development in plants and will lay the foundation for future *BSD*-based molecular breeding studies in apricot.

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Comparing nuclear DNA content, pollen viability, pollen production and seed retention of lavender and lavandin

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ABSTRACT

Lavender (*Lavandula* spp.) is one of the most widely grown essential oil crops in the world. This study aimed to determine the nuclear DNA contents using flow cytometry, pollen viability using TTC (2,3,5-Triphenyl Tetrazolium Chloride) and IKI (Iodine Potassium Iodide) tests, pollen production quantities using hemacytometric method, and seed retention rates per spike and flower of lavender (*L. angustifolia* var. Raya) and lavandin (*L. intermedia* var. Super) grown under ecological conditions in Isparta province of Turkey. The nuclear DNA contents were 2.11 and 2.54 pg 2C⁻¹, respectively in the lavender and lavandin cultivar. The flowers of the lavender cultivar produced abundant pollen grains (average 5800 pollen per flower and 1450 pollen per anther) with high viability (60.65-65.05%) and seed retention rate per spike (91.57% on average). The lavandin cultivar, which had very low pollen viability (1.08-3.32%) and pollen grains (average of 2350 pollen per flower and 587.5 pollen per anther) gave very low seed retention rates per spike (0.60% on average). While each flower had four ovaries with the potential to produce four nutlets, lavandin flowers produced only trace numbers (0.15% on average) of seeds. As a result, the lavandin cultivar had more nuclear DNA content, longer stem and spike, smaller size but more numerous flowers, less and lighter anthers, lower pollen grains and viability, and very few inviable seeds compared to the lavender cultivar. It has been observed by eye that honey bees do not visit lavender and lavandin flowers for collecting pollen, but solely for collecting nectar.

1. Introduction

Lavender (*Lavandula* spp.), a semi-shrub and perennial plant from the Lamiaceae family, has a total of 39 species that are predominantly of Mediterranean origin and are distributed in 6 sections (*Lavandula*, *Dentatae*, *Stoechas*, *Pterostoechas*, *Subnuda*, and *Chaetostachys*) around the world (Upson and Andrews 2004). The most valuable commercially cultivated species, whose essential oils are utilized, are lavender (*L. angustifolia* Mill.), lavandin (*L. x intermedia* Emeric. ex. Loisel) and spike lavender (*L. latifolia* Medik.) in the *Lavandula* section (Beetham and Entwistle 1982). Among these, lavandin (syn. *L. hybrida*), also called "hybrid lavender", is a natural hybrid of *L. angustifolia* and *L. spica*, and was first identified in 1828 (Tucker 1985).

The essential oils obtained by steam distillation of the lavender and lavandin fresh/dried flowers are rich in terpenic compounds such as linalool, linalyl acetate, and camphor, and have a wide range of uses including perfume, cosmetics, medicine, and food additives (Lawrence 1994). Compared to lavender varieties, lavandin varieties bloom later and have higher flower yield and oil content (Baydar 2022). However, lavender essential oil is more suitable for the perfumery industry due to its lower camphor and 1,8-cineole content and higher levels of linalyl acetate, lavandulyl acetate and lavandulol (Karik et al. 2017). The camphor contents of the essential oils obtained from Bulgarian lavender cultivars are below the upper limit value

(<0.6%) required by ISO 3515:2002 standard (Stanev et al. 2016).

Both lavender and lavandin are cultivated in many countries of the world, especially in the Mediterranean and Balkan countries. The first commercial lavandin cultivation in Türkiye (Turkey) began in Kuyucak village in the Isparta province in the middle of the 1970s (Kara and Baydar 2011). Nowadays, Kuyucak village has created an agrotourism identity by attracting thousands of local and foreign tourists every year with the "The village with Lavandin Scent" and "Lavandin Festival" events and has become a role model for sustainable rural development (Tarhan 2020). *Lavandula* flowers which are purple in color, due to the pigments such as delphinidin and malvidin, and very fragrant due to the mono/sesquiterpenic essential oils, are of great value in terms of both ecotourism and beekeeping activities.

In recent years, lavender and lavandin production areas have reached up to 5000 hectares across Türkiye (TUİK 2023). Due to the lack of cultural practices such as irrigation, chemical fertilization and pesticide application, producers prefer *Lavandula* cultivation. Although lavandin (var. Super) is widely cultivated in the Western Mediterranean Region, lavender varieties (Bulgarian varieties such as Sevtopolis, Yubileina, Hemus, Hebar, Drujba, Raya and Karlova) are widely cultivated in other regions, especially in the Aegean, Marmara,

Mediterranean and Central Anatolian regions. An average of 7500 kg ha⁻¹ fresh (with stems) and 1500 kg ha⁻¹ dry (without stems) flowers are produced from the Super lavandin cultivar grown in Isparta province, and 1 kg of lavandin oil is obtained by steam distillation of 60-70 kg of fresh lavandin flowers (Baydar and Kineci 2009).

While lavandin cultivars, which have seed sterility, are only propagated vegetatively, lavender cultivars can be propagated both vegetatively and generatively (Urwin 2014). In addition, since lavender is a cross-pollinated plant, its seeds show a high degree of genetic variation, and seed-grown seedlings exhibit heterogeneous growth and development, as well as reaching economic yield age later (Baydar 2022). Due to these disadvantages, plantations are commonly established using rooted cuttings under *in vivo* and *in vitro* conditions (Kara and Baydar 2020). Although various opinions have been expressed as to why lavandin varieties show seed sterility, there is no exact data on this aspect (Beetham and Entwistle 1982). Therefore, it is necessary to understand the flowering, pollination, and fertilization biology of the lavender/lavandin plants for the plant breeding methods and the seed certification processes. The aim of this research was to compare the floral, pollination and fertilization characteristics of lavender and lavandin cultivars, in particular to obtain findings that will help to understand the problem of high seed sterility in lavandin scientifically.

2. Materials and Methods

This research was conducted at the Department of Field Crops, Faculty of Agriculture, Isparta Applied Sciences University in Türkiye. Lavender (*Lavandula angustifolia* Mill. var. Raya) and lavandin (*Lavandula x intermedia* Emeric ex Loiseleur var. Super) cultivars grown under ecological conditions in Isparta province were used as material in the flowering season of 2021.

2.1. Nuclear DNA content by flow cytometry

Since pollen and seed sterility in plants are closely, although not directly, related to the genome structure, the DNA contents of both varieties were determined. (Van Oost et al. 2021). The nuclear DNA content (pg 2C⁻¹) was determined using flow cytometry according to a protocol explained by Tuna et al. (2016). Healthy, young and fresh leaves of sample and standard plants were chopped in petri dishes containing 500 µL nuclei extraction buffer. The extract was passed through a 30 µm CellTrics filter, and transferred into a tube containing 2 mL staining solution (prepared by mixing 6 µL RNase stock solution

and 12 µL propidium iodide stock solution). The solution was incubated in the dark for 30-60 minutes before being read on a PARTEC Flow Cytometer device. The nuclear DNA content of the sample was calculated based on the relative positions of the G1 peaks of the sample and standard (*Vicia sativa* was used as the standard).

2.2. Pollen viability and productivity tests

During the flowering season of the cultivars (Raya bloomed from June 10 to July 15, 2021, and Super bloomed from June 25 to August 25, 2021), a sufficient number of mature flower buds were collected from the plants whose flowers were expected to open one day later. Anthers were separated from these flowers (each flower has 4 anthers) and spread over the petri dishes, which were kept at room temperature under a 60-watt lamp overnight. The pollen productivity of hexacolpat-structured (6-pore) pollen obtained from exploding anthers was tested using the hemacytometric method, and pollen viability was tested with 2,3,5-triphenyl tetrazolium chloride (2 hours in 1% TTC solution) and iodine-potassium iodide (1.5% in IKI solution) maximum 5 minutes (Eti 1990 1991). For each cultivar, flower pollen grains were observed under a microscope (Nikon SE, 400×) using two slides, with four observations made on each slide for the TTC test, which stained the grains dark red, and the IKI test, which stained them dark brown as seen in Figure 1. Pollen grains that stained dark red or dark brown were considered viable, while those that remained unstained or appeared yellow/brown were considered non-viable (Norton 1966). Additionally, the width and length (in µm) of one hundred randomly selected pollen grains were measured during pollen viability tests.

Mature anthers of 10 flowers were used to determine the average pollen amount (number) per anther by the hemocytometric method. Forty anthers were soaked overnight in 3 ml of distilled water to which one drop of Tween-20 was added. Later, a suspension containing a drop of pollen was placed onto each of the two counting chambers on a hemocytometric slide and covered with a coverslip. Under a microscope (Nikon SE), the flower pollen grains, in four randomly selected large squares, were counted in each counting chamber. Using the volume of the space between the hemocytometer slide and coverslip (0.1 mm³) and the total number of pollen grains in a 3 ml suspension (Eti 1990), the average number of pollen grains per anther was determined by dividing the values obtained by 10, and the number of pollen grains per anther was multiplied by 4 to determine the average number of pollen grains per flower.

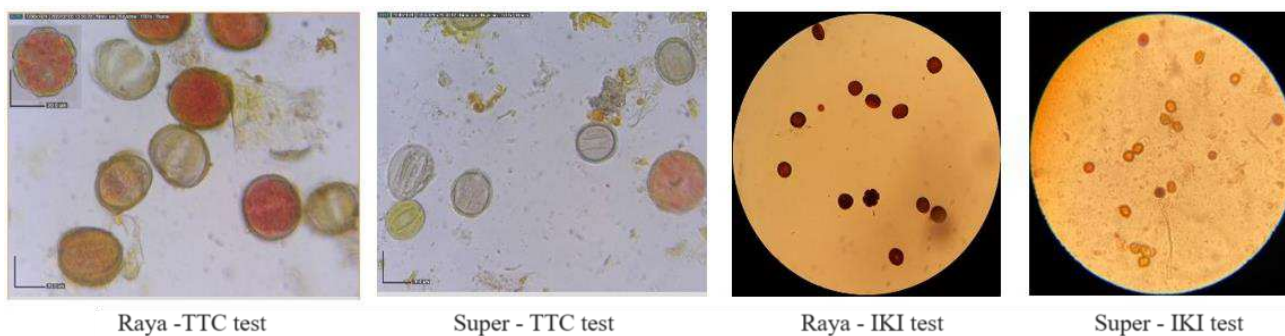


Figure 1. Pollen viability tests of lavender (var. Raya) and lavandin (var. Super).

2.3. Floral and scent characteristics

Various parameters explained by Kara (2011) were determined for each cultivar including flower stem height (cm), spike length (cm), number of nodes per spike, number of flowers per node, number of flowers per spike, flower width and length (mm), anther width and length (μm), anther weight (g 1000 flowers⁻¹), seed retention (setting) rate per spike [(total number of seeds/total number of flowers) x100], seed retention rate per flower [(number of seeds/number of ovaries) x100], 1000-seed weight (g), and seed germination rate (%). Since lavender plants use the attractive properties of scents to attract pollinator insects (Benachour 2017; Valchev et al. 2022), the essential oil contents of both lavender species were determined. At the full flowering stage, the essential oil content (%) of fresh flower with stem was determined by water distillation for 3 hours using a Clevenger apparatus (Kara and Baydar 2011, 2012, 2013).

2.4. Statistical analysis

The data obtained from the examined characteristics of the cultivars were presented as mean \pm standard deviation, and the significance of the differences between means was checked using the Student t-test (SAS 1998).

3. Results

The average nuclear DNA contents measured in triplicate on fresh leaf samples taken from each species and flow cytometry histograms of the lavender and lavandin cultivars are shown in Figure 2. The sample peaks were clearly distinguishable from the standard plant G1 peak. The nuclear DNA contents were $2.11 \pm 0.05 \text{ pg } 2C^{-1}$ and $2.54 \pm 0.08 \text{ pg } 2C^{-1}$, respectively in the lavender (var. Raya) and lavandin (var. Super) cultivar (Figure 2). The floral characteristics of lavender (var. Raya) and lavandin (var. Super) are presented in Table 1. Statistically significant differences were found between the cultivars Raya and Super in terms of flower stem length (31.80 ± 8.63 and 61.00 ± 6.16 cm), spike length (11.40 ± 2.27 and 15.10 ± 3.25 cm), number of nodes per spike (5.50 ± 0.53 and 10.20 ± 0.79), number of flowers per

node (7.90 ± 1.45 and 18.10 ± 2.56), number of flowers per spike (43.20 ± 7.64 and 184.50 ± 29.70), pollen width (37.64 ± 2.01 and $25.31 \pm 1.40 \mu\text{m}$) and length (41.81 ± 3.18 and $27.18 \pm 1.58 \mu\text{m}$), pollen viability (60.65 ± 11.31 and 1.08 ± 0.85 in TTC test, 65.05 ± 21.51 and 3.32 ± 1.95 in IKI test; Figure 1), pollen production (1450 ± 287.9 and 587.5 ± 356.1 per anther), seed retention rate per spike ($91.57\% \pm 27.68$ and $0.60\% \pm 0.27$), seed retention rate per flower ($22.89\% \pm 6.92$ and $0.15\% \pm 0.07$), and essential oil content ($1.25\% \pm 0.10$ and $1.75\% \pm 0.25$), respectively (Table 1). However, no statistically significant differences were observed between both varieties in terms of flower width (2.45 ± 0.25 mm and 2.30 ± 0.15 mm) and length (12.05 ± 2.00 mm and 11.68 ± 1.70 mm), anther width (0.60 ± 0.10 mm and 0.50 ± 0.10 mm), anther length (1.00 ± 0.10 mm and 0.80 ± 0.15 mm), anther weight (0.08 ± 0.01 g and 0.07 ± 0.01 g 1000 pcs⁻¹), and 1000 seed weight (1.15 ± 0.12 g and 0.09 ± 0.17 g, respectively (Table 1).

4. Discussion

According to the results of the flow cytometer analysis, it was determined that the nuclear DNA amount was $2.11 \text{ pg } 2C^{-1}$ in the lavender cultivar and $2.54 \text{ pg } 2C^{-1}$ in the lavandin cultivar (Figure 2; Table 1). Indeed, in a comprehensive karyotype study conducted on a total of 82 genotypes in the *Lavandula*, *Stoechas*, *Dentatae*, *Pterostoechas*, and *Subnudaeda* sections of lavender, it was reported that the genome size ranged from 0.76 to 4.80 $\text{pg } 2C^{-1}$ and the chromosome numbers varied between 22 and 100 (Van Oost et al. 2021). In the same study, it was explained that the $2n$ chromosome number of *L. x intermedia* 'Heavenly Angel' genotype was 100. The chromosome numbers of species belonging to *Lavandula* subsection have been determined as $2n= 34, 36, 42, 48, 50, 54,$ and 75 (Garcia 1942; Darlington and Wylie 1955; Upson 2004; Rice et al. 2015). It has been reported that there is a close relationship between the genome size ($2C$ values) and chromosome number of the species in this section, and that those with high nuclear DNA amounts also have a higher number of chromosomes (Van Oost et al. 2021).



Figure 2. Flow cytometer histograms and generative reproductive organs of lavender (var. Raya) and lavandin (var. Super).

Table 1. Nuclear DNA contents and floral characteristics of lavender (var. Raya) and lavandin (var. Super)

Characteristics	Lavender (var. Raya)	Lavandin (var. Super)	t- value
Genome size			
DNA content (pg 2C ⁻¹)	2.11 ± 0.05 ^a	2.54 ± 0.08	
Flower properties			
Flower stem height (cm)	31.80 ± 8.63	61.00 ± 6.16	8.70**
Spike length (cm)	11.40 ± 2.27	15.10 ± 3.25	2.95**
Number of nodes per spike	5.50 ± 0.53	10.20 ± 0.79	15.67**
Number of flowers per node	7.90 ± 1.45	18.10 ± 2.56	10.97**
Number of flowers per spike	43.20 ± 7.64	184.50 ± 29.70	14.57**
Flower dimensions			
Flower width (mm)	2.45 ± 0.25	2.30 ± 0.15	1.49
Flower length (mm)	12.05 ± 2.00	11.68 ± 1.70	0.44
Anther dimensions			
Anther width (mm)	0.60 ± 0.10	0.50 ± 0.10	0.59
Anther length (mm)	1.00 ± 0.10	0.80 ± 0.15	0.68
Anther weight (g 1000 pcs ⁻¹)	0.08 ± 0.01	0.07 ± 0.01	0.17
Pollen dimensions			
Pollen width (400x) (µm)	37.64 ± 2.01	25.31 ± 1.40	17.44**
Pollen length (400x) (µm)	41.81 ± 3.18	27.18 ± 1.58	14.27**
Pollen production			
Pollen number per anther	1450 ± 287.9	587.5 ± 356.1	8.42**
Pollen number per flower	5800 ± 1151.7	2350 ± 1424.4	8.42**
Pollen viability			
TTC test (%)	60.65 ± 11.31	1.08 ± 0.85	23.43**
IKI test (%)	65.05 ± 21.51	3.32 ± 1.95	12.78**
Seed fertility			
Seed retention rate per spike (%)	91.57 ± 27.68	0.60 ± 0.27	10.39**
Seed retention rate per flower (%)	22.89 ± 6.92	0.15 ± 0.07	10.39**
1000 seed weight (g)	1.15 ± 0.12	0.09 ± 0.17	0.79
Essential oil content (%)	1.25 ± 0.10	1.75 ± 0.25	2.49*

^aAll values are means ± standard deviation (n= 10). Asterisk marks significant differences (*P≤0.05 and **P≤0.01) between lavender and lavandin for that parameter according to Student's t-test.

Since lavandin (*L. x intermedia*) is a natural hybrid between lavender (*L. angustifolia*) and spike lavender (*L. latifolia*), it would be expected to have an intermediate 2C value between the amounts of their two ancestral species. Indeed, in the karyotype study conducted by Van Oost et al. (2021), which confirms this view, genome sizes were determined as 2.02-2.25 pg 2C⁻¹ in *L. angustifolia* (2n= 48), 2.48-2.59 pg 2C⁻¹ in *L. latifolia* (2n= 50), and 2.28-2.37 pg 2C⁻¹ in *L. x intermedia* (2n= 50). However, in our findings, while the 2C values of *L. angustifolia* were similar, the 2C DNA amount of *L. x intermedia* was found to be close to the value of *L. latifolia*, not between the values of *L. angustifolia* and *L. latifolia* determined by Van Oost et al. (2021) (Table 1). Therefore, more detailed karyotype analyses and molecular phylogenetic research are needed to dispel doubts on the *Lavandula* species.

While lavandin produces little to no seeds due to its sterility, lavender and spike lavender which are the ancestral species of lavandin are fertile and produce seeds (Tucker 1985). In our study, it was found that the lavender cultivar produced a large

number of pollen (an average of 5800 grains per flower) with high viability (60.65% to 65.05%). However both pollen viability (1.08% to 3.32%) and pollen production (an average of 2350 grains per flower) were found to be relatively lower in the lavandin cultivar (Table 1). Due to the very low pollen productivity and viability, it was observed that the seed setting (retention) rate of the lavandin variety was very low (average of 0.6% per spike). Additionally, lavender seeds germinated on average at a rate of 35% in pure water and 50% in 0.1% GA₃ solution within a week (not tabulated).

Due to the lack of regular pairing between homologous chromosomes during meiosis in the lavandin genome, which has different chromosome numbers from its parents, gametes with aneuploid chromosome numbers that can cause infertility or gametophytic sterility have been formed. Pollen sterility and seed sterility are also widespread in English mint, such as lavandin. English mint (*Mentha x piperita*, 6x= 72), which is a natural hybrid of water mint (*M. aquatica*, 8x= 96) and garden mint (*M. spicata*, 4x= 48), is an allohexaploid species (Tucker 2012).

However, instead of producing $6x=72$ (48+24) chromosome offspring through normal meiotic fertilization, different ploidy levels such as 48, 60, 72, 84, and 96 (cytomixis) can occur (Tucker and Fairbrothers 1981).

Each lavender or lavandin flower carries 4 male organs (stamens) and 1 female organ (pistil) with 4 ovaries (Figure 2) with a potential to produce 4 nutlets (seeds). However, it was observed that these four ovaries could produce about one seed (22.89% on average) in lavender, and trace number of seeds (0.15% on average) in lavandin flower. It has been determined by Urwin (2014) that autotetraploid lavandin (var. Grosso and Seal) plants with doubled chromosome numbers through colchicine application were able to become fertile and produce seeds.

In our study, the lavandin cultivar has shown narrower but longer leaves, longer stems and spikes, smaller but more numerous flowers, smaller and lighter anthers, pollen and seeds compared to the lavender cultivar. As a result of the "gigas effect" that occurs parallel to the increase in chromosome number and nuclear DNA amount in polyploid plants, there are increases in the size of organs such as leaves, flowers, fruits, and seeds, as well as the numbers of stomata and chloroplasts, and the amounts of secondary metabolites (Simmonds 1980; Sattler et al. 2016; Eng and Ho 2019). At least for now, based on the scientific findings and interpretations, it is believed that lavandin is not a polyploid species but rather a natural hybrid of lavender and spike lavender. Moreover, it is still a matter of curiosity whether lavandin shows the gigas effect since it has a higher chromosome number and nuclear DNA amount than lavender. The findings obtained from these studies are valuable at least in terms of providing insights on this issue.

Lavender flowers are pollinated, especially by bumble and honey bees (Valchev et al. 2022). However, we observed in this study that honey bees (*Apis mellifera*) visit lavender and lavandin flowers only to collect nectar, not pollen. The honey bee lands on the narrow flowers that are too small to fit in, and sucks nectar from the 6-7 mm long tongue through the 7-8 mm long corolla tube in an average of 2.32 seconds, only transferring pollen by contact with the fuzzy front of its head to the anthers (Benachour 2017). Although the bees help with pollination by carrying pollen on their bodies, their contribution to pollination is lower in lavandin flowers, which produce approximately three times less pollen than lavender flowers.

5. Conclusion

As a result, the lavandin genome contains a higher amount of nuclear DNA than lavender. Lavandin also blooms later, forms a wider and taller habitus, produces longer flower stems and spikes, and yields more flowers. Finally, it contains a higher percentage of essential oil. However, due to low pollen viability and production, its seed-setting rate remains very low in lavandin compared to lavender. The findings from this research will be a valuable tool for evaluating the opportunities and possibilities of the lavender and lavandin breeding approaches involving hybridization.

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Effects of salt stress on germination, seedling growth, and ion content of sweet sorghum

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ABSTRACT

Salinity is one of the most common abiotic stresses in the world. It negatively affects the growth and development of sweet sorghum (*Sorghum bicolor* L. Moench). It significantly reduces germination and seedling growth parameters. The present study was carried out to evaluate the impact of four salinity levels (0, 100, 200, and 300 mM) on the germination and seedling growth parameters of four sweet sorghum genotypes (Erdurmus, Uzun, Srg 156, and BSS 424) and on their ion content (Na, K, Ca, and Mg). The results indicate that under nonsaline conditions, the germination percentage (GP) of all genotypes was 100%, and Erdurmus was identified as the earliest germinating genotype. The BSS 424 genotype showed a significant reduction in germination index (GI), ranging from 8.33% at 100 mM to 0.89% at 300 mM, while Erdurmus and Srg 156 showed the lowest decreases, with mean values of 15.801 and 13.901, respectively. The highest root fresh weight (RFW) value was observed in the control for all the genotypes, while Erdurmus showed the lowest decrease. Moreover, the highest decrease in Mg (0.24%) and Ca (0.17%) content was observed in Uzun, and the lowest K content was identified in BSS 424 (0.5%), whereas the highest Na content was also determined in Uzun (3.12%). Considering all the results, salt stress above 200 mM significantly affected the germination and seedling growth parameters. Therefore, lower concentrations should be taken into consideration for sustainable sorghum production.

1. Introduction

Global warming and climate change, considered triggers for factors such as soil salinity, are two of the most important problems in the world (Özyazıcı and Açıkbay 2021). The salinity of soil affects areas of agricultural production, particularly in arid, semi-arid, and coastal regions (Pankova and Konyushkova 2013; Corwin 2021). It is responsible for losing over 7% of arable land and 33% of irrigated land worldwide (Chele et al. 2021). It also contributes to the loss of 27.3 billion US dollars per year (Wichelns and Qadir 2015; Kumar and Sharma 2020). Salinity negatively affects plant growth and development (Rajabi Dehnavi et al. 2020; Sabagh et al. 2021), which is associated with some morphological, physiological, and biochemical aspects, particularly osmotic stress in young leaves and ion toxicity in older leaves (Munns and Tester 2008). It significantly reduces the length of the shoot and root, as well as the dry weight of the roots and shoots (Hamada and Al-Hakimi 2001; Ashrafuzzaman et al. 2002). In addition, salinity causes slower and dysfunctional germination (Ekmekçi et al. 2005) and reduces the absorption of mineral components by plants (Xu et al. 2016).

Sorghum (*Sorghum bicolor* L. Moench) is one of the most economically important cereals in the world (Tigabu et al. 2012). It ranks fifth in cereal production after wheat, rice, maize, and barley (Bakari et al. 2022). More than 100 countries (Hao et al. 2021) cultivate it on around 40.7 million hectares (FAOSTAT 2024), with an annual production of 57.58 million tons in 2022

(FAOSTAT 2024). Sorghum is an important crop cultivated for its seeds, fodder, and bioenergy production (Steduto et al. 1997; Bakari et al. 2022). Sweet sorghum is a type of sorghum characterized by high sugar concentrations in the stalks (Atokple et al. 2014). Salinity stress limits sorghum productivity (Wang et al. 2003). It represents a serious problem and a major obstacle for global agriculture (Flowers 2004). This reduces the germination ability and affects seedling growth factors, such as the length of the root and shoot and the weight of the fresh shoot (Norlyn and Epstein 1984). Germination and seedling characteristics remain the most commonly used factors in plant salt tolerance selection because of their importance to the crop (Tigabu et al. 2012).

Germination parameters, such as the germination index and germination percentage, are crucial and vulnerable stages in plant development (Hakim et al. 2010). Moreover, seedling growth parameters, such as dry and fresh shoot weight, dry and fresh root weight, and length of root and shoot are the factors commonly used in genotype selection, which is essential for the management of saline conditions (Bybordi and Tabatabaei 2009). Although sweet sorghum tolerates salt stress better than other cereals (e.g., wheat and rice) (Ratanavathi et al. 2004), its germination can be affected by severe salt stress, which reduces its productivity (Zhu et al. 2019). Many studies have shown significant differences in sorghum genotypes in response to various salinity concentrations. For example, Özyazıcı and Açıkbay (2021)

demonstrated that different varieties were more sensitive after a 100-mM salt dose. In contrast, [Netondo et al. \(2004a\)](#) and [Gökkaya and Arslan \(2023\)](#) reported that sorghum plants were sensitive to NaCl above 150 mM.

Selecting salt-tolerant sorghum genotypes by assessing different morphological, biochemical, and physiological plant characteristics contributes to the improvement of crop yields and the sustainability of agriculture ([Sagar et al. 2023](#)). In saline conditions, sorghum has the capacity to exclude certain minerals, such as Na ([Yang et al. 2018](#)), and reduce its transport from the roots to the leaves by discharging it from the root xylem ([Shakeri and Emam 2017](#); [Yan et al. 2015](#)). Specific translocation and absorption of K and Ca compared to Na are determined as another mechanism of salt tolerance in the plant ([Shakeri et al. 2020](#)). High concentrations of sodium cause a toxic accumulation in sorghum leaves, affecting the absorption and translocation of K, Ca, and Mg ([Netondo et al. 2004a](#); [Bavei et al. 2011](#)), which influences the development and photosynthetic activity of the plant ([Netondo et al. 2004b](#); [Joardar et al. 2018](#)).

Generally, increasing salinity significantly reduces germination and seedling growth parameters ([Jamil et al. 2006](#); [Okumuş et al. 2023](#); [Okumuş and Şekerci 2024](#)). Identifying the genotype that exhibits resistance to various levels of salinity stress at the early seedling stage is crucial for the advancement of salt-tolerant plants and the achievement of optimal agricultural yields in saline environments ([Hakim et al. 2010](#)). Thus, the objective of our study is to investigate the response of four sweet sorghum genotypes to different levels of salinity for (i) germination and seedling growth parameters and (ii) to determine the ion contents (Na, K, Ca, and Mg) in the shoots of these genotypes.

2. Materials and Methods

2.1. Plant materials

In this study, four different sweet sorghum genotypes were used as a genetic material; Uzun, Erdurmus, BSS 424 and Srg156. Erdurmus and Uzun are sweet sorghum cultivars that are registered by the Western Mediterranean Agricultural Research Institute of Türkiye. The BSS 424 is a sorghum elite line originating from the United States (ID number IS 20697). Srg156 is an elite sweet sorghum line obtained by crossing sweet sorghum (Erdurmus) and grain sorghum (Ogretmenoglu) cultivars, reaching the F8 generation.

2.2. Germination experiment

A Petri dish experiment was conducted using a complete randomized design. The experiment involved a factorial design, with four salt treatments (0, 100, 200, and 300 mM NaCl) and four sweet sorghum genotypes. The experiment was performed in three replicates in a growth chamber at Akdeniz University, Faculty of Agriculture, in the Field Crops Department Laboratories. The growth chamber maintained a temperature of 25°C and a 16-hour light period. 48 Petri dishes were prepared with Whatman No. 2 filter paper and filled with varying NaCl solutions. Each dish contained ten sorghum seeds. Observations were performed in Petri dishes for ten days until germination was completed.

A modified version of seed sterilization process [Özyazıcı and Açıkbaş \(2021\)](#) was adapted. Firstly, the seeds were surface sterilized in a solution of 70% C₂H₅OH (ethanol) for 5 minutes, and the seeds were washed with ultra-distilled water. Then the

seeds were kept in a solution of 10% NaClO (sodium hypochlorite) for 10 minutes. Afterwards, sorghum seeds were washed with ultra-distilled water. We added 10 ml of saline (NaCl) solution to each Petri dish, except for the control group.

2.3. Germination and seedling growth assessments

Germination number, root, and shoot length of the seeds were observed, and results were recorded daily. At the end of the study, fresh weights of roots and shoots were measured for each sample. Afterwards, the samples were dried at 65 °C for two days and their dry weight was recorded.

We evaluated the following variables:

Germination Percentage (GP): $GP = \frac{NGS}{TS} \times 100$, NGS: Number of normal germinated seeds, TS: Total number of utilized seeds ([Scott et al. 1984](#)).

Germination Index (GI): $GI = \sum \frac{Gi}{Tt}$, Gi: Germination percentage at the *i*th day, Tt: Days of germination test duration ([Wang et al. 2004](#)).

Total Sodium, Magnesium, Calcium and Potassium Content: 0.5 g of the dried shoot samples were taken and 3 ml of HCl (37%), 9 ml of HNO₃ (65%) were added. Wet combustion was performed in a digiblock (Labtech ED 36S) combustion unit ([U.S. EPA 2007](#)) The total concentration of Na, Mg, Ca and K was determined by ICP-OES (PEOptima 7000 DV).

2.4. Data analysis

The analysis of variance (ANOVA, PROC GLM) was performed with SAS version 9.2.

3. Results

All measured parameters showed statistically significant main effects of salinity and genotypes, as well as the combined effects of their interactions. However, there were no significant differences in genotypes for RDW, SDW and SL, and in the combined effects of genotypes and salinity interactions for RDW, SFW, GI and SL ([Table 1](#)).

3.1. Germination parameters

There were significant variations among sorghum genotypes in terms of germination parameters, GI and GP in saline conditions.

Statistical analysis showed that in non-saline conditions, there were no significant differences between genotypes in terms of GP; however, there were significant variations in GI ([Table 1](#)). The results indicated that in non-saline conditions, the GP of all genotypes achieved 100% ([Table 2](#)). In non-saline condition, Erdurmus was identified as the earliest germinating genotype, while BSS 424 was determined to be the latest genotype ([Figure 1](#)). With salinity increasing to 100 mM NaCl, all genotypes showed a decrease in GP between 3.4% and 43.4%. GP decreased significantly in all sweet sorghum genotypes that were exposed to NaCl concentrations of 200 mM and 300 mM. Under 300 mM NaCl, the highest decrease was observed in BSS 424, while the lowest was identified in Erdurmus.

Salinity also significantly decreased GI. Under saline conditions, the maximum reduction was identified in genotype BSS 424, ranging from 8.33% under 100 mM to 0.89% at 300 mM. Erdurmus had the lowest decreases, with a mean of 15.800, followed by Srg 156 with a mean of 13.900 ([Table 2](#)).

Table 1. Analysis of variance (*F* value) of four sweet sorghum genotypes in four salinity levels

Traits		NaCl	Genotypes	NaCl X Genotypes
Germination Parameters	GI	51.04**	38.04**	0.79 ^{ns}
	GP	81.80**	39.07**	5.59**
Seedling Growth Parameters	RFW	153.89**	13.35**	3.50*
	RDW	13.39**	0.10 ^{ns}	1.00 ^{ns}
	SFW	136.34**	8.96*	1.00 ^{ns}
	SDW	77.60**	5.08 ^{ns}	5.54**
	RL	131.17**	5.22*	3.25*
	SL	122.41**	2.36 ^{ns}	1.80 ^{ns}
Ion Assimilation Parameters	Mg	1980.05**	302.18**	68.32**
	Ca	1044.57**	30.07**	27.17**
	Na	975057**	13272.30**	11135.80**
	K	2219.02**	5269.60**	226.55**

Root Fresh Weight; RFW, Root Dry Weight; RDW, Shoot Fresh Weight; SFW, Shoot Dry Weight; SDW, Germination Index; GI, Germination Percentage; GP, RL; RootLength, SL; Shoot Length *; $P < 0.05$, **; $P < 0.001$, ns; non-significant.

3.2. Seedling growth parameters

The salinity levels had a significant impact on the seedling growth parameters of the sweet sorghum genotypes (Table 1). All seedling growth parameters decreased significantly as salinity levels increased. No observations were obtained at the 300 mM level for RFW, RDW, and SDW, as well as at the 200 mM level for RDW (Table 2).

The highest value for RFW was observed in the control condition for all genotypes. BSS 424 had the highest decrease relative to the control in each level, followed by Srg 156, while the genotype Erdurmus showed the lowest decline. The lowest RDW was recorded with a 300 mM NaCl level, while the highest was recorded with 0 mM NaCl for all genotypes; however, there is no significant difference among genotypes.

Other measured seedling growth parameters, SFW and SDW, showed similar trends: there was a significant reduction in SFW and SDW with increasing levels of NaCl concentration (Table 2). However, there was no significant variation observed among the different genotypes for SDW. In the control concentration, the data on SFW showed that the cultivar Srg 156 achieved the maximum value, followed by Erdurmus and BSS 424, while the lowest value was obtained in Uzun.

In the control condition, RL among sorghum genotypes ranged between 4.02 and 6.26 cm, and Srg 156 was identified as having the highest value. (Table 2) The root length exceeded the shoot in Erdurmus, while in the BSS 424, the shoot length exceeded the root length in the control condition (Figure 2). Moreover, a drastic decrease in RL was identified at 200 and 300 mM NaCl for all genotypes. BSS 424 had the highest decrease relative to the control in each level, while the genotype Erdurmus showed the lowest decline. The SL also declined with the higher salinity effect; however, there is no significant difference among genotypes (Table 1).

3.3. Ion content

We identified Na, K, Ca, and Mg concentrations in shoot samples at two NaCl levels: 0 and 100 mM NaCl, because samples at other doses were insufficient. All Na, K, Ca, and Mg parameters were influenced by the salinity level (Table 3). The highest decrease in Mg (0.24%) and Ca (0.17%) content was observed in Uzun, and the maximum decrease in K content was identified in BSS 424 (0.5%), while the highest increase in Na content was also determined in Uzun (3.12%). Compared to other

genotypes, Erdurmus and Srg 156 showed a relatively lower loss in ion content despite their high sodium accumulation.

4. Discussion

Seed germination is one of the most important indicators for the successful development of resistant cultivars under salinity stress conditions. In general, when the salt concentration increases, the water potential around the seeds drops. This leads to a smaller difference in water potential between the inside and outside of the seed, which restricts the absorption of water by the seeds (Munns 2002). The impact of salinity on seed germination and plant growth can differ based on the plant species and the various genotypes within a species (Rajabi Dehnavi et al. 2020). Therefore, it is crucial to assess various genotypes in order to understand the mechanism of salt tolerance under different salt concentrations (Ranjbar et al. 2008). For this reason, in this study, we evaluated four sweet sorghum genotypes to understand the response to various salinity concentrations. Our results indicated that the sweet sorghum genotypes exhibited different responses to salinity in germination and seedling growth parameters. The findings of our study indicate that as the level of salt stress increased, there was a noticeable decline in all the seedling growth parameters of the sweet sorghum genotypes. The study found that as the salt concentration increased, the sorghum genotypes experienced a decrease in SL and RL. In saline conditions, it is common for plants to experience a decrease in the lengths of their seedling shoots and roots. This is due to the fact that roots are the first organs to come into contact with salinity, as they are in direct contact with the soil (Asaadi 2009). This finding aligns with previous studies conducted by Bashir et al. (2011), Nimir et al. (2014), Rajabi Dehnavi et al. (2020) and Özyazıcı and Açıkbay (2021). Furthermore, we discovered that the impact of salinity on root parameters was more severe than its influence on shoot parameters (Figure 2). For example, after the 6th day, the shoot continued to grow while the root length remained almost unchanged for Erdurmus. This difference might be attributed to the greater inhibitory impact of NaCl on root development compared to shoot growth (Rahman et al. 2001). Furthermore, the decrease in RFW, RDW, SFW, and SDW might perhaps be attributed to the toxic impact of Na⁺ on the rate of photosynthesis, particularly at elevated concentrations (Kawasaki et al. 1983). The results confirmed the observation of Rajabi Dehnavi et al. (2020) that salinity negatively affects plant growth parameters.

Table 2. Germination and seedling growth parameters of four sweet sorghum genotypes in four salinity levels

Genotypes	Salinity Concentrations (mM NaCl)				
	0	100	200	300	Mean
GP (%)					
Erdurmus	100.00	96.66	86.66	63.33	86.66
Uzun	100.00	76.66	53.33	46.66	69.16
Srg 156	100.00	96.66	80.00	53.33	82.49
BSS 424	100.00	56.66	33.33	10.00	49.99
Mean	100.00	81.66	63.33	43.33	72.08
GI					
Erdurmus	22.380	18.900	13.700	8.210	15.800
Uzun	13.960	12.740	6.060	5.470	9.550
Srg 156	19.520	17.980	11.570	6.520	13.900
BSS 424	10.380	8.330	3.640	0.890	5.810
Mean	16.563	14.492	8.747	5.277	11.265
RFW (g)					
Erdurmus	0.110	0.080	0.026	0.000	0.054
Uzun	0.096	0.073	0.010	0.000	0.045
Srg 156	0.116	0.056	0.006	0.000	0.045
BSS 424	0.056	0.036	0.000	0.000	0.023
Mean	0.095	0.061	0.010	0.000	0.041
RDW (g)					
Erdurmus	0.011	0.007	0.000	0.000	0.0045
Uzun	0.008	0.010	0.000	0.000	0.0045
Srg 156	0.012	0.007	0.000	0.000	0.00475
BSS 424	0.013	0.004	0.000	0.000	0.00425
Mean	0.011	0.007	0.000	0.000	0.0045
SFW (g)					
Erdurmus	0.360	0.276	0.123	0.046	0.201
Uzun	0.320	0.190	0.026	0.003	0.135
Srg 156	0.406	0.236	0.113	0.016	0.193
BSS 424	0.340	0.153	0.006	0.000	0.125
Mean	0.356	0.214	0.067	0.016	0.164
SDW (g)					
Erdurmus	0.030	0.027	0.012	0.000	0.0173
Uzun	0.025	0.021	0.001	0.000	0.0118
Srg 156	0.041	0.026	0.011	0.000	0.0195
BSS 424	0.030	0.017	0.003	0.000	0.0125
Mean	0.031	0.022	0.006	0.000	0.0153
RL (cm)					
Erdurmus	5.620	2.830	1.060	0.590	2.520
Uzun	4.770	3.560	0.730	0.240	2.330
Srg 156	6.260	2.570	0.810	0.340	2.740
BSS 424	4.020	1.990	0.450	0.230	1.670
Mean	5.167	2.74	0.760	3.350	2.320
SL (cm)					
Erdurmus	5.260	4.490	1.930	1.070	3.190
Uzun	5.340	4.060	0.780	0.250	2.600
Srg 156	6.490	3.640	2.120	1.320	3.390
BSS 424	6.860	4.020	1.080	0.400	3.090
Mean	5.980	4.050	1.480	0.760	3.070

GP: Germination percentage; GI: Germination Index; RFW: Root Fresh Weight; RDW: Root DryWeight; SFW: Shoot Fresh Weight; SDW: Shoot Dry Weight; RL: RootLength; SL: Shoot Length.

Seedling growth measures, such as GP and GI, exhibited a similar pattern to that reported for germination parameters. Salinity is well recognized to have a negative impact on GP and GI (Rehman et al. 2000). The effect of salinity varies depending on the degree of salt content. Low levels of NaCl induce seed

dormancy, whereas high levels of NaCl impede seed germination due to the impact of increased osmotic potential and the toxicity of particular ions (Khan et al. 2008). In this study, we did not identify a salinity effect on GP at a relatively low salinity level of 100 mM NaCl in two genotypes, Sr 156 and Erdurmus.

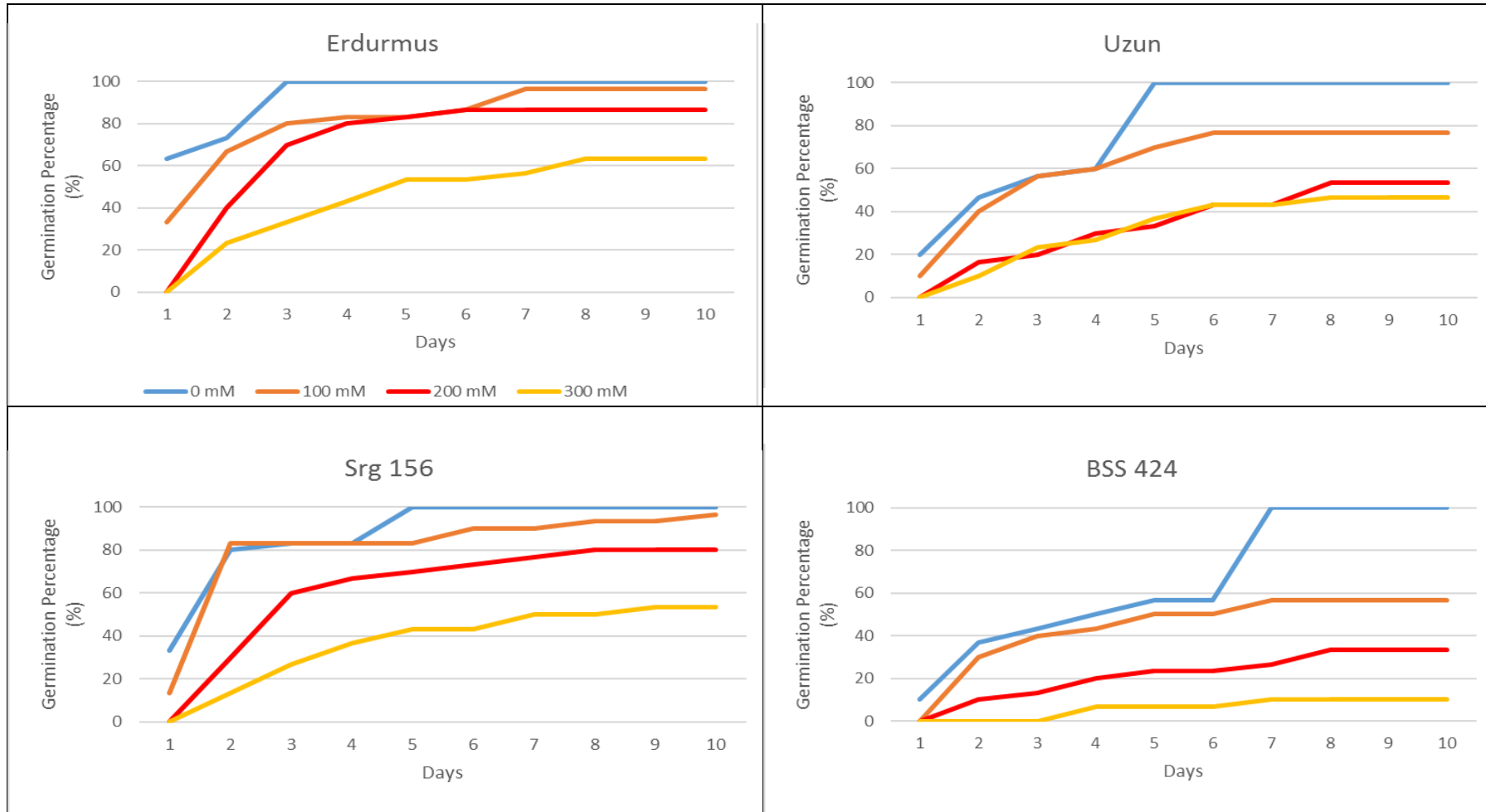


Figure 1. Effect of different levels of salinity stress on germination percentage.

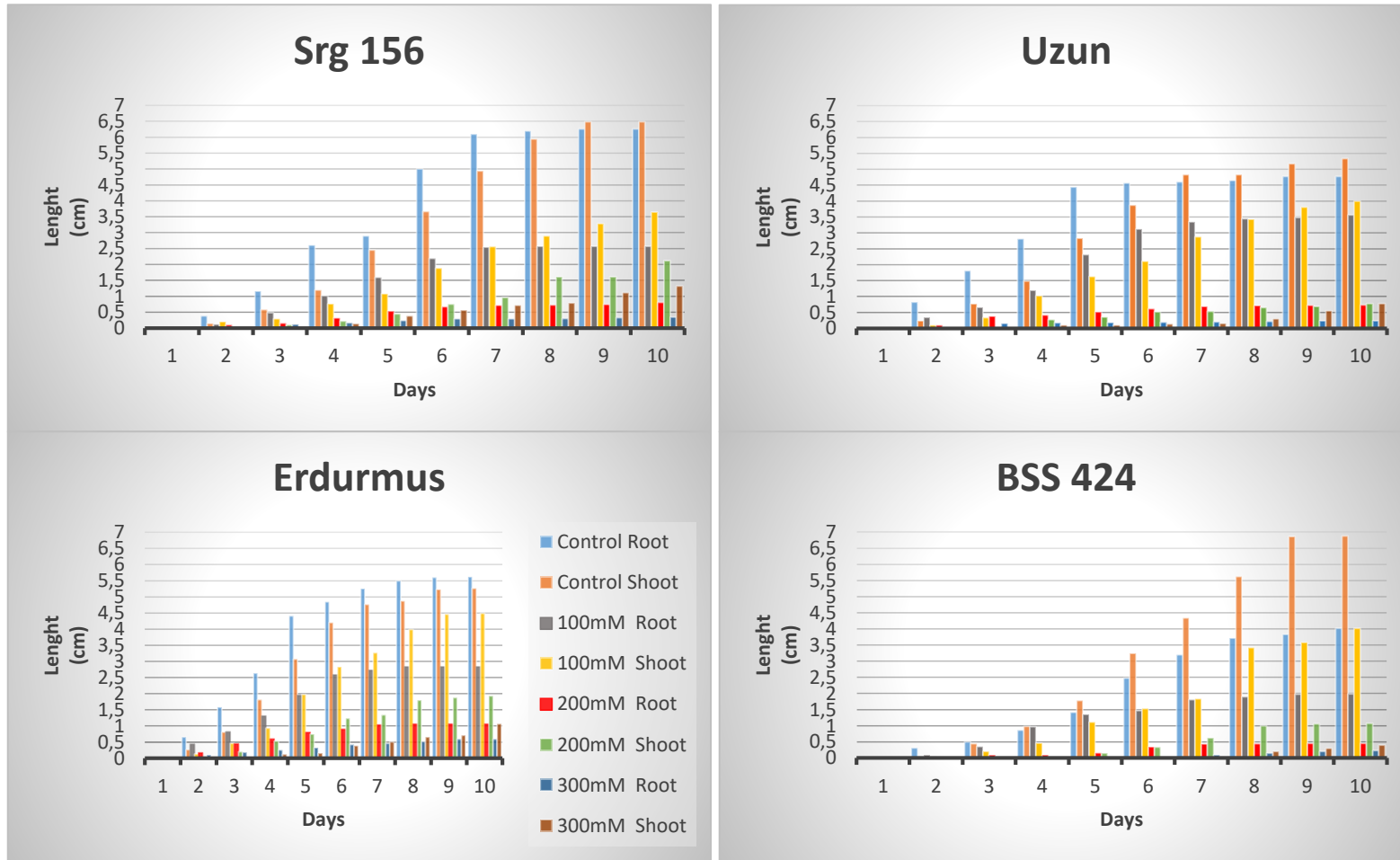


Figure 2: Effects of salt stress on root and shoot lengths of different genotypes of sweet sorghum.

Table 3. Mean Mg, Ca, Na, and K concentrations in shoots at two NaCl levels (%) for four sweet sorghum genotypes

Genotypes	Mg (%)		Ca (%)		Na (%)		K (%)	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Erdurmus	0.376	0.190	0.226	0.066	0.136	3.250	1.083	0.916
Uzun	0.420	0.180	0.220	0.050	0.160	3.280	1.016	0.893
Srg 156	0.260	0.120	0.156	0.066	0.090	2.890	1.146	0.883
BSS 424	0.220	0.123	0.146	0.050	0.090	1.973	2.050	1.550

However, salinity significantly influenced the germination parameter GI (Table 1). Moreover, among all genotypes examined in the present study, Erdurmus, Uzun, and Srg 156 exhibited the lowest reductions in GP and GI, whereas genotype BSS 424 had the most reductions, in comparison to the control group. Several investigations have shown that genotypes that exhibit better germination rates in salty conditions are considered salt resistant and tend to have more biomass and yield (Ashraf et al. 2006; Shakeri and Emam 2017; Rajabi Dehnavi et al. 2020). Therefore, based on germination parameters, the genotypes Erdurmus, Uzun, and Srg 156 may be considered salt tolerant, whereas the genotype BSS 424 can be considered salt sensitive. The variations in germination parameters across sorghum genotypes seem to be attributed to genetic factors and variations in inheritance (Kausar et al. 2012).

Excessive levels of Na can result in an accumulation of toxic compounds in sorghum leaves (Netondo et al. 2004a) which can negatively impact the absorption and movement of essential nutrients such as K, Ca, and Mg (Bavei et al. 2011; Joardar et al. 2018). Uzun exhibited the highest salinity accumulation, while also demonstrating the lowest ion (except for K) uptake in comparison to the control conditions and other genotypes. This result aligns with the research conducted by Calone et al. (2020), which stated that higher Na accumulation was associated with lower Ca and Mg accumulation (Table 3). Moreover, Uzun was determined to be one of the genotypes showing low FSW in response to increasing salinity stress. Erdurmus and Srg 156 exhibited a comparatively lower decline in ion content despite their elevated sodium accumulation, and both had higher FSW as compared to other genotypes (Table 2, 3). These findings confirm the conclusion that plants exposed to salt stress have a lower biomass because of increased respiration (Ashraf 2004). Increased ethylene release during periods of stress may have hindered the development of both roots and shoots, resulting in a decrease in their growth rates (Sagar 2017).

5. Conclusion

The results of this study showed that as salinity concentration increased, germination and seedling growth parameters, as well as ion uptake, decreased. The genotypes Erdurmus and Srg 156 have shown better performances, particularly in terms of germination seedling growth parameters. These genotypes should be further assessed in field conditions to determine their agro-morphological characteristics.

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Incubation characteristics and their relation to the age of the parent flock for broiler production

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ABSTRACT

The aim of this study was to examine the incubation characteristics of hatching eggs from one of the widely distributed hybrid combinations of classic broilers globally and to trace the dynamics of their changes depending on the age of the parental flock. The study covered a 12-year period - between 2011 to 2023. The share of quality chicks hatched ranged from 76.8% to 91.6%, with average values of 84.1-84.3%, while the share of culled chickens was within the normal range (0.4 to 1.7%). The proportion of culled eggs during candling on the 7th day varied between 4.3% and 14.7%, with average values of 7.64-8.24%. With the increase in the age of the parental flock, there was a stable tendency to increase the share of culled eggs at the first test egg cull, particularly due to the increase of the share of infertile eggs. The age of the parental flock determined more than 38% of the manifestation of the egg fertility trait and about 45% of that of quality chicks. Its influence on total embryonic mortality was significantly weaker ($R^2= 0.1456$). The study of the relationship between the traits "share of quality chicks" and "share of infertile eggs" showed that in the examined dataset, the correlation between them was moderate, negative ($r=-0.472\pm 0.100$ at $P<0.001$), with a linear character ($L= 0.046\pm 0.062$).

1. Introduction

The hatchery, in its simplest definition, is an enterprise for egg incubation, hatching, and the delivery of chicks (EC 617/2008). It plays a crucial role in the breeding center – hatchery – broiler production chain (Yassin et al. 2008). According to definitions, industrial hatcheries, depending on their purpose, can operate as enterprises with closed or open production cycles (Official Gazette 2006). Closed-type hatcheries are integral parts of poultry complexes or large farms with a clearly defined production specialization and productivity direction. The process of incubation and hatching is a part of the overall technological process on the farm. Large, specialized poultry facilities for broiler chicken meat production usually have their own facilities for raising parental birds, hatcheries, feeding centers, and even their own slaughterhouses. In this way, their hatcheries always work with fresh eggs (up to 7 days after laying), providing direct information about hatchability and incubation performance related to the flock and the age of the birds.

Non-specialized industrial hatcheries do not have a clearly defined production specialization and productivity direction. They are usually not linked to their own production of hatching eggs and operate as independent enterprises. Throughout different periods of the year, depending on the specific market needs, eggs from various productive directions (egg and meat production or dual-purpose) of the same bird species can be hatched, intended for both industrial farms and small producers, and even for personal use in the backyard poultry farming (Genchev and Lukanov 2023).

Farms specializing only in the production of hatching eggs can be standalone units or more often are found in combination with a hatchery. In these conditions, they operate only in the hatching egg market, and for economic reasons, their hatcheries are primarily loaded with eggs that cannot be sold on the market. As a result, they do not always have objective and reliable information about the real incubation qualities of the eggs they offer on the domestic or international market. In such a work scheme, the only way to have reliable information is through feedback with trusted hatcheries to which they sell guaranteed fresh hatching eggs obtained from parental flocks of a certain known age.

Considering these circumstances, the aim of this study was to examine the incubation characteristics of the hatching eggs and track the dynamics of their change depending on the age of the parent flock for one of the widely distributed hybrid combinations of classic broilers on a global scale.

2. Material and Methods

The study covered a 12-years period: from 2011 until 2023. The presented results are based on samples of over 243000 incubated eggs from the Ross 308 hybrid (Aviagen®). The study explored the dynamics in the incubation characteristics of eggs obtained from parent stock between 28-49 weeks of age.

During the specified period, two farms, significant producers of broiler eggs in Bulgaria, supplied the hatchery with hatching eggs. From 2011 to 2016, the supplier was "Farm 1", based in

Northwestern Bulgaria. From 2017 to 2023, hatching eggs were supplied from “Farm 2”, located in the central part of Southern Bulgaria. Both facilities provided information on the age of the parental flocks and the date of egg collection.

Transport conditions, egg storage until setting into the incubator, incubation and hatching regimen corresponded to the requirements for meat type hybrids of the chicken species (Genchev and Lukanov 2023). During loading into incubation trays, eggs with compromised shell integrity, those with contamination, deformities, and other shell-related issues, as well as deviations in the egg shape and weight, were separated as “unsuitable for incubation eggs”. All loaded eggs were candled on the 7th day of incubation, at which point the incubation cull was recorded as “culled eggs at first candling”. The number of hatched healthy chicks (“quality chicks”) and those culled due to deviations from the norm (“culled chicks”) was documented during hatching. The quantity of unhatched eggs was recorded at the end of incubation. According to the technology used in the country for chicken quality, chickens are classified as good (class A) or acceptable (class B) quality if they meet the following requirements: vital and active, without visible developmental disorders; the down - dry, clean, sufficiently long and shiny; the eyes - wide open, shiny and protruding; the legs - without swelling and hemorrhages; the navel - clean and completely closed. All day-old chicks that do not meet these requirements are classified as class C (culling).

The results obtained were compared to the quantity of eggs loaded for incubation, calculated the share of:

- culled eggs at first candling (%) – unfertilized eggs and eggs with embryos dead by the 7th day.
- quality chicks (%) – quality chicks suitable for rearing.
- culled chicks (%) – hatched chicks with various physical defects, deformities, and other quality deviations incompatible with further rearing.
- unhatched eggs (%) – eggs with embryos dead between the 7th -21st days. Mortality in the middle and at the end of incubation, technological culling from eggs with compromised shell integrity during the incubation period, were covered.

Statistical analysis was conducted using specialized software IBM® SPSS® Statistics (V26). The statistical comparison was made by the least significant difference (LSD) test at the 95% probability level. All data were reported as mean \pm standard error of the mean ($\bar{x} \pm \text{SEM}$). Statistical significance was established at $P < 0.05$. Dispersion analysis and calculations for elucidation of

the strength of the influence of individual factors on the determination of the studied traits were made. The graphical design was created by using Microsoft Excel 16.0 (2018, for Windows).

3. Results and Discussion

The results presented in Figure 1 show that the average share of eggs not suitable for incubation purchased from Farm 1 varied between 0.24% - 0.91%. Although within normal limits, this number of culled eggs reflects on the value of the purchased hatching eggs, increasing their relative price on average by 0.49%. For eggs purchased from Farm 2, the share of culled eggs was more than 2 times higher and, over the study period, averages up to 1.20%, with variation in the average values over the individual years ranging from 0.69% to 1.71%. This leads to an increase in production costs at the hatchery by an average of 1.46%. If we consider a comparable average value of 0.28 EUR for a hatching egg based on international prices, the difference between the two farms reached 2.88 EUR per 1000 eggs set.

Comparing the results of the culled eggs at first candling we did not find a significant difference between both farms (Table 1). The percentage of culled eggs during candling on the 7th day in the individual hatching batches ranged from 4.3% to 12.6% for Farm 1 and from 5.7% to 14.7% for Farm 2. The analysis of the degree of dispersion of values for the studied characteristic shows that the sample is approximately homogeneous (CV 26.4% for Farm 1 and 22.4% for Farm 2). The result obtained is within normal limits for the hybrid (Yousaf et al. 2018), considering the fact that over different years, egg from parents of different ages have been loaded into the hatchery.

The most crucial factor determining the efficiency of a hatchery is the hatchability from eggs set, particularly the share of quality chicks. The current understanding of the limits of the hatchability from eggs significantly differs from that of 30 years ago. Until the early 1990s, it was considered normal, in broiler production, for hatchability to be within the range of 78-80%, but not lower than 75% (Markaryan et al. 1986; Danilova et al. 1987; Fisinin et al. 1988; Burtov et al. 1990). However, for modern high-productivity broiler hybrids, cumulative hatchability below 80% for the whole productive period of parental birds is deemed unacceptable (Cobb-Vanress 2020; Aviagen 2021; Hubbard Breeders 2022). According to Pizzari (2017), the goal is to achieve an average hatchability of 83% for the 40-week productive period of the parent’s stock of the Ross 308 hybrid.

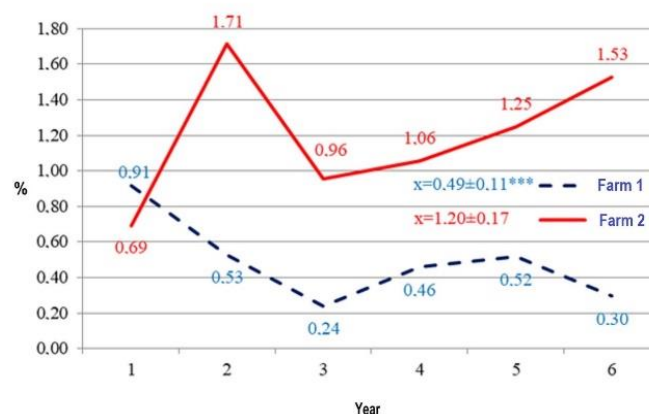


Figure 1. Share of the unsuitable eggs for incubation, %.

Table 1. Incubation characteristics, % from the eggs set

Years	Parameters*			
	1	2	3	4
	Farm 1			
2011	9.34±0.43	83.10±0.57	0.74±0.06	6.82±0.37
2012	5.58±0.27	87.94±0.64	1.10±0.01	5.38±0.49
2013	7.22±0.67	84.72±0.67	0.80±0.06	7.25±0.20
2014	5.05±0.31	83.05±0.76	1.18±0.14	7.54±0.63
2015	6.83±0.36	83.27±1.19	1.07±0.02	8.73±1.02
2016	9.57±0.63	80.41±0.94	1.08±0.02	8.94±0.99
Average	7.64±0.25	84.07±0.42	0.99±0.03	7.28±0.28
min-max	4.33÷12.63	76.84÷91.60	0.37÷1.77	1.79÷14.57
	Farm 2			
2018	7.23±0.31	84.84±1.04	1.09±0.02	6.84±0.75
2019	9.43±0.49	82.92±0.39	1.02±0.03	6.63±0.50
2020	7.75±0.65	86.02±1.06	1.08±0.02	5.14±0.55
2021	7.76±0.58	84.81±0.71	1.09±0.01	6.35±0.47
2022	7.74±0.43	84.33±0.49	0.98±0.06	6.95±0.30
2023	10.37±1.44	81.86±1.44	0.75±0.16	6.98±0.40
Average	8.24±0.28	84.33±0.37	1.02±0.02	6.41±0.22
min-max	5.70÷14.74	78.48÷89.22	0.23÷1.16	3.59÷10.08

Note: * 1. culled eggs at first examination; 2. quality chicks; 3. culled chicks; 4. unhatched eggs.

Our results indicate that for individual hatching batches, over the entire study period, the proportion of hatched quality chicks ranged from 76.8 to 91.6% for eggs from Farm 1 and 78.5-89.2% from Farm 2. The average values for this trait between both farms are practically indistinguishable, and the low coefficient of variation indicates high uniformity in the sample (CV 2.9-3.9%). The proportion of hatched but culled chicks for both farms ranged between 0.23 and 1.7%, which is within the normal range for hatchery practices (Orlov 1987; Mymrin 1989), complies with standards for meat-type hybrids (Buryan 2006; Mailyan 2009; Genchev and Lukanov 2023).

Reviewing literature on embryonic mortality in the Ross 308 hybrid between the 7th and 21st days, we found that the norms are highest during the chick hatching phase, ranging from 2.5-3.5% (Tullet 2009). This mortality is influenced by the age of the parental flock, with higher mortality at the beginning (up to peak of egg laying) and at the end of the productive cycle (after 50 weeks of age) (Yousaf et al. 2018). Cumulative culling from “unhatched eggs”, according to hybrid producer, may reach 4.5-6.5% of eggs set (Tullet 2009). Other authors suggest a lower cumulative culling for the entire productive period, up to 4% (Dyadichkina 2016), but it can also reach 8-10% of eggs set (Markaryan et al. 1986; Burtov et al. 1990). Comparing our results, we found that average values during the study period varied between 5.12 and 8.94%, consistent with those presented in cited literature.

Analyzing the results, accounting for the age of the parental flock, reveals a consistent trend of increasing culling rates upon initial inspection (7th day) overlapping with that of the share of unfertilized eggs (Figure 2). The studied production period shows that early embryonic mortality ranges from 2.1 to 4.3% of eggs set, comparable to published Ross hybrids norms (Tullet 2009), confirmed and by Yousaf et al. (2018).

One of the most important components of egg culling up to the 7th day is the share of unfertilized eggs. In Figure 2, it is observed that up to the age of 39 weeks, their percentage slowly changes from 2.2-2.3% at 30-33 weeks of age to 3.7-3.9% in the period of 35-39 weeks. The results obtained during the peak laying phase of the parental flock are comparable to the norms

for the same period outlined in the technological documentation of the other two leading broiler hybrid producers, Cobb and Hubbard (Cobb-Vantress 2020; Hubbard Breeders 2022). From the age of 40 weeks, the proportion of infertile eggs increases more intensively and reaches its peak levels between 42 and 44 weeks of age. Usually, this is the age at which, in practice, some age roosters from different housing areas are exchanged, or some of the less active roosters in the flock are replaced with younger ones. The purpose of such measures is to maintain hatchability at a satisfactory level by increasing the fertility of the eggs in the parental flock (Pizzari 2017). The observed decrease in the percentage of infertile eggs during the 46-49 weeks studied age period confirms the aforementioned.

Another trait with a high relative “weight” for the final incubation result is the embryonic mortality between the 8th and 21st days. While mortality in the middle stage of incubation (8-18 days) is insignificant and usually within the range of 0.5-1% of eggs set, embryonic mortality in the 18-21 day period reaches 3-6% (Dyadichkina 2016; Yousaf et al. 2018). Figure 3 presents the results of embryonic mortality for the 21-week period studied, including early mortality and the share of unhatched eggs.

During the specified period, the total embryonic mortality was most commonly within the range of 9 to 11% of eggs set, with two prominent peaks at 31 and 39-42 weeks of age of the parental flock. The reasons for these peaks vary. The first peak may be attributed to permissible errors in temperature control during both in egg cooling at farm level or in egg transport from the farm to the hatchery. The second peak's cause may be linked, on one hand, to the quality of the hatching eggs and, on the other hand, to a potential mistake during the hatching period. The total embryonic mortality for the studied period is close to the norm (7.5-11%) for the Ross 308 hybrid (Tullet 2009), and generally corresponds to this cull in the other two world leading broiler hybrids Cobb 500 (6.7-11.3%) and Hubbard (6.6-10.7%) (Cobb-Vantress 2020; Hubbard Breeders 2022). Comparing embryonic mortality over different periods, we found that the share of early mortality constitutes 21.8-35.8% of the total embryonic mortality. A significantly larger percentage of

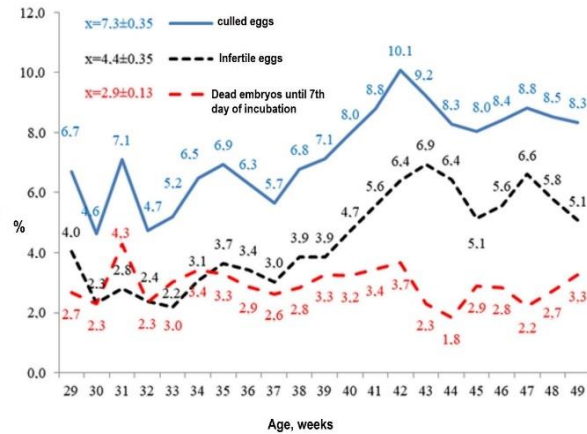


Figure 2. Culled eggs at first candling (7th day), % of eggs set.

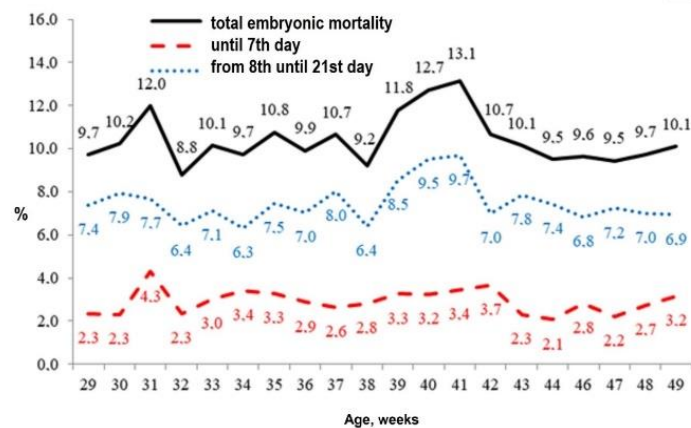


Figure 3. Embryonic mortality, % eggs set.

embryos die during the 8-21 day period, accounting for about 64.2-78.2% of the total mortality. Our result notably differs from literature data for this meat hybrid, with early embryonal mortality at 57.5% and late embryonal mortality at 38.4% (Peñuela and Hernandez 2018).

The culling due to infertile eggs and embryonal mortality, determines the hatchability of the eggs set (Figure 4). The percentage of hatched broilers during the period studied varied between 82.4 and 88.8% of eggs set, which is close to the results of the best flocks of the Ross 308 hybrid (84.4-89.3%) (Aviagen 2021). Similar hatchability from the eggs set is also demonstrated by the best flocks of the other two widely used broiler hybrids globally (83.9-87.4% for Cobb 500 and 82.6-89.4% for Hubbard) (Cobb-Vantress 2020; Hubbard Breeders 2022). The cumulative hatchability rate in this study was over 85% of hatching eggs set, with the lowest values recorded during the 40-43 week of age (82.4-83.6%), coinciding with the peak of infertile eggs. This trait is dependent on the number of quality broilers, suitable for placement and further rearing, and the quantity of the low-quality chicks (culled chickens). The proportion of culled chickens is an indicator of compliance with the incubation and, most importantly, the hatching process. Over the study period, the reported percentage of culled chickens in the hatchery was within the range of 0.8-1.3%, fully aligning with modern standards (0.5-1.5%) for this trait (Dyadichkina 2016).

The share of quality broilers hatched, suitable for placement and further rearing, as well as hatchability, was negatively

influenced by the age of the parental flock. The figure clearly shows that up to 39 weeks of age, the percentage of quality chicks hatched remained sustainably high (above 84% of hatching eggs set). After this age, the share decreased and was usually below 84%. A conducted analysis of variance for the results over the studied 21 week period shows that the influence of the factor "age of the parents" on the trait "quality chicks (%)" can be estimated at around 45% of the total variance ($R^2 = 0.4494$). To a greater extent, the result is influenced by random factors such as the management of the parent flock, conditions and duration of egg transport and storage, fertility, embryonal mortality, incubation and hatching regimen, etc. (Genchev and Lukanov 2023).

Another trait dependent on the age of the parent flock is "egg fertility". Evidence of this could be found in the correlation between the decline in hatchability and the peak values of infertile eggs (Figure 2 and 4). The analysis of variance showed that the strength of the influence of the age of the parent flock can be estimated at over 38% of the total variance of the trait ($R^2 = 0.3878$). Its influence on total embryonic mortality was significantly weaker ($R^2 = 0.1456$). Studying the relationship between the traits "quality chicks (%)" and "infertile eggs (%)" revealed a moderate negative correlation in the analyzed dataset ($r = -0.472 \pm 0.100$ at $P < 0.001$), with a linear character ($L = 0.046 \pm 0.062$). The valid equation for linear regression is $y = -0.531x + 89.685$. The analysis indicates that the share of quality chicks is a dependent trait on egg fertility, and the strength of this influence can be estimated at around 22% ($R^2 = 0.223$).

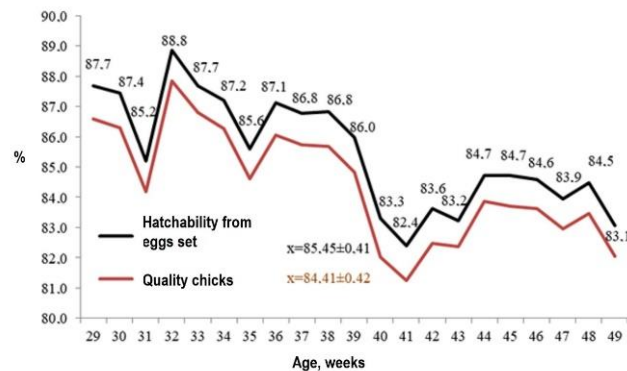


Figure 4. Hatchability and chick quality, %.

4. Conclusion

Based on the results obtained and the conducted analysis, we can conclude that the broiler chicken hatching eggs offered on both the domestic and international markets are of high quality. The proportion of quality chicks hatched is comparable to the target set by the hybrid combination's manufacturer, while culled chickens and culled hatching eggs fall within normal ranges. As the parental flock ages, there is a consistent trend of an increase in the culled eggs share during the first egg cull, primarily due to a rise in the percentage of infertile eggs. This constitutes a primary reason for the decrease in hatchability from the eggs set, which in turn affects the share of quality chicks.

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Developing a machine learning prediction model for honey production

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ABSTRACT

Türkiye, with its rich flora diversity, holds a significant share in global honey production. However, honey bee populations, essential for agricultural ecosystems, face multifaceted threats such as climate change, habitat degradation, diseases, parasites, and exposure to pesticides. Alongside the increasing global food demand driven by population growth, there is a pressing need for a substantial increase in honey production. In this context, advances in machine learning algorithms offer tools to predict future food needs and production levels. The objective of this work is to develop a predictive model using machine learning techniques to predict Türkiye's honey output in the next years. To achieve this goal, a range of machine learning algorithms including K-Nearest Neighbor, Random Forest, Linear Regression, and Gaussian Naive Bayes were employed. Following investigations, Linear Regression emerged as the most effective method for predicting honey production levels ($R^2=0.97$).

1. Introduction

Honey is the main product of beekeeping, which makes a great contribution to the development of rural areas. Honey produced by honey bees is derived largely from flower nectar and is a sweet, natural food transformed by a group of enzymes found in the saliva of worker bees. Honey is aerated to evaporate its water and then stored in hives (Atanasov et al. 2023). Also, honey is one of the most important agricultural products for the Turkish economy. Considered the agricultural beekeeping center of the future due to the richness of its flora (75% of the world's species and varieties) and geographical structure, Türkiye is the world's largest honey producer after China (Coşkun 2019; Atanasov et al. 2023). However, honey bees, which play a critical role in agricultural production and the environment, are threatened by various factors such as climate change, habitat loss, diseases, ecto- and endoparasites and pesticides (Brown et al. 2016; Potts et al. 2016; Pătruică et al. 2021). Biotic and abiotic factors that affect the health status of honey bees also affect honey production (Olate-Olave et al. 2021). Previous studies have shown that climate parameters such as temperature, humidity and rainfall affect honey production and honey quality (Oroian et al. 2017; Clarke and Robert 2018; Fatima et al. 2022; Şengül et al. 2023). In addition, honey bee losses caused by pesticide use and the decrease in foraging activity, resulting from diminishing the amount of agricultural and forest areas, lead to a decrease in honey production (Ferreira et al. 2015; Alqarni et al. 2021; Abay et al. 2023).

The exponential and unregulated growth of the global population will lead to a corresponding surge in the demand for food. For instance, it is stated that world food production must increase by approximately 60% to feed the world population in 2050 (Van Dijk et al. 2021). When we look at Türkiye specifically, it is expected that the country's population will reach 100.4 million by the year 2050 (TÜİK 2023). Considering the

current population of 85 million, at least a 20% increase in honey production is required to maintain the supply-demand balance. Given the challenges honey bees are now facing, it is crucial to enhance honey output and productivity in order to fulfill the future need for food.

Obtaining data on agricultural production before harvest provides significant advantages in both obtaining information about the production process and achieving sustainable development goals. In production estimates, reliable statistical procedures such as multivariate statistical methods are generally applied (Niazian and Niedbala 2020). Nowadays, with the rapid development of technology, approaches such as machine learning algorithms (MLA) based on statistical methods are widely used for production estimates and similar business processes (Ahmed and Hussain 2022). Machine learning (ML) is an important sub-branch of artificial intelligence, and its main purpose is to be able to handle complexities in large data sets and make such estimations because of their learning abilities (Kononenko 2001). While there is limited knowledge regarding the interaction of factors influencing honey production and their impact on honey production, one of the most significant approaches in this regard is the evaluation of honey production estimation within a ML model.

Looking at the literature, although a lot of work has been done on various processes in different species using machine learning, there are few studies on honey bees. Nevertheless, studies have shown that machine learning techniques are quite suitable and useful for analyzing beekeeping data. Prešern and Smodiš Škerl (2019) used the Gradient Boosting Machine algorithm of the machine learning software H2O to estimate the parameters affecting queen body mass. They developed three different models using different parameter combinations and in

their results, they determined that "ovary mass" and "breeder" parameters are the most important factors in model estimations. [Campbell et al. \(2020\)](#) used machine learning to evaluate the capacity of both weather data and satellite-derived vegetation data to develop a predictive model for Marri honey harvest in South Western Australia. Regression Trees were able to predict Marri honey harvested per hive to a Mean Error (MAE) of 10.3 kg. [Calovi et al. \(2021\)](#) aimed to evaluate the importance of weather, topography, land use and management factors on winter mortality in honey bee colonies using the Random Forest algorithm and to estimate survival given the existing factors. Random Forest estimated overwintering survival with 73.3% accuracy for colonies and 65.7% accuracy for beehives with managed Varroa mite populations. Additionally, growing degree days and precipitation in the warmest quarter of the previous year were the most important determinants at both levels. [Veiner et al. \(2022\)](#) tested three supervised learning algorithms (Random Forests, Lasso and Elastic net Regularized Generalized Linear Model, and Support Vector Machine) for their performance in characterizing transcriptomic patterns and identifying genes associated with honey bee waggle dance. By matching the analysis results with differential gene expression outputs, they identified two candidate genes for the neural regulation of waggle dance. [Braga et al. \(2023\)](#) developed a machine learning model to estimate temperature drops in honey bee colonies, the Long Short-Term Memory algorithm, which was applied to five real data sets with input factors of internal temperature, internal humidity, mean fanning, mean noise, mass and external temperature. Their results showed that they could predict the temperature 24 hours in advance with a Root Mean Square Error (RMSE) of 0.5%.

Previous studies have explored the impact of some environmental factors and climatic conditions on honey production ([Ferreira et al. 2015](#); [Oroian et al. 2017](#); [Clarke and Robert 2018](#); [Alqarni et al. 2021](#); [Fatima et al. 2022](#); [Abay et al. 2023](#); [Şengül et al. 2023](#)). Drawing on the information obtained from these studies, this study attempted to develop a predictive model using machine learning techniques to predict Türkiye's honey output over the next years.

2. Material and Methods

2.1. Material

This study incorporates eight distinct attributes: honey production volume, number of enterprises, number of colonies, pesticide application, agricultural and forest land coverage, as well as temperature and rainfall. The selection of these attributes was informed by their relevance to honey production dynamics. Given the systematic recording of agricultural data in Türkiye since the early 2000s, this study utilized annual data collected between 2000 and 2022 as the TrbalDataSet training set. Agricultural data was sourced from the Republic of Türkiye Ministry of Agriculture and Forestry website, while meteorological data was obtained from the Turkish State Meteorological Service website. There is no null value in the data set. In the study, the min-max normalization method was applied to normalize the data set values between 0 and 1.

2.2. Methods

The study employed the Python programming language, along with popular libraries such as Pandas, Numpy, Matplotlib, and Scipy, for coding machine learning algorithms. A 75% portion of the dataset was allocated for the training phase of each

algorithm, while the remaining 25% served as the test set to assess the accuracy of predictions derived from this training.

The creation of estimation models involved the selection of commonly used machine learning methods, outlined below:

- K-Nearest Neighbors (KNN): KNN is a non-parametric supervised learning algorithm employed in classification and regression domains. Its popularity has surged across diverse fields due to its simplicity of application and reliance on a straightforward mathematical foundation. The algorithm identifies the nearest neighbors to a given point and utilizes these points for estimations. At its core, KNN operates on the concept that the outcome of an event mirrors that of its closest neighboring events. The parameter "K" signifies the number of closest points considered in the estimation process ([Hai et al. 2023](#)).

- Random Forest (RF): This algorithm operates as an ensemble learning method founded on decision trees, a type of supervised learning technique commonly utilized in machine learning to construct estimation models. Specifically, it forms a random forest, which is an assembly of decision trees trained through the bagging method. The rationale behind employing the bagging method is to enhance overall results by combining multiple learning models ([Breiman 2001](#)). The Random Forest approach proves highly effective when dealing with datasets featuring a multitude of predictors, especially in cases where variable relationships are nonlinear or intricate. This is because it offers flexibility and is not constrained by specific distributions ([Shoemaker et al. 2018](#)).

- Linear Regression (LR): Linear Regression stands as a machine learning technique employed to quantify the association between two variables. The objective of this method is to model the linear correlation between the independent variable (x) and the dependent variable (y). Its primary aim is to predict the dependent variable based on the values of the independent variables ([Maulud and Abdulazeez 2020](#)).

- Gaussian Naive Bayes (GNB): Developed on the principles of Bayes theorem, Naive Bayes stands out as one of the most straightforward, comprehensible, and practical machine learning algorithms employed in classification tasks. The term "naive" is attributed to the algorithm due to its assumption of independence among features during the classification process ([John and Langley 2013](#)).

The test set was contrasted with the values generated by the algorithms' estimations, and the evaluation encompassed the coefficient of determination (R^2) along with error metrics, including Root Mean Square Error (RMSE), Mean Absolute Percentage Error (MAPE), and Mean Absolute Error (MAE) ([Rahman et al. 2021](#)). Previous studies ([Gültepe 2019](#); [Rahman et al. 2021](#)) indicate that algorithms exhibiting R^2 values nearing 1 and error values approaching 0 are considered the most effective.

3. Results and Discussion

In tests conducted with machine learning, metrics such as R^2 , MSE, RMSE and MAE were taken into account in evaluating the performance of the algorithms. Accordingly, in [Table 1](#), the lowest RMSE (2966.83) value among the algorithms for honey production estimation was calculated for LR. Similarly, in terms of MAE (2455.34), MAPE (0.03), MedAE (2953.96) values, the lowest was calculated for the LR algorithm. Another important metric that measures the performance of the ML model is R^2 .

Table 1. Comparison of the results of machine learning algorithms used on TrbalDataSet

Algorithms	R ²	RMSE	MAE	MAPE	MedAE
K-Nearest Neighbors (KNN)	0.60	11635.88	9265.17	0.10	6998.50
Random Forest (RF)	0.58	11864.28	9678.50	0.10	8238.50
Linear Regression (LR)	0.97	2966.83	2455.34	0.03	2953.96
Gauss Naive Bayes (GNB)	0.60	11635.88	9265.17	0.10	6998.50

* R², coefficient of determination; RMSE, Root Mean Square Error; MAE, Mean Absolute Error; MAPE, Mean Absolute Percentage Error; MedAE, median absolute error.

Looking at the table 1, R² values for KNN, RF, LR and GNB are calculated as 0.60, 0.58, 0.97, 0.60 respectively. The highest R² value was obtained for LR, indicating higher accuracy.

After data learning, the regression graphs obtained for trains and tests are shown in Figure 1. The obtained graphs demonstrate the concordance between the algorithm and real data. When examining the graphs, it is evident that the Linear Regression algorithm is the most compatible with the real data.

According to the scores obtained, the Linear Regression method showed the highest performance and provided convincing evidence that annual honey production estimation is possible. When we estimate honey production in 2050 with the data between 2000 and 2022 using this algorithm, it is expected that 218,271.34 tons of production will occur. This amount is more than 60% of the 2022 honey production amount and meets the previously mentioned food production increase required by 2050 (Van Dijk et al. 2021). Honey production is affected by environmental factors (Ferreira et al. 2015; Oroian et al. 2017; Clarke and Robert 2018; Alqarni et al. 2021; Fatima et al. 2022; Abay et al. 2023; Şengül et al. 2023). Suitable areas are required to carry out beekeeping activities, and forest areas are ideal regions for beekeeping in terms of climate conditions and vegetation (Güngör and Ayhan 2016). In particular, the honey forest action plan, which was implemented by the Türkiye Ministry of Agriculture and Forestry in 2013 to increase honey production and is still ongoing (Karaağaç and Bulut 2023), is thought to have had a significant impact on the increase in the honey production amount, number of enterprises, number of colonies, and amount of forest area over the last 10 years. Considering these effects, similar projects should be increased in the coming years and Türkiye's beekeeping potential should be utilized more. Thus, by increasing honey production, positive results will be achieved in terms of food security.

No prior studies have been identified in Türkiye where the quantity of honey production was estimated using machine learning techniques. While various traditional statistical methods have been employed to estimate honey production, these approaches provide shorter-term and comparatively less reliable predictions. For instance, Burucu and Gülse Bal (2017) conducted a 7-year (2017-2023) estimation using the ARIMA model with data sourced from TURKSTAT, projecting a continuous increase in honey production in Türkiye, reaching 121,216 tons in 2023. In a separate study, the ARIMA model was utilized to predict honey supply and demand in Türkiye for the period 2016-2023. The study estimated honey supply per capita to be 1.43 kg in 2017 and 1.54 kg in 2023 (Naseri et al. 2016). Çukur and Çukur (2021) aimed to estimate the quantity of honey production in Türkiye using the Box Jenkins ARIMA model, incorporating honey production data from 1990-2019. The results suggested an estimated honey production of 123420 tons in the year 2025. However, these models tend to produce

inaccurate estimations when dealing with multidimensional input data. Recognizing this limitation, machine learning is now widely adopted, offering highly accurate predictions for complex activities, such as agricultural production, by considering numerous ecological variables (Rahman et al. 2021).

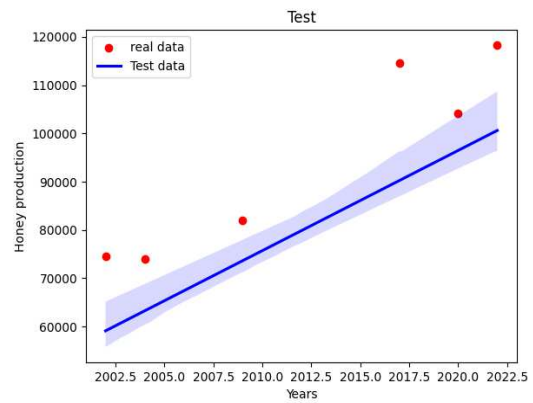
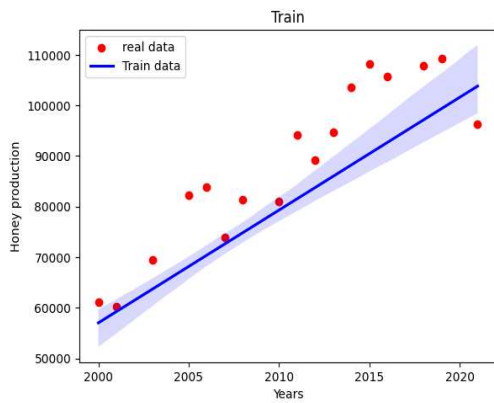
Nevertheless, the pervasive issue of climate change poses a significant challenge to the accuracy of future honey production estimates. Climate change has far-reaching implications for ecological systems and honey bee populations, being closely linked to colony collapse disorder (Pătruică et al. 2021; Şengül et al. 2023). The adverse effects on pollination activities and overall productivity, coupled with the potential increase in infectious diseases and parasites, such as Varroa destructor, highlight the vulnerability of honey bee colonies (Klein et al. 2007; Switaneck et al. 2015). Unfortunately, these challenges are expected to intensify in the years to come (Varol and Yücel 2019). To mitigate these risks, various adaptation strategies have been proposed, including reforestation, hive sterilization improvement, queen bee replacement, artificial feeding, breeding, migratory beekeeping, honey crop cultivation, changes in apiary management, adoption of good beekeeping practices, seeking technical assistance, and maintaining comprehensive records. However, obstacles such as insufficient funding, limited availability of suitable beekeeping land, and bureaucratic challenges in migratory beekeeping impede the widespread adoption of these strategies by beekeepers (Şengül et al. 2023).

4. Conclusions

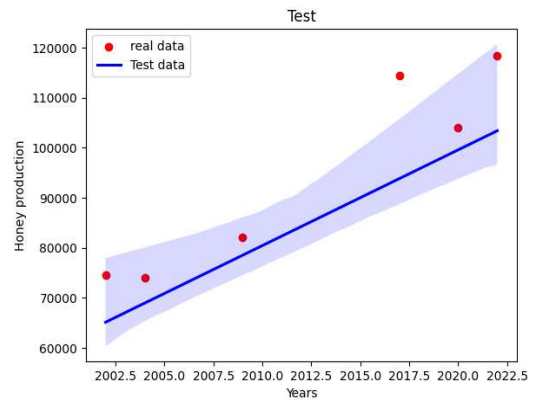
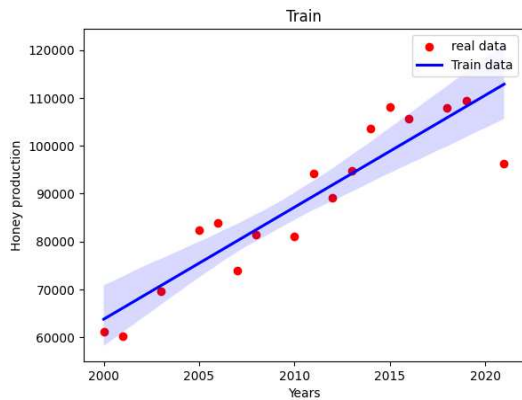
This study has utilized machine learning to create a prediction model for Türkiye's honey production, offering a fresh perspective to help understand the intricate dynamics of honey production. Despite the reliability of the metrics obtained from our analysis, it is advisable to consider additional production dynamics to offer a more comprehensive outlook for the upcoming years. Nevertheless, shortcomings exist in beekeeping production data within Türkiye, particularly in health records.. To improve production forecasts and realistically portray food supply and demand in the future, a meticulous record-keeping approach is essential. Moreover, it is crucial to embrace climate change adaptation strategies and to advocate for these strategies to be part of agricultural policy in order to sustain economic activities in beekeeping, especially honey production, in Türkiye.

In summary, while machine learning, specifically the Linear Regression method, proves invaluable in refining the accuracy of honey production estimates, the urgent challenges presented by climate change highlight the necessity for ongoing research, innovation, and collaborative endeavours. These efforts are critical for ensuring the sustainability of beekeeping practices and addressing food security concerns in the face of evolving environmental dynamics.

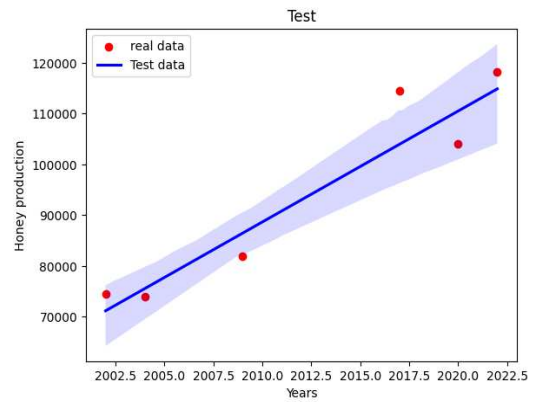
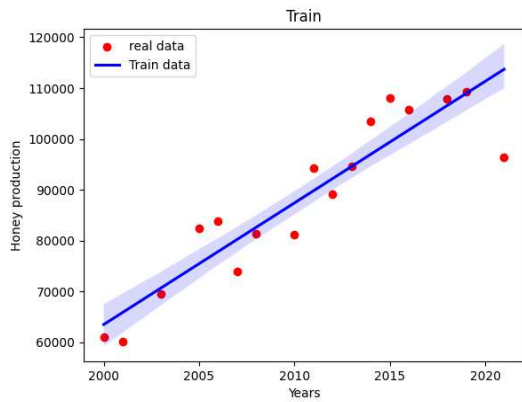
a)



b)



c)



d)

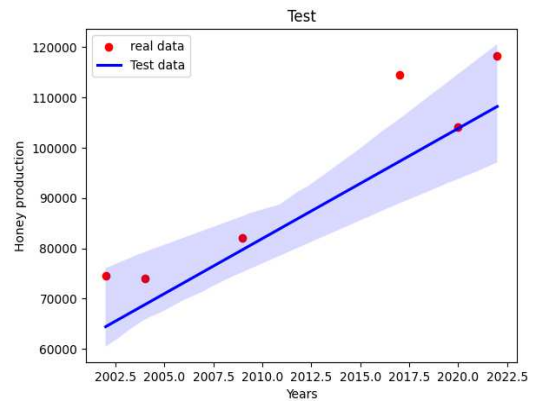
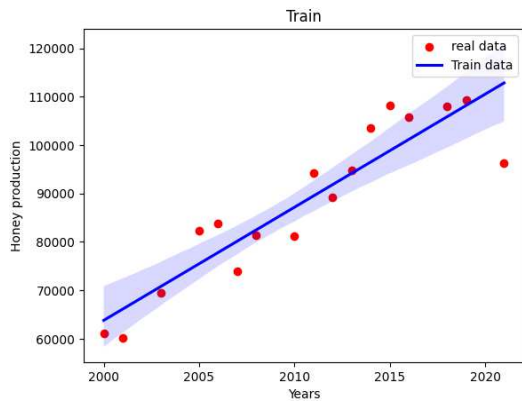


Figure 1. The train and test regression graphs obtained from the algorithms. The red dots represent real data, while the blue curves represent estimated data. a) KNN train and test graph b) RF train and test graph c) LR train and test graph d) GNB train and test graph.

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Keeve R, Loupser HL, Kruger GHJ (2000) Effect of temperature and photoperiod on days to flowering, yield and yield components of *Lupinusalbus* (L.) under field conditions. Journal of Agronomy and Crop Science 184: 187-196.

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Theses:

Sever Mutlu S (2009) Warm-season turfgrass species: Adaptation, drought resistance and response to trinexapac-ethyl application. PhD Thesis, The University of Nebraska, Nebraska.

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