



# RESILIENT AGRICULTURE WITH THE HELP OF CROP DIVERSIFICATION

EDITOR

Dr. Feyza Döndü BİLGİN



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Publishing House

# RESILIENT AGRICULTURE WITH THE HELP OF CROP DIVERSIFICATION

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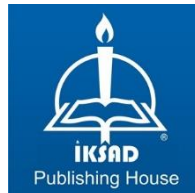
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Researches Publications®

(The Licence Number of Publicator: 2014/31220)

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Iksad Publications – 2022©

**ISBN: 978-625-8323-39-9**

Cover Design: İbrahim KAYA

July / 2022

Ankara / Turkey

Size = 16 x 24 cm

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## **PREFACE**

Proteins requirements in the diet is a challenge in feeding the global population. Protein rich crops such as legumes contribute to reduce environmental risk of agricultural production. Legumes are an important part of sustainable ecosystem services. Sustainable intensification of grain legumes optimizes food security on smallholder farms. Plant factors are affecting forage quality and grassland biodiversity. Climate change impact the adaptation and mitigation in agriculture and effect plants in complex multi-functioning landscapes. Resilient agriculture is targeting cultivation of food systems but also quality and utilization of agroecological resources for sustainable livestock farming. Improving the productivity of livestock with warm-season legumes will help to reduce climate change impact, adaptation, and mitigation in temperate grazing systems. As a result nutrition, feeding and management of beef cattle in intensive and extensive production systems get progress.

Apart from legumes, agricultural species diversification with crop such as sunflowers, vegetables, beets and many other plants from different families may help resilient agriculture in agricultural regions.

Here in this section, some of these subjects are presented for the agricultural experts and researchers.

Dr. Feyza Döndü BİLGİN



## CHAPTER 1

### DETERMINATION OF POMOLOGICAL AND SOME BIOCHEMICAL CHARACTERISTICS OF NATURALLY GROWING ROSE HIP (*Rosa canina* L.) GENOTYPES IN DIFFERENT LOCATIONS OF VAN PROVINCE

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## **INTRODUCTION**

The fruits of the *Rosa canina* plant are called as rose hips. The widely used fruits of *Rosa canina* are known as a rich source of antioxidants such as ascorbic acid and polyphenols. Rosehip has been used for food, medicinal and landscaping purposes since ancient times. In terms of its positive effects on human health, rosehip is in high demand today. *Rosa canina* L. (rosehip) is rich in sugars, vitamin C, pectin, tannins, amino acids, phenols, antioxidants, macro and micro elements and many other biologically active components (Demir and Özcan 2001; Orhan et al., 2009; Chai and Ding 1995; Ercisli 2007). Rosehip has a high phenolic content. These components, which are potential antioxidants, provide positive effects on human health (Kılıçgün and Altner 2010; Egea et al. 2010). Although it is rarely consumed fresh, rosehip fruit is usually dried and processed to be consumed as tea, nectar, wine or marmalade (Uggla, et al., 2005; Yıldız and Alpaslan, 2012).

The chemical content of rosehip fruit may vary according to species, cultivar, genotype, growth ecology, altitude, harvest season and fruit ripening stage. It is widely used in the food, pharmacy and cosmetics industry (Yıldız and Çelik, 2011). It is used in many European countries to enrich fruit jelly, baby food, fruit juice, marmalade, tea, pastry fruit and vegetable juices in terms of vitamins and as an additive in the confectionery industry (Kızılcı, 2005; Dölek, 2008). In Turkey, it is processed as fruit juice, marmalade, pulp and tea in factories and establishments in Gümüşhane, Erzincan and Tokat (Kızılcı, 2005). Rosehip species, which have not yet been adequately cultured in our country, are cultivated to a significant degree in the today's world.



(Ercisli, 2005). The fact that rose hips have been used by many civilizations for thousands of years is due to their positive effects on human health. Rosehip, which has a wide range of uses, especially in nutrition in terms of human health, unfortunately did not receive the necessary importance in our country until recent years. Today, as a result of intensive and comprehensive scientific research on this plant, it has been revealed that it is an important source of nutrients and is also rich in vitamins, minerals and phytochemicals. On the other hand, with the expansion of the product range, it is seen that our low-income farmers can obtain an important source of income from rosehip cultivation. However, for the production of abundant and high quality rosehips, it is necessary to create suitable environment and conditions.

## **1. MATERIAL AND METHOD**

The rosehip genotypes constituting the material of this study were selected from naturally grown populations in different regions of Van (Gevaş, Erciş and Çatak) at altitudes ranging from 1450 to 1789 m (Table 1). No fruit samples were taken from genotypes with very small, extremely prickly or contaminated fruit. Fruit samples selected from 30 different genotypes were harvested in triplicate during the ripening period. Some physical, chemical and biochemical analyzes were carried out on harvested fruit samples.

**Table 1.** Altitudes of the locations where *Rosa canina* L. genotypes were taken.

<b>Genotype</b>	<b>Altitude (m)</b>	<b>Genotype</b>	<b>Altitude (m)</b>	<b>Genotype</b>	<b>Altitude (m)</b>
<b>GE-KS-1</b>	1691	<b>ER-KS-1</b>	1772	<b>ÇA-KS-1</b>	1550
<b>GE-KS-2</b>	1691	<b>ER-KS-2</b>	1770	<b>ÇA-KS-2</b>	1578
<b>GE-KS-3</b>	1691	<b>ER-KS-3</b>	1765	<b>ÇA-KS-3</b>	1530
<b>GE-KS-4</b>	1688	<b>ER-KS-4</b>	1772	<b>ÇA-KS-4</b>	1620
<b>GE-KS-5</b>	1692	<b>ER-KS-5</b>	1784	<b>ÇA-KS-5</b>	1548
<b>GE-KS-6</b>	1690	<b>ER-KS-6</b>	1786	<b>ÇA-KS-6</b>	1660
<b>GE-KS-7</b>	1688	<b>ER-KS-7</b>	1785	<b>ÇA-KS-7</b>	1475
<b>GE-KS-8</b>	1683	<b>ER-KS-8</b>	1787	<b>ÇA-KS-8</b>	1450
<b>GE-KS-9</b>	1693	<b>ER-KS-9</b>	1789	<b>ÇA-KS-9</b>	1542
<b>GE-KS-10</b>	1695	<b>ER-KS-10</b>	1789	<b>ÇA-KS-10</b>	1582

### **Physical Parameters**

50 fruits taken randomly from each replication were weighed with 0.001 g precision digital scale and calculated as average fruit weight and expressed as g. The fruit pulp ratio was obtained by subtracting the seed weight from the fruit weight and dividing it by the fruit weight (Ercişli, 1996). Dry matter (DM) ratio was determined as % according to AOAC (1990).

### **Water Soluble Dry Matter, Titratable Acid Content, pH Value**

The water-soluble dry matter (WSDM) content from the droplets obtained from the juice taken by squeezing the rosehip fruits was determined using a digital refractometer (Karaçalı, 2012). Titratable acid content was measured from the NaOH content consumed by titration of 5 ml rosehip juice with 0.1 N NaOH to pH 8.1 (Karaçalı, 2012). The pH value was determined from the fruit juice using a pH meter (MP220, Mettler Toledo, Germany).

### **Vitamin C (L-Ascorbic Acid)**

Vitamin C contents of rosehip fruit samples were determined (Cemeroğlu, 2007).

### **Analysis of Phenolic Compounds in Fruit Juice**

In the separation of phenolic compounds by HPLC, Rodriguez-Delgado et al. (2001) was used by modifying the method.

### **Identification of the Species**

Flower and fruit samples were taken from the plants in order to determine the species to which they belong. German flower and fruit samples were examined with the naked eye and under a microscope in the Herbarium of the Faculty of Science and Letters of Yüzüncü Yıl University.

### **Statistical analyzes**

Descriptive statistics in terms of the features emphasized are expressed as mean and standard error. Duncan's multiple comparison test was used to identify different varieties following analysis of variance.

## **2. RESULTS and DISCUSSION**

*Rosa canina* genotypes, which grow naturally at an altitude of 1450-1789 m in the Gevaş, Erciş and Çatak districts of Van province, were investigated for two years (2015-2016). In this study, 30 promising genotypes with the highest WRM scores were selected and fruit characteristics were determined. The fruit weight, seed weight, seed

number, fruit flesh weight, fruit flesh ratio, fruit length, fruit width, fruit shape index recorded for promising genotypes are presented in Table 2. Fruit weight was recorded between 1.36-2.31 g. Eight genotypes (GE-KS-2, GE-KS-4, GE-KS-9, GE-KS-10, ER-KS-1, ER-KS-8, ER-KS-9 and ÇA-KS-1 ) varied in the range of 2.36-2.42 g. It was determined that seed weight was 0.20-0.50 g, 6-24 seeds per fruit, flesh weight 1.12-1.82 g, fruit pulp ratio between 77% and 93% (Table 2).

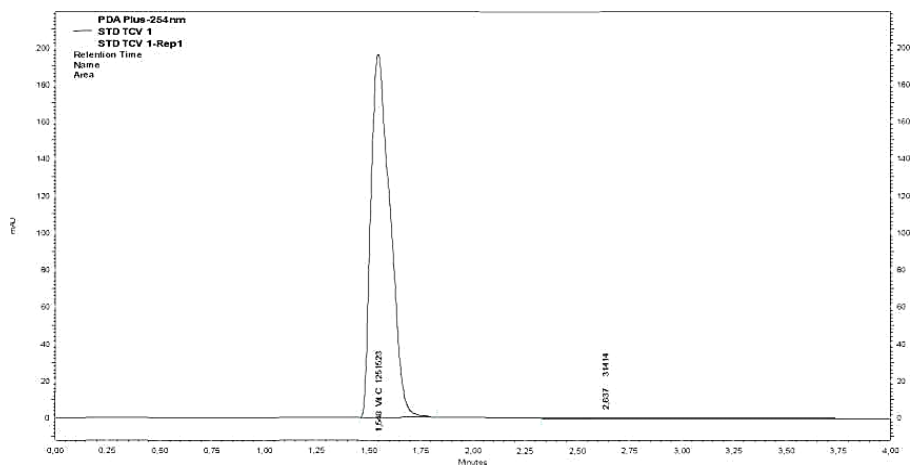
**Table 2.** Some fruit characteristics of some *Rosa canina* genotypes from different locations.

Genotype	Fruit Weight (g)	Seed Weight (g)	Seed Number	Fruit Flesh Weight (g)	Fruit Flesh Ratio (%)	Fruit Length (mm)	Fruit Width (mm)	Fruit Shape Index
GE-KS-1	2.16 abc	0.40 ab	18.2 cd	1.46 abcd	81.48 efghi	25.13 bcd	13.13 abc	1.91 bcdef
GE-KS-2	2.38 a	0.50 a	14.3 fg	1.84 ab	78.99 ghi	25.65 bc	12.75 abc	2.01 bcde
GE-KS-3	2.24 ab	0.40 ab	11.0 hi	1.60 abc	82.14 efgh	25.42 bc	12.42 abc	2.05 bcd
GE-KS-4	2.36 a	0.40 ab	20.4 c	2.12 a	83.05 efg	25.47 bc	13.52 ab	1.88 cdef
GE-KS-5	2.10 bc	0.40 ab	11.2 hi	1.60 abc	80.95 fghi	28.42 a	10.96 cd	2.59 a
GE-KS-6	1.96 cd	0.30 b	10.3 ij	1.48 abc	84.69 ef	25.36 bc	11.43 cd	2.22 b
GE-KS-7	1.85 cde	0.40 ab	16.5 de	1.54 abc	78.38 hi	21.98 def	10.50 cd	2.09 bc
GE-KS-8	2.19 ab	0.50 a	32.0 a	1.43 bcd	77.17 i	23.65 cd	11.87 c	1.99 bcde
GE-KS-9	2.38 a	0.50 a	15.8 ef	1.86 a	78.99 hi	26.34 b	12.01 bc	2.19 b
GE-KS-10	2.46 a	0.20 c	12.4 h	1.60 abc	91.87 b	22.80 d	10.35 d	2.20 b
ER-KS-1	2.38 a	0.30 b	06.41	1.85 a	87.39 de	24.60 cd	13.22 ab	1.86 def
ER-KS-2	1.76 ef	0.20 c	10.3 ij	1.76 ab	88.64 cd	21.42 efg	12.76 abc	1.68 f
ER-KS-3	1.64 fgh	0.20 c	11.4 i	1.48 abc	87.80 cde	21.36 efg	12.52 abc	1.71 ef
ER-KS-4	1.94 cd	0.30 b	08.6 k	1.64 ab	84.54 ef	21.20 fg	12.39 bc	1.71 ef
ER-KS-5	2.12 bc	0.30 b	09.2 j	1.63 ab	85.85 e	23.85 cd	13.24 ab	1.80 def
ER-KS-6	1.56 gh	0.10 d	03.9 m	1.25 cd	93.59 a	22.60 d	12.42 bc	1.82 def
ER-KS-7	1.92 cd	0.20 c	06.51	1.56 abc	89.58 c	20.32 ghi	11.87 c	1.71 ef
ER-KS-8	2.36 a	0.30 b	17.4 d	1.64 ab	87.29 de	23.44 cd	13.45 ab	1.74 ef
ER-KS-9	2.42 a	0.20 c	06.61	1.90 a	91.74 b	25.16 bc	12.36 bc	2.04 bcd
ER-KS-10	1.79 e	0.10 d	03.2 m	1.80 ab	94.41 a	23.15 d	12.94 abc	1.79 def
ÇA-KS-1	2.31 a	0.50 a	24.4 b	1.65 ab	78.35 hi	21.45 ef	13.20 ab	1.63 f
ÇA-KS-2	1.76ef	0.20 c	20.2 c	1.20 d	88.64 cd	22.26 de	13.54 a	1.64 f
ÇA-KS-3	1.94 cd	0.30 b	19.7 c	1.25 cd	84.54 ef	20.50 gh	12.63abc	1.62 f
ÇA-KS-4	1.74 ef	0.30 b	13.4 g	1.12 d	82.76 efg	19.76 hij	12.58 abc	1.57 f
ÇA-KS-5	1.58 gh	0.30 b	17.0 d	1.25 cd	81.01 fghi	19.50 ijk	13.66 a	1.43 g
ÇA-KS-6	1.82 de	0.30 b	16.2 de	1.34 cd	83.52 ef	19.69 hij	12.63 abc	1.56 f
ÇA-KS-7	1.56 h	0.30 b	15.7 f	1.25 cd	80.77 ghi	21.09 fg	12.15 bc	1.74 ef
ÇA-KS-8	1.65 fg	0.30 b	18.4 d	1.39 cd	81.82 efgh	21.20 fg	11.87 c	1.79 def
ÇA-KS-9	1.42 i	0.20 c	06.41	1.82 ab	85.92 e	19.20 jk	11.40 cd	1.68 f
ÇA-KS-10	1.36 i	0.20 c	16.8 de	1.76 ab	85.29 e	18.85 k	11.70 c	1.61 f

\* Duncan P < 0.05

The examined rosehip berries contained TTS between 19 and 24 brix° in seven genotypes (GE-KS-1, GE-KS-4, GE-KS-6, ER-KS-2, ER-KS-

4, ER-KS-5 and ER-KS-10) total soluble dry matter content was found to vary between 25.12-26.12%, TA 1.68% - 3.64%, pH value 3.12-4.05, vitamin C content between 122.10-685.16 mg/100g<sup>-1</sup> (Table 3; Figure one). All genotypes were found to have red, light orange and orange colored, oval, conical and cylindrical fruits. In terms of plant productivity, the genotypes in Erciş location were high, the genotypes in Gevaş location were medium and the genotypes in Çatak location had low productivity (Table 3).



**Figure 1.** Standard chromatogram of vitamin C.

In the study, the amounts of Elagic, Protocatechive, Rutin, Quercetin, Gallic, Catechin, Chlorogenic, Caffaik, Syring, P-Cumarik, Ferulic, Phlorodizin in the fruits of *Rosa caninas* taken from different regions were determined by HPLC. After determining the chromatogram of the standards of phenolic compounds in the study, the calculations were made according to these standards (Figure 2). Differences of *Rosa canina* genotypes in terms of Protocatechive contents were found to be

statistically significant. The highest value was determined as 96.2  $\mu\text{g/g}$  FW in the *Rosa canina* genotype taken from Gevaş at an altitude of 1691. The lowest value was determined as 9.8  $\mu\text{g/g}$  FW in the *Rosa canina* genotype taken from Çatak at an altitude of 1582.

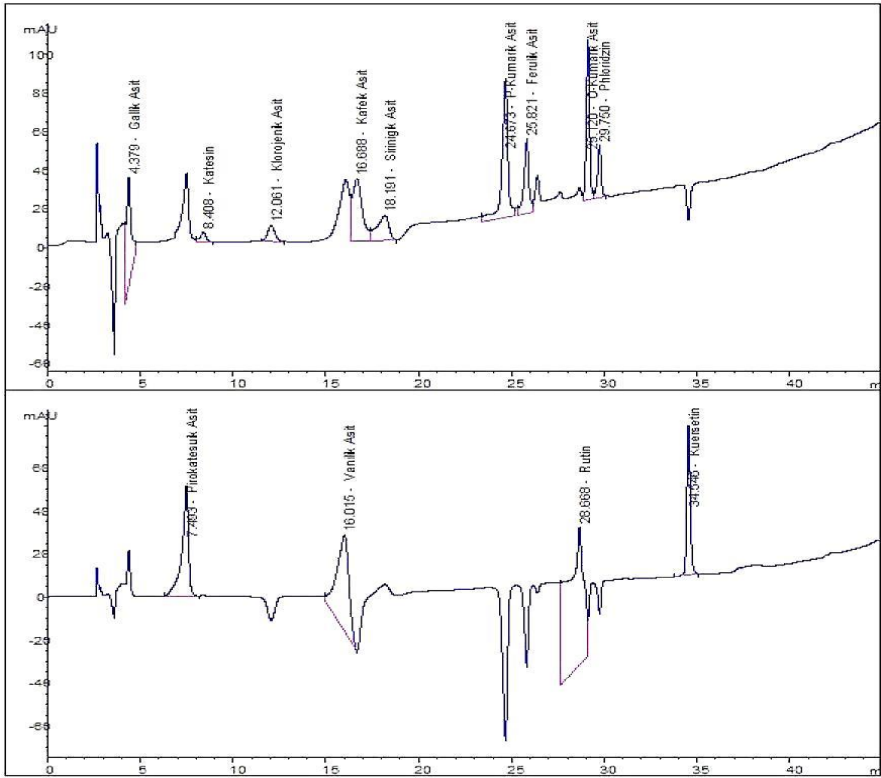
**Table 3.** The total soluble solids (TSS), pH, vitamin C, titratable acidity (TA), Fruit Color, Fruit Shape, Fruit Exterior Pubescence and Fruit fullness values for 30 rose hip (*Rosa spp.*) genotypes \*

Genotype	TSS (Brix <sup>o</sup> )	TA (%)	pH	Vitamin C (mg 100g <sup>-1</sup> )	Fruit Color	Fruit Shape	Fruit Exterior Pubescence	Fruit Fullness
GE-KS-1	26.12 a	2.65 bcdef	3.50 abc	520.14±1.87 e	Light Orange	Cylindrical	Absent	Good
GE-KS-2	24.56 ab	3.26 abc	3.36 abc	656.41±2.53 b	Light Orange	Cylindrical	Absent	Medium
GE-KS-3	22.16 bcdef	3.34 ab	3.12 bc	510.22±2.85 e	Light Orange	Conical	Absent	Medium
GE-KS-4	25.36 a	3.58 ab	3.60 abc	460.40±3.84 h	Light Orange	Conical	Absent	Medium
GE-KS-5	23.58 abc	2.96 bcd	3.12 c	585.60±2.25 d	Orange	Cylindrical	Absent	Medium
GE-KS-6	26.05 a	2.48 def	3.40 abc	659.10±2.96 b	Orange	Cylindrical	Absent	Medium
GE-KS-7	21.16 efg	2.59 cdef	3.12 c	685.16±2.35 a	Light Orange	Cylindrical	Absent	Medium
GE-KS-8	22.15 cdef	3.64 a	3.40 abc	380.26±4.25 k	Red	Elliptic	Absent	Medium
GE-KS-9	19.70 hi	2.76 bcde	3.28 bc	612.80±5.34 c	Red	Cylindrical	Absent	Medium
GE-KS-10	23.24 abc	2.55 cdef	3.54 abc	470.19±4.28 g	Red	Cylindrical	Absent	Medium
ER-KS-1	22.26 bcde	2.46 ef	3.90 a	396.40±5.21 j	Orange	Cylindrical	Absent	High
ER-KS-2	25.12 a	2.20 f	3.48 abc	484.80±3.40 f	Orange	Cylindrical	Absent	High
ER-KS-3	23.26 abc	2.90 bcd	3.91 a	465.30±2.36 h	Red	Conical	Absent	High
ER-KS-4	26.12 a	1.94 fg	3.56 abc	427.87±2.85 i	Red	Conical	Absent	High
ER-KS-5	25.15 a	2.15 f	3.65 ab	232.80±3.91 p	Red	Conical	Absent	High
ER-KS-6	23.40 abc	2.36 f	3.80 a	246.30±4.22 o	Orange	Conical	Absent	High
ER-KS-7	24.20 ab	2.58 cdef	3.76 a	157.56±1.85 r	Orange	Cylindrical	Absent	High
ER-KS-8	22.00 def	3.05 bc	3.82 a	252.80±2.35 n	Light Orange	Cylindrical	Absent	High
ER-KS-9	23.60 ab	2.65 cdef	4.05 a	122.10±1.48 t	Light Orange	Cylindrical	Absent	High
ER-KS-10	25.38 a	2.20 f	3.67 ab	260.21±3.65 m	Orange	Elliptic	Absent	High
ÇA-KS-1	19.40 i	1.79 fg	3.98 a	232.80±1.22 p	Red	Conical	Absent	Low
ÇA-KS-2	21.50 ef	2.05 f	3.80 a	285.30±2.37 l	Red	Conical	Absent	Low
ÇA-KS-3	20.40 gh	2.12 f	3.55 abc	185.56±3.20 q	Light Orange	Cylindrical	Absent	Low
ÇA-KS-4	22.50 bcde	2.45 ef	3.76 a	252.80±3.47 n	Light Orange	Cylindrical	Absent	Low
ÇA-KS-5	24.00 ab	2.34 f	3.92 a	123.20±1.33 t	Red	Elliptic	Absent	Low
ÇA-KS-6	23.24 bc	2.60 cdef	3.70 ab	147.21±2.30 t	Red	Cylindrical	Absent	Low
ÇA-KS-7	20.19 ghi	2.29 f	3.68 ab	246.30±1.21 o	Orange	Conical	Absent	Low
ÇA-KS-8	19.52 i	1.68 g	3.46 abc	157.56±1.32 r	Orange	Conical	Absent	Low
ÇA-KS-9	20.36 gh	2.57 cdef	3.51 abc	252.80±3.10 n	Red	Conical	Absent	Low
ÇA-KS-10	21.10 fg	2.80 bcd	3.78 abc	122.10±2.00 t	Red	Cylindrical	Absent	Low

\* Duncan P < 0.05

Differences in routine contents were found to be statistically significant. Considering the routine contents, the highest values were determined as 96.8  $\mu\text{g/g}$  FW in GE-KS-4 genotypes taken from Gevaş at an altitude of 1688 m. The lowest value was determined as 17.9  $\mu\text{g/g}$  FW in the ÇA-KS-7 genotype taken from Çatak, which has an altitude of 1582 m.





**Figure 2.** Chromatography of phenolic compound standards.

Considering the quercetin contents, the highest values were determined as 28.5  $\mu\text{g/g}$  FW in the ER-KS-3 genotype taken from Erçiş at an altitude of 1698 m. The lowest values were determined as 13.0  $\mu\text{g/g}$  FW in the ÇA-KS-8 genotype taken from Çatak at an altitude of 1582 m. Considering the gallic acid contents, the highest value was determined as 80.4  $\mu\text{g/g}$  FW in the GE-KS-8 genotype taken from Gevaş at an altitude of 1683 m. The lowest value was determined as 12.3  $\mu\text{g/g}$  FW in the ER-KS-2 genotype taken from Erçiş, which has an altitude of 1785 m.

**Table 4.** Phenolic compounds in rosehip fruits ( $\mu\text{g/g}$  FW)\*

Genotype	Protocatechive	Elagic Acid	Rutin	Quercetin	Galic Acid	Catechin
GE-KS-1	18.4 $\pm$ 0.8 hijk	296.4 $\pm$ 4.2 jk	47.6 $\pm$ 2.4 def	15.3 $\pm$ 0.8 defg	16.9 $\pm$ 0.9 lmn	86.2 $\pm$ 6.2 cd
GE-KS-2	28.6 $\pm$ 1.7 fgh	324.7 $\pm$ 5.5 gh	58.0 $\pm$ 1.2 d	24.7 $\pm$ 1.0 ab	63.0 $\pm$ 1.5 b	53.8 $\pm$ 5.3 fgh
GE-KS-3	96.2 $\pm$ 2.2 a	353.1 $\pm$ 2.4 e	48.6 $\pm$ 0.9 de	18.5 $\pm$ 1.1 cd	20.0 $\pm$ 0.7 kl	48.5 $\pm$ 5.4 gh
GE-KS-4	41.0 $\pm$ 1.9 def	246.5 $\pm$ 4.3 op	96.8 $\pm$ 2.5 a	13.5 $\pm$ 1.4 fg	32.4 $\pm$ 0.8 fg	134.8 $\pm$ 7.9 a
GE-KS-5	18.6 $\pm$ 0.9 hij	284.7 $\pm$ 4.6 k	41.1 $\pm$ 2.3 ef	14.6 $\pm$ 1.0 efg	15.2 $\pm$ 1.2 mn	78.5 $\pm$ 6.3 d
GE-KS-6	25.4 $\pm$ 1.7 h	312.5 $\pm$ 5.1 hij	53.8 $\pm$ 1.3 d	20.8 $\pm$ 1.0 bcd	25.1 $\pm$ 1.3 hij	76.6 $\pm$ 5.9 d
GE-KS-7	49.7 $\pm$ 2.2 d	364.6 $\pm$ 3.2 e	48.4 $\pm$ 1.7 de	13.9 $\pm$ 0.7 fg	23.6 $\pm$ 1.0 ijk	53.3 $\pm$ 4.6 gh
GE-KS-8	36.0 $\pm$ 2.8 efg	212.5 $\pm$ 3.7 r	85.4 $\pm$ 2.2 b	18.9 $\pm$ 1.2 cd	80.4 $\pm$ 3.5 a	96.4 $\pm$ 7.8 c
GE-KS-9	25.4 $\pm$ 1.3 h	254.2 $\pm$ 2.5 no	40.7 $\pm$ 1.4 ef	15.6 $\pm$ 1.5 def	26.8 $\pm$ 1.6 h	52.0 $\pm$ 8.2 gh
GE-KS-10	36.0 $\pm$ 1.8 efg	238.4 $\pm$ 3.3 p	56.8 $\pm$ 2.5 d	13.5 $\pm$ 0.7 fg	35.4 $\pm$ 0.8 f	104.1 $\pm$ 6.1 c
ER-KS-1	28.5 $\pm$ 0.9 gh	279.7 $\pm$ 2.9 kl	41.2 $\pm$ 1.8 ef	22.0 $\pm$ 1.4 b	18.5 $\pm$ 1.1 l	98.6 $\pm$ 8.1 c
ER-KS-2	75.4 $\pm$ 1.6 b	679.8 $\pm$ 3.2 a	84.0 $\pm$ 2.1 b	16.8 $\pm$ 1.6 de	12.3 $\pm$ 1.5 n	57.8 $\pm$ 8.7 fg
ER-KS-3	59.4 $\pm$ 1.0 c	401.0 $\pm$ 2.7 c	44.1 $\pm$ 1.6 ef	28.5 $\pm$ 0.9 a	9.4 $\pm$ 0.6 o	43.5 $\pm$ 7.3 h
ER-KS-4	35.4 $\pm$ 2.1 efg	284.5 $\pm$ 3.6 k	43.0 $\pm$ 1.2 ef	24.0 $\pm$ 1.3 b	27.0 $\pm$ 1.0 h	58.5 $\pm$ 8.9 fg
ER-KS-5	28.5 $\pm$ 1.9 h	325.6 $\pm$ 3.1 g	72.0 $\pm$ 1.5 c	26.3 $\pm$ 1.6 a	15.0 $\pm$ 0.9 n	103.1 $\pm$ 7.2 c
ER-KS-6	41.2 $\pm$ 1.6 de	340.0 $\pm$ 5.8 f	32.2 $\pm$ 2.3 fgh	14.9 $\pm$ 1.4 efg	12.9 $\pm$ 1.8 n	43.3 $\pm$ 6.4 h
ER-KS-7	25.9 $\pm$ 1.0 h	530.2 $\pm$ 4.3 b	47.5 $\pm$ 1.2 def	23.0 $\pm$ 1.6 b	14.0 $\pm$ 0.4 p	34.9 $\pm$ 5.6 i
ER-KS-8	24.6 $\pm$ 1.4 h	314.2 $\pm$ 3.4 hi	56.3 $\pm$ 2.0 d	18.0 $\pm$ 1.3 d	17.4 $\pm$ 1.2 lm	68.7 $\pm$ 4.6 de
ER-KS-9	21.7 $\pm$ 2.7 h	208.7 $\pm$ 2.9 r	29.0 $\pm$ 0.8 hi	9.0 $\pm$ 0.9 h	17.0 $\pm$ 1.1 lm	40.0 $\pm$ 7.0 hi
ER-KS-10	43.8 $\pm$ 1.8 d	271.2 $\pm$ 2.8 m	35.2 $\pm$ 1.2 fg	21.0 $\pm$ 0.7 bc	23.2 $\pm$ 1.4 jk	49.6 $\pm$ 6.2 gh
ÇA-KS-1	15.7 $\pm$ 0.5 jk	192.3 $\pm$ 1.7 s	28.1 $\pm$ 1.6 hi	16.5 $\pm$ 1.4 de	24.0 $\pm$ 1.2 ij	64.0 $\pm$ 7.9 ef
ÇA-KS-2	20.8 $\pm$ 0.9 hi	228.1 $\pm$ 1.9 q	25.0 $\pm$ 1.1 ij	8.5 $\pm$ 0.8 h	32.0 $\pm$ 1.4 g	60.0 $\pm$ 8.5 fg
ÇA-KS-3	16.0 $\pm$ 1.0 ijk	354.5 $\pm$ 2.2 e	28.6 $\pm$ 2.0 hi	15.0 $\pm$ 0.8 efg	25.4 $\pm$ 1.3 hi	95.6 $\pm$ 6.7 c
ÇA-KS-4	14.9 $\pm$ 1.2 jk	313.8 $\pm$ 2.9 hi	26.2 $\pm$ 1.7 ij	14.0 $\pm$ 0.9 fg	29.6 $\pm$ 1.7 g	76.0 $\pm$ 8.2 d
ÇA-KS-5	9.8 $\pm$ 2.1 k	316.3 $\pm$ 3.5 h	31.0 $\pm$ 1.3 fgh	18.5 $\pm$ 0.6 d	49.2 $\pm$ 1.7 d	60.0 $\pm$ 6.8 fg
ÇA-KS-6	31.2 $\pm$ 2.0 fgh	260.0 $\pm$ 1.6 n	35.4 $\pm$ 2.4 f	16.5 $\pm$ 0.5 de	51.3 $\pm$ 1.4 c	62.0 $\pm$ 4.9 f
ÇA-KS-7	24.3 $\pm$ 1.5 h	276.5 $\pm$ 2.1 l	17.9 $\pm$ 1.1 k	NS	30.0 $\pm$ 1.9 g	63.0 $\pm$ 6.2 ef
ÇA-KS-8	18.5 $\pm$ 1.0 hij	325.4 $\pm$ 3.5 g	30.9 $\pm$ 1.4 gh	13.0 $\pm$ 1.2 g	20.5 $\pm$ 1.9 kl	123.7 $\pm$ 8.7 b
ÇA-KS-9	14.9 $\pm$ 0.9 jk	380.0 $\pm$ 3.7 d	22.5 $\pm$ 1.7 j	15.6 $\pm$ 0.8 def	23.6 $\pm$ 1.4 jk	74.5 $\pm$ 6.9 d
ÇA-KS-10	18.4 $\pm$ 1.3 hijk	310.6 $\pm$ 1.5 ij	24.8 $\pm$ 1.2 ij	14.1 $\pm$ 0.4 fg	38.5 $\pm$ 2.7 e	67.2 $\pm$ 5.5 def

\* Duncan P &lt; 0.05

Considering the catechin contents, the highest value was determined as 134.8  $\mu\text{g/g}$  FW in the GE-KS-4 genotype taken from Gevaş at an altitude of 1688 m. The lowest value was determined as 34.9  $\mu\text{g/g}$  FW in the ER-KS-7 genotype taken from Erciş, which has an altitude of 1785 m. Considering the chlorogenic acid contents, the highest value was determined as 84.0  $\mu\text{g/g}$  FW in the Rosa canina genotype ÇA-KS-3 taken from Çatak at an altitude of 1530 m. The lowest value was determined as 9.1  $\mu\text{g/g}$  FW in the ER-KS-3 genotype taken from Gevaş at an altitude of 1691 m.

**Table 5.** Table 3. Phenolic compounds in rosehip fruits ( $\mu\text{g/g}$  FW) \*

Genotype	Chlorogenic Acid	Caffeic Acid	Syring Acid	P-Coumaric Acid	Ferulic Acid	Phlorodizin
GE-KS-1	13.3±2.4 lmno	25.8±1.4 a	6.0±0.9 ghi	18.0±1.4 bcd	8.10±0.9 hi	8.6±0.8 jk
GE-KS-2	Ns	NS	Ns	19.6±1.8 bc	10.0±1.2 ghi	23.5±1.5 cd
GE-KS-3	12.8±2.0 mno	25.5±2.2 a	9.1±0.4 fgh	16.0±1.3 cd	19.0±1.2 bc	31.0±1.3 ab
GE-KS-4	19.8±4.0 jk	20.4±2.2 abc	11.3±0.2defg	15.9±1.3 cd	12.0±1.1 efgh	12.1±1.4 j
GE-KS-5	13.3±2.2 mno	25.8±1.6 a	6.0±0.9 hi	13.5±1.4 de	8.12±1.2 i	14.4±1.5 ghij
GE-KS-6	12.2±3.1 no	18.5±1.5 bcd	15.4±0.4 bc	17.4±1.6 cd	14.0±1.4 defgh	25.0±1.3 c
GE-KS-7	15.6±1.3 lm	18.0±2.2 cd	9.1±0.004 gh	13.8±1.2 de	13.5±1.5 efgh	18.4±1.2 ef
GE-KS-8	20.6±1.1 j	13.6±2.2 e	11.3±0.8 efg	14.6±0.9 d	12.0±1.2 fgh	16.0±1.3 fgh
GE-KS-9	18.5±1.8 jkl	23.5±2.1 a	9.1±0.4 gh	16.4±0.8 cd	17.5±1.1 c	13.9±0.9 hij
GE-KS-10	16.8±2.6 kl	21.0±2.3 ab	11.3±1.2 efg	17.1±1.3 cd	14.6±0.9 defg	10.6±1.0 jk
ER-KS-1	10.0±1.7 o	18.5±1.2 cd	10.6±1.0 fg	16.8±1.4 cd	14.7±1.3 def	18.7±0.8 e
ER-KS-2	17.0±2.8 kl	18.7±1.9 bc	17.1±1.2 ab	11.0±1.2 ef	12.4±1.6 efgh	13.4±1.4 ij
ER-KS-3	9.1±1.6 o	12.0±1.2 e	17.9±1.4a	12.8±1.4 de	9.8±0.9 hi	20.5±1.4 de
ER-KS-4	9.6±1.3 o	23.8±1.5 a	9.6±0.9 fg	16.0±1.4 cd	11.5±1.2 gh	8.4±1.5 k
ER-KS-5	12.5±1.7 no	12.9±1.4 e	13.5±1.1 cd	9.0±1.2 f	17.1±1.4	16.4±1.2 fg
ER-KS-6	13.6±1.8 lmn	16.5±1.2 d	17.4±1.2 ab	20.6±1.5 b	12.6±1.5 efgh	13.1±1.5 j
ER-KS-7	12.5±1.6 no	14.0±1.2 e	13.0±1.4 de	11.4±1.2 ef	8.4±0.8 hi	18.9±1.5 e
ER-KS-8	10.0±1.3 o	20.0±5.2 abc	10.6±1.3 fg	15.0±1.4 d	8.9±1.1 hi	12.4±1.1 j
ER-KS-9	9.8±1.6 o	18.0±1.5 cd	13.0±1.5 de	13.3±0.9 de	14.3±1.2 defg	19.6±1.2 de
ER-KS-10	12.6±1.2 no	13.4±1.2 e	9.8±0.9 fg	14.0±1.4 d	12.5±1.2 efgh	8.5±0.6 k
ÇA-KS-1	55.4±2.6 e	20.9±1.4 ab	6.4±0.5 gh	19.4±1.0 bc	14.6±1.3 defg	32.0±1.9 a
ÇA-KS-2	59.7±1.5 d	21.6±1.2 a	Ns	15.0±1.5 d	18.7±1.5 bc	29.5±1.2 b
ÇA-KS-3	84.0±1.3 a	23.4±1.4 a	9.1±0.6 gh	16.3±1.3 cd	15.0±1.3 de	32.2±1.5 a
ÇA-KS-4	77.1±2.8 b	22.0±2.3 a	8.5±1.8 gh	21.4±1.3 b	13.5±1.2 efgh	19.1±1.1 e
ÇA-KS-5	64.5±3.0 c	22.1±1.4 a	6.2±0.7 gh	22.0±1.4 b	14.7±1.4 def	30.6±2.1 ab
ÇA-KS-6	53.8±2.2 f	23.6±3.2 a	8.0±1.2 gh	24.0±1.6 a	20.0±1.3 b	15.2±1.7 ghi
ÇA-KS-7	47.5±3.1 g	17.8±1.4 cd	9.2±0.8 fg	15.3±1.5 d	23.0±1.4 a	20.0±1.3 de
ÇA-KS-8	42.0±1.9 h	18.5±1.1 cd	Ns	17.2±1.3 cd	16.0±1.3 cd	19.4±1.5 de
ÇA-KS-9	40.0±1.4 i	13.5±1.2 e	10.1±0.8 fg	20.0±1.3 b	14.0±1.2 efgh	14.0±1.3 hij
ÇA-KS-10	39.6±2.0 i	19.6±1.3 bc	12.0±1.7 def	12.0±1.4 e	17.4±1.1 c	15.7±1.4 gh

\* Duncan P &lt; 0.05

Differences in caffeic acid contents were found to be statistically significant. Considering the caffeic acid contents, the highest value was determined as 25.8  $\mu\text{g/g}$  FW in GE-KS-1 and GE-KS-3 Rosa canina genotypes taken from Gevaş at an altitude of 1692 m. The lowest value was determined as 13.5  $\mu\text{g/g}$  FW in the ÇA-KS-9 genotype taken from Gevaş at an altitude of 1691 m.

Differences in syring acid contents were found to be statistically significant. Considering the syring acid contents, the highest value was determined as 17.9  $\mu\text{g/g}$  FW in Rosa canina genotypes ER-KS-3 taken from Erciş, which has an altitude of 1765 m. The lowest value was

determined as 6.0 µg/g FW in the GE-KS-1 genotype taken from Gevaş at an altitude of 1691 m.

The differences in terms of P-Cumaric acid contents were found to be statistically significant. Considering the P-Cumaric acid contents, the highest value was determined as 24.0 µg/g FW in the Rosa canina genotype taken from Çatak at an altitude of 1660 m. The lowest value was found in those taken from Erciş district. It was determined as 9.0 µg/g FW in the Rosa canina genotype taken from Erciş at an altitude of 1784 m.

Differences in ferulic acid contents were found to be statistically significant. Considering the ferulic contents, the highest value was determined as 23.0 µg/g FW in the Rosa canina genotype taken from Çatak at an altitude of 1475 m. The lowest values were determined as 8.12 µg/g FW in the Rosa canina genotype taken from Gevaş at an altitude of 1692 m.

The highest value in terms of phlorodizin contents was determined as 32.2 µg/g FW in the Rosa canina genotype taken from Çatak at an altitude of 1530 m. The lowest value was determined as 8.4 µg/g FW in Rosa canina genotypes taken from Erciş, which has an altitude of 1772 m.

## **CONCLUSION**

There are many studies on rosehip genetic resources in different regions of Turkey (Ercişli and Equalken, 2004; Türkben et al., 2005; Doğan and Kazankaya, 2006; Çelik, 2007, Çelik et al., 2009; Sağır, 2010; Güneş ,

2011; Yıldız and Çelik, 2011; Ekincialp and Kazankaya, 2012; Çelik et al., 2015; Akkuş, 2016; Ersoy and Özen, 2016; Eroğlu and Oğuz, 2018; İpek and Balta, 2020). In this study, which was carried out on *Rosa canina* populations in different locations (Gevaş, Erciş and Çatak) in the province of Van, fruit, some plant characteristics and phytochemical characteristics of 30 genotypes selected according to selection criteria were determined.

The fruit weight of the determined genotypes was between 1.36 g and 2.31 g. Eight genotypes (GE-KS-2, GE-KS-4, GE-KS-9, GE-KS-10, ER-KS-1, ER-KS-8, ER-KS-9 and ÇA-KS-1) had values in the range of 2.36-2.42 g. Fruit flesh weight varies between 1.12-1.82 gr. The fruit pulp ratio ranged between 77% and 93%, and it was found to be over 85% for 13 genotypes. In genotypes obtained from different regions in Turkey, fruit weight range and fruit pulp ratio were 2.04-6.10 g and 46.8-79.9% from Bitlis, from Hakkari and Van region (Kazankaya et al., 2005), 2.21-6.16 gr and 61.3-80.4% from Gümüşhane-Erzincan (Kızılcı, 2005), 1.37-3.04 gr and 45.8-79.4% from Amasya (Dölek, 2008), 1.79-4.95 gr and 66.4-100% from Van Lake Basin (Çelik et al., 2009), 1.65-2.78 g and 63.8-75.0% from Sivas, Akıncılar (Sagir, 2010), 1.82-4.09 g and 55-2-85.0 % from Muradiye (Yıldız and Çelik, 2011), 1.52-3.92 gr and 59.3-76.6 % from Hakkari (Ekincialp and Kazankaya, 2012), 1.44-4.69 gr and 60-79% from Ağrı, Hamur (Akkuş, 2016), 1.40-2.77 gr and 64-9-82.8% (Ersoy and Özen, 2016), 1.22-3.47 gr and 62-72% from Ordu, Akkuş (İpek and Balta, 2020) values are reported. There are similarities between the data obtained in the study and the

data reported by different researchers.

It has been reported that factors such as species, variety, genotype, ecology, altitude, harvest time and ripening stage affect the chemical composition and vitamin C content of rosehip fruit. Rosehip, which has an effective blood purifier, intestinal softener, and worm-reducing feature, is used against hemorrhoids, rickets, rheumatism, scurvy and hemorrhoid diseases, as well as regulating the development of the body due to its high vitamin C content, and against fever and colds (Doğan et al., 2006, Çelik et al., 2009).

Vitamin C content of genotypes in different locations was determined in the range of 122.10-685.16 mg/100g-1. The vitamin C content ranges of rosehip (100 g FW) in different studies are given below; 1074-2557 mg (Ercişli and Equalgen 2004), 301-1183 mg (Kazankaya et al., 2005), 305-945 mg (Kızılcı, 2005), 108-908 mg (Dölek, 2008), 517-1032 mg (Çelik et al., 2009), 438-766 mg (Deaf, 2010), 616-867 mg (Rosu et al., 2011), 330-535 mg (Yıldız and Çelik, 2011), 414-916 mg (Ekincialp and Kazankaya, 2012), 372-737 mg (Cheikh-Affene et al., 2013), 332-1603 mg (Ersoy and Özen, 2016), 53-563 mg (Soare et al., 2015), 540-1315 mg (Akkuş, 2016), 402-511 mg (Alp et al., 2016) and 560-1025 mg (İpek and Balta, 2020). In this study, there are similarities with the values obtained in *Rosa canina* genotypes, and it is seen that they are lower than the values reported by some researchers. It is thought that there are differences between the *Rosa canina* genotypes in terms of vitamin C content, and it is thought to be effective in the region where it is taken and environmental factors.



Dry matter ratio in the examined rosehip fruits ranged between 19-24 brix°. Fruit weight, fruit width, fruit length, fruit pulp ratio, seed weight and dry matter content of the examined rosehip genotypes are generally within the limits of previous studies (Ercişli, 1996; Mısırlı et al. 1999; Güneş and Şen, 2001; Uğla et al. 2005; Güneş and Dölek, 2010; Yıldız and Çelik 2011; İpek and Balta, 2020).

The main phenolic compounds in the fruits of *Rosa canina* ecotypes are catechin, elagic acid, protocatechive, gallic acid, chlorogenic acid, followed by rutin, p-coumaric acid, caffeic acid and others. Anthocyanins, one of the phenolic substances, provide the formation of unique colors of fruits and vegetables (Cemeroğlu et al., 2004).

As a result of the analysis made by HPLC, it shows that the *Rosa canina* fruit samples examined contain phenolic acids, caffeic acid and chlorogenic acid (Heleno et al., 2015). Phenolic acids are potent antioxidant (Kumar & Goel 2019), anti-inflammatory (Taofiq et al., 2015), renoprotective (Buys-Gonçalves et al., 2019), hepatoprotective (Saha et al., 2019) and anti-diabetic (Kumar & Goel 2019) features. In our study, quercetin content was 8.5-28.5 µg/g FW, gallic acid 14.0-80.4 µg/g FW, catechin 34.9- 134.8 µg/g FW, chlorogenic acid 9.8-84.0 µg/g FW, caffeic acid 12.0-25.8 µg/g FW, syring acid 6.0-17.9 µg/g FW, p-cumaric acid 9.0-20.6 µg/g FW, ferulic acid 8.1-23.0 µg/g FW and phlorodizin 8.4-32.2 µg/g FW. Flavonoids affect the aroma and color values of fruits, as well as biotic and abiotic stress factors (Samanta et al., 2011). Medveckiene et al., (2020) reported that the total flavonoid content in rose hips depends on the species and the highest

total flavonoid content was found to be 415.9  $\mu\text{g/g}$  DW. In another study, the total flavonoid content of rose hips is reported to vary between 520-560  $\mu\text{g/g}$  (Winther et al., 2016).

Demir et al., (2014) Rosa L., reported that the (+)-catechin content varies between 7.18-50.46  $\mu\text{g/g}$ . Most of the Rosa canina fruit samples evaluated in our study contained higher proportions of this compound. In a study conducted in Poland, (Fetni et al., 2020, Nadpal et al. 2016), (+)-catechin content (2.37–7.83  $\mu\text{g/g}$ ) were found to be significantly lower than the levels of this compound determined in our Rosa canina fruit samples. Bozhöyük et al. (2021) determined the chlorogenic acid content as 27.8-813  $\mu\text{g/g}$  FW and the gallic acid content as 10.9-49.3  $\mu\text{g/g}$  FW  $\mu\text{g/g}$  FW in a study they conducted on different ecotypes of R. canina and R. dumalis.

The highest p-coumaric acid was found to be 33.2  $\mu\text{g/g}$  FW, and the caffeic acid content was 14.2  $\mu\text{g/g}$  FW. There are general similarities between the findings obtained by the researchers in terms of phenolic substances as a result of their studies on rosehip species and the findings obtained in our study.

In Iran, Shameh et al. (2019) report that the fruits of rosehip species mostly contain chlorogenic acid and gallic acid, and they found high variability among species in these compounds. In the studied extracts of rosehip fruit samples, flavonols were the most abundant group of phenolic compounds. These compounds are important for human health due to their strong antioxidant and anticancer activities (Sharma et al.,

2018). Consumption of products rich in quercetin and its glycosides reduces the risk of cardiovascular (Ferenczyova et al., 2020) and neurodegenerative diseases (Khan et al., 2019).

The results of the research prove the existence of rich rosehip genetic resources in Van province. Determination of phenol content, antioxidant activity and sugar composition of genotypes in recent studies on rosehip in Turkey is very important in terms of being a resource for future improvement and breeding efforts. In addition, it is thought that the results of the study will contribute to a better understanding of rosehip fruits in terms of health. Significant differences were determined among rosehip fruits in terms of physico-chemical properties, species, genotype and ecological conditions. This study provides new information about the phenolic content of *Rosa canina* fruit of cultivars grown in Van (Turkey) climatic conditions. It also allows the results obtained to be compared with the results of other studies.

#### **ACKNOWLEDGEMENTS**

This research was produced from the findings of the project numbered 2015-ZF-B099 supported by the Scientific Research Projects Unit of Van Yüzüncü Yıl University. We thank Van Yüzüncü Yıl University Scientific Research Projects Unit for their financial support.

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## **CHAPTER 2**

### **PEA (*Pisum sativum* L.): A CURRENT REVIEW**

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## 1. Introduction

*Pisum sativum* L. is a *Fabaceae* family legume plant that grows in winter season. It is grown in over 95 countries largely for its protein-rich seeds, which are used for food and feed (Gondalia et al., 2022). The pea is a temperate legume that is grown as a vegetable and a pulse crop (Javaid et al., 2022). One of the most significant self-pollinating legume crops is the garden pea (*Pisum sativum* L.). Peas are a valuable crop that can be used for food, feed, and industrial purposes all around the world. Garden peas are an annual winter-season crop that is grown from winter to early summer in different parts of the world. A vast genetic resource collection of pea germplasm has been preserved via gene banks. *Pisum* is diverse in terms of biological status, geographical locations, and morpho-agronomic characteristics (Abdel-Hamid & Salem, 2021).

It is an important component of low-input cropping systems because of its symbiosis with nitrogen-fixing bacteria. Dry pea (*Pisum sativum* L.) is a good source of low-digestible carbohydrates, protein, and micronutrients in terms of nutrition (Thavarajah et al., 2022). *Pisum sativum* is a leguminous crop that may be grown everywhere. It's utilized in animal feed as a fodder or dry seed supplement, and, more recently, as a non-traditional oilseed (Addo et al., 2022). *Pisum sativum* sprouts appeared to be a promising health-promoting food, according to the findings (Concha et al., 2022).

Green pea hulls are a byproduct of green pea manufacturing and are high in phenolic compounds. The ultraperformance liquid

chromatography-linear ion trap orbitrap tandem mass spectrometry (UHPLC-LTQ-OrbiTrap-MS) technology was used to quantify a total of 12 phenolic components (Guo et al., 2022). Over many decades, mutants have been used in the improvement of pea as a crop. Pea (*Pisum sativum* subsp. *sativum*) has hundreds of distinct mutations, some of which are useful for breeding. Depending on the obstacles, various countries may use different breeding tactics. Numerous spontaneous and intentional mutations have been used in breeding pea (Sinjushin et al., 2022).

## **2. Agronomy & Biotic Stress**

Prusinski and Borowska (2022) investigated the impact of planting density and row spacing on pea plant growth, yield, structural seed yield components, and various plant indices in Poland's central and northern regions. Three seeding densities (70, 90, and 110 seeds per 1 m<sup>2</sup>) were used, with two row spacings (16 and 32 cm). Peas were extremely sensitive to precipitation deficits; seed yields were about half as high in rainy seasons as they were in dry years. Except in 2019, there was no influence of planting density or row spacing on pea yield.

Plant proteins can be found in field peas (*Pisum sativum*) and faba beans (*Vicia faba*) for human consumption. Their regional cultivation has seen a resurgence in recent years across Europe. Walter et al. examined the effects of genetic variables, cultivation period, meteorological parameters, soil composition, and cultivation conditions (conventional vs. organic) on the variations in protein content of peas (*Pisum sativum*) and beans (*Vicia faba*) cultivated under real-world conditions (2022).

The cultivar and the cultivation time were shown to have the greatest impact on the protein content of the two legumes. However, several ecophysiological conditions influenced protein content to some extent. High temperatures and limited rainfall, in particular, were linked to a greater protein content. Furthermore, organic beans have a greater protein content than conventionally grown beans. The findings suggest that genetic and ecophysiological factors may alter pea and faba bean protein content. This makes it possible to use the protein content of legumes as a basic payment plan for farmers based on their harvest.

Pea, also known as vegetable pea, is a nutritious cool-season, frost-hardy leguminous vegetable that is commonly farmed for its green pods all over the world. It is widely planted in temperate zones as a cool-season crop. Garden pea and field pea are other names for it. Pea is a fast-growing annual herbaceous vine that grows best when supported by a trellis. Apart from a small amount of iron, it is a good source of protein (25%), amino acids, sugars (12%), carbohydrate, vitamins A and C, calcium, and phosphorus. Peas are a valuable vegetable because they are high in protein. It's eaten as a vegetable or in soup, and it's available in canned, frozen, or dried form. It's served as a side dish with or without potatoes. Pea straw is a nutrient-dense feed. After China, India is the world's second-largest pea grower. Peas are susceptible to a variety of fungal, bacterial, and viral diseases, resulting in significant production reductions (Sharma et al., 2022).

Green peas are susceptible to a variety of diseases, some of which can result in major damage or loss. Disease-related annual losses vary from



year to year, typically dependent on local meteorological conditions. Root rots and diseases like ascochyta and bacterial blights can cause significant losses if the soil is saturated from copious rainfall and the weather is chilly. Total loss may occur if the soil is heavily contaminated with root-rotting organisms (Jagadeesh et al., 2022).

Pulses offer a variety of useful characteristics that make them appealing to farmers. However, large-scale legume cultivation confronts a number of challenges, including the vulnerability of currently available cultivars to a variety of diseases that severely reduce yields and seed quality. Powdery mildew (a common name for parasitic fungi of the *Erysiphales* order) is one of the most devastating legume diseases (Sulima & Zhukov, 2022).

Powdery mildew, caused by *Erysiphe pisi*, is one of the most common pea diseases worldwide. In addition, two additional species, *Erysiphe trifolii* and *Erysiphe baeumleri*, have been discovered as pea plant pathogens. To date, three resistance genes, er1, er2, and Er3, have been found on linkage groups VI, III, and IV, respectively (Devi et al., 2022).



**Fig. 1.** Powdery mildew on *Pisum sativum* L. (a): fungus growth on stems and leaves; (b): affected pods of commercial cultivar 'PC-531' from India; (c): susceptible and resistant lines growing under natural epiphytic conditions at ICAR-IIVR, Varanasi, India (Devi et al., 2022).

Root rot infections have been reported to reduce the quality and production of common bean (*Phaseolus vulgaris* L.) and pea crops (*Pisum sativum* L.). *Fusarium solani* and *Rhizoctonia solani*, among other soil-borne fungal infections, cause this aggressive crop disease that has a negative economic impact. Synthetic pesticides have been used to control destructive plant diseases such as root rot in recent decades. It's critical to find cost-effective and environmentally acceptable ways to control aggressive soil-borne fungal infections that cause large yield losses. *Trichoderma* has emerged as a promising pathogen antagonist that inhibits microorganisms that cause root rot disease. In vitro and in vivo studies using different *Trichoderma* species (*T. harzianum*, *T. viride*, *T. hamatum*, *T. asperellum*, *T. koningii*, *T. atroviridae*, *T. virens*, *T. lignorum*, *T. cerinum*, *T. longibrachiatum*, and *T. album*) for managing common bean and pea root rot disease were reported with plant growth promotion. The use of specific metabolites produced by *Trichoderma* spp. on a large scale to induce host resistance and/or increase crop output could be a significant tool for implementing integrated pest management techniques. *Trichoderma* species and their secondary metabolites are effective in the establishment of root rot resistance in common bean and pea crops, resulting in good yields (Ketta & Hewedy, 2021).

It's been proven that rhizobia and plant-growth-promoting rhizobacteria work together. The effect of pre-sowing versus post-emergence co-inoculation with *Azotobacter chroococcum* and *Rhizobium leguminosarum* bv. *viceae* on growth, yield, and quality attributes of *Pisum sativum* plants was investigated by Ibrahim & El-Sawah, (2022).

When compared to control plants inoculated alone with *Rhizobium*, the application of *A. chroococcum* and *R. leguminosarum* bv. *viciae* dramatically improved plant biomass, nitrogen absorption, and leaf photosynthetic pigments. Furthermore, compared to control plants, inoculation with *A. chroococcum* increased nodulation indices (number of nodules, nodule diameter, and nodule dry weight), as well as nitrogenase enzyme activity. Furthermore, co-inoculation with *A. chroococcum* increased seed yield while also improving ascorbic acid, protein, and carbohydrate content in the seeds. Furthermore, there were no significant differences between pre-sowing and post-emergence application, according to the findings. Adding *A. chroococcum* to pre-soaked seeds at the same time as *Rhizobium* could be a simple, cost-effective, and efficient way to improve pea plant development, yield, nutrient uptake, and yield quality attributes.

### **3. Agronomy & Abiotic Stress**

Physiological breeding is a technique that supplements traditional breeding by identifying and characterizing features in breeding populations. This gives breeders the option of selecting crosses based on desirable and adaptable features, which may be more dependable than selecting just on yield (Bourgault et al., 2022).

Due to a variety of biotic and abiotic challenges, including heat, water scarcity, waterlogging, frost, saline soil, and soil nutrient deficiencies, anthropogenic climate change has had a negative impact on field pea yield. For abiotic stress tolerance, it will be ideal to use all gene pools (primary, secondary, and tertiary) of pea for genetic advancements in

order to develop genetic choices for the development of climate resilient pea. There are a variety of traditional breeding approaches as well as newly developed technologies such as genome wide association mapping, genomic selection, gene editing, marker assisted breeding, and nanobiotechnology. TILLING technology can be used to detect mutants in pea populations that have been physically or chemically mutagenized. Because the plants generated using this process are non-GMO, they are more widely accepted. Major quantitative trait loci associated with distinct abiotic stress tolerances have been established through the identification of molecular markers and genome-wide association studies, which can be useful in detecting potential genes. The recent release of the pea draft genome may assist in emphasizing the genetic basis of agronomically important features. Bioinformatics resources like as gene and genome databases, as well as comparative gene expression databases, provide essential information regarding abiotic stress resistant candidate genes, while protein and metabolome databases provide details on functional proteins and gene pathways (Gondalia et al., 2022). Plant productivity is a direct result of the numerous biotic and abiotic stresses to which it is subjected in its environment (Hakeem et al., 2022).

High temperatures affect reproductive growth and lead to yield loss in many crops. Field pea is heat sensitive. High temperatures disturb ovule development in field pea (Osorio et al., 2022).

Apart from drought stress, salinity is regarded the greatest limiting factor of productivity in arid and semiarid settings (El-Aidy et al.,

2021). Peas are a type of legume vegetable crop that is an important part of human nutrition. Salinity inhibits legume crop development and productivity through disturbing the ionic and osmotic equilibrium as well as hormonal control. Plant growth-promoting rhizobacteria (PGPRs) reduce the negative effects of salinity on pea seedling growth and yield, and can be employed as bio-inoculants to boost crop productivity in saline conditions (Sapre et al., 2022). Salinity stress is a severe environmental concern to the world because it restricts plant growth, resulting in lower crop yields. Salt has negative impacts on the photosynthetic process, which has an impact on plant productivity. Under high salt circumstances for pea, the net photosynthetic rate, transpiration rate, and stomatal conductance all decrease significantly, however the water usage efficiency increases dramatically. In high salt, the photochemical efficiency of photosystems (PS) I and II is reduced because their donor and acceptor sides are inhibited (Dhokne et al., 2022).

The pea is an excellent protein source for livestock and human nutrition. However, its growth is hampered by several factors including lodging in irrigation areas (Yang et al., 2022).

Symbiotic nitrogen fixation is a sustainable green technique to maintain fertility in modern agriculture, based on the symbiotic link between rhizobia and plants. However, a lack of a complete knowledge of the mechanisms prevents this biological method from being used to boost agricultural production (Li et al., 2022). Molybdenum (Mo), boron (B), and iron (Fe) all play a part in legume plants' symbiotic nitrogen

fixation. The strength of this process varies depending on the stage of growth of the legume, and the variations are accompanied by changes in the content and translocation of these micronutrients inside the plant (Wysokinski et al., 2022).

Copper (Cu) is important for cellular redox state, and silicon (Si) makes plant tissue stronger. Molybdenum (Mo) is important for nitrate uptake, and boron (B) is required for peas (*Pisum sativum* L.) growth. Depending on the concentration, SiO<sub>2</sub>, Cu, Mo, and B NPs can have both beneficial and negative impacts on pea growth, antioxidant system, and mineral uptake (Sutuliene et al., 2022).

Heavy metals are common soil contaminants that have been found in agricultural regions. In many situations, their pollution levels are much higher than the maximum allowable levels (Erofeeva, 2021). Ca and citrate are effective in instilling Cu tolerance in pea germinating seeds, suggesting that supplementing Cu-polluted environments with Ca or citrate could be a cost-effective method of reducing heavy metal toxicity and accumulation in crops (Massoud et al., 2022). Cadmium (Cd) buildup in leaves decreases photosynthetic capability by destroying photosynthetic pigments, lowering photosystem II activity, and generating reactive oxygen species (ROS). Heavy metal (HM) stress tolerance in plants has been shown to be induced by the application of Methyl Jasmonate (MeJA). When pea plants were exposed to Cd stress, MeJA's mitigating impact was related to its role in cellular redox balance and photosynthetic machinery (Manzoor et al., 2022).

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## CHAPTER 3

### **COWPEA (*Vigna unguiculata* L. Walp): A CURRENT REVIEW**

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## **1. Introduction**

Providing safe and secure food for an ever-increasing number of people in the world is getting more difficult. The current agricultural production system is not agro-ecologically friendly or sustainable to deal with such a big population. Cowpea (*Vigna unguiculata* L. Walp.) is a versatile legume with several uses. It is rich in protein for cattle fodder and contains high-quality protein for human use. It enhances the soil by recycling nutrients through nitrogen fixation in collaboration with nodulating microorganisms (Omomowo & Babalola, 2021).

Cowpea is a warm-season diploid ( $2n = 22$ ) legume that is extremely important for food and nutritional security. For millions of individuals in Sub-Saharan Africa, it is an important source of nutritional protein, fiber, minerals, and vitamins (MunozAmatrian et al., 2021). This crop was domesticated in Africa and is widely cultivated throughout the world's tropical and subtropical zones (Che et al., 2021).



**Fig. 1.** Completely developed cowpea pods and seeds, intermediate pods and seeds, and immature pods and seeds are shown from left to right (Weiss et al., 2018).

Cowpea is an annual herbaceous legume crop with a high protein content (18%–25%) that is comparable to certain types of meat. Because this crop is resistant to heat and drought, it is used in a variety of farming systems around the world. *Vigna unguiculata* var. *unguiculata* and *Vigna unguiculata* var. *spontanea* are its two botanical variants. The cultivated cowpea has five cultivar groups and *unguiculata* is the largest group which is further subdivided into 11 sub species complex. Various biotic and abiotic stressors have an impact on cowpea yield. For genetic improvement of cowpea, the nature of inheritance of qualitative and quantitative features has been extensively explored. Resistant genotypes have been developed for drought, heat stress, aluminum toxicity tolerance, high seed protein content and resistance

to aphids, flower thrips, pod borer, bruchids and disease resistance against cercospora leaf spot, bacterial blight, bean common mosaic virus and parasitic weeds. Several genetic markers linked to quantitative trait loci for drought response, maturity, pod characteristics, bacterial blight resistance, leaf spot, thrips, and striga have been identified. For the benefit of the farming community, a number of high producing cultivars with desired plant type, seed coat color, and nutritional value have been released. Integration of classical breeding and marker assisted selection can be a viable program in the introgression of all the desirable gene combinations into a single cultivar towards the development of climate resilient cowpea varieties (Narayana & Angamuthu, 2021).

## **2. Agronomy**

The cowpea is an important crop in arid and semi-arid areas (Tavares et al., 2021). Cowpea is a staple leguminous crop farmed mostly by smallholder farmers in Sub-Saharan Africa for food and animal feed. They are mostly produced in West Africa, which accounts for 60% of global production in Sub-Saharan Africa. The crop is susceptible to a variety of pests and diseases, which frequently result in crop loss (Nkomo et al., 2021).

Cowpea is a multipurpose crop that feeds people and cattle while also regulating and supporting ecological functions. Cowpea is grown largely for grain and as a cover crop in developed countries; in developing countries, it is used as a dual-purpose crop for grain and



feed. Root-knot nematodes (*Meloidogyne* spp.) constitute a widespread danger to cowpea production (Dareus et al., 2021).

*Callosobruchus maculatus*, a cowpea seed bruchid, has proven a major big obstacle to its storage. Ashamo et al., (2021) demonstrated that some agro-wastes (rice husk, groundnut pod, and wheat husk pulverized separately and another portion burned to ashes at 525°C) have promising insecticidal potential against *C. maculatus* and can be used as possible alternatives to synthetic chemical insecticides for the control of stored product insects. The cowpea weevil, *Callosobruchus maculatus* F. (*Coleoptera: Chrysomelidae*) is a destructive pest of cowpea grains in the field and storage (Naseri & Hamzavi, 2021).

Cowpea is mostly grown in a semi-arid climate, and it commonly experiences intermittent dry periods during various phases of plant development, reducing its yield significantly. Cowpea performance under changing climates may benefit from osmopriming and biochar application (Farooq et al., 2021). Cowpea development can be hampered by high temperatures, resulting in a variety of problems during the vegetative and reproductive phases of the crop. High temperatures have a major impact on photosynthesis, stomatal conductance, leaf transpiration, and enzyme activity (Barros et al., 2021). *Vigna unguiculata* is a heat and drought-tolerant legume that can be consumed at any stage of the plant's development. Drought is a severe environmental stressor that has a significant impact on plant growth and output (Carvalho et al., 2021).

### **3. Breeding**

Plant breeding programs must incorporate new sources of genetic variety to continue to increase yield and quality, as well as tolerance to abiotic and biotic stressors (MunozAmatriain et al., 2021). One of the most common causes of yield losses in cowpea in dry and semi-arid locations is pod shattering, which results in the explosive release of seeds from the pod. Reduced shattering has thus been a significant selection aim for cowpea, among other species, during domestication and development (Lo et al., 2021).

It would be critical to improve cowpea cultivars to make them more resistant to abiotic stress like drought. A multi-parent advanced generation intercross (MAGIC) population has been demonstrated to be effective in boosting the frequency of rare alleles that may be linked to significant agricultural features. Furthermore, the drought tolerance index has been shown to be a trustworthy metric for determining crop tolerance to water shortages (Ravelombola et al., 2021). It has been developed to generate types that are hardy to shifting climates and is drought and heat tolerant. Flowering timing has a significant impact on plant adaptability to changing conditions and production. To develop cowpea breeding, it's crucial to understand the genetic basis of flowering timing (Paudel et al., 2021).

The success of any hybridization outcome in cowpea is dependent on temperature and humidity, which affect flower initiation, pollen fertility and pod setting (Amusa et al., 2022). Drought that occurs during the reproductive stage has the greatest impact on cowpea yield. Cowpea

productivity can be severely harmed by drought during the reproductive period. This is due to a decrease in the length of the reproductive stage and the number of branches, which results in a decrease in pod number and seed weight. Drought priming during the early stages of growth can help cowpeas cope with drought stress throughout the post-anthesis drought period and increase water productivity. Drought priming during the early growth period is a promising approach for conserving water for irrigation while enhancing crop WP in water-scarce regions (Tankari et al., 2021).

Cowpea production has been enhanced for decades, including the development and marketing of improved cultivars that are high producing and pest and disease resistant or tolerant. Diseases were shown to be both positively and adversely related to cowpea yields. It is necessary to create and/or promote suitable high-yielding cultivars, as well as to spread excellent agricultural practices to reduce the occurrence of pests and diseases, in order to boost cowpea output (Baoua et al., 2021).

Characterization and evaluation of cowpea germplasm collections based on seed yield for genotype  $\times$  environment interactions can assist in improving the adaptability and stability across environments (Mbuma et al., 2021).

Cowpea can develop successful tripartite symbiotic associations with nitrogen-fixing bacteria (*Sinorhizobium meliloti*) and arbuscular mycorrhizal fungi, albeit appropriate arbuscular mycorrhizal fungi

species and rhizobial strains that promote cowpea growth must be chosen (Kavadia et al., 2021).

Abiotic variables, such as pre-harvest insect pests like aphids, reduce cowpea output. Aphids lower yields through encouraging the formation of sooty moulds and honeydew, as well as reducing photosynthetic processes and rates. A variety of strategies have been used to try to control aphid damage in cowpea plants. Chemical insecticides, cultural, mechanical, biological, and integrated pest management are among the approaches used. However, because smallholder farmers planting cowpea in marginal locations cannot afford these approaches, they have not shown to be very effective. As a result, the best and most successful strategy for managing aphids in cowpea plantations is host plant resistance/genetic control (Mofokeng & Gerrano, 2021).

The cowpea seed beetle, *Callosobruchus maculatus*, is a major post-harvest pest in dry and semi-arid regions of Africa, Asia, and South America, where it is an important grain and fodder crop. Cultivars resistant to *C. maculatus* population growth in storage could boost grain production and quality while reducing insecticide use (Messina et al., 2021). The principal pest that attacks cowpea seeds during storage is the cowpea weevil (*Callosobruchus maculatus*), which causes nutritional and economic losses in the cowpea crop (Ferreira et al., 2021). The most destructive bugs that cause considerable losses to cowpea seeds during storage are *Callosobruchus chinensis* (azuki bean weevil) and *Callosobruchus maculatus* (cowpea weevil). A primary goal of the cowpea breeding program is cultivar resistance to bruchids.

One or two recessive genes are in responsibility of resistance. The resistance is caused by the additive gene effect and the additive dominance gene interaction. According to correlation research, the genes that govern resistance to *C. chinensis* and those that provide resistance to *C. maculatus* are unrelated (Thandar et al., 2021).

Soil salinity is becoming a greater danger to cowpea productivity, necessitating the development of salt-tolerant cowpea cultivars (Ravelombola et al., 2022).

#### **4. Food & Feed**

Cowpea is a tropical lowland plant that grows well in dry locations and mild temperate climates. Cowpea is a starchy legume that is commonly consumed in Sub-Saharan Africa as a low-cost source of protein, essential amino acids, digestible and nondigestible carbohydrates, and polyphenols. Cowpeas are frequently blended with cereal grains in weaning diets (Phillips et al., 2022).

Cowpea is a high-protein, high-carbohydrate food legume that could be used to alleviate food and nutritional problems in many regions of the world. Some cowpea types, on the other hand, are difficult to cook, resulting in higher energy consumption and greater nutrient loss. Cowpea starch, which makes up the majority of the carbohydrate in the plant, could be used as an alternative starch source in a variety of industrial applications, especially because it's high in resistant starch, which has several physiological benefits (Oyeyinka et al., 2021).

In comparison to common beans, the novel biofortified cowpea varieties have a high Fe and Zn content. Cowpea seeds that have been biofortified deliver higher Fe and Zn for gastrointestinal absorption. For 1–3-year-old children, 50 g of biofortified Aracê cowpea provides 27% and 48% of the Fe and Zn, respectively (Coelho et al., 2021).

Higher levels of quercetin and quercetin glycosides, kaempferol diglucoside, and other tetrahydroxylated flavones and flavonols were found in cowpea pod samples compared to seed extracts, which could be due to higher levels of quercetin and quercetin glycosides, kaempferol diglucoside, and other tetrahydroxylated flavones and flavonols (Avanza et al., 2021).

Legume stovers could be a valuable animal feed source, particularly in Mediterranean farming systems. However, antinutritional substances such as lignin in these stovers may impact the animals' growth performance. White-rot fungi have been shown to boost the nutritional content of a variety of agricultural wastes by altering the lignin structure, allowing access to potentially digestible structural polysaccharides. The nutritional content of cowpea stover was improved by white-rot fungi. The life weight of rabbits fed treated cowpea stover diets increased. In rabbits fed treated cowpea stover, blood cholesterol levels were shown to be lower (Andrade et al., 2021).

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## **CHAPTER 4**

### **AVIAN SYSTEMA NERVOSUM CENTRALE**

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## **INTRODUCTION**

The nervous system takes part in almost all processes in the body. The nervous system is divided into two parts, the central nervous system and the peripheral nervous system (Aslan 2018). Nerve cell bodies are located in the brain, spinal cord, cranial and spinal ganglia, and gray matter of ganglia that are included in the autonomic nervous system (Dursun 2008). These nerve cell bodies are classified as unipolar, bipolar, and multipolar. The unipolar cell body has only one extension and can easily be divided into two. Found in spinal ganglia and cranial ganglia. Most ganglion cells are multipolar. The nerve cell has two parts, the axon, which is long, and the dendrite, which surrounds the nerve cell like a net. Each nerve cell and its extensions are called neurons. The complex structure between neurons is called synapse. Thanks to the synapse, the passage of impulses between neurons is ensured (Dursun 2008, Nickel et al. 1977). In order to evaluate the abnormal functions of the nervous system, it is necessary to know the anatomy of the winged nervous system well. A neurological examination is performed on the bird with abnormal signs. The objectives of the neurological examination are to confirm whether there is a neurological abnormality and to specifically localize the abnormality within the nervous system.

**Table 1:** Intracranial lesion localisation and clinical signs (Simon et al. 2011)

Clinical Syndrome/ Lesion Localization	Characteristics of the Location	Specific Clinical Signs
Cerebrum	Damage to cerebrum affects intellectual, learned and sensory activities (vision, hearing, touch, pain)	<ul style="list-style-type: none"> <li>i. Altered mental state</li> <li>ii. Seizures</li> <li>iii. Behavioral change</li> <li>iv. Pleurothotonus and adversion</li> <li>v. Head pressing</li> <li>vi. Central blindness</li> </ul>
Diencephalon	Damage affects autonomic visceral functions and endocrine regulation	<ul style="list-style-type: none"> <li>i. Altered mental state</li> <li>ii. Seizures</li> <li>iii. Behavioral change</li> <li>iv. Endocrine disturbances</li> <li>v. Abnormalities of temperature</li> </ul>
Midbrain	Damage affects control of alert status, as well as CN III and CN IV function	<ul style="list-style-type: none"> <li>i. Altered mental state</li> <li>ii. Opisthotonus</li> <li>iii. Contralateral paresis</li> <li>iv. Ipsilateral CN III dysfunction (mydriasis/ventrolateral strabismus)</li> <li>v. Contralateral CN IV dysfunction (dorsomedial strabismus)</li> </ul>
Pons and Medulla	Damage affects multiple cranial nerves (V-XII) as well as cardiorespiratory centers	<ul style="list-style-type: none"> <li>i. Altered mental state</li> <li>ii. Ipsilateral paresis</li> <li>iii. Irregular respiration</li> <li>iv. Ipsilateral CN V dysfunction (decreased beak strength, decreased palpebral reflex, decreased facial sensation)</li> <li>v. Ipsilateral CN VI dysfunction (third eyelid protrusion and medial strabismus)</li> <li>vi. Ipsilateral CN VII dysfunction (decreased tone in facial musculature, decreased taste, decreased lacrimation)</li> <li>vii. Ipsilateral CN VIII damage (ipsilateral deafness, vestibular dysfunction)</li> <li>viii. Ipsilateral CN IX-XII damage (dysphagia, regurgitation, tongue deviation, inspiratory dyspnea)</li> </ul>
Cerebellum	Damage affects coordinating and reinforcing actions	<ul style="list-style-type: none"> <li>i. Normal mental status and behavior</li> <li>ii. Normal strength</li> <li>iii. Opisthotonus</li> <li>iv. Dysmetric ataxia</li> <li>v. Head (intention) tremors</li> </ul>

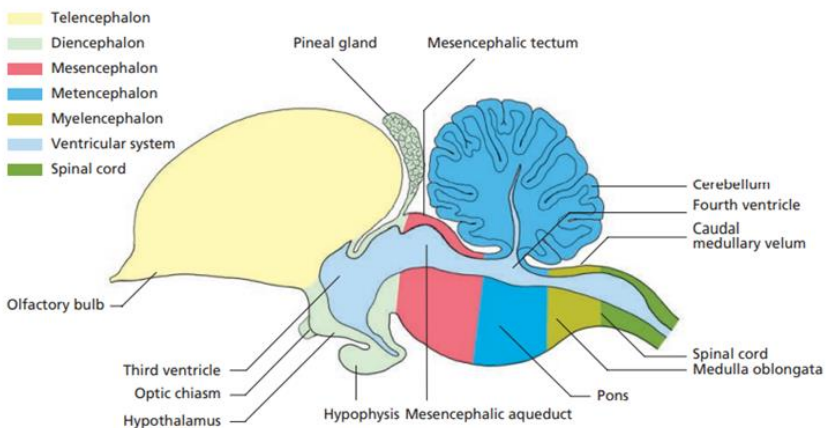
## 1. Encephalon (Brain)

The fully developed winged brain is divided into three parts by two knuckles. The first part is the prosencephalon (forebrain), the second part is the mesencephalon (midbrain), and the third part is the

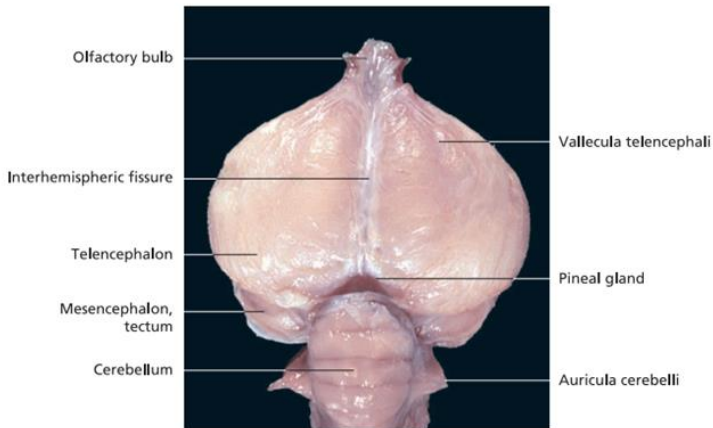
rhombencephalon (hindbrain). The prosencephalon consists of three parts, the telencephalon, the rhinencephalon, and the diencephalon. The rhombencephalon is divided into two parts, the metencephalon and the myelencephalon (Aslan 2018). Although the hindbrain and midbrain are similar in mammals and poultry, there are many differences in the forebrain. As in mammals, the brain and spinal cord are covered by three membranes. These membranes are duramater, arachnoidea and piamater. Duramater (pachymeninx) fused with the periosteum of the cavum cranii. There is a semicircular dura duplicator between each hemispherium and optic lobe. Other duplications are found between the cerebellum and optic lobe, and between the hypothalamus and hypophysis. Piamater and arachnoidea cover the surface of the brain (Dursun 2008). In birds, both arachnoidea and piamater membranes are called leptomeninx. Arachnoidea is a veinless layer. Piamater, on the other hand, is rich in veins. Piamater also participates in the structure of the plexus choroideus formed in the brain ventricles. Between these two membranes is a space called the cavitas subarachnoidea, where the cerebrospinal fluid is located. It is very difficult to collect this liquid from live avian. In domestic avian, approximately 0.5 ml can be taken from the space in the foramen magnum. However, the risk of hemorrhage due to venous sinuses in this region should not be forgotten (König et al. 2016). Hemispheriums do not have gyri and sulci. It is separated from the dorsal by a long and deep cleft. In mammals, the ventriculus quartus is associated with the cavitas subarachnoidea through a pair of lateral openings. Pigeons and chickens do not have these holes. Transitions happen by diffusion. The mammalian corpora



quadrigemina corresponds to the corpora bigemina and is also called the optic lobe (Dursun 2008). The corpora bigemina is covered by hemispheriums. Fissura longitudinalis cerebri is a longitudinal median slit. It divides the brain into two halves called hemispherium. Fissura transversa cerebri is the cleft between the cerebrum and the cerebellum. The pineal gland develops from the diencephalon. On the ventral surface of the brain, the bulbus olfactoriuses extend from the basal surfaces of the hemispheriums. Caudal contains paramedian protuberantia. This region is called the basal area in the lateral. Chiasma opticum and optic nerves are located in the diencephalon. The caudal of these formations is the pituitary gland. The caudal of the pituitary gland contains the optic lobes, the third and fourth cranial nerves, and the rod-shaped medulla oblongata. The continuation of this region is divided into two by the fissura mediana ventralis. The fifth to twelfth cranial nerves emerge from both sides, forming two crura pedunculi cerebri (Dursun 2008).



**Figure 1:** Brain schematized in chicken (König et al. 2016)



**Figure 2:** Dorsal view of brain and cerebellum in chicken cadaver (König et al. 2016)

## **1.1. Prosencephalon (Forebrain)**

### **1.1.1. Telencephalon (End-brain)**

It is characterized by the size of the hemispheriums. It is the part of the brain that participates in the development of the rhinencephalon to a small extent. It consists of the corpora striata and basal ganglia and fills the lateral ventricles. Median septum, ventrolateral corpus striatum, dorsolateral and medial pallium or cortex cerebri can be distinguished in hemispheriums. Corpus striatum; consists of paleostriatum, neostriatum, and archistriatum. Paleostriatum is found in the basal and contains large cells with basal nuclei (Dursun 2008). The archistriatum, which is the most caudal, is located on the very large neostriatum. Paleostriatum develops from the laterobasal wall of the primitive forebrain, while neostriatum develops only from the lateral wall. The

corpus striatum is similar to that in mammals and is associated with the diencephalon and mesencephalon parts of the brain. Pallium or cortex; divided into paleocortical, archicortical, and neocortical parts. The cortex lobe olfactorii, area priformis, and the cortical part of the archistriatum are of paleocortical origin (Dursun 2008, Nickel et al. 1977). The most important structural difference that separates the mammalian brain from the avian brain is the neocortex. Neocortex is a replacement for the general cortex of the multilayered cortex, which in mammals is overextended with 5 or 6 layers. Avians do not have neocortex. Avians have large areas of gray matter under the surface of the cerebral hemispherium (König et al. 2016). The telencephalic complex of the avian has many features in common with the neocortex of mammals, both functionally and anatomically. These features are:

1. Both receive tectothalamic alerts.
2. They receive direct retinal impulses. It is formed by the optic nucleus of the thalamus in avians.
3. They receive warnings about hearing.
4. They form motor pathways to the forebrain and spinal cord (Dursun 2008).

### **1.1.2. Rhinencephalon**

The term rhinencephalon is used to fully describe the telencephalon. Despite their well-developed eyesight, the avian's sense of smell is not well developed, with some exceptions. The neurites of primary neurons originating from the receptors on the olfactory mucous membrane are

collected in the olfactory nerve. These neurites are connected with secondary cells, namely mitral cells. The dendrites of the mitral cells form the glomerular olfactoria in the bulbus olfactorius (Dursun 2008). In the bulbus, granular cells as well as mitral cells function as internuncial cells. All these nerve fibers and cells are known as the "formatio bulbaris". Within the tractus olfactorius, the neurites of the mitral cells, which are shaped as the secondary tractus olfactorius, enter the corte prepiriformis caudally and surround the olfactory lobe. Tractus corticoarchistriatum and tractus parolfactoarchistriaticus archistriatum receive signals from the secondary olfactory center, which is a very important connection area in the sense of smell in avians. It also associates with tractus olfactorius septi, area prepiriformis and primordium hippocampi. Connection to the nucleus habenularis is also provided by the tractus corticohabenularis and olfactohabenularis.

### **Diencephalon (Tween brain)**

It is a pink, conical structure located in the triangular region between the cerebellar hemispheriums and the cerebellum. It is 3.5mm long and 2mm wide in domestic avian (Dursun 2008).

The diencephalon, which is related to the telencephalon, consists of a large number of descending and ascending fibrils and is examined in three parts.

1- epithalamus or roof

2- Thalamus or thickened lateral walls

### 3- Hypothalamus or floor

These structures surround the ventriculus tertius with a vertical cleft and are in contact with the ventriculus lateralis caudally through the aqueductus mesencephali and rostrally through the foramen interventriculare (Nickel et al. 1977). The epithalamus is the thin epithelial layer of the diencephalon and bears the plexus choroideus ventriculi III located within the ventriculus tertius. The pineal gland that separates the mesencephalon from the diencephalon and is located dorsally in the fissura transversa produces melatonin. Thalamus, the main part of the diencephalon, is divided on each side into its dorsal and ventral parts and contains many nuclei aligned side by side. Nucleus geniculatus lateralis is in the dorsal part of the thalamus. It receives fibrils from the tractus opticus and sends fibrils to the tectum opticum and other mesencephalic nuclei. In the ventral part of the thalamus is the ganglion opticum basale. This part receives the fibrils from the optic part and sends a bundle of fibrils to the nucleus spiriformis. Nucleus spiriformis is a relation center for optical and static stimulation. Nucleus intercalatus occurs in the same region and contains fibrils from tractus bulbothalamicus (Dursun 2008). The fibrils known as commissura optica dorsalis and commissura optica ventralis cross at the base of the ventricle of the diencephalon caudal to the chiasma opticum. The ventral part of the diencephalon is formed by the hypothalamus. Its rostral end is located in the lamina terminalis grisea. Both optic parts of the chiasma opticum, which lie opposite the outer surface of the hypothalamus, continue in the tectum opticum through the corpus

geniculatum lateralis. The caudal of chiasma is the hypophyseal stem or the infundibulum of the pituitary. Several masses have been described in the hypothalamus, including supraoptic, paraventricular, preoptic, and infundibular nuclei. The paraventricular, supraoptic, and infundibular nuclei join the hypothalamo-hypophyseal region, which innervates the pituitary. Like mammals, it has all autonomous functions, including reactions such as temperature regulation, respiration, circulation, eating, drinking, reproduction, protection and attack (König et al. 2016).

### **1.2. Mesencephalon (Mid brain)**

In avian, the midbrain is the junction of vestibular, acoustic, optic, and protopathic stimuli. The most important structure in the midbrain is the tectum mesencephali. This is also called the optic lobe. The size of the volume indicates the degree of vision of the avians. The counterpart of colliculus rostralis in the corpora quadrigemina in mammals is the optic lobe in avian. It is located ventrolaterally on the brain stem in avian. Under the dorsolateral surface of the mesencephalon is a colliculus caudalis-like structure (Nickel et al. 1977). The nuclei of the midbrain originate from the wing and base parts. The tectum opticum develops from the wing part and the tegmentum opticum develops from the base part. In avian these are the last parts of the tractus opticus and are quite well developed as all the formations of the midbrain are related to vision and balance. It is present in the basal layer of the nervus trochlearis and nervus oculomotorius tegmentum. The nervus oculomotorius arises from the lateral aspect of the mesencephalon, and the nervus trochlearis

from its dorsal (Dursun 2008). Nucleus reticularis tegmenti is located in the midbrain towards the rostral as a continuation of its similar formation in the medulla oblongata. In this region, various nuclei differentiate and include the nucleus ruber. Nucleus ruber; It receives a significant amount of fibrils and contains large cells from the nervus vestibulocochlearis and the pedunculus cerebelli rostralis of the cerebellum. Tectum mesencephali includes nucleus semilunaris, nucleus isthmo-opticus, and nucleus mesencephalicus lateralis (N.A.A. 1993). These three nuclei and their ends are associated with the center of the vestibular nerve. It is present at the rostral end of the commissura caudalis tectum. It contains a longitudinal bundle of fibrils that form the bond between the nucleus spiriformis and nucleus praetectalis, which are distant from each other in the midbrain. The nucleus spiriformis is associated with the nucleus mesencephali lateralis. Both nuclei are connected to the cerebellum via tractus cerebellaris and to the corpus striatum via tractus striomesencephalicus. The efferent fibers of the tectum extend into the formatio reticularis of the medulla oblongata and possibly the spinal cord (Dursun 2008).

### **1.3. Rhombencephalon (Hind brain)**

The rhombencephalon consists of the cerebellum and the medulla oblongata.

#### **1.3.1. Cerebellum (Metencephalon)**

It is a central organ for balance and movement functions. Due to these functions, avians have a larger and more stable structure compared to

other sections. The appearance of the arbor vitae (tree of life) seen in mammals is similar in avian. The cerebellum has reached a significant size and degree difference in avian (Dursun 2008). Because it is a vital organ for balance and movement. The cerebellum joins the dorsal aspect of the medulla oblongata via the pedunculus cerebelli caudalis and the pedunculus cerebelli rostralis. The cerebellum consists of a large to medium structure and a small cerebellar hemispherium on each side. The main structure of each hemispherium is the flocculus and paraflocculus, which protrude from the caudal end of the vermis. The cerebellum is connected to the midbrain via the velum medullare rostrale. In the caudal velum medullare provides its connection with the medulla oblongata (McLelland 1990). The cerebellum is divided into three lobes by a transversal and a deep cleft. These lobes; lobus anterior, lobus medius and lobus posterior. Numerous lobules and folia are present in these lobes and folds. The divisions seen in the winged cerebellum are termed similar to the mammalian cerebellum. The basal part of the anterior lobe is called the "lingula" and the posterior lobe is called the "uvula". In general, the classification is made by giving numbers from 1 to 10 of the lobes from the anterior lobe to the posterior lobe. Flocculus is formed by the lateral expansion of the 10th lobe, while paraflocculus is formed by the lateral expansion of the 9th lobe. In the section made from the midline part of the cerebellum, it is seen that the outer cortical layer is dark and the inner medulla layer is white. These layers contain afferent and efferent fibers as well as inter cerebellar fibrils in the metencephalon. When the sagittal section of the cerebellum is made, an image similar to the tree of life called "arbor



vitea" appears due to the ratio of gray and white matter in the lobes (Dursun 2008). The cortex of the cerebellum has 3 layers from the outside to the inside. These layers are the molecular layer, the large layer with Purkinje cells, and the granular layer. In avian, there are two nuclei in the cerebellum. The nucleus fastigi (nucleus cerebellaris medialis) is located medially, and the other is nucleus cerebellaris lateralis located laterally. Among the afferent tracts of the cerebellum is tractus spinocerebellaris, where the fibrils originate entirely from the spinal cord. This part reaches the anterior and posterior lobes of the cerebellum via the pedunculus cerebelli caudalis. Tractus olivocerebellaris follows this pathway. The efferent pathways of the cerebellum contain neurites of Purkinje cells that terminate in the nuclei of the cerebellum. True efferent parts arise from these nuclei.

### **1.3.2. Medulla oblongata (Myelencephalon)**

It is a larger and club-shaped organ than the spinal cord in the brain cavity. After the level of the foramen magnum, it continues as the spinal cord. Although fissura mediana ventralis is quite prominent in avians, it is not as prominent as in pyramis and pons mammals. Where the caudal part of the fossa rhomboidea ends, there is a pointed projection called the calamus scriptorius. Fossa rhomboidea forms the floor of the ventriculus quartus and the roof of the velum medullare caudale. The ventriculus quartus caudal also opens into the ventriculus tertius through the central wing of the spinal cord and the aqueductus mesencephali in the rostral. Medulla spinalis; It controls the circulation, respiratory mechanism, all functions related to the food taken into the

body and the statoacoustic devices that regulate all the motor functions of the body (Dursun 2008).

## **2. Medulla spinalis (Spinal cord)**

The medulla spinalis extends evenly along the vertebral column in avian. It grows to the end of the vertebral canal and enters the filum terminale, tapering in the form of thread, without making the cauda equina (Demiraslan and Orhun Dayan 2021). In avian, the spinal cord is long, narrow and thin. We can divide the spinal cord into cervical, thoracal, lumbal, sacral and coccygeal regions according to the sections of the columna vertebralis. Each segment of the spinal cord that connects with the appropriate body segment has a pair of spinal nerves. Bilaterally, the spinal cord is symmetrical. A cleft is seen on its ventral surface, which continues medianly from one end to the other. It is called the fissura mediana ventralis. On its dorsal surface, just opposite this cleft, there is a barely noticeable groove called "sulcus medianus dorsalis". In the cross-section of the avian spinal cord, gray and white matter are seen. Unlike mammals, gray matter contains masses known as marginal nuclei (nucleus marginalis) (Demiraslan and Orhun Dayan 2021). The gray matter consists of ganglion cells and neurites, and afferent, unmyelinated or slightly myelinated nerve fibers (Dursun 2008). The gray matter runs the entire length of the spinal cord as a four-sided column with a butterfly-like cross-section. The places where the medulla spinalis shows two different enlargements are the regions where the plexus brachialis and plexus lumbosacralis are formed. The enlargement on the cranial side is greater than the caudal (König et al.

2016). In the upper midline, where there is lumbosacral enlargement, there is the avian rhomboidal sinus. The right and left parts of this sinus are surrounded by a gelatinous substance. The gelatinous substance consists of glial cells rich in glycogen. The cornu ventrale of gray matter increases in volume where there is lumbosacral enlargement. In this way, many projections formed by the ventral rootlets on the ventrolateral surface of the spinal cord are formed. It is also found in the lumbosacral enlargement of small lateral projections from the lateral column (Dursun 2008, Nickel et al. 1977). The spinal cord is surrounded, protected, and supported by three membranes called the dura mater, arachnoidea, and piamater, as in mammals (Dyce et al. 2018). The outermost layer is the duramater, relatively thick and durable. In the cervical and thoracic regions, it is separated from the periosteal sheath of the canalis vertebralis by an epidural space. This space is filled with a gelatinous substance. Toward the caudal end of the thoracic region, the periosteum and duramater unite. This union continues until the end of the canalis vertebralis (Dursun 2008). In the foramen magnum, the periosteum and the duramater are united. More or less in contact with the duramater, the arachnoidea is a delicate membrane. Piamater tightly surrounds the spinal cord and is rich in blood vessels. Cavity subarachnoidea is located between the pia mater and arachnoidea. White matter; It is divided into three parts: ventral, dorsal and lateral. Between the septum dorsale medianum and the dorsal rootlets of the spinal nerves and the cornu dorsale is the funiculus dorsalis. Funiculus lateralis; It is the region of white matter between the dorsal and ventral rootlets and the cornu dorsale and cornu ventrale.

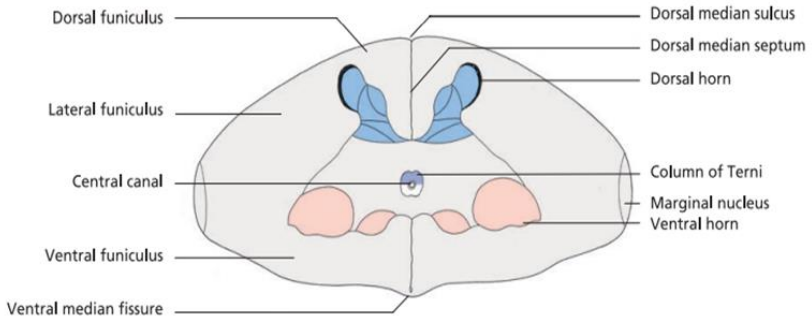
Funiculus ventralis; It is located between the cornu ventrale and ventral rootlets and fissura mediana ventralis (König et al. 2016). The ventral and lateral branches of the white matter are usually large, and the dorsal branch is small. Septum dorsale medianum and fissura mediana ventralis do not reach the gray matter. There is a dorsal white commissura above the gray matter and a ventral white commissura below the gray matter (Dursun 2008, Nickel et al. 1977). Each of these commissura separates nerve fibers that pass diagonally from one side of the spinal cord to the other. The dorsal commissura includes the ascending fibers of the primary afferent neurons in the dorsal roots and the fibers arising from the stems of the nerve cells in the gray matter. The ventral commissura (like the spinothalamic and spinoreticular tracts) contains nerve fibers that come together to form ascendens tracts (Nickel et al. 1977).

#### The roads to descendens

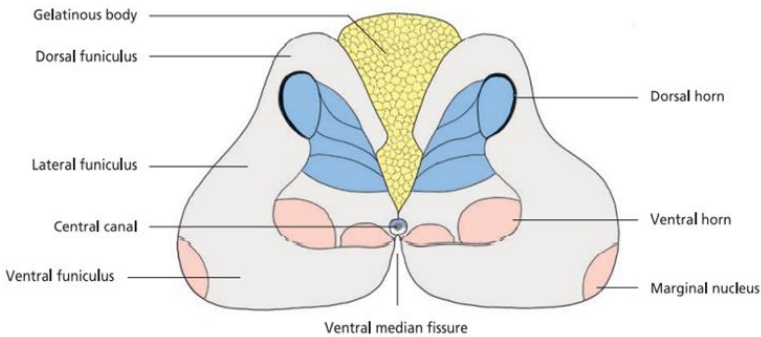
Funiculus dorsalis contains cell bodies in the dorsal root ganglion and ascending fibers originating from primary afferent neurons. These fibrils are arranged as in mammals. The fact that the volume of the dorsal arm does not change along the length of the spinal cord indicates that the axons are quite short. After entering the spinal cord, these axons do not terminate here, but extend to the medulla oblongata. Ascending fibrils originating from the funiculus dorsalis form part of the somato-sensory system of the avian. These pathways found in avian are thought to have the same function (touch, pressure, etc.) as in mammals (Dursun 2008). The connection activated by the muscle receptors of the

ventrolateral ascending bundle and the dorsolateral ascending bundle include the tractus spinocerebellaris dorsalis. In avian, this pathway is limited to the wing region of the spinal cord, whereas in mammals it is limited to the hind legs and trunk. Therefore, the similarity is not fully seen. In avian, the ventrolateral ascending bundle includes the tractus spinocerebellaris ventralis. This pathway is activated by the afferent muscles in both poultry and mammals and exits the hind leg region of the spinal cord to the cerebellum. It is the main pathway connecting the spinal cord to the cerebellum. A different group of fine fibrils covers the tip of the cornu dorsalis of the avian and is known as the fasciculus dorsolateralis. The tip of the cornu dorsale is the nucleus of the substantia gelatinosa. Degeneration studies have shown that the ascending fibrils in pigeons, after crossing from the cornu dorsale, descend into the funiculus lateralis to project directly into the thalamus. This suggests that the mammalian tractus spinothalamicus has an avian counterpart (Dursun 2008). Although it only transmits the sensation of pain, heat and touch like pinprick, only tactile transmission is seen for avians. Again in degeneration trials, it was observed that well-developed spinoreticular tracts were also found in avian. This pathway ascends bilaterally from the spinal levels to the reticular formation of the medulla oblongata, pons, and mesencephalon. The ascending pathways in the ventral arm appear to be limited to the short intersegmental fibers of the propriospinal system (Dursun 2008, Nickel et al. 1977).

Funiculus lateralis; includes the tractus rubrospinalis and the tractus reticulospinalis lateralis. The tractus rubrospinalis in mammals is similar to the tractus rubrospinalis in avian. Its fibers originate from the red nucleus in the mesencephalon. It descends along the entire length of the spinal cord and terminates in the motor cells of the cornu ventrales. These endings are close to the motor neurons that mainly innervate the flexor muscles. The fibers of the tractus reticulospinalis terminate in the nucleus intermedius. This is probably why it has a visceral motor function. The descendens tracts form most of the ventral branch. It contains vestibulospinal, reticulospinal, and tectospinal tracts, and vestibulospinal fibers project from the medial longitudinal bundle of the brain to the spinal cord. These fibrils extend in the ventral arm as two branches medial and lateral (Dursun 2008, Nickel et al. 1977). Both extend along the entire length of the spinal cord and terminate in association with extensor motor neurons. The medial drupe is larger than the lateral group. The medial group resembles the tractus vestibulospinalis ventralis of mammals, and the lateral group resembles the tractus vestibulospinalis lateralis. Alpha and gamma motor neurons organize lateral groups. This is probably why movement is important in somatic motor control. The ventral arm, tractus reticulospinalis medialis, is also visible. Its function in avian is unknown. It is believed to contain mesencephalic and vestibular components and affect the visceral and somatic motor control systems. The tectospinal tract extends from the tectum mesencephali to the upper segments of the spinal cord, to the oculomotor nucleus. It is presumed to control eye and neck movements when following moving objects (König et al. 2016).



**Figure 3:** Schematic view of the cervicothoracic part of the spinal cord (König et al. 2016)



**Figure 4:** Schematic view of the lumbar part of the spinal cord (König et al. 2016)

## BRAIN VENTRICULICIES

The central nervous system has developed from the simple neural tube with increasing volume and differentiation and is considered a system of cavities that continues along its entire length. While the spaces in the medulla spinalis are expressed by the narrow central canal, there are brain ventricles of different volumes and sizes in different regions of

the brain. *Canalis centralis* and *ventriculus* are limited by special ependymal (glial) cells (Dursun 2008). Brain *ventriculus* consists of *ventriculus lateralis* located in cerebral hemispheres, *ventriculus tertius* in diencephalon and *ventriculus quartus* in rhombencephalon. In some parts of these spaces, the roof of the brain consists of a single layer, or *lamina epithelialis* (N.A.A. 1993). Arterial plexuses are formed separately within the *ventriculus*. The arteries covered by the *piamater* are called *tela choroidea* and the structure that covers them is called the "plexus choroideus". Choroid plexuses are located caudal to the cerebellum and are known as "plexus choroideus ventriculi IV". The plexus choroideus ventriculi III, located in the *ventriculus* of the diencephalon, passes over the lateral ventricle through the *foramen interventriculare* and is defined as the plexus choroideus ventriculi lateralis. The plexuses secrete the *liquor cerebrospinalis*. This fluid fills the brain cavities and *canalis centralis* (Dursun 2008). The shape and volume of the *ventriculus lateralis* are determined by the *corpora striata* in avians. *Cornu rostrale* extend into *bulbus olfactorius*. Both *ventriculus lateralis* are connected via the *foramen interventriculare*. Due to the absence of the *adhesio interthalamica*, the third ventricle is a narrow slit in the diencephalon with a median continuity. On the basal edge of the *ventriculus quartus* are *recessus infundibuli*, *recessus postopticus*, and *recessus suprapinealis*. The connection between the third and fourth ventricles is provided by the *aqueductus mesencephali*. The *fossa rhomboidea* is located in the fourth *ventriculus*. From the dorsal to the cerebellum, the fourth *ventriculus* extends as *recessus lateralis* and *recessus cerebelli*. Each *recessus lateralis* of the fourth



ventriculus shows an opening called "apertura laterales Luschkae". In this way, the internal cerebrospinal fluid flows into the subarachnoid space and contains the liquor cerebrospinalis externus (Dursun 2008, Nickel et al. 1977).

### **The membranes of the brain and spinal cord**

The brain and spinal cord are covered by the leptomeninx and pachymeninx. Pachymeninx or dura mater cerebri are connected to each other and to the bone, forming a durable structure with the endosteum of the cranial bones forming the cranial cavity (Gofur 2020). The sinus venosus, the efferent blood system of the brain, is surrounded by the duramater. These sinuses can be very wide where they are. Falx cerebri and tentorium cerebelli are not well developed in avian. The endostiva or endoarchis is not attached to the spinal cord of the duramater. In this region, the duramater is in the form of a larger tube than the spinal cord. Where they pierce the duramater, the spinal nerves are fed by the connective tissue sheath. They surround the spinal ganglia between the foramen intervertebrale (Dyce et al. 2018). In avian, the leptomeninx in the brain and spinal cord consists of two thin layers of connective tissue. The outer avascular membrane is called "arachnoidea". The inner membrane completely surrounds the spinal cord and brain and is rich in blood vessels. This layer is also called piamater. The intermediate or subarachnoid space is narrowly located between the two membranes and is filled with cerebrospinal fluid (Dursun 2008, Nickel et al. 1977). Thin connective tissue fibrils act as bridges in this gap. The ligamentum denticulatum provides the suspension of the spinal cord arising from

the spinal pia mater and within the dural tube. This ligament is the bilateral bands of the pia mater interrupted by the spinal nerves. The plexus choroideus, which are formed in the ventricles of the brain, form the pia mater cerebri. The tela, which is surrounded by a capillary network, forms the choroidea and extends to the opposite wall of the brain. There is a single lamina epithelialis layer in this region (Dursun 2008).

## **CONCLUSION**

Avian medicine has become an important branch that veterinarians turn to, in parallel with the preference of avian as food, their breeding as ornamental animals and the increasing interest in wild avians. It is necessary to take samples from nervous system organs in some viral, bacterial and infectious diseases in avian. In order to correctly evaluate the condition of the sick animal, it is necessary to have sufficient knowledge about the anatomy of the organs of the relevant system. There are studies in avians. However, in the literature reviews, it has been seen that there are limited number of studies that synthesize traditional and current information about the anatomy of the poultry nervous system. We think that with the book section presented in line with this need, it will contribute to this deficiency to some extent.

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## **CHAPTER 5**

### **INSECTS IN THE MODERN WORLD**

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## **INTRODUCTION**

Human civilization has mentioned insects since the time immemorial. They have been economically and aesthetically important to us. Several useful products are produced by insects such as honey, lac, silk, etc. However, insects have also been associated with unwanted consequences for humans such as being carriers of diseases, threats to hygiene, or harming the agricultural crops, timber and stored food products.

All insects belong to phylum Arthropoda and include four subphyla i.e. Hexapoda (largest subphylum in terms of described species) which includes insects; Chelicerate which includes spiders, scorpions, mites etc.; Crustacea which includes crabs, shrimps, prawns etc., and Myriapoda which includes millipedes, centipedes etc.

The class Insecta has 29 orders and based on the stages of development during their life cycles, insects belong to any of the three groups i.e. holometabolous (show complete metamorphosis i.e. egg, larva, pupa, adult), hemimetabolous (show incomplete metamorphosis i.e. do not show a proper pupal stage) and ametabolous (no nymphs). Of these, the largest group is holometabolous which comprises of orders such as Coleoptera, Lepidoptera, Hymenoptera, Hemiptera, Diptera and others. Each one of these groups have more than a million species and many of them have yet not been recognized and described. However, Coleoptera is the group which includes largest described species. Ten insect orders include aquatic species, which are either totally aquatic or some stage

of their life cycle is aquatic. There are five orders which include aquatic larvae or nymphs/naiads: Odonata (Ex. Dragonflies and damselflies), Ephemeroptera (Ex. Mayflies), Plecoptera (Ex. Stoneflies), Megaloptera (Ex. Hellgrammites and dobsonflies), and Trichoptera (Ex, caddisflies).

## **RELATIONSHIP BETWEEN INSECTS AND HUMANS**

The association between humans and insects is ancient, which has evolved with time. The perception of people towards insects and nature has also changed. Insects have co-existed with humans since time immemorial, hence we find their mentions in many traditional beliefs and mythology (Duffus et al., 2021). Insects find mention in the ancient texts of many civilizations and history in general. The most well-known and earliest examples of insects come from 8 AD from Greece, about their metamorphosis. Back in around 3000 BC, Egyptians equated butterfly with soul and tied them to the philosophy of rebirth (Duffus et al., 2021). Ancient Egyptians considered dung beetles sacred. In Medieval era, insects were associated with death and decay or hard work and persistence. In India, insects were mentioned in *Vedas*, the sacred texts, where they either provided useful products or were carriers of diseases. Thus, there were many stories from ancient civilizations that showed close associations between humans and insects. Insects were considered valuable as they provided many useful ecosystem services such as pollination, provided food, pest control or simply had aesthetic value. However, recently their contribution in ecosystem services has been more seriously thought upon. For instance, many

insects such as butterflies, moths, wasps and flies pollinate crops. Hymenopterans and Coleopterans help in pest control because they feed on pest species such as aphids, white flies etc. Soil insects such as dung beetles and termites help in soil fertility. The ecosystem services provided by insects have been valued at \$47 billion per year (Eggleton, 2020).

Social insects such as ants, wasps, bees, termites etc. are major components of ecosystem communities and are vital ecosystem service providers (ESPs). Social insects provide at least 10 ecosystem services and the major traits of these insects that make their contribution as ESPs more valuable are: their high biomass, higher abundance, diverse mutualistic associations, ability to build biogenic structures, their ability to produce variety of chemical defences, simultaneous delivery of several ecosystem services, division of labour and presence of several castes, efficient communication system and coordination, ability to store food for future, and long life span. They can also tolerate stress and are easy to manage (Elizalde et al., 2020).

Insects have also entered our culinary traditions. They are widely consumed across many countries. For instance, there are at least 1681 species from 14 orders that are consumed across 102 countries. In Mexico, 29 indigenous groups consume around 67 species of Lepidoptera. Lepidopterans have higher calorific density than vertebrate food sources and entomophagy is more than just a nutrition. This practice is learnt and passed through generations and thus, holds a valuable cultural heritage. Furthermore, Australian Aboriginal



communities have been using secretions of psyllid species, known as 'lerps', as a food source for generations. Lerps have high amylose content which makes them an excellent source of energy. Culture of these people is also influenced by lerps. For example, their calendars, religious practices, marital and initiation ceremonies are influenced by lerp seasons (Reviewed by Duffus et al., 2021).

However, there is another side of insects too. They are agricultural pests and vectors for many diseases. Crop-feeding insects have been a vital component of agroecosystems for over millennia. Mutual and complex relationships exist between crops and insect pests. They cause huge crop losses (20-40% globally per year). The loss to global economy due to insect pests is estimated to be around \$220 billion (FAO, 2020). The common insect pests include aphids, scales, thrips, mealybugs, whiteflies, spider-mites, leaf beetles and weevils, caterpillars, cut worms, leaf-miners, termites, leaf rollers, red pumpkin beetles, stem borers, cabbage diamond-black moth, jassids, etc. In addition to being the crop pests, many of the insects, which feed on other smaller crop pests, have the potential to act as biocontrol agents, thereby reducing the chemical burden of earth which is due to use of chemicals in agriculture. Some of the common biocontrol agents include ladybird beetles, social wasps, ground beetles, rove beetles, lacewings, hoverflies, flower bugs, parasitoids, etc.

Insects act as vectors for many pathogens that cause various diseases (Table 1). The insect vectors are found more in tropical and subtropical regions i.e. South America, Africa, South East Asia and the Pacific.

Almost half of the world's population is facing risk of insect-borne diseases and thousands of them die (Shaw and Catteruccia, 2019). The economic burden on the countries due to insect borne diseases is very difficult to assess, but it would be much heavier in tropical and subtropical countries, especially economically weaker ones.

**Table 1:** Insect vectors for human diseases

<b>Insect vectors</b>	<b>Human/animal Diseases</b>	<b>Causative organisms/Pathogens</b>	<b>References</b>
<i>Anopheles</i> (Mosquito)	Malaria	<i>Plasmodium</i> (Protozoa)	Shaw and Catteruccia, 2019
<i>Culex</i> , <i>Aedes</i> , <i>Anopheles</i> (in Africa) <i>Mansonia</i> (Mosquito)	Lymphatic filariasis	<i>Wuchereria bancrofti</i> , <i>Brugia timori</i> , <i>Brugia malayi</i> (Nematods)	Shaw and Catteruccia, 2019
<i>Glossina</i> (Tsetse fly)	Sleeping sickness in humans (African trypanosomiasis) and nagana in cattle	<i>Trypanosoma brucei</i> (Protozoa)	Shaw and Catteruccia, 2019
<i>Rhodnius</i> , <i>Triatoma</i> (Reduviid bug/Kissing bug)	Chagas disease (American trypanosomiasis)	<i>Trypanosoma cruzi</i> (Protozoa)	Shaw and Catteruccia, 2019
<i>Phlebotomus</i> , <i>Lutzomyia</i> (Sand fly)	<i>Leishmaniasis</i> (Kala azar)	<i>Leishmania</i> (Protozoa)	Shaw and Catteruccia, 2019
<i>Simulium</i> (Black fly)	River blindness (Onchocerciasis)	<i>Onchocerca volvulus</i> (Nematode)	Shaw and Catteruccia, 2019
<i>Aedes</i> (Mosquito)	Dengue	DENV (Flavivirus)	Shaw and Catteruccia, 2019
<i>Aedes</i> , <i>Haemogogus</i> (Mosquito)	Yellow fever	YFV (Flavivirus)	Shaw and Catteruccia, 2019
<i>Aedes</i> (Mosquito)	Zika	ZIKV (Flavivirus)	Shaw and Catteruccia, 2019

<i>Aedes</i> (Mosquito)	Chikungunya	CHIKV (Togavirus)	Shaw and Catteruccia, 2019
<i>Culex</i> (Mosquito)	West Nile fever	West Nile virus	Müller et al., 2019
<i>Aedes, Culex</i> (Mosquito)	Japanese encephalitis	Japanese encephalitis virus	Müller et al., 2019
<i>Aedes</i> (Mosquito)	Rift Valley fever (RVF) in domestic animals	RVF virus (arbovirus)	Müller et al., 2019
<i>Pediculus humanus corporis</i> (Body louse) or <i>P. humanus capitis</i> (Head Louse)	Louse-borne relapsing fever	<i>Borrelia recurrentis</i> (Spirochaete)	Warrell, 2019

## DECLINE OF INSECT SPECIES ON EARTH

Insects being the largest diverse group, their decline is noticeable and it is a reason to worry for humans. The insects' decline might have been happening much earlier than the Industrial Revolution, and the decline was more noticeable in the developed countries. The first key proof of the insect decline came from studies on Lepidopterans i.e. butterflies and moths, in the 1870s, as they were extensively studied for centuries in developed countries, particularly Europe. There have been other similar studies on insect decline, but these have been carried out more recently (Hallmann et al., 2017; Wagner et al., 2021).

Several reasons account for the insect decline, and majority of them are anthropogenic. Some of the reasons include urbanization, shifting from natural to agricultural systems, climate change, habitat loss due to deforestation, overexploitation, pollution, fire, drought, increasing nitrification (fossil fuel burning or usage of synthetic fertilizers), and

invasive species (Cardoso et al., 2020; Raven and Wagner, 2021; Wagner et al., 2021). Numerous studies have reported the decline in numbers, abundance and biomass of insects from different regions worldwide. After World War II, numbers of many butterfly species were reported to have declined due to change in agricultural practices, the decline being more in Europe than other countries (Eggleton, 2020; Wagner et al., 2021). A 27-year study (1989-2016) in 63 naturally protected areas in Germany reported a 76% seasonal decline and 82% mid-summer decline in biomass of winged insect (Hallmann et al., 2017). The study also suggested that factors such as type of habitat, land use, and climate change did not influence this decline. The study only provided information on biomass decline but did not talk about decline in their numbers (Eggleton, 2020).

The decline/loss of not only the rare insects but also the abundant ones raises concerns about the functioning of ecosystems. Currently, the yearly decline rate of insect species is 1-2% but all insect species are not declining (Reviewed by Wagner et al., 2021). Some insects such as many moth species in Great Britain are declining, while others are increasing in abundance. Many temperate insect species, especially those whose numbers used to be limited due to cold temperatures, are increasing in numbers due to increase in average temperature of earth i.e. global warming. The global decline in insect population can be risky for several ecosystem services that are regulated by insects such as soil formation, nutrient cycling, water purification, decomposition,

biocontrol, insect pollination and maintenance of a healthy food web that is necessary for human survival (Sluijs, 2020).

The study by Carvalheiro et al. (2020) in three European countries (Great Britain, Belgium, and Netherlands) between 1930-1990 showed extensive loss of bumblebee species before 1990. They also reported decline in richness of butterflies in these countries from 1950 to 2009. Another study reported that 132 (32%) of 413 recorded species of butterflies were extirpated in Singapore since 1854 (Theng et al., 2020).

Although several studies have shown decline in insect biomass or abundance, the global decline in insect species is very difficult to assess as insect taxa are highly diverse and include millions of species, many of which are yet not described. The reports of decline in insect species from different regions offer two sentiments: one, the decline is real in certain regions and second, the studies reporting insect decline so far are inadequate to give substantial conclusions for global insect decline. Many studies have reported a decline in different insect species in different regions globally, which is a cause of concern. The studies reporting insect decline in some countries might have weightage for that region, however, the same can't be extrapolated for other regions. There is dearth of comparable studies targeting selected insect species in different regions or countries which can help us to come to a realistic conclusion about the decline in insect numbers. Tremendous work needs to be done in this direction. But, one thing is for sure human pressure has been impacting ecosystems and thus, is affecting insect diversity and abundance.

The studies that report the decline are biased, as a majority of them report about taxa that are more common and closer to humans like those associated with agriculture, and data is primarily collected from developed countries. To have some conclusive information on decline or increase in abundance of insects, decadal census data should be thoroughly studied. However, again it is very challenging to amass and maintain such data for all insect taxa as it is labor and time-intensive as well as expensive (Wagner et al., 2021). The plummeting numbers of insects and subsequently their loss might have long-lasting and irreparable consequences. Some of these include loss of abundance and biomass, differences in the occurrence of insects in space (different hierarchy in soil or plants) and time (diurnal or nocturnal), loss of phylogenetic diversity, loss of functional diversity, and loss of crucial roles insects play in ecological networks (Cardoso et al., 2020).

The diversity of insects is a major hurdle in identifying and describing them. Hence, it seems unfeasible, at the moment, to collect such kind of data and come to any conclusion with regard to changes in their numbers with time. Whatever information we receive about the decline pertains to only the known insect species and that too from the countries which are actively involved in research in this area. So, we are still in dark about the changes in numbers of insect species over decades and centuries. However, based on whatever little information about their decline we have, it is ostensible that we should take steps to minimize human interference in the natural ecosystems so that insects can thrive well and continue to support ecosystems.

## **PONDERING OVER STUDIES ABOUT PLUMMETING INSECT NUMBERS**

Several studies have conveyed decline in the insect diversity since the late 19<sup>th</sup> century. The information provided by some such studies are convincing while for many others it is not so. The methods adopted by some noteworthy studies have been criticised by academia. For example, the study by Hallman et al. (2017) reported a decrease in insect biomass over a period of 27 years (1989-2016). Though the study is substantial, there are two significant factors missing in the study i.e. there is no information on decrease in insect numbers and secondly, simple linear models used in the study, can't be applied to data collected unevenly from few data points. Another study, on tropical insects, from Puerto Rico reported decrease in arthropod biomass by 4-30 times between 1976 and 2012 (Lister and Garcia, 2018). They correlated this decrease to temperature increase and also talked of the resulting food web collapse. Again, the study was criticised for considering only one factor i.e. temperature, for this decline. Also, the data was not analysed properly. Upon reanalysis of the data, it was found that there was no correlation between declining biomass and temperature increase or collapsing food web (Willig et al., 2019). One review (Sánchez-Bayo and Wyckhuys, 2019) summarized the data of insect decline from 73 studies, almost all from United States and Europe. These studies concluded that insect orders such as Lepidoptera, Coleoptera, Hymenoptera, Odonata, Plecoptera, Ephemeroptera, and Trichoptera are at greater risk than others. They showed that there has been more

than 41% decline in insect numbers and also warned that there is risk for 40% of global insect species to become extinct within decades. However, the studies encompassed in the review, were biased as they focussed more on the ecologically and economically important insects, had limited geographical range and their study design was flawed. A quantitative study by Seibold et al (2019) observed 150 grassland sites and 140 forest sites from 2008 to 2017 in Germany. They reported a 67%, 78%, and 34% decline in biomass, abundance, and number of insect species in grasslands. In 30 forest sites, there was a decline of 41% and 36% in biomass and species number respectively, but they did not observe any reduction in abundance. Rare insect species were more affected than abundant species but abundant species were also significantly affected. Again in this study, focus was on flying insects, and although it showed broader geographical declines it dealt with a relatively small area.

### **ANCIENT INSECT SPECIES AND POSITION OF INSECTS IN THE NEW WORLD ORDER**

Based on the traditional methods, the existence of hexapods dates back to early the Devonian period (416 to 359 mya; mya= million years ago), and by Carboniferous period (354 to 290 mya) they evolved into diverse winged forms. However, between the Early Devonian and Late Carboniferous periods, their fossil records are rare. Thus, this period that lacks sufficient fossil records of hexapods is known as ‘hexapod gap’. The first insect abundance is reported from the Late Carboniferous period and this period was dominated by pterygotes (Giribet and



Edgecombe, 2019). Nonetheless, insects are the most successful animals in the 450-million-year history of terrestrial existence.

If palaeodiversity is considered as a measure of the number of families recorded, insects have been the most successful group. There are 1454 insect families in fossil record while 1067 are living (Jarzembowski, 2016). Out of the 40 insect orders, 30 have living representatives. All insects are put into two major groups i.e. apterygota and pterygota. Apteriygota includes 3 orders and one of them (Monura) is extinct while in pterygota, 9 orders have become extinct.

More than a million insect species have been identified and described, but still there are an estimated 4.5 to 7 million species of living insects that are still undescribed (Amendt, 2019). Speculation is that probably most of these species are in tropical regions as the environment in these regions is more favourable for their sustenance. Many factors dictate the distribution of insects in different regions such as latitude (lower latitudes have higher diversity), geographic area (tropical regions have larger area than temperate), productivity, climatic stability, speciation speed, higher pathogen-predator pressure and geometric constraints (Eggleton, 2020).

Traditionally, systematic relationships between insects were constructed through morphological and/or ultrastructural characters. A relatively accurate insect phylogeny was produced in 1904. However, many aspects of the phylogeny were controversial and there have been vigorous debates over insect phylogeny for over a century. However,

with the latest advances in insect phylogenomics, attempts are being made to understand the evolutionary lineages of insects in a better and more convincing way. Phylogenomics started in 1980s and made use of few dominating genes, but it was not sufficient to not answer the controversies regarding insect phylogeny (Behura, 2015).

Next-generation sequencing techniques generate genomic and transcriptomic data which has helped in better understanding of these relationships. For example, a small subunit of ribosomal RNA (rRNA) is conserved in different insect species, and hence, it is used as a reference for comparing these species. Phylogenomics is highly useful for insects, as they are a diverse group and in about one million species of animals that are identified, insects form the highest proportion i.e. 75%. Insect phylogenomics is carried out by three methods i.e. sequence alignment, comparing existing 'DNA strings' and comparing gene content. A large number of insects, such as Hymenopterans, mosquitoes, holometabolic insects, Lepidopterans, and Neuropterans have been studied using phylogenomics. Along similar lines, phylogenetics is also an important area that makes use of integrating large amount of sequence data, computational tools, and evolutionary principles to give better explanations for the evolutionary lineages of insects (Behura, 2015). Due to these advances, insect phylogeny is now understood with more confidence.

The molecular and morphological data when integrated, will not only improve insect phylogeny but will also incorporate fossil species within the insect phylogeny (Tihelka et al., 2021) and it will fill up the gaps in

our understanding of the origin and evolution of insects over millions of years. For example, through molecular evidence we have come to understand that hexapods are more closely related to Crustaceans (both included under Pancrustacea, also known as Tetraconata), unlike the previous notion that hexapods are a sister group of Myriapoda (millipedes and centipedes) or sister taxon of Myriapod subgroup under monophyletic Tracheata (Myriapoda + Hexapoda) (Kjer et al., 2016). Molecular techniques have also resolved previously controversial phylogenetic relationships among insects. Information obtained from phylogenomics and phylogenetics by 1K Insect Transcriptome Evolution (KITE) team, which studied just 1478 protein-coding genes, retraces the origin of insects to Early Ordovician period (~479 mya), flight in insects to Early Devonian period (~406 mya), major extant insect lineages in Mississippian period (~345 mya) and major diversification of holometabolous insects to Early Carboniferous period (Misof et al., 2014). Misof et al. (2014) generated transcriptome sequence data for 144 insect species and using maximum likelihood models showed that insects diversified into four groups i.e. Palaeoptera, Polyneoptera, Holometabola, and Condylgnatha. They also showed that diversification within the modern-day winged insects started in Paleozoic era. The KITE team has collected transcriptomes from over 1400 insect taxa and many similar projects are in pipeline.

Although the next-generation molecular techniques are a great tool to unearth the evolutionary relationships between insects, many times the large-scale data produced by them is redundant and in isolation does

not convey much meaningful information. Integrating molecular techniques with the traditional methods of insect phylogeny is way ahead of putting many controversies, regarding insect phylogeny and evolutionary relationships, to rest.

## **FUTURE OF ENTOMOLOGY**

The traditional methods of entomology have provided us huge information about insects and their contribution in ecosystem sustenance and benefits to humans. However, in the past few decades, accelerated changes in the environment and repercussions of advent of new technology, have accelerated the adverse effects on biota, including insects. After centuries of our association with insects, we do not know about even half the insect species. Much work is required but at a faster pace. The new generation genomic techniques, together with phylogenetics might fill in the gaps in our knowledge about insects. However, that is still not sufficient as insects are highly diverse. Advances in computer vision and deep learning have great potential to monitor the diversity and abundance of insects in a given region. Cameras and other sensors such as Internet of Things (IoT) can perform entomological observations throughout day and night and the entire year, in an effective, continuous and non-invasive manner. The physical appearance of insects can be captured through camera in laboratory and deep learning models can tell us about insect abundance, biomass and diversity. They can also quantify variations in phenotypic traits, interactions and behaviour. Deep learning can also give taxonomic identification of the specimen captured as image. Another method

known as DNA barcoding is now an established and powerful method which can reliably assess insect biodiversity (Høye et al., 2021).

## **CONCLUSION**

Insects have had a complex and deep-rooted association with humans, other animals, and plants through ages. While many of them are beneficial, others are detrimental. Nonetheless, they are an integral part of food webs and ecosystems. Their absence will dangerously impact the structural integrity of life on earth as we would be devoid of many ecosystem services which are so central to existence of life. Astonishingly, insects are so diverse that we are not able to assess the changes in their diversity and abundance, except for few studies that report decline in their abundance and biomass over a while. Still, these studies hold significance as they are indicators of the changing face of the earth. Some scientists are even hinting at the ‘sixth mass extinction’ in near future due to drastic anthropogenic pressure on the environment. It is high time that we realize this and understand the issue and work towards minimizing the human influence on earth. Knowledge about insects is very essential as they support ecosystems that are directly related to the source of food for us. A decline in insect species would be accompanied by other drastic changes on earth, which would seriously impact humans and other animals in the long run.

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## **CHAPTER 6**

### **MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION STUDIES ON PEPPER GENOTYPES**

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## INTRODUCTION

Peppers from the *Solanaceae* family are among the most important vegetables in Turkey and world production. Its homeland is reported to be Central and South America (Verit et al., 2001; Ahmed, 2013). There are 20-30 species in the genus *Capsicum* belonging to the *Solanaceae* family (Greenleaf, 1986; Krug, 1986). It is known that the *C. annuum* L type of pepper is consumed in the world and in our country, and it is also used in breeding studies (Bosland and Votava, 2005). It has been reported that five species of pepper from the *Capsicum* genus (*C. annuum* L, *C. baccatum*, *C. pendulum*, *C. chinense*, *C. frutescens*, *C. pubescens*) are cultured (Ortiz and Delgado 1990; Eshbaugh, 2012; Barboza et al, 2019; Bozkalfa and Eşiyok, 2006).

It is known that the entry of pepper to our country was at the beginning of the 16th century, and it spread to Anatolia over time (Vural et al., 2000). As a result of the researches, it was determined that the pepper is very rich in vitamins, in 100 g of fresh green pepper; 1.1 g protein, 29 calories, 0.2 g fat, 4.2 g carbohydrate content, however, the antioxidant rate is high due to the capsaicin (bitterness) substance contained in the pepper.

Ancient civilizations knew that these properties of pepper were good for most diseases and used it for therapeutic purposes. For example, it is reported that pepper accelerates the intestines and stomach and facilitates digestion due to its capsaicin content (Kaur and Kapoor, 2001; Günay, 2005; Eşiyok, 2012).

According to the statistics of the United Nations Food and Agriculture Organization (FAO) in 2019, the world production of pepper was 1,990, and according to the results, the average yield per m<sup>2</sup> in the world in 2019 was 1.91 kg. has taken place. Turkey's pepper cultivation area is 92,089 with a production of 2,588 kg million tons, ranking 3rd after China and Mexico (FAO 2019). In our country in 2020; Capia, which is used in tomato paste production, was produced with 1,291,0912 tons, bell pepper with 389,957 tons, green pepper with 838,890 tons, and red pepper with 116,967 tons (TÜİK, 2021).

Pepper is an important type of vegetable that is well adapted to grow in every region of our country. Although our country is not the gene center of pepper, micro gene centers have become genetic diversity centers for pepper over time. Although Turkey is not the homeland of pepper, the climate of our country is suitable for pepper cultivation and it has shown a significant diversity, as in most vegetable species, since it adapts to each region. The fertile Anatolian lands have caused many civilizations to live in these lands and due to the ecological diversity, variations have occurred in pepper over the years, as in most plant species. There are many locally grown genetic materials in pepper with different characteristics. In our country, it is very important to know the genetic resources of the pepper species and to determine its change with environmental interaction.

While characterizing plant genetic resources, it is aimed to define the properties of the studied material, to reveal the genetic differences between populations, the rate and distribution of genetic variation

within populations. Morphological diversity is very important in plant breeding research. Because the higher the variation, the more successful the breeding work will be (Bliss, 1980). It is very important in gene banks as well as in breeding research of gene resources. The plant characteristics of the genetic resources collected for this purpose should be known. While characterizing plant gene sources, it also shows the rate of variation between populations. Unless the gene sources are characterized, they cannot be used in any breeding studies and may be lost over time.

Genetic variation is of great importance in the genetic evaluation of agronomic characteristics in vegetable breeding studies. The high rate of these variations will provide a better determination of the genetic characteristics of the population. Cultivation of the plant; Adaptation to the characteristics of the region where it grows and its cultivation for many years causes local populations. Local genotypes are reported to be ideal in cultivar breeding studies due to their adaptability to different regions from the regions where they are grown, resistance to diseases and pests, and many demanded quality characteristics (Hawkes, 1983). For this reason, it is necessary to collect and protect plant genetic resources.

The information obtained in breeding studies, such as the status of the population formed according to the characterization results, the determination of the genetic distance between the genotypes, the duplication status in the population, directs the breeding studies (Karataş et al., 2017; Açıkgöz, 2004). Knowing the morphological

variation in breeding studies forms the basis of plant breeding programs (Bliss, 1981; Gil and Ron, 1992; Escribano et al., 1998; García-Neria and Rivera-Bustamante, 2011). In many studies; Molecular analysis methods, one of the morphological and evolving biotechnological methods, are used to determine genetic diversity, genetic similarity or differences in plants precisely and clearly (Geleta et al., 2005).

Characterization of the plant material to be studied; It has been reported that the identification of the material by utilizing morphological and molecular methods by creating gene populations is of great importance in preventing the loss of local genotypes, in cultivating cultivars, in improving cultivar characteristics and in further breeding studies. (Conicella et al., 1990; Lefebvre et al., 1993; Zewdie and Zeven, 1997; Balkaya and Yanmaz, 2001; Geleta et al., 2004; Karaağaç and Balkaya, 2017). In this study, studies conducted with morphological and molecular methods in pepper gene populations in our country and in the world and recent developments are summarized. The level of variation and morphological characteristics in pepper populations are determined according to UPOV (International Union for Conservation of New Plant Varieties) and IPGRI (International Plant Genetic Resources Institute) criteria. At the same time, the variation rate of populations can be determined by using molecular methods (SRAP, ISSR, SSR, RAPD). Recently, characterization studies have been carried out in local pepper populations using morphological and molecular methods. In this study, researches made by morphological and molecular

methods in pepper gene populations in the world and in our country and recent developments are summarized.

## **MORPHOLOGICAL STUDIES**

It is reported that the first studies on the collection, identification, preservation and evaluation of plant gene resources in our country were started in 1929 (Karaağaç and Balkaya, 2009). In the morphological characterization study of the local hot pepper population, where the researchers gathered in the Black Sea region (Kar et al., 1999), genotypes were found to differ. In another study, 25 local pepper genotypes grown in Bornova conditions, generally fresh and industrially produced, were collected and some phenotypic and biochemical characteristics were examined (Duman and Düzyaman 2004). It is reported that table genotypes, table and industrial types, capia type peppers preferred industrially, Jalapeno peppers, which are in high demand in sauce and pickle production, and tomato peppers that are used in brine, are in other groups.

Morphological characterization analysis was carried out on 185 pepper species from different regions of Turkey. The characterization mentioned here is based on the definition list of IPGRI and the feature list of UPOV. 45 features were found in pepper (Mutlu et al., 2009). An evaluation was made on 48 pepper genotypes including thirty peppers and eighteen commercial varieties obtained from different locations of our country between 2004-2005. These species have been characterized in terms of 68 morphological and agronomic features (Bozokalfa and

Eşiyok, 2010); Morphological characterization research was conducted on 26 pepper populations and 3 different pepper species collected from Marmara, Black Sea, Mediterranean, Central Anatolia and Eastern Anatolia Regions in Turkey between 2005-2007. The characterization mentioned here is based on the definition list of IPGRI and the feature list of UPOV. Populations, on the other hand, were examined for 54 character traits. A wide variety was found in the study, and it was concluded that the pepper variety in Turkey is quite high (Binbir and Baş, 2010).

It is seen that 56 different populations were included in the study, where local red peppers were collected from Samsun's Bafra Plain and its surroundings between 2004-2005 and examined in terms of morphology of gene sources. As a result of the study, it was revealed that the morphological variability among the red pepper genotypes was high (Karaağaç and Balkaya, 2010). The determination and development of agronomic characterization of the morphology of pepper lines in Red Jalapeno pepper commercially grown in Brazil was investigated. A two-stage study was conducted; It was characterized by the Single Seed Progeny method in the first stage and by the Capsicum descriptive feature developed by IPGRI (1995) in the second stage. As a result, significant differences were detected between inbred lines as a result of qualitative and morphological evaluations (Ulhoa et al., 2017). The study conducted by Ventura et al., (2018) in the Mexican state of Tabasco was carried out to determine the morphological characteristics of wild peppers. According to this study, 131 collections were accessed.

Accordingly, it was concluded that wild peppers in the Mexican state of Tabasco have morphological features that should be protected.

Within the scope of a research, 240 pepper genotypes in 2014 and 313 pepper genotypes in 2015 were collected in Kırşehir city center and its villages, and 99 sharp peppers were collected. When the scope of the research is wanted to be examined (Basak, 2019); Forty-eight morphological and agronomic features were analyzed based on IPGRI and UPOV criteria. They were determined as groups classified on the basis of morphological and agronomic features, which were divided into 15 clusters. The local pepper population collected in and around Aydın province was compared with commercial varieties and investigated in terms of some morphological, pomological and phytochemicals. In the study, it was reported that some characteristics of local pepper genotypes were more successful than commercial varieties (Gülcan, 2020). In another study carried out in some of the central villages of Diyarbakır, 18 local melon genotypes were analyzed. In the study based on UPOV (2006+2014) TG/104/5 Rev criteria, fruits were divided into two as summer and winter melon genotypes (Tatar and Şensoy, 2020).

## **MOLECULAR STUDIES**

In the molecular marker method; They are DNA regions that show genetic difference, similarity, polymorphism rate in genetics, and they are preferred because the analyzes are reproducible, unaffected by environmental conditions, short-term labor requirement, and give 100%



accurate results (Liu, 1998). For the purpose of performing both morphological and molecular characterization of 504 pepper accessions in the gene collection of the Vegetable Department of the Czech Republic Crop Research Institute (Stavelikova et al. 2010); Duplications were determined in the generated population. Eight SSR markers were analyzed with 54 morphological features. As a result of the morphological and molecular analyzes of the obtained data, it was stated that the duplications that complement each other were in four groups.

Tilahun et al. (2013) used RAPD and SSR markers to determine the genetic relationship in 30 pepper genotypes. In the study, it was reported that both RADP and SSR methods showed genetic variability, but the SSR method was able to detect the genetic relationship more comprehensively than the RAPD method. In another study, ISSR technique was used to determine genetic diversity in 5 pepper species. It was found to be polymorphic in 13 primers in 15 ISSR primers, and two groups were reported in cluster analysis (Lijun and Xuexiao 2012). ISSR and SSR markers were used for genetic characterization of 2 Serrano, 2 Jalape and 1 Capsicum pubescens pepper cultivars in Mexico (Ibarra-Torres et al. 2014). 38 bands from 8 ISSR primers, between 150 and 6000 bp, mean values of 0.77 for PIC, 0.74 for MI and 16.08 for Rp, allele count on SSR marker 1 to 10 range, PIC value 0.5, SSRs with amplification in *C. pubescens* It has been reported that there is a grouping between cultivars.

To determine genetic relatedness (Liu and Li 2015); ISSR markers were used on 8 pepper (*Capsicum annuum*) cultivars. As a result of the 30 ISSR primary analyzes, 382 bands were obtained and it was reported that 270 bands (70.6%) had polymorphism, as well as a clustering map was obtained according to the analysis of the SPSS16.0 software system. 372 pepper accessions collected from different locations in China and 31 pepper accessions collected from different countries were screened with 28 SSR primers to determine the genetic relationship. The results of the study were evaluated in general and it was reported that the genetic diversity of pepper grown in China was determined to be varieties that were formed by natural selection and adapting to climatic conditions over time (Zhang et al., 2016).

In the study conducted by Adalı (2017), 26 SRAP markers were used to determine the genetic relatedness between 26 pepper genotypes. López-Espinosa et al. (2018), genetic characterization of 60 Habanero pepper genotypes collected from Yucatan, Campeche, Quintana Roo and Tabasco regions of Mexico was aimed using the ISSR marker method. As a result of the study, it was reported that 32 bands were obtained in ISSR, 98% polymorphism was detected, and the cumulative variation was 95.5% in the UPGMA method and principal component analysis. The ISSR marker method was used to determine genetic diversity in 53 hot pepper (*Capsicum annuum*) genotypes by Nibret (2018) in Ethiopia. As a result of the study, it was reported that there was a high level of variation.

Between 2017-2018, genetic characterization was made between pepper genotypes by applying 19 SRAP primer combinations on 38 pepper genotypes containing 35 peppers and 3 commercial varieties, which were pure line obtained by inbreeding (Okay 2019); In addition, resistance to PVY, TSWV and PMMoV was screened by molecular methods, 5 main groups were formed in the dendrogram formed according to SRAP marker analysis, similarity between 0.35 and 0.97 and cumulative variation in principal component analysis was reported to be 84.9%. In the study conducted by Taş (2020), it was aimed to determine the vegetative characteristics of UPOV of 75 pepper genotypes from *Capsicum chinense* species collected from different regions of the world and the variation and genetic relatedness in the population created by using the SSR marker method. In terms of genotypes and morphological features, the presence of high phenotypic variation, cumulative variance of 70.99% in cluster analysis and principal component analysis (PCA) was determined. In molecular studies, using 14 SSR primers, 66 out of 115 bands have polymorphism with 57.4%, and according to the data, 3 different groups with genetic similarity have been formed and it has been reported that there is a genetic distance between 0.15 and 0.75.

In order to morphologically and molecularly characterize (Dilfuruz 2021) the recombinant inbred lines (RIL) developed by crossing the *Capsicum annuum* L. X *Capsicum frutescens* populations, 96 F2 individuals were inbred from the established population up to the F6 stage. The lines coming to the F6 level were characterized by SRAP

and SSR molecular marker method, and as a result of the study, in the population created *Capsicum annuum* X *Capsicum frutescens*, wide variation was observed in both of the analysis results made by morphological and molecular methods, and this situation will be determined in the future, especially in terms of resistance to disease and pests, yield and productivity. It has been reported that it will be useful for breeding studies such as mapping some quality traits.

## **RESULTS**

Numerous studies have been carried out on local genotypes in our country and in the world. There are many studies using phenological, morphological and molecular methods for the collection and identification of local pepper genotypes. When literature studies are examined, characterization studies have been carried out by examining morphological features for many years, but it has been determined that the results obtained are inevitably affected by environmental conditions.

It is known that morphological features are easy to study, but it is labor intensive and takes a lot of time. Morphological evaluations are made according to the definition from the descriptor published for pepper by UPOV (International Union for Conservation of New Plant Varieties) and IPGRI (International Institute of Plant Genetic Resources). In studies conducted in molecular methods, the kinship relationship of genotypes such as RAPD, SSR, ISSR, AFLP, SCAR, SRAP, from which gene pool, and their resistance to diseases are determined.

It is accepted by most researchers that the observation of morphological features alone is not sufficient for the characterization of genotypes. In morphological observations, it is reported that the material is affected by the environmental conditions in which it is grown, but the results obtained are certain since the plant DNA is examined in molecular analyzes and neither the environment nor any conditions are affected. Accordingly, since both methods reveal the genetic relationship between genotypes, the application of these two methods together will increase the success of the study.

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## CHAPTER 7

### **DETERMINATION of WEED EMERGENCE DIFFERENCES and DENSITIES in SUNFLOWER (*Helianthus Annuus L.*) FIELDS in DIFFERENT REGIONS**

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## **INTRODUCTION**

Fats are vital nutrients for many functions in the human body. For a sufficient energy absorption and intromission of fat-soluble vitamins, ensuring it with energy and forming as constructive components in cell walls. They are also the source of the flavor of the foods (Lichtenstein et al., 1998). FAO and WHO draw attention to the necessity of 20-35% of total energy intake from fats. Fats differ according to their nutritional and physical properties. They are categorised as saturated, polyunsaturated, and monounsaturated in terms of fatty acids.

Saturated fatty acids are considered harmful to health, especially cardiovascular disease outcomes, whereas some unsaturated fatty acids play a beneficial role in the human body (Gobbo et al., 2016).

Unsaturated fatty acids are abundant in vegetable oils such as olive oil, hazelnut, canola, corn, soybean, and sunflower oil, especially in fish such as mackerel, tuna, and salmon living in cold waters (Şahingöz, 2007).

Sunflower oil is one of the most crucial field crop oils rich in unsaturated fatty acids. Due to its high amount of oil, sunflower meets a critical raw material in the edible oil industry. Sunflower is widely used in mixed feed production because it is consumed as a snack, contains vitamin E and linoleic acid, and has high protein in its pulp. In addition, it is an influential plant for feed support because it is used in animal nutrition by making green fodder or silage and is a significant source of pollen for honey (Arioğlu, 2007).

Sunflower takes second place after cotton in terms of oil crop cultivation areas in our country. Oil sunflowers are mostly produced in the provinces of Tekirdağ (18.63%), Konya (14.66%), Edirne (12.65%), Kırklareli (11.91%) (TUIK, 2021). While 44% of the world's crude edible oil is obtained from sunflowers, 39.35% of it is met from sunflowers in Turkey (TUIK, 2021). The production amount of Sunflower for Oil has been determined as 2 370 000 tons for 2021 (TUIK, 2021).

Sunflower is an indispensable crop for rotations in ICLS (Brighenti and Castro, 2013) because it is highly adaptable and versatile (Amabile et al., 2003; Silva et al., 2012). In addition, sunflower plants have desirable features, e.g., increased drought tolerance and good capacity of using waste from previously fertilized crops, thereby increasing the ability to use the soil, machinery, and factors of production (Brighenti and Castro, 2013). Additionally, sunflowers ( *Helianthus annuus* L. ) are one of the most important oil and industrial crops worldwide, and have been planted in several Mediterranean countries ( Andrade, 1995 ; Archontoulis et al., 2007 ). It is generally considered among the most prevalent crops, with many uses and high adaptability to different soil and climatic conditions (Beard and Geng, 1982; De la Vega and Hall, 2002).

The knowledge of the emergence of dominant weed species in a crop growing area is critical, primarily since the emergence times of weeds can be used in strategies to prevent crop losses in the relationship of weed crop competition (Benjamin et al. 2010; Grundy 2003). The most

commonly used investigation to assess crop losses in studies is weed competition and critical period determination for weed control (Knezevic et al., 2002; Swanton et al., 2015). These investigations offer acceptable descriptions of yield loss and enounce the period when to control weeds, but the experiments are expensive and uncertain and are difficult to generalize for different environments (Otto et al., 2009). The importance of knowing and predicting the emergence of weeds rather than critical period determination studies has been accepted for years, and various studies on weed emergence models aim to reach more precise results in a short time. (Colbach et al. 2007; Dorado et al. 2009). Only weeds pose almost 33% of yield losses also caused by agricultural pests. Weeds are an important responsible for yield reduction because the weeds be able to compete for nutrients, space, light, and water, (Roa et al., 2018).

Including such models in decision support programs can reduce herbicide use and weed control costs compared to standard management practices (Forcella et al. 2000). Appropriate timing of weed control is particularly important given the increasing frequency of POST control in sunflower fields. These models provide the cumulative percentage of weed species achieved each day, and farmers can use this information to choose the best timing of mechanical or chemical control (Masin et al., 2011; Chantre et al., 2012).

A well-managed weed control plan is essential for successful crop production (Nishanthan et al., 2018). Weeds are considered among the most severe constraints on the success of the sunflower crop since they

can cause substantial yield losses (Breccia et al., 2011; Alberio et al., 2015). In the study carried out for weed control in sunflower, it is pointed out that as a result of the study carried out on the fields of different soil types on different development stages of sunflower, weed damage in sunflower causes crop losses of 29-75% (Dharam et al., 1993). if weeds are not controlled, they properly are also capable of reducing sunflower's yield by up to 90%.

Chemical weed control can produce 40–97% higher yields as compared to un-weeded crops (Li et al., 2018). Controlling weeds with herbicides ensures efficient weed control (Chaudhry et al., 2017). Nevertheless, the higher cost of herbicides and their negative impact on human and environmental health are some of the drawbacks. Herbicides need to be repeatedly applied because weeds re-emerge from their seed bank (Mehdi et al., 2017).

Tillage destroys already germinated weeds and buries the seed present on the surface by decreasing the weeds population afterwards. Profound tillage implements like moldboard ploughs have weed seed burying ability. Seeds buried in lower soil layers are less likely to grow due to the physical hindrance of above soil layers. In other words, a moldboard plough reduces the probability of the emergence of weed seeds (Nishanthan et al., 2018).

The first condition for excellent and successful control of weeds is determining the weeds in sunflower planting areas and their density—

another critical factor in estimating the emergence of weeds in sunflower fields.

This study was carried out to determine the widespread weeds in three different sunflower plantations and determine the prevalence and emergence densities of weeds that can emerge during the growing season, depending on the climatic characteristics.

## **MATERIALS AND METHODS**

The first field experiment was carried out in the Gönen district of Balıkesir province (40°08'34.1"N 27°41'22.5"E and 28 m) in 2013 and 2014. The second field experiment was conducted in the Bergama district of İzmir province (39°04'05.0"N 27°03'44.4"E and 24 m) in 2016 and 2017. The third field experiment was performed in the Uzunköprü district of Edirne province (41°15'23.5"N 26°42'03.6"E and 36 m) in 2019 and 2020 in Turkey.

Weather conditions during the growing season (March, April, May, June, July, August, September) of sunflower were collected from the Republic of Turkey General Directorate of Meteorology. (Tab. 2). The sites have almost the same subhumid climatic conditions. The average annual temperature of all sites is 18.28 °C, increasing from an average minimum: of 13.28 °C to an average maximum: of 28.67 °C). Annual rainfall is about 350 mm and is uniformly distributed throughout the year.



## **Assessment of data**

After counting the weeds with 2-4 leaves emerging during the growing season, they were destroyed with a herbicide containing glyphosate. Afterwards, it was expected that weeds would emerge again. In the trial area, this practice was treated every month. This application was repeated six times until harvest time.

Experiment Design, Treatments, and Studied Traits were designed following a randomized complete block design with three replications. The quantity of water and herbicide was calculated before spraying. Hand operated Knapsack/sprayer fitted with a nozzle was used. Agronomic practices (excluding those understudy) were kept uniform. Data were recorded on weed density (plants m<sup>-2</sup>).

Weeds that could not be identified in weed detection studies were brought to the laboratory, making diagnoses according to Flora of Turkey (Davis, 1965-1988).

## **RESULTS**

### **The Comparison of Average Climate Data in Regions**

Edirne had the lowest average temperature in 2019. The statistical comparison of the climate data shows that the average daily temperatures in İzmir and Balıkesir are approximately 28.0% higher, the average air humidity is approximately 28.5%, and the dew point degree is 22.0% higher than the province of Edirne in April. In the examination of the measurements in May, the average temperatures of

Balıkesir province were 17.8% higher, the dew point 24.2%, and the air humidity 17.8% higher than the other provinces. According to the climatic values in June, the average temperatures in İzmir and Balıkesir provinces were 44-54% higher than in Edirne province. However, Edirne province had more than two times higher air humidity than İzmir province and 21.7% higher than Balıkesir province. In July, the average temperature in İzmir was measured to be 10.1% higher than in other provinces. While the average temperature and dew point in August contains values close to each other, it was determined that the air humidity in Balıkesir was 12.0% higher than in other provinces.

**Table 1.** Comparison of Average Climate Data in Regions

REGION	APRIL			MAY			JUNE		
	Avg. Temp.	Awg. Dew	Avg. Hum.	Avg. Temp.	Awg. Dew	Avg. Hum.	Avg. Temp.	Awg. Dew	Avg. Hum.
I.	17.05 a	9.13 a	61.60 b	22.45 a	13.93 a	60.45 b	25.46 <sup>a</sup>	16.04 a	48.93 <sup>a</sup>
II.	17.10 a	9.08 a	62.92 b	19.06 b	10.57 b	60.93 b	26.47 a	13.76 b	27.32 b
III.	13.33 b	7.10 b	69.50 a	19.00 b	12.94 <sup>a</sup>	69.35 <sup>a</sup>	25.71 a	16.57 a	59.58 a
Sig. (P=0.05)	0.004	0.019	0.009	0.000	0.000	0.000	0.410	0.001	0.000

REGION	JULY			AUGUST			SEPTEMBER		
	Avg. Temp.	Awg. Dew	Avg. Hum.	Avg. Temp.	Awg. Dew	Avg. Hum.	Avg. Temp.	Awg. Dew	Avg. Hum.
I.	27.23 b	15.65 a	50.62 a	27.33 b	17.12 a	54.63 a	28.63 ab	14.19 a	56.10 a
II.	29.07 a	13.99 b	54.50 a	28.68 a	16.35 a	49.36 b	29.83 a	11.85 b	49.65 b
III.	26.10 c	15.84 a	56.84 a	28.13 a	15.03 b	48.03 b	27.97 b	12.97 ab	55.40 a
Sig. (P=0.05)	0.001	0.008	0.839	0.005	0.000	0.001	0.869	0.014	0.035

## The Comparison of Weeds Emergence

Balikesir (2016), Izmir (2019) and Edirne (2021), which in were established experimental sunflower fields. Determined as the dominant three types of family Poaceae, 3 species of Polygonaceae family, 2 species of Amaranthaceae family, 2 species of the Boraginaceae family, 2 species from the Solanaceae family, type 1 from Asteraceae family, 1 species of Convolvulaceae family, type 1 of Cyperaceae family, type 1 from the Malvaceae family, 1 type from Plantaginaceae family, type 1 of Portulacaceae family, type 1 from Lamiaceae family, 1 type from Zygophyllaceae family in the experimental areas. In total, twenty species continued to emerge as the dominant weed.

The weeds that were emerged most intensively emergence were respectively; *Cyperus roduntus* (175.3 weed/m<sup>-2</sup>), *Datura stramonium* (92.0 weed/m<sup>-2</sup>), *Portulaca oleraceae* (80.7 weed/m<sup>-2</sup>), *Convolvulus arvensis* (76.7 weed/m<sup>-2</sup>), *Xanthium strumarium* (76.0 weed/m<sup>-2</sup>), *Chenopodium album* (77.3 weed/m<sup>-2</sup>). *A. theophrasti*, *A. retroflexus*, *C. album*, *C. arvensis*, *C. roduntus*, *D. stramonium*, *E. crus-galli*, *P. oleraceae* and *X. strumarium* were able to re-emergence at almost all counting times in the established trials,

June was determined as the month when both annual and perennial weeds appeared at the highest rate. Compared to weeds in the sunflower experiment area in Balikesir (region I) had, 13,68% more weeds emerged than II. region (İzmir), had 17.14% more weeds than Edirne (III. region). It was determined that perennial weeds in the İzmir region are 38.12% more than in the Balikesir region and 35.91% more than in the Edirne region.

**Table 2.** Weeds emerging throughout the sunflower vegetation in the regions (2013-2016-2019)

WEEDS	REGION	NUMBER OF WEEDS EMERGENCE DURING THE VEGETATION PERIOD (Number/m <sup>2</sup> )							AVERAGE WEED NUMBER (Number/m <sup>2</sup> )
		APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER		
<i>Abutilon theophrasti</i> L.	I.	52	28	120	52	40	28	53.3	
	II.	28	28	112	56	0	0	37.3	
	III.	16	72	28	44	32	20	37.5	
<i>Alopecurus myosuroides</i> Huds.	I.	16	0	20	12	8	16	12.0	
	II.	8	0	16	8	0	0	5.3	
	III.	12	40	16	8	0	0	12.7	
<i>Amaranthus retroflexus</i> L.	I.	108	24	136	52	32	48	66.7	
	II.	96	24	160	64	0	0	57.3	
	III.	48	36	92	40	28	0	40.7	
<i>Chenopodium album</i> L.	I.	132	24	144	60	64	40	77.3	
	II.	88	44	96	56	0	12	49.3	
	III.	68	36	100	36	28	12	46.7	
<i>Convolvulus arvensis</i> L.	I.	148	44	112	64	48	44	76.7	
	II.	128	68	96	48	36	20	66.0	
	III.	80	120	100	56	36	44	72.7	
<i>Cyperus roduntus</i> L.	I.	100	24	196	36	52	28	72.7	
	II.	96	448	172	56	204	76	175.3	
	III.	24	92	184	112	44	36	82.0	
	I.	148	36	176	96	68	28	92.0	

<i>Datura stramonium</i> L.	II.	60	36	164	76	0	0	56.0
	III.	48	68	172	72	24	12	66.0
	I.	140	20	96	60	40	48	67.3
<i>Echinocloa crus-galli</i> L.	II.	128	52	76	60	0	8	54.0
	III.	84	80	68	44	0	8	47.3
	I.	84	12	72	8	12	8	32.7
<i>Heliotropium europaeum</i> L.	II.	40	12	84	0	0	0	22.7
	III.	28	24	88	24	16	12	32.0
	I.	0	0	0	0	0	0	0
<i>Hordeum murinum</i> L.	II.	8	0	0	0	0	0	1.33
	III.	15	9	0	0	0	0	4
	I.	116	8	88	0	0	0	35.3
<i>Lamium purpureum</i> L.	II.	68	72	0	0	0	0	23.3
	III.	40	36	0	0	0	0	12.7
	I.	80	8	16	0	0	0	17.3
<i>Lithospermum officinale</i> L.	II.	8	24	20	0	0	0	8.7
	III.	32	4	4	4	0	0	7.3
	I.	156	28	40	0	0	0	37.3
<i>Polygonum aviculare</i> L.	II.	80	20	32	0	0	0	22.0
	III.	20	28	40	16	0	0	17.3
	I.	76	8	16	16	16	16	24.7
<i>Polygonum lapatifolium</i> L.	II.	52	8	16	0	0	0	12.7
	III.	36	8	4	12	0	0	10.0
	I.	64	12	0	12	8	0	16.0
<i>Polygonum persicaria</i> L.	II.	0	12	12	16	0	0	6.7

	III.	4	12	0	20	0	0	0	6.0
<i>Portulaca oleracea</i> L.	I.	20	144	48	84	56	36	64.7	
	II.	24	168	72	80	72	68	80.7	
	III.	20	24	56	32	48	68	41.3	
<i>Solanum nigrum</i> L.	I.	92	40	12	0	0	0	24.0	
	II.	32	40	24	0	0	36	22.0	
	III.	48	40	16	0	0	0	17.3	
<i>Tribulus terrestris</i> L.	I.	0	0	16	28	48	28	20.0	
	II.	0	88	0	36	40	0	27.3	
	III.	0	0	8	40	8	0	9.3	
<i>Veronica</i> spp.	I.	84	16	24	0	0	0	20.7	
	II.	0	24	40	0	0	0	10.7	
	III.	28	16	12	0	0	0	9.3	
<i>Xanthium strumarium</i> L.	I.	144	64	124	32	32	60	76.0	
	II.	52	64	12	32	0	0	26.7	
	III.	20	64	68	56	32	20	43.3	

Regions; I. Balıkesir-Gönen. II. Bölge; İzmir-Bergama. III. Bölge: Edirne-Uzunköprü

## DISCUSSION and CONCLUSION

Examined the climate and weed emergences, there were differences in average temperature, humidity and dew point characteristics in April. The average temperatures in the trial areas in Balıkesir and İzmir provinces were 28% higher than those in Edirne province. This situation caused an increase in the emergence rates of *A. theophrasti*, *A. retroflexus*, *C. album*, *C. arvensis*, *C. roduntus*, *D. stramonium*, *H. europaeum*, *P. aviculare*, *P. lapatifolium*, *P. oleraceae*, *S. nigrum*, *T. terrestris*, *X. strumarium*, *L. purpureum* and *Veronica* spp. It was also observed that some annual winter weeds can emerge until June. In addition, *L. purpureum*, *L. officinale*, *P. lapatifolium*, *P. oleraceae*, *S. nigrum* and *Veronica* spp. tended to decrease significantly during the vegetation period. The emergence of weeds in sunflower fields, their power of emergence and their continuity during vegetation vary greatly. This indicates how weeds can be affected by changing climatic characteristics.

In addition, the effect of direct or indirect result of climate change that affects growth or changes the adaptability of weeds will be affected by climate change. Therefore, competition components will change with the interaction between weeds and crops (Singh, et al., 2011). In addition, significant changes are seen in weed spread, population dynamics, life cycle duration, infestation pressure and the overall occurrence of the majority in the number of agricultural (Bale et al., 2002; Petzoldt and Seaman, 2006). Because climate change affects the growth and reproduction of



some weed species, it requires different application methods (Mahajan et al., 2012). Knowing the emergence characteristics of weeds, which is an essential finding in controlling weeds and determining the duration of the activity, is expected to give a great impetus to integrated control. Especially in the sunflower, a hoe plant, it is applied that 10-15 cm tall and 25-30 cm were tall before flowering. The changing characteristics of weeds under environmental and climatic changes have become very important in determining the hoeing time, increasing the effectiveness of estimation methods and observations control methods.

Although the emergence times of many weeds have been determined in terms of temperature and soil temperatures in the studies, there is not much information about the re-emergence rates of weeds during vegetation. (Werle et al., 2014; Roya-Esnal et al., 2022).

Weed seeds mature and disperse due to insufficient control activity or timing error. In weedy sunflower areas, the particular flowering period of sunflowers can be quite long. In addition, cultural practices limit flowering periods and thus the variability of individual breeding time: it must occur post-emergence or pre-emergence. Thus, as a whole, our results have three simultaneous implications: breeding time is heritable, gene flow from crop to weed occurs, and breeding time variation within weed populations requires partial reproductive isolation from the crop. Similar approaches have been identified, using a fixed base T to accumulate heat units across all species, making it possible to

compare the order and duration of emergence between species under field conditions (Myers et al. 2004; Werle et al. 2014). These determining temperature values vary considerably between different environmental conditions, and weed species may be understandable and not applicable to farmers. Therefore, all factors should be carefully reviewed, and applications should be made throughout the vegetation.

Ultimately, it is crucial to recognize that weed emergence timing should be used as a general guide rather than an absolute predictor. In different studies, seed bank and weed emergence in the soil has been observed. However, it was determined that the seeds of many species remained viable in the seed bank and germinated in the following season. Differences in environmental factors over the years affected total seedling emergence.

Information and computing model comparison approaches were a powerful tool for selecting adequate baselines and models to predict the emergence of different weed species based on observational field studies.

All the research results can help growers predict the timing of the emergence of multiple weed species. This information can better plan management strategies and identify possible changes in the composition of weed species resulting from consistent crop management practices. For instance, early emerging species, especially those with a short spawn time. After most seedlings emerge, they can be managed using residual activity or tillage-

depleted herbicides prior to crop planting.

A single management strategy can control species with a short emergence period (post-herbicide application). At the same time, weed species with longer emergence times may require more than one treatment. In contrast, after crop establishment, mid and late-emerging species will need to be managed by selective post-herbicide or interrow cultivation.

Sowing dates can also be a reliable strategy for weed control. Delayed planting will allow chemical or mechanical control of early emerging weeds prior to crop planting. However, it can additionally reduce crop yield potential. Constantly delaying planting annual summer crops can shift species composition to species that emerge later in the season. Early planting causes the canopy to close earlier. It results in better weed suppression of mid and late-emerging crop varieties. However, consistent early planting can shift species composition towards species that appear simultaneously as crops. However, it will be challenging to control the weeds that have the power to emerge continuously throughout the sunflower vegetation. All factors should be carefully reviewed and best management practices applied throughout the vegetation. Best practices should be made, considering the changes in the seed reserve in the soil and the different germination frequencies of weed species.

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## **CHAPTER 8**

### **HAPLOTYPE SOURCED RECESSIVE DEFECTS AFFECTING FERTILITY IN DAIRY CATTLE**

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## **INTRODUCTION**

After the publication of the first whole-genome sequencing of cattle, detailed genetic study of cattle became easy and more convenient (Zimin et al., 2009). Along with the improvement in animal biotechnology and cattle genomics, understanding genetics controlling production and reproduction characteristics in the dairy cow is more precise. Similarly, the genetic reasons behind dairy cattle fertility are more understandable than before.

Fertility of dairy cattle is a complex phenomenon that starts with the insemination of dairy cows and ends with a successful live birth of calves (Han, 2016). Because of their complexity in nature, these phenotypes require the implementation of all available resources and tools, including genetic methods, in understanding and improvement. Generally, various factors are responsible for the expression of fertility or reproductive traits like the nutrition status of animals, herd management, environment, and individual genetics. The genetic reasons behind fertility range from simple monogenic effects of recessive lethal mutations to complex mechanisms involving multiple genes with additive and non-additive effects (Ma et al., 2019). Although the heritability of familiar reproductive traits is considered as low (1 to 10%), therefore, a noticeable phenotypic difference and genetic correlation in the fertility traits have been found in the literature, for example, in the lactating cows, significant genetic variation for fertility has been proved (Norman et al., 2009; Spencer et al., 2014).

Dairy cow fertility has a significant impact on the industrial level. Lower fertility results in decreased milk output, longer calving intervals, greater insemination costs, lower calving rate, increased animal culling, higher replacement costs, and shorter effective herd life (Rahbar et al., 2016; Kiser et al., 2019). At the farm level, it has been reported that reproductive issues are the most common reason for culling (Höglund et al., 2015).

The fertility of dairy cattle has started to decline over the past centuries all over the world, reported in the USA, European countries, South Africa, and Australia (Norman et al., 2009; Buchanan et al., 2013; Fritz et al., 2013; Soydan & Kuran, 2017). Although reproductive management of dairy cattle has improved dramatically in the last twenty years, substandard reproductive performance exists in the current dairy herd, accounting for huge financial losses to the dairy industry (Rezende et al., 2018).

Low fertility of dairy cattle includes multiple aspects such as anovulation, improper expression of heat, irregularity of oestrus cycles, and embryo loss after insemination (Hansen, 2011). Poor fertility is a result of the combined effects of various genetic and non-genetic factors. Non-genetic factors include the growing size of the dairy herd, extensive use of the intensive breeding practice, manpower shortages on farms, and global warming (Lucy, 2001; Walsh et al., 2011; Zahedi et al., 2021). Genetic factors that may cause fertility problems are chromosomal defects, individual genes, and genetic interactions (Spencer et al., 2014). Since the development of modern cattle breeds,

the cattle genome has constantly been under selection pressure that affects reproduction negatively. In the dairy cattle, selection processes concentrated on milk production for a long time. Interestingly, a negative genetic correlation exists between milk yield and fertility in dairy cattle. Thus, intensive selection in production traits over the years without selection for fertility has contributed to lower fertility (Spencer et al., 2014). It showed that genetic selection for milk production in the Holstein breed has resulted in genome modifications that adversely affect fertility traits and disease resistance genes. High inbreeding rates are another major cause of low fertility in dairy cattle breeds. Popular dairy cattle breeds possess high inbreeding rates and continuing upward trends (Brito et al., 2021). The prevalence of lethal genes causing early embryonic mortality is high while breeding animals have higher inbreeding percentages (Cole et al., 2016; Howard et al., 2017). It was reported that early embryonic loss is one of the two main factors responsible for lower fertility (Han and Peñagaricano, 2016).

A haplotype is a group of alleles inherited together at close intervals on a chromosome. Haplotypes are common in the genome and have positive, negative, or neutral effects on any trait. In dairy cattle, scientists have discovered many haplotypes that regulate fertility in dairy cows connected with early embryonic loss. Haplotypes showing greater heterozygosity with a lack of homozygous conditions have the possibility of bearing lethal alleles. In 2011, a scientist group identified five haplotypes with significant carrier frequencies without homozygous animals in Holstein, Jersey, and Brown Swiss breeds by

analysing their genotype data (VanRaden et al., 2011a). The name of these defects is given in a defined manner where the first letter stands for a specific breed name and an “H” for haplotype and the number of defects in the breed orderly (VanRaden et al., 2011a). After that, a list of lethal haplotypes was reported in different cattle breeds (Cooper et al., 2013; Sahana et al., 2013; Fritz et al., 2013; Schwarzenbacher et al., 2016; Null et al., 2017). The probability of discovering recessive abnormalities is increased by routine genotyping of hundreds of animals every year for genetic examination in different countries (Hozé et al., 2020). In the lethal haplotypes, specific mutations were identified from the haplotype region, which explained the reason for embryonic death and made it possible to screen the carrier animals more precisely. As dairy cattle use artificial insemination more widely, these deleterious mutations spread to other parts of the world through the carrier bulls.

This chapter will discuss various defects in Holstein Friesian, Ayrshire, Brown Swiss, Montbeliarde, and Jersey breeds that cause embryonic mortality and calf death.

## **1. DEFECTS IN HOLSTEIN FRIESIAN (HF) BREED**

The Holstein breed is undoubtedly the most well known, with its unique black and white or red and white markings. These cattle are known for their higher milk productivity, although milk fat and protein content are lower than other milk breeds. Holstein cows were evolved in the Netherlands over 2000 years ago by crossing black Batavians with

white Friesians cattle (Elischer, 2014). The Holstein breed has more recessive defects affecting fertility among all dairy breeds. Seven defects reported in Holstein and their crossbreed are discussed below.

### 1.1 Holstein Haplotype 1(HH1)

HH1 is a lethal defect that causes pregnancy losses between 60 and 200 days of gestation in the Holstein Friesian cows (Adams et al., 2016). This defect sources from a highly prolific bull named Pawnee Farm Arlinda Chief, which contributes 14% of the genome in the current HF population and is one of the two most popular bulls in the Holstein industry (VanRaden et al., 2011b; Yue et al., 2015; Adams et al., 2016). HH1 is a result of a point mutation in the apoptotic peptide-activating factor 1 (*APAF1*) gene.

**Table 1:** Common Lethal Defects of Dairy Breeds

Defects	Earliest genotyped bull (s)	BTA (interval, MB)	Affected gene	Mutation / Polymorphism site
HH1	Pawnee Farm Arlinda Chief (1962)	5 (62-68)	Apoptotic peptide activating factor 1 gene ( <i>APAF1</i> )	g.63150400 C>T
HH2	Willowholme Mark Anthony (1975)	1 (94.8- 96.5)	Intraflagellar protein 80 ( <i>IFT80</i> )	g.107172616delT
HH3	Gray View Skyliner (1954)	8 (92-97)	Structural maintenance of chromosomes 2 ( <i>SMC2</i> )	g.95410507T>C
HH4	Besne Buck (1986)	1 (1.9-3.3)	Glycinamide ribonucleotide transformylase protein ( <i>GART</i> )	g.1277227A>C
HH5	Thornlea Texal Supreme (1957)	9 (93.23-93.37)	Dimethyl-adenosine transferase 1 ( <i>TFB1M</i> ).	138kb deletion
HH6	Mountain (1987)	16 (29.2-30.3)	SDE2 telomere maintenance homolog ( <i>SDE2</i> )	g.29773628 A>G
HH7	Secret (1980), Dutch Boy (1996), Merdrignac (1996)	27 (13-14.4)	Centromer protein U ( <i>CENPU</i> )	g.14168130delTACT

AH1	Selwood Betty's Commander (1953)	17 (65.9-66.2)	Ubiquitin protein ligase E3B ( <i>UBE3B</i> )	g.65921497G>A
AH2	Oak Ridge Flashy Kellogg (1961), Oak-Ridge Lightning (1958)	3	RNA polymerase 2 associated protein ( <i>RPAP2</i> )	g.51267548 Splice variant
BH2	Rancho Rustic My Design (1963)	19 (10.6-11.8)	Tubulin delta 1 ( <i>TUBD1</i> )	g.11063520 T>C
MH1	Boislevin	19 (27.6-29.4)	Phosphoribosylformylglycinamide synthase ( <i>PFAS</i> )	g.28511199C>T
MH2		29 (27.9-29.1)	Solute carrier family 37 member 2 ( <i>SLC37A2</i> )	g.28879810C>T
JH1	Observer Chocolate Soldier (1962)	15(11-16)	CWC15 spliceosome associated protein homolog ( <i>CWC15</i> )	g.15707169C>T

The discovery of the causative mutation was completed by whole-genome sequencing of the haplotype founder bull Chief and his three famous sons. Consistent with the lethality, there were no homozygotes (5299 heterozygotes) in a population of 246773 Holstein individuals (Adams et al., 2016).

Cattle *APAF1* gene contains 28 exons and 89600 nucleotides and encodes 1248 aa long protein called apoptotic protease-activating factor 1 isoform X2 (Adams et al., 2016; NCBI, 2021a). It is a cytoplasmic protein that commences apoptosis and contains a few copies of the WD40 domain, an ATPase domain (NB-ARC) and a caspase recruitment domain (CARD) (NCBI, 2022a). Causative mutation in HH1 is a change of CAA to a stop codon TAA in exon 11 of the *APAF1* gene occurs at position 579 of the polypeptide chain. Mutation cuts 670 C-terminal amino acids (53.7%) containing a WD40 domain (Adams et al., 2016).

APAF1 protein plays a direct role in cancer etiology, disorders related to growth, and neurodegenerative illness. Expression of *APAF1* during murine development begins early in development in different embryonic tissues and is required for central nervous system development. Homozygous *APAF1* knockout gene in mice leads to embryonic death on 16.5th day, and deficiency of this gene has shown excessive brain growth and craniofacial malformations. Deletion of 670 amino acids from the APAF1 removes 15 WD40 repeats that are predicted to form a functional WD40 domain in bovine protein. Many proteins involved in signal transduction, transcriptional control, and apoptosis contain WD40 domains. WD40 domains are also necessary for protein-protein interactions. Without the functional WD40 domain, bovine embryos will be unable to initiate apoptotic pathways that untimely result in various developmental abnormalities and embryos aborted spontaneously (Adams et al., 2016).

The estimated number of abortions for HH1 was approximately 525000 all over the world and 140000 in the USA in the last 35 years, which accounts for 420 million dollars in loss (Adams et al., 2016). HH1 was present in almost all countries; only Ussenbekov et al. (2022) reported that HH1 was absent in a sample of 164 imported Canadian HF in Kazakhstan. The prevalence of HH1 has been reported in different cattle populations with various samples that are summarized in table 2.



## 1.2 Holstein Haplotype 2 (HH2)

HH2 results in pregnancy termination before 60 days of gestation with some stillbirths. This defect showed  $3\pm 0.8\%$  lower fertility in carrier-to-carrier mating (VanRaden et al., 2011a; VanRaden et al., 2011b). HH2 is located in the chromosomal interval from 94.8 to 96.5 MB in the BTA1 (McClure et al., 2014). Yang et al. (2021) reported a base deletion that causes a frameshift mutation in the intraflagellar transport protein 80 (*IFT80*) gene. In HH2, thymine deletion occurs at genomic position 107172616 in exon 11 of the *IFT80* gene (Yang et al., 2021).

The cattle *IFT80* gene is 131907 nucleotides long, having 21 exons and 20 introns (NCBI, 2020a). *IFT80* encodes a 760 amino acid protein (Yang et al., 2021). A frameshift mutation in the HH2 changes valine to a stop codon, causing a loss of the remaining 377 amino acids. The *IFT80* protein consists of seven WD40 beta-propeller repeat domains that serve as a platform for protein-protein or protein-DNA interactions (Yang et al., 2021). Early cessation of the *IFT80* gene interrupts the last WD 40 domains and a weak polyampholyte domain from the original protein (Yang et al., 2021; Ortega et al., 2021). *IFT80* knockout cattle embryos were produced by CRISPR-Cas9 technology, and the results showed that development consistently stopped at the 8-cell stage of embryonic development. Furthermore, the *IFT80* protein structure was significantly altered due to the loss of functional domains (Ortega et al., 2021). It proved that *IFT80* gene expression is indispensable for progression through the 8 to 16 cell developmental phase (Rix et al., 2011; Ortega et al., 2021).

IFT80 protein is a part of intraflagellar transport (IFT) complex B required in the formation, maintenance, and functionality of cilia (Rix et al., 2011). Through the Hedgehog-GLI and Wnt signalling pathways, IFT80 plays a key role in the formation of bone and differentiation of osteoblast. In addition, the *IFT80* gene is ubiquitously expressed in the cattle tissues (Yang et al., 2021). In humans, mutations in the *IFT80* gene are associated with Jeune asphyxiating thoracic dystrophy (JATD) and short rib polydactyly (SRP) type III (Beales et al., 2007; Rix et al., 2011). Deletion of *IFT80* in mice disrupts the fibroblast growth factor 2 (FGF2) signalling pathway (Yuan et al., 2019). During mammalian embryo preimplantation, FGF2 signalling is necessary for lineage commitment and blastocyst formation. The disruption of FGF2 signalling in mice study is comparable to the developmental arrest observed in bovine *IFT80* knockout embryos (Ortega et al., 2021). The economic losses due to HH2 in the USA were estimated at 2 million dollars (Ortega et al., 2021). The carrier frequency of HH2 ranges from 0.99 to 2.64% in different Holstein population (Table 2).

**Table 2:** Prevalence of Different Holstein Haplotypes in Different Countries

Country	HH1	HH2	HH3	HH4	HH5	HH6	HH7	References
USA	2.56	2.42	5.28	0.46	4.78	0.88		Cole et al., (2022)
France	1.6	1.7	3.1	4.2	1.8	1.6	1.1	Fritz et al., (2013); Hozé et al., (2020)
Canada	2.3	2	2	0.1	6.5	0.6		Van Doormaal, (2021)
Italy	3.24	2.64	5.27	1.1	6.88	0.72		Internizoo, (2022)
Uruguay	4.44		3.13	1.04	0.26			Briano-Rodriguez et al., (2021)

China	6.92	5.76	00	4.3	1.84	Khan et al., (2021)
Germany	1.8	5.1	4	5.5		Schütz et al., (2016)
India	3.34	3.34	3.34			Kumar et al., (2020)
Russia	2.96	0.99	2.88	1.14	2.23	Khatib et al., (2020)
Kazakhstan	0	3				Ussenbekov et al., (2022)
Japan	2.9					Ghanem et al., (2018)

### 1.3 Holstein Haplotype 3 (HH3)

Lethal defect of Holstein cattle, HH3 is responsible for 3.26% lower fertility and results in conception loss before the first trimester of cow pregnancy (VanRaden et al., 2011b; McClure et al., 2014). The earliest genotyped ancestor of HH2 is a prolific American bull named Gray View Skyliner (VanRaden et al., 2011b). HH3 was identified in chromosome 8 in the 92-97Mb interval, and the causative mutation is a single nucleotide substitution in the 24th exon of the *SMC2* gene (VanRaden et al., 2011b; McClure et al., 2014).

The 50063 nucleotide long bovine *SMC2* gene consists of 26 exons (NCBI, 2020b) and encodes structural maintenance of chromosome protein 2. T to C changes in the HH3 cause a serine to phenylalanine substitution at amino acid 1135, which changes the NTPase domain in the *SMC2* protein (McClure et al., 2014). This protein is 1191 aa long, and Phe1135 residue in the *SMC2* protein is crucial for normal functioning as it is conserved in all eukaryotes (Daetwyler et al., 2014; NCBI, 2020b). In humans, mutations in the *SMC2* and *SMC4* were

reported in pyothorax-associated lymphoma (Downen & Young, 2014). SMC2 has a critical role in DNA repair, mitotic chromosome condensation, and chromosome segregation in cell division (NCBI, 2022b). In mutated HH3 embryos, the ATP hydrolyzing capacity is greatly weakened, hampering normal condensation activity that causes structural abnormalities of chromosomes incompatible with embryonic development (McClure et al., 2014). The embryonic lethality of HH3 was also supported by genotype data of 5606 Holstein individuals for the T>C mutation. These data resulted in no individuals with the CC genotype (homozygous HH3) and 171 carriers with the TC genotype (heterozygous HH3) (Daetwyler et al., 2014).

HH3 reported moderate to high carrier status in different countries, and the high frequencies were 5.28%, 5.27%, 5.1%, and 5.76% in the USA, Italy, Germany, and China, respectively (Table 2).

#### **1.4 Holstein Haplotype 4 (HH4)**

HH4 was first reported in the French Holstein cattle, showing a 3% decrease in the conception rate (Fritz et al., 2013). HH4 homozygous embryo aborts before the 60th day of pregnancy (Fritz et al., 2013; Cooper et al., 2014). The first heterozygous carrier bull of HH4 is a French bull named Besne Buck, born in 1986 (Cole et al., 2016). HH4 haplotype covers the 1.9-3.3 Mb region of BTA1, and lethality is caused by a mutation in the *GART* gene (Fritz et al., 2013).

The bovine *GART* gene is 26291 nucleotides long with 22 exons (NCBI, 2022c). The *GART* gene encodes 1010 aa long protein name

trifunctional purine biosynthetic protein adenosine-3 (NCBI, 2022c). In HH4, lethality is due to e of A to C transversion at base position g.1277227, which changes the amino acid asparagine to threonine in the manganese-binding site of the encoded protein (Fritz et al., 2013). The GART protein is fully conserved in all eukaryotes and is required for the primary synthesis of purines, which are important components of hereditary molecules. An embryo without *GART* gene function is likely to abort in the early gestation due to its inability to synthesize nucleic acid (Fritz et al., 2013).

The lower carrier frequencies of HH4 were observed in the USA, Canada, Italy, Uruguay, and Russia. Although, carriers are more frequent in France, Germany, and India, reported at 4.2%, 4%, and 3.34%, respectively (Table 2).

### **1.5 Holstein Haplotype 5 (HH5)**

In HH5, the homozygous embryos abort before 60 days of gestation and are responsible for a 3.5% conception rate with some stillbirths (Cooper et al., 2013; Schütz et al., 2016). The first genotyped heterozygous ancestor of HH5 is a Canadian bull named Thornlea Texal Supreme (Cooper et al., 2013; Cole et al., 2016). HH5 mutation is a 138 kb deletion that contains the *TFB1M* gene (Schutz et al., 2016).

Cattle *TFB1M* gene encodes a protein called mitochondrial dimethyladenosine transferase 1 isoform X1, which is 341 aa long (NCBI, 2022d). The main role of TFB1M is the dimethylation of adenine residues located in the hairpin loop at the 3' end of

mitochondrial 12S rRNA. This dimethylation process is critical for the biosynthesis and function of the small ribosomal subunit. Loss of *TFB1M* gene function destabilizes ribosomes, resulting in the cessation of mitochondrial protein translation. Lack of protein translation in mitochondria, and cell-specific destruction confirmed a severe lethal effect on mammalian cells (Schütz et al., 2016). Loss of *TFB1M* in human  $\beta$ -cells results in mitochondrial malfunction and, as a result, diabetes due to a loss of both function and mass of  $\beta$ -cells Sharoyko et al., (2014).

Among the reported countries, HH5 showed lower carrier status in France and Uruguay. Besides this, other countries had moderate to high carrier frequencies in a range of 2.23% (Russia) to 6.88% (Italy) (Table 2).

### **1.6 Holstein Haplotype 6 (HH6)**

Lethal haplotype HH6, causes pregnancy loss in a very early stage of gestation (before 35 days) and has shown a 5% less conception rate in cows. HH6 spread through an American bull named Mountain, used widely for artificial insemination. HH6 was mapped to a 1.1 Mb region on chromosome 16 and found the causative mutation in the *SDE2* telomere maintenance homolog gene (Fritz et al., 2018).

The Cattle *SDE2* gene has a 15504 base long ssequence comprising seven exons and encodes a 453 aa long protein (NCBI, 2020c). The change of A to G changes the codon ATG (Met) codon to ACG in the

nucleotide sequence of the *SDE2* gene that would be truncated by 83 amino acids when translation began at the nearest in-frame Met codon. These missed amino acids contain an 8-amino-acid motif (called SDE2-C) which is conserved among eukaryotes (Fritz et al., 2018). SDE2 helps cells to relieve replication stress and ensures a complete DNA replication process in cultured human HeLa cells. In addition, SDE2 supports the splicing of selected pre-mRNAs in an intron-specific manner in *Schizosaccharomyces pombe*. The mutated HH6 embryos having a lack of SDE2-C motif may have a significant impact on the SDE2 protein function, which can cause embryo death after several cell divisions (Fritz et al., 2018).

In the reported countries, HH6 has lower frequencies compared to other defects only China and France reported frequencies above 1% (Table 2).

### **1.7 Holstein Haplotype 7 (HH7)**

In the Holstein breed, HH7 has been reported to be responsible for embryonic death before the 35 days of pregnancy. Three bulls, two of US origin, Secret and Dutch Boy, and French origin Merdignac have been reported as the earliest carriers for HH7 (Hozé et al., 2020).

HH7 is the result of a four-base deletion in the *CENPU* gene. This deletion removes four nucleotides (g.14168130 to 14168133 TACT) in the *CENPU* gene, and the resulting mutation changes the splicing pattern of the gene. CENPU gene has 13 exons, 47546 base sequence, and produces 409aa long protein (NCBI, 2021b). CENPU is a

centromere component required for proper chromosome segregation during mitosis. Disruption of the *CENPU* gene in humans and mice showed mitotic defects and lethality, respectively. This haplotype has a carrier frequency of 1.1% in the French cattle population (Hozé et al., 2020).

## **2. DEFECTS IN AYRSHIRE BREED**

Ayrshire is one of the most beautiful dairy breeds that originated in the Ayr County of Scotland and is reddish-brown and white cattle. This breed is medium in size, has a strong structure, and is suitable for commercial farming. The milk of this breed has moderate fat and high protein content (Anonymous, 2022a). Two defects associated with fertility called AH1 and AH2 in the Ayrshire breed are described below.

### **2.1 Ayrshire haplotype 1 (AH1)**

The recessive haplotype AH1 in Ayrshire breed was initially identified as lower conception rate ( $4.3\% \pm 2.5\%$ ) but recent studies report that this fertility haplotype causes late pregnancy loss which increases stillbirth rate (1.4% for cows and 2.1% for heifers) (Cooper et al., 2014; Van Doormaal, 2017; Guarini et al., 2019). Interestingly, homozygous AH1 is related with the development of a syndrome in calves known as PIRM (ptosis, intellectual disability, growth retardation and death). AH1 live animals die little after birth at an early age either are euthanized because of the progression of pathologies that are inconsistent with life (Venhoranta et al., 2014). The earliest heterozygous carrier ancestor of AH1 was a bull named Selwood



Betty's Commander and this bull had the highest 11.1% inbreeding rate in the Ayrshire breed. This bull with some of his sons were very popular in the Ayrshire industry and used widely in AI resulting higher carrier frequency among the Ayrshire cattle (Cooper et al., 2014). AH1 is caused by a synonymous mutation (G>A) occurred in the last nucleotide of exon 23 of *UBE3B* gene (Venhoranta et al. 2014).

The 46475 nucleotides long cattle *UBE3B* gene has 29 exons and encodes 1068 aa long protein called ubiquitin-protein ligase E3B isoform X1 (NCBI, 2020d). Mutation in *UBE3B* affects the splicing process of gene and leads to incomplete truncation of the HECT domain in the coded protein. *UBE3B* is a member of the ubiquitin E3-ligase family and is involved in a variety of biological processes throughout organogenesis and neurodevelopment. In humans, loss-of-function mutations in the *UBE3B* gene cause severe permanent neurodevelopmental disorders those are phenotypically manifested as developmental delay, mental retardation, facial dysmorphisms, hypotonia, microcephaly, neonatal respiratory distress, gastrointestinal pathologies, and hypocholesterolemia (Venhoranta et al. 2014).

In Russia, in a sample of 186 bulls, the proportion of AH1 carriers was found 16.66% (Pozovnikova et al., 2020). The frequency of AH1 has been reported at 22.32% in the US Ayrshire population, and this frequency was approximately 17.4% in Canadian heifers born in 2020 (Van Doormaal, 2021; Cole et al., 2022).

## **2.2 Ayrshire haplotype 2 (AH2)**

Another recently identified fertility haplotype AH2 in the Ayrshire breed expressed as a 6.1% reduction in sire pregnancy rate and a more than 5% reduction in 56-day non-return rate (Null et al., 2017; Guarini et al., 2019). The earliest heterozygous source of AH2 is two bulls named Oak Ridge Flashy Kellogg and Oak-Ridge Lightning (Van Doormaal, 2017). AH2 haplotype locates on bovine autosome 3, and causal mutation was found as a splicing acceptor variant at 51267548 bp in the *RPAP2* gene (Null et al., 2017; Guarini et al., 2019).

*RPAP2* gene consists of 127,627 bases with 25 exons in cattle. *RPAP2* encodes 562 aa long protein called putative RNA polymerase II subunit B1 CTD phosphatase RPAP2 isoform X3 (NCBI, 2022e). This protein is an important component of the RNA polymerase 2 holoenzyme, needs for snRNA transcription. A knockout mouse experiment showed that RPAP2 is required for embryonic development (Guarini et al., 2019).

The AH2 carrier frequency has been reported as 21% in current US Ayrshire cattle and 22.1% in heifers born in 2020 in Canada (Van Doormaal, 2021; Cole et al., 2022).

## **3. DEFECTS IN BROWN SWISS BREED**

The light brown colour Brown Swiss or Braunvieh breed is considered the oldest dairy breed that originated in the northeastern part of Switzerland. These cattle are suitable in the dairy production system

for their well-structured feet and legs, good temperament, and long productive life. In addition, the milk of this breed possesses the best fat-to-protein ratio (Anonymous, 2022b). Two haplotypes BH1 and BH2 were found to be associated with fertility in the Brown Swiss breed, but BH1 has now been removed for its negligible effects (Cole et al., 2022).

### **3.1 Brown Swiss haplotype 2 (BH2)**

BH2 is a recessive defect reported in the Brown Swiss or Braunvieh, Fleckvieh and Nordic Red cattle. This haplotype is related to lower fertility as well as growth related difficulties, causing 4.6% higher mortality of calves born from carrier-to-carrier mating of BH2 (Schwarzenbacher et al., 2016; Häfliger et al., 2021). BH2 was identified in the BTA19, and mutation was reported in the *TUBD1* gene (Schwarzenbacher et al, 2016).

Cattle *TUBD1* gene has 9 exons and a whole gene length is 23905 nucleotide long and encodes protein called tubulin delta chain of 453aa long (NCBI, 2020e). The change of T to C creates a missense mutation that causes a histidine to be replaced by an arginine at a conserved position of the gene and damage normal function of protein. BH2 Homozygous calves suffer from chronic airway disease caused by defective cilia in the airways (Schwarzenbacher et al, 2016).

The BH2 frequency was 1.7% and 6.5% in Fleckvieh and Braunvieh animals, respectively, and a homozygous animal was also reported in the Braunvieh cattle (Schwarzenbacher et al, 2016). The frequencies of this lethal defect have been reported as 4.42% in Nordic Red Dairy

cattle (Wu et al., 2020). BH2 carrier frequency has been reported as 6.65% in Brwon Swiss cattle in the USA and 4.22% in Braunvieh populations in Switzerland (Häfliger et al., 2021; Cole et al., 2022). In addition, heifers born in Canada in 2020 had a BH2 carrier frequency of 9.6% (Van Doormaal, 2021).

#### **4. DEFECTS IN MONTBÉLIARDE BREED**

The Montbéliarde is a red and white color breed that evolved from the Haute Saône-Doubs region of France and is well known for their hard feet and strong udder structure. Montbéliarde cattle are placed in the highest rank for their breeding qualities and functional characteristics, such as mastitis resistance, good fertility, longevity, and calving ease (Anonymous, 2022c). Montbéliarde breed has two lethal haplotypes called MH1 and MH2 and have also been found in the Vorderwald cattle.

##### **4.1 Montbéliarde haplotype 1 (MH1)**

Montbéliarde haplotype 1 is one of the fertility defects in the Montbéliarde dairy cattle, reported by Fitz et al., (2013) with 9% haplotype frequency in the French Montbéliarde population. Carrier to carrier mating for MH1 showed 5.96% and 4.84% less calving rate in heifer and cows, respectively. Homozygous MH2 embryos fail to continue their development after 35 days of gestation.

This haplotype locates in bovine chromosome 19, and causal mutation was reported in *SHBG* (g.27956790C>T) gene, but the presence of

homozygous carrier (TT) in the Montbéliarde and Vorderwald cattle invalidated this mutation as lethal. Finally, one mutation in the *PFAS* gene was found that is strongly associated with embryonic mortality in MH1. Cytosine to thymine substitution results in an entirely conserved in eukaryotes arginine to cysteine changes in the protein structure which is essential for structural stability or activity of protein function (Michot et al., 2017).

Cattle *PFAS* gene has an exon count of 29 and the whole gene covers a 18424 nucleotide length sequence. This gene encodes 1367aa long protein named phosphoribosylformylglycinamide synthase isoform X3 after translation (NCBI, 2022f). This protein is involved in the 4th step of de novo synthesis of purine which is an essential component of genetic material and energy molecules of cells. Furthermore, de novo purine synthesis is so crucial in early embryonic development. Several mutations of this pathway were also reported including *GART* gene mutation in HH4. Mutations in the *PFAS* cause short head and short nose in mice and the deficient *ade2* gene (ortholog of *PFAS*) was lethal in *Drosophila* (Holland et al., 2011; Palmer et al., 2016). This base substitution is also segregated at a very low frequency in the human population (Michot et al., 2017). In humans, disorders related to de novo purine synthesis such as adenylosuccinate lyase (ADSL) deficiency, aminoimidazolecarboxamide-ribosiduria (AICA-ribosiduria) usually expressed as wide, undefined neurological symptoms and embryonic loss occur in cases with a severe phenotype (Baresova et al., 2016).

MH1 has a carrier frequency of 6.7% in the Montbéliarde cattle of France and 5.8% in the Vorderwald cattle of Germany that have the blood of the Montbéliarde breed (Michot et al., 2017; Reinartz & Distl, 2020).

#### **4.2 Montbéliarde haplotype 2 (MH2)**

Fritz et al., (2013), have first identified another lethal defect of the Montbéliarde breed by analyzing genomic data of the French cattle population for a lack of homozygosity. This haplotype had an adverse effect on the calving rate of heifers (5.26%) and cows (4.85%). The mutation for this defect was identified in the *SLC37A2* gene, where a nonsense mutation causes an early cessation of the amino acid chain.

The cattle *SLC37A2* gene has an exon count of 21, and nucleotide length of 53694 base pairs. This gene encodes the protein name glucose-6-phosphate exchanger SLC37A2 isoform X3 and 468 aa length (NCBI, 2021c). The *SLC37A2* gene belongs to the solute carrier superfamily, which consists of over 300 members divided into 51 families. This gene family is involved in the transmembrane transport of a variety of solutes. Deficiency in solute carrier protein has been linked to a variety of genetic abnormalities in humans, mice, and livestock (Fritz et al., 2013). SLC37A2 is a phosphate-linked glucose-6-phosphate (G6P) antiporter and it is a critical chemical in cellular energy metabolism, and impairment in multiple G6P metabolic enzymes, which causes embryonic death in mice (Pan et al., 2011; Fritz et al., 2013). The carrier

of heterozygous MH2 was 7.8% in Vorderwald and 5.1% in the Montbéliarde cattle.

## **5. DEFECT IN JERSEY BREED**

The fawn shade or cream color cattle known as the Jersey breed is one of the oldest dairy cattle breeds, evolved about 200 years ago on the English Channel Jersey Island off the coast of France (Huson et al., 2020). This breed has a moderate milk production capacity, but milk has higher fat and protein content compared to other dairy cattle (Norman et al., 2020). JH1 is the lethal defect in this breed responsible for embryonic mortality.

### **5.1 Jersey Haplotype 1 (JH1)**

JH1 1 has a negative effect on fertility in the Jersey cattle, where analysis of fertility records showed a pregnancy rate of 33.3% for at-risk mating, as opposed to 37% in the normal mating (Sonstegard et al., 2013). Investigation of 60, 100, and 140-day non-return rates revealed that most embryonic losses occur before 60 days of gestation (VanRaden et al., 2011b). The first heterozygous carrier of JH1 was a high-performance bull named Observer Chocolate Soldier that sired a large number of offspring (VanRaden et al., 2011b; Sonstegard et al., 2013).

JH1 was found in the 11-16Mb interval of *Bos taurus* autosome 15 (VanRaden et al., 2011b). Causative mutation of JH1 was discovered by whole-genome sequencing of one son of "Soldier" and ten other

carrier bulls. Of the 15 primary identified SNPs and one INDEL in the haplotype domain, a highly effective nonsense mutation in the *CWC15* gene was identified as causative. Mutation in JH1 causes C to T transition that changes arginine to a stop codon in exon 3 of the *CWC15* gene (Sonstegard et al., 2013).

The bovine *CWC15* gene has 9028 nucleotides containing eight exons and produces a 231 amino acid long protein named spliceosome-associated protein CWC15 homolog isoform X1 (NCBI, 2021d). The JH1 mutation reduces the size of the CWC15 protein product from 231aa to 54 amino acids (Sonstegard et al., 2013). CWC15 protein presents in the nucleus, mitochondria, and nuclear speck (NCBI, 2021d). This protein is involved in the pre-mRNA splicing process as a component of the spliceosome. CWC15 is a component of the PRP19-CDC5L complex that forms an integral part of the spliceosome and is required to activate pre-mRNA splicing (NCBI, 2021d; Uniprot, 2022). *CWC15* gene expression in all eighty-seven bovine tissues supports its role as an essential gene for cell function. The highest relative expression of the *CWC15* gene includes parts of the placenta and the uterine attachment to the placenta. The mouse *CWC15* gene expresses during early embryonic development (Sonstegard et al., 2013). CWC15 is essential for plant development, including embryo development, as the splices are affected at the whole genome level. Downregulation of *CWC15* results in developmental defects in the plant *Arabidopsis*. Loss of *CWC15* function is embryonic lethal in the yeast *Schizosaccharomyces pombe* (Slane et al., 2020). Cattle JH1



homozygous embryos survive for a short time until the presence of this gene is completely essential for the efficient alternative splicing required for proper development (Sonstegard et al., 2013).

The JH1 has a carrier frequency of 18.42 % in the USA and 14.7% in the heifers born in 2020 in the Canadian Jersey herd (Van Doormaal, 2021; Cole et al., 2022). The carrier of this defect was reported as 23.3% in the Indian Jersey and 6.9% in the imported Jersey cattle in China (Zhang et al., 2015; Kumar et al., 2021).

## **CONCLUSION**

In case of lethal defects, 25% of embryos die while carrier sires and dams are mated. This situation causes cows to repeat breeding and affects the overall fertility of herds. Therefore, the detection of carrier animals is crucial to control and eliminate these defects at the population level. Due to the high incidence and the growing number of lethal defects, comprehensive culling of carriers of lethal recessive alleles is impractical.

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## **CHAPTER 9**

### **NUTRITIONAL VALUE AND USE OF SUGAR BEET LEAVES IN ANIMAL NUTRITION**

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## **INTRODUCTION**

Sugar beet leaves (SBL) (*Beta vulgaris ssp. vulgaris*) are obtained as a by-product remaining after sugar production. It is grown in regions with cool climates, especially in Europe. Approximately 120 million tons of sugar beet leaves are produced in Europe each year (Undiandeye et al. 2022). In Turkey, 38,238 producers planted in 2020, and 69.11 thousand tons/ha root yield and 8,582.038 million tons of sugar beet were produced from 124,279.2 hectares of land. Sugar beet leaves production constitutes 80-85% of root yield per decare. Our country has an important share in sugar production among the other producer countries of the world (Türkşeker 2021). One of the most important problems to be solved in the development of the livestock in our country is to meet the need for quality and cheap roughage. A large part of the leaves obtained after the sugar beet harvest is mixed with the soil as green manure in the field. Very little of it is fed to animals. Sugar beet leaves, which is consumed by animals willingly as fresh, dried and silage due to the nutrients it contains, and which has a great potential in terms of animal nutrition, which does not require any extra expenditure for its production, cannot be utilized sufficiently (Kılıç 1986; Alhan and Can 2017).

### **Nutrient Content of Sugar Beet Leaves**

The nutrient contents of the sugar beet leaves are given in Table 1. The dry matter content and crude cellulose content of the sugar beet leaves are low, while the ash content is high. The ash content varies depending



on the contamination rate with the soil. Approximately 2% of the ash content is mineral matter and 8% is soil contaminated with leaves. If the stem content is high in the leaves, an increase in dry matter and nutrient content is observed (Akyıldız 1983; Kılıç 1986).

**Table 1.** Chemical composition of sugar beet leaves (DM%)

	DM	OM	CP	Ash	EE	NDF	NDS	ADF	HC	CEL	S	Phenolic
SBL	10.0	78.9	21.9	21.0	3.5	42.3	57.8	21.1	21.2	11.4	24.9	2.9

DM: dry matter; OM: organic matter; CP: crude protein; EE: Ether extract; NDF: neutral detergent fiber; NDS: neutral detergent solubles; ADF: acid detergent fiber; HC: hemicellulose; CEL: cellulose; S: sugar. Wadhwa and Bakshi (2013)

Sugar beet leaves (SBL) contain 21.9% CP, 78.9% OM, 3.5% EE and 21.0% ash. SBL is also rich in sugar content (Table 1). Protein fraction in SBL was in order albumin (60.6%), glutelin (14.7%), globulin (12.7%) and prolamin (12.0%) (Wadhwa and Bakshi 2013).

**Table 2.** Macro and micro element contents of SBL (ppm)

	SBL	MTL
	Macro Elements	
Ca	0.88	1.00
P	0.20	1.00
Mg	0.82	0.40
Na	1.76	1.60
K	0.54	3.00
S	0.31	0.40
	Micro Elements	
Fe	677.0	1000
Cu	8.5	40
Zn	32.5	540
Mn	67.8	1000
Mo	1.4	10
Co	2.3	10

MTL: maximum tolerance limits, Madhwa and Bakshi (2013)

SBL are rich source of macro and micro elements (Table 2.) Only Na and Mg contents of SBL were higher than maximum tolerance for ruminants animals.

The heavy metal content of the sugar beet leave is well below the limits that may adversely affect the health of ruminant animals.

**Table 3.** Heavy metal content of sugar beet leaves (ppm)

Heavy Metal	SBL	MTL
Al	958.0	1000
As	6.6	50
Cr	3.5	1000
Ni	9.8	50
Cd	0.42	0.50
Pb	6.16	30

Madhwa and Bakshi (2013)

### **Use of Sugar Beet Leaves in Animal Nutrition**

SBL can be fed to animals in the barn by grazing the animals or cutting the leaves directly in the field. The effect of harmful substances such as oxalic acid contained in SBL disappears with withering. For this purpose, the leaves should be used for feeding after wilting for one day (Kılıç 1986). SBL is highly digestible by ruminant animals. However, when given in high amounts, it causes digestive disorders. Maximum half of the ration dry matter should be met with SBL. It's not used for feedings piglet due to its oxalic acid content. A maximum of 15 kg per day can be given to cattle, and a maximum of 2 kg to sheep (Kılıç 1986; Akyıldız 1983; Karabulut 1995; Wadhwa and Bakshi 2013). Since the dry matter content of SBL is low, when it is used in the diet as the main source of roughage, it should be given to animals together with forages

such as alfalfa fodder or straw. It's not used for feedings piglet due to its oxalic acid content.

According to the study of Wadhwa and Bakshi (2013) SBL has been determined and specified in terms of dry matter digestion kinetic parameters, UF (undegradable fraction) 9%, ED (effective degradability) 81.9%, TD (true degradability) 75.9%, RF (Rumen fill) 15.4% They found PDMI (potential dry matter intake) as 11.2% and NIV (nutritive index value) as 79.6%. In their studies, using the vitro gas production technique, the same researchers determined SBL's (gas production) 20ml/24 h/g DM, NDFD (notral detergent fiber digestion) as 61.9%, OMD (organic matter digestion) as 83% and metabolic energy content as 7.3 MJ/kg DM.

Due to the fact that SBL is usually harvested at the end of September and the beginning of October (autumn), it is not possible to store it by drying because excessive precipitation causes spoilage and mold. It is more appropriate to make silage in order to meet the green and water-rich feed needs of animals in winter (Kılıç, 1986). The feed value changes depending on the amount of beet that SBL contains and the soil contamination rate. The more it is polluted with the soil, the higher the density of contamination with bacteria and the more negatively affected the silage fermentation. The material to be ensiled must be clean (Kılıç 1986). Pimlot (1991) states that there is no need for any additives in the ensiling of SBL. However, hay, straw, sugar beet pulp, and grain crushes are added in order to prevent nutrient losses caused by silo water and to increase dry matter content (Corporal 1987; Keady 2003).

The nutrient content of ensiled sugar beet leaves is given in Table 4. SBL silage contains 76.02 OM 15% CP and 3.3% EE in % dry matter.

**Table 4.** Chemical composition of sugar beet leaves silages (DM%)

	DM	OM	CP	CF	EE	N-Free Extract	Ca	P
SBL	17.7	76.2	15.5	17.0	3.30	42.3	21.1	21.2

DM: dry matter; OM: organic matter; CP: crude protein; EE: Ether extract; Akyıldız (1986)

Ethanol is produced in silage of feeds rich in sugar content, mostly by yeast, but a small part of it is produced by lactic acid bacteria, enzymes and other microorganisms (Mc Donald et al.1991; Rooke and Hatfield 2003).

Thaysen et al. (2012) reported that ensiling sugar beet leaves without additives caused the dry matter losses. As high as 20% sugar was converted into ethanol. Gerlach et al. (2017) found that formic acid and propionic acid additions to sugar beet leaves silages limited ethanol production and the sugar content of the silage was largely preserved.

Ak and Uzaticı (2000) found the SBL organic matter digestibility to be between 59.8-82.8% and the ME content of 2366 Kcal/kg ME in dry matter in their study on the silage of sugar beet leaves.

Gürbüz and Kaplan (2008) determined the nutrient content and in vitro (gas production) organic matter digestibility of SBL silages: they found organic matter digestibility in the range of 36.02-57.44% and ME content in the range of 5.82-8.77%. They found that the dry matter

content of the unadded SBL silage was lower than that of the added SBL silage, and the protein content was higher.

Suliman et al. (2013) investigated the effects of sugar beet leaves, green maize stems, sugar cane tops silages given to lambs in the growing period on the yield performance of lambs in the growing period, the average Daily gain, feed conversing, dressing percentage and the other carcass traits were determined similar among the CFM+ berseem hay, CFM+SGMS, CFM, SSCT and CFM+SBL treatments group.

Hellwing et al. (2017) in their study to determine the effects of SBL silages with and without additives on the performance of dairy cows, they found that milk yield was higher in silages without SBL than in SBL silages with and without additives, but the fat concentration of milk was higher in SBL silages with and without additives.

Alhan and Can (2017), in their study to determine the effect of adding wheat straw and inoculant to SBL on silage quality, the highest dry matter content was SBL+10% WS+4 mL /kg Inoculant, SBL+10% WS+2 mL /kg, Inoculant respectively., SBL+10% WS groups found the highest protein content in SBL, SBL+10% WS+4 mL /kg Inoculant, SBL+10% WS+2 mL /kg, Inoculant groups, respectively. The in vitro dry matter digestibility of the study was highest in 80.82%, 79.40%, 75.82, SBL, SBL+10% WS+2mL /kg, Inoculant, SBL+10% WS+4 mL/kg Inoculant groups, respectively. The LA contents of the study were also determined as 80.29%, 75.27 and 74.12 in the SBL,

SBL+10% WS+4 mL/kg, Inoculant, SBL+10% WS+2 mL/kg Inoculant groups.

Most of the SBL, which is of good quality in terms of nutrient content, remains in the field. Meeting the roughage deficit, which is needed by our country's livestock, with SBL silage both contributes to the elimination of the problem and is economical since it is obtained as a by-product during the production of sugar beet, and there is no extra production cost. Additive and without additive sugar beet leaves are an ideal feed source for dairy cows.

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## **CHAPTER 10**

### **GRAFTING STUDIES ON VEGETABLES**

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## **INTRODUCTION**

Contrary to what is known, the grafting method in plant breeding is not a new technology, but has a very old history. Grafting is a technique used in fruit production since ancient times. The history of herbal vaccination is in the inscriptions BC. It is reported that it was made on trees in the Far East in the 1500s and successful results were obtained. B.C. It is reported that in the 1560s, the Chinese applied the grafting technique on trees and it was successful (Garner, R.J., 1979). On the other hand, inoculation in vegetables started in 1920 (Ashita, E., 1927; Yamakawa, B., 1983), for the first time in Korea and Japan in the 1920s, it was applied against *Fusarium* wilt on the rootstock of watermelon (*Citrullus lanatus*) and gourd (*Lagenaria siceraria*). success has been achieved (Ashita, E., 1927; Yamakawa, B., 1983; Grows et al., 2004; Lee et al., 1992; Davis et al, 2008; Ulaş and Grow, 2016). After the success in Japan and Korea, there has been an increase in production and yield in the production areas and vegetable types made with the vaccine.

Asian countries are leading in the production of grafted vegetable seedlings. Mediterranean countries and other European countries follow after Asian countries (Traka-Mavrona et al., 2000; Lee and Oda, 2010; Huang et al., 2015). It has been reported that grafting on vegetables is seen as an innovative technique in the Mediterranean region, where extensive and continuous land use is common (Pogony et al., 2005; Flores et al., 2010; King et al., 2010; Khah et al., 2012). Grafting technique is mostly applied in tomato, zucchini, eggplant and

watermelon. In the 1950s, grafted seedlings of tomato and eggplant were used commercially in Asia.

The first studies on grafted vegetables in Turkey started in the 1980s and the first research was done by grafting eggplant to tomato. Later, vaccination studies on watermelon and melon followed (Vuruşkan, 1989; Yetişir, 2001; Yarsi, 2003). In one of the first studies, the effects on morphological characteristics such as quality, yield and plant growth in watermelon and melon were investigated by using different rootstocks at Çukurova University (Yetisir, H., 2001; Yarsi, G., 2003).

The first commercially grafted vegetable production in Turkey started in 1998 with the production of 70,000 tomatoes and reached 120 million plants in 2012. In vegetable grafting, watermelon was the first, followed by tomato and eggplant with 9% (Balkaya, 2013; Yelboğa, 2014). In our country, the production of grafted seedlings, the climate structure of vegetable growing, especially the Mediterranean region, where it is done even in winter, is the province of Antalya. Grafted with watermelon, tomato, eggplant, cucumber, melon in Turkey. Seedlings, where there are 33 seedling companies, 77 million watermelons and 74 million tomatoes were vaccinated.

Grafting in perennial plants is done in order to provide vegetative reproduction, to prevent seed loss by maintaining preservation, to benefit from the superior characteristics of the rootstock, to repair plant damage and to save time in the selection of varieties. Grafting in vegetables is a propagation technique that combines two vegetative

parts of the plant (rootstock, scion) with appropriate techniques so that they can grow and develop as a single plant (Ağaoğlu et al., 1995; Edelstein et al., 2004). The main purpose of grafting is to control soil-borne diseases, disease tolerance, high and low temperature tolerance, water absorption, nutrient absorption and resistance to root nematodes, and to eliminate negativities such as salinity and excessive humidity. (Balaz, F., 1982; Tachibana, S., 1988; Kato, T., and Lou, H., 1989; Choi, J.S, et al., 1992; Zerki, M. and Parsons L. R., 1992; Lee, J. M., 1994; Ruiz, J.M., 1997; Yetişir, H., 2001; Han, J.H., et al., 2003; Yetişir, H., et al., 2003. While grafting is effective against biotic and abiotic stress in vegetables, yield and product quality are increased with other rootstock features such as resistance, high root absorption, strong roots and high adaptability. It also improves flowering, fruit quality, prolongs harvest time, increases growth rate, high yield and tolerance to certain environmental stresses (Lee et al., 2008).

Plants grafted on strong rootstocks have strong broad roots, reduce the need for irrigation and provide higher yields. The compatibility between rootstock and cultivar and the success of grafting increased the yield of crops such as tomato, pepper, eggplant, cucumber, melon and watermelon (Bletsos et al., 2003; Passam et al., 2005; Rauphael et al., 2010; Lee, 2010; Savvas et al., 2010). Researchers have determined that grafting plants onto the correct rootstock can be effective against diseases caused by fungi, bacteria, viruses and nematodes.

Grafting vegetables to healthy rootstocks can increase yield, strength and tolerance to environmental stresses such as temperature and soil

salinity. However, it has been reported that grafting in the wrong rootstock selection does not increase yield (Romano and Paratore, 2000). For these reasons, rootstock selection is of great importance in grafted seedling production. The rootstock used should have good hypocotyl properties and uniform, homogeneous seedlings. At the same time, rootstocks that are resistant to diseases and pests should be selected. Although there are many purposes of using a grafted seedling, there are also many similar advantages and disadvantages.

**Advantages of vaccination;**

Effective, easy and clean struggle with soil-borne diseases such as Fusarium, tolerance to low soil and air temperatures, better intake and more effective use of water and plant nutrients, prolonging the economic harvest period as a result of increasing plant power, increasing plant power and prolonging the harvest period. As a result of the reduction of the time required for breeding, the decrease in the chemicals to be used in soil disinfection and plant protection, and the better uptake of plant nutrients in the soil by removing the characteristics such as resistance to diseases provided by the rootstock, tolerance to low temperatures and adverse soil conditions from the variety breeding program. prevention of damage to the environment.

**Disadvantages of vaccination;**

Grafting needs extra time, place and plant material, requires sufficient experience for hanging and post-plant care, incompatibility problems, quality deterioration depending on rootstock, especially when hybrid

rootstock is used, the cost increases. Studies have shown that the strong root system of rootstocks is more effective in absorbing water and nutrients from grafted plants than ungrafted plants. As a result, it was determined that rootstock growth performance had a positive effect on yield increase and disease control. On the other hand, due to poor compatibility of rootstock and cuttings, it causes negative effects such as loss of product, decrease in fruit quality and plant death (Lee, J. M., 1994).

The use of grafted seedlings is widely practiced in many countries after these countries, due to the advantages such as a significant increase in yield by providing resistance to various soil-borne diseases and abiotic stress conditions. In our country, its use has increased in recent years. Different grafting methods were applied in vegetable species; Tube grafting techniques are known as English Tongue (Snapping), Splitting, Inlay (Seat), Slant Cut (slant-cut), Horizontal Cut (horizontal-cut). In recent years, countries such as Korea and Japan have been vaccinating vegetables with vaccine robots by making use of the latest technologies. The purpose of this review; reasons of grafting in vegetable growing, its place in the world and in our country, developments in grafted seedling production, researches done in the world and in our country and recent developments are summarized.

## **VACCINATION STUDIES ON VEGETABLES**

The yield of grafted plants depends on rootstock compatibility, environmental conditions and production methods (Cohen et al., 2005).



Plants grafted with strong rootstocks have stronger root systems, thus reducing water requirements and providing higher yields. Compatible, successful rootstock – variety relationships increase yields in vegetables such as tomato, pepper, eggplant, cucumber, melon and watermelon (Bower and Nel, 1981; Mavrona and Pritsa, 2000; Lee and Oda, 2003; Lee, 2010; Savvas et al., 2010). ; Schwarz et al, 2010; Balkaya, 2014; Nawaz et al,2016; Suchoff et al, 2017; Saribas et al, 2019).

Researchers have found that grafting plants onto the correct rootstock is beneficial for fungal, bacterial, viral and It is effective against nematode diseases, strong. They reported that inoculating rootstocks with the right grafting technique increased yield, strength and tolerance to environmental stresses such as soil temperature and soil salinity. However, it was also stated in the results of the research that the grafting did not give the desired quality even if the rootstock selection was not done correctly (Romano and Paratore, 2001). The success of rootstock and scion in grafting; The nutritional status of the rootstock and scion are also effective on the grafting technique (split graft, tongue, etc.) and the maintenance conditions in the planting period (Philippines, 1990).

They reported that *S. torvum* rootstock was a very effective and superior rootstock against the disease in grafted plants sensitive to *Solanum torvum* and *S.aethiopicum* rootstocks in soils contaminated with *Verticillium dahliae*, and that the disease resistance of the rootstock increased the yield even under the stress conditions of the commercial variety (Johnson et al., 2014).

He grafted the eggplant on four different tomato rootstocks and found that two rootstocks increased the yield compared to the control plants, because the increase in yield increased the fruit weight. They reported that it is due to the increase in the number of fruits and this increases the number of fruits (Passam et al., 2005). He investigated the effect of reducing nutrient concentrations on eggplants inoculated with *Solanum torvum* on yield and plant growth.

Bletsos and Olympios (2008) published the vaccine study in a comprehensive publication. They reported that it is effective against soil pathogen, increases yield and quality, and that some biochemicals secreted by the vaccine carrier can be transported to the grafted plant. Abu Glion et al. (2019), Beaufort, He-man and Resistar commercial rootstocks Belladonna they were vaccinated with the F1 variety. Highest fruit yield under three different salt stress conditions it has been reported that although the rootstocks increased the yield in plants grafted on He-man rootstock, the content of vitamins and total phenolic substances, which are quality characteristics, decreased significantly.

Karaagac et al. (2020), in their study on pepper rootstock breeding, pepper belonging to *Capsicum annuum*, *Capsicum chinense* and *Capsicum baccatum* species examination of root structures of genotypes and rooting characteristics intended to be compared. In the results of working; *C. baccatum* and *C. chinense* species generally have more root characteristics than *C. annuum*. It has been found to be strong and superior. *C. annuum* in terms of root length and root surface area. *C. chinense*, root volume and root dry weight, *C. baccatum* species is

more prominent root morphology when its features are evaluated; CB73 and CB4 in *C. baccatum* species genotypes and CC47, CC61 and CC76 genotypes in *C. chinense* species. performance has been reported.

## RESULTS

The use of grafted seedlings is used in most places where agriculture is intense. Grafting and the use of grafted seedlings in vegetables, especially preference against soil-borne pathogens though it is; It is also preferred because it increases tolerance and resistance to salinity, drought, low and high temperature, biotic and abiotic stress effects. At the same time, since it provides earliness, reduces the use of fertilizers with high water and plant nutrient intake, it prevents damage to the environment, harvesting The increase in yield with the prolongation of the period increases the use of grafted seedlings day by day. Due to the benefits provided by the use of grafted seedlings, companies that produce vegetable seedlings have started to produce grafted seedlings, which has led to significant developments in the grafted seedling sector.

In our country, grafted seedlings are mostly in watermelon, zucchini and tomato, and the production of grafted seedlings will increase the demand for other types of vegetables in the coming years. However, the lack of sufficient studies in the breeding of rootstock varieties used in grafted seedlings is a deficiency, and it is very important to increase studies in this direction.

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**CHAPTER 11**

**RESPONSES OF C3 AND C4 GRASSES TO  
ENVIRONMENTAL FACTORS RELATED TO GLOBAL  
CLIMATE CHANGE**

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## **Introduction**

C3 and C4 grasses in rangelands respond differently to global climate change with forage yield and quality features. Increases in atmospheric CO<sub>2</sub> will stimulate C3 photosynthesis more than C4 and most C3 plants will respond positively to photosynthetic changes. C4 species respond positively to nutrient-restricted conditions along with high temperature and drought. The increase in water use efficiency due to the increase in the amount of CO<sub>2</sub> and the decrease in drought stress will increase the growth and yield of C4 plants. A decrease in stomatal conductivity will be observed in both C3 and C4 plants. This reduction will be more pronounced in C4 plants. Thus, leaf temperatures and heat damage will increase due to less transpiration. The increase in the amount of CO<sub>2</sub> causes the closure of stomata, the efficiency of water use, the increase in leaf expansion, the promotion of photosynthesis, and the increase in the amount of solute required for osmotic adjustment, especially when it occurs with arid conditions. However, increasing CO<sub>2</sub> will cause a decrease in photorespiration and stomatal conductivity thus increasing the yield in C3 plants. The water use efficiency of C3 species will increase as a result of the increase in the amount of CO<sub>2</sub> causing a decrease in the transpiration rate of the plants and an increase in the rate of CO<sub>2</sub> assimilation. Due to drought stress, low stomatal conductivity and higher intercellular CO<sub>2</sub> ameliorate the adverse effects of high CO<sub>2</sub> conditions. In this case, in high CO<sub>2</sub> interaction with drought stress the increase in CO<sub>2</sub> will cause an increase in osmotic adjustment. As a result, unlike C3 plants C4 plants will gain an advantage. C4 plants have

lower stomatal conductivity in arid conditions and maintain the CO<sub>2</sub> assimilation rate. Therefore, in arid conditions with high temperatures, C<sub>4</sub> plants are widely available. C<sub>4</sub> forage crops will show higher adaptation to hot and dry season conditions than C<sub>3</sub> plants at high temperature and light values.

Extreme weather conditions such as higher CO<sub>2</sub>, temperature and drought trigger growth and stress responses in rangeland plants. With stress, the composition of forage crops and hence the nutritional value for livestock decreases with increasing temperatures (Lee et al., 2017; Loka et al., 2019).

Cool climate grasses employ the C<sub>3</sub> photosynthesis while hot climate grasses employ the C<sub>4</sub> photosynthesis, which occurs in lower soil moisture and lower CO<sub>2</sub> conditions (Osborne and Sack, 2012). C<sub>4</sub> photosynthesis is common in rangeland plants and accounts for approximately 42% of all rangeland species (Pardo and VanBuren, 2021). In temperate climates, C<sub>3</sub> grasses are known as the invasive plants in C<sub>4</sub>-based rangelands (Dekeyser et al., 2013).

Plants of *Bambusoideae*, *Oryzoideae* and *Pooideae* families use the C<sub>3</sub> pathway of photosynthesis while some of (*Panicoideae*, *Aristidoideae*, *Arundinoideae*, *Chloridoideae*, *Micrairoideae*, and *Danthonioideae*) families use the C<sub>4</sub> pathway and are more resistant to drought (Pardo and VanBuren, 2021).

Fructan synthesis takes place in C<sub>3</sub> plants and fructan storage increases especially under low temperature and intense light conditions. In fact

more than 30% increase in fructan in dry weight is ascertained in C3 exposed to low temperatures (Chatterton et al., 1989). However C3 plants are not expected to produce high levels of fructans unless other environmental factors such as low temperature interact with high carbon dioxide levels (Barbehenn et al., 2004).

With the increase in the concentration of CO<sub>2</sub> in C3 plants a meaningful increase was observed in the amount of sugar, starch and fructan. On the other hand, there was a significant decrease in protein level and C4 plants were less affected by the increase in CO<sub>2</sub>. C4 photosynthesis is effective in many combinations of anatomical, biochemical, and physiological modifications that concentrate CO<sub>2</sub> in the bundle sheath and effectively saturate Rubisco at ambient CO<sub>2</sub> concentrations (Figure 1.). This almost eliminates photorespiration and C4 plants provide CO<sub>2</sub> fixation at equal or higher rates than C3 plants resulting in a decrease in stomatal opening (Percy and Ehleringer, 1984). In C4 plants such responses and the resulting increased water use efficiency reveal that C4 photosynthesis is insensitive to arid conditions and can be exploited in arid areas due to its advantage (Taiz and Zeiger, 1991; Haxeltine and Prentice, 1996). Bundle sheath cells of C4 plants either do not inhibit protein digestion or show a small decrease in livestock. In general, it was observed that increases in CO<sub>2</sub> amount increased protein, non-structural carbohydrate and water content in C3 plants while fiber, endurance and total carbohydrate/protein ratio decreased compared to C4 plants. Therefore, C3 pasture plants are generally considered to be more nutritious than C4 plants (Barbehenn et al., 2004).

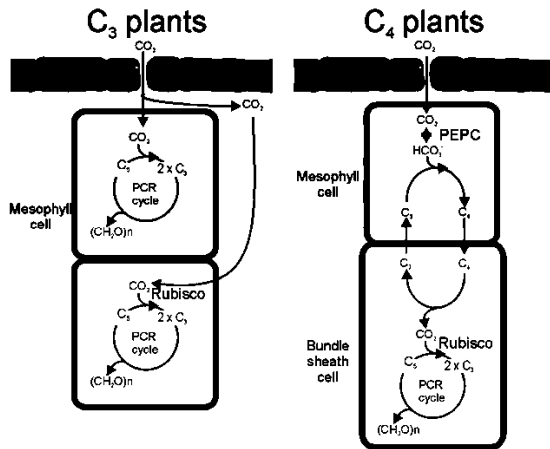


Figure 1. C<sub>3</sub> - C<sub>4</sub> plants carbon fixation pathway (Derived from Lara et al., 2011).

In hot climates, whether in arid or humid areas C<sub>4</sub> plants predominate in pasture lands and vice versa in cold climates. In temperate climates, arid conditions help C<sub>4</sub> vegetation stay at temperatures that lead to C<sub>3</sub> dominance. It is reported that this may be due to either having higher photosynthetic water use efficiency or indirect effects on leaf temperature (Lattanzi, 2010).

## Temperature

Today, the average temperature is increasing all over the world and there are changes in vegetation due to climate change. Heat stress causes a change in forage quality and adversely affects plant quality characteristics. With stress, starch content decreases in maize varieties oil and protein content varies depending on the variety used and crude fiber content increases (Mahmood et al., 2010). In high-temperature stress conditions, tannin synthesis which affects the nutritional quality

of forage plants also increases (Gebrehiwot et al., 2002). Heat stress caused a decrease in lignin content in C3 species such as *Festuca arundinacea* and *Dactylis glomerata*, lignin/N ratio in *Festuca arundinacea* and an increase in non-cellulosic carbohydrate content in *Dactylis glomerata* (Sanaullah et al., 2013).

There is less information about the impact of high values of temperature on forage quality (Dumont et al. 2015). Increases in temperatures affect forage quality in various ways. Warming of the soil increases the soil nitrogen content (Rustad et al., 2001, Dieleman et al., 2012) while drying due to the increase in temperature decreases the plant nitrogen uptake (Dijkstra et al., 2010). Although there are insufficient studies on increases in both temperature and CO<sub>2</sub> amount, studies have shown that plant nitrogen content may decrease due to interaction (Dieleman et al., 2012, Mueller et al., 2016). Due to high temperature the structure of chloroplasts in plants changes and photosynthesis is prevented by inactivating chloroplast enzymes due to oxidative stress (Dekov et al., 2000; Cui et al., 2006). Heat shock in a C3 plant *Vigna unguiculata* increased the amount of  $\gamma$ -aminobutyric acid (GABA),  $\beta$ -alanine, alanine and proline (Mayer et al., 1990). Similarly, heat stress causes the accumulation of various metabolites (galacturonic acid, threonic acid, succinic acid, gluconic acid, proline, aspartic acid, serine, valine, methylmalonic acid, fructose, galactose, xylose, glucose) in the *Poa pratensis* (C3) (Du et al., 2011). Although *Festuca arundinacea* is drought-avoidant due to its deep root structure, it has limited tolerance to high-temperature stress (Fry and Huang, 2004). Temperature



increases also caused citric acid accumulation in *Cynodon* sp. (C4) and *Poa pratensis* (C3) (Du et al., 2011).

Given these, C3 and C4 grasses has an important role in the development of sustainable rangeland in future Mediterranean and temperate summer-arid environments. The current role of C3 grasses in livestock farming is critical and while the feed contribution of C4 species may increase, the role of C3 species is always greater (Norton et al., 2016).

Temperature is a significant factor confining the growth of C3 forage crops. In increased CO<sub>2</sub> conditions plants exposed to heat stress significantly increased organic acids (shikimic acid, malonic acid, threonic acid, glyceric acid, galactaric acid and citric acid), amino acid (serine, valine and 5 -oxoprolin) and carbohydrate (sucrose and maltose) content. Due to the increase of metabolites with important biological functions under stress conditions it may be possible to mitigate heat stress damage by increasing CO<sub>2</sub>. Therefore, high CO<sub>2</sub> conditions may show better forage quality and shoot growth and stress tolerance in plants exposed to heat stress. Taking advantage of this effect of CO<sub>2</sub> will be beneficial in forage crops production especially for the adaptation of the plant to increasing temperatures in summer (Yu et al., 2012). Hot climate C4 grasses work better than C3 plants in high temperatures and light values. In hot climates, C4 forage crops give higher yields than C3's (Gherbin et al., 2007) but high drought tolerance is not always observed (Ripley et al., 2007).

## **Carbon dioxide**

Atmospheric carbon dioxide concentration is predicted to increase from almost 400 ppm to 900's ppm depending on the 2100 emission scenarios (IPCC, 2013). The combined effect of increased CO<sub>2</sub> and temperature caused an increase in feed production by 38% (Augustine et al., 2018). With the increase in CO<sub>2</sub> concentration photosynthesis will increase in plants. It is observed that increasing atmospheric CO<sub>2</sub> concentration, drought and heatwave effect have opposite effects on forage quality (Niderkorn et al., 2021). An important reduce in respiration was observed in *Dactylus glomerata* at 15 and 25°C, as a result of the effect of high CO<sub>2</sub> (Ziska and Bunce 1993). High CO<sub>2</sub> can reduce the negative effects of heat stress or increase temperature tolerance (Yu et al., 2012). CO<sub>2</sub> increase generally leads to a decrease in soil nitrogen content and plant nitrogen therefore it is thought that there is a tendency to decrease in forage quality (Augustine et al., 2018). Increases in atmospheric CO<sub>2</sub> and annual average temperatures are expected to affect crop production, species composition, biogeochemistry, and thus the amount and quality of forage in rangeland ecosystems (Augustine et al., 2018). Atmospheric CO<sub>2</sub> and temperature cause significant changes in crop production. Increases in the amount of atmospheric CO<sub>2</sub> or increases with high temperatures have important and generally positive effects on the productivity of temperate and semi-arid pastures. This causes an increase in the dominant grasses consumed by livestock (Morgan et al. 2004; Polley et al. 2011; Mueller et al. 2016). Grassland areas cover more than 25% of

the world's land area, but there are limited studies on the response of grassland areas to climate change conditions. Under good irrigation conditions, the increase in CO<sub>2</sub> increased the amount of dry matter harvested from a C3 legume (*Arachis glabrata* Benth) and tended to increase in the C4 plant (*Paspalum notatum* Flüggé). Both species showed positive responses to increased temperatures (Newman et al., 2001). CO<sub>2</sub> increases significantly reduced in vitro dry matter digestibility and nitrogen content except during heavy rainfall. This was associated with an increase in acid detergent fiber (ADF) content (Augustine et al., 2018). Forage quality decreases under high CO<sub>2</sub> conditions but increases in drought stress because plant tissues contain less water and more dry matter (Dumont et al., 2015).

High CO<sub>2</sub> caused an increase in biomass in *Festuca arundinaceae*, a C3 plant, and it was revealed that the variation in the physical and chemical properties of the soil has important effects on the response of plants to global climate change, and due to high light, the soil factor should also be taken into account against global change (Nord et al. et al., 2015). High CO<sub>2</sub> promotes plant growth especially reducing heat stress damage in C3 species including perennial forage crops such as *Festuca arundinacea* (Yu et al., 2012). CO<sub>2</sub> increase has high direct and indirect effects on protein levels in C3 plants. Since protein is a limiting nutrient for herbivores, the decrease in protein level in C3 plants will disappear after high CO<sub>2</sub> conditions (Barbehenn et al., 2004).

Although the increase in the amount of CO<sub>2</sub> in the atmosphere significantly improves the efficiency of the underground organs in

high-yielding pastures, this increase is limited in low-yielding pastures. Biomass in pastures will indirectly affect root activity due to increases in temperature and CO<sub>2</sub> content. High CO<sub>2</sub> levels improve the rate of photosynthesis in plants, increase water retention, carbon fixation and fructan accumulation, and consequently alleviate the effects of drought stress (Taub, 2010; Volaire et al., 2020).

The increase in the amount of CO<sub>2</sub> caused a decrease in the C3 plant, *Poa pratensis* in areas dominated by C4 plants (Owensby et al., 1993). Roots and shoots of C4 plants *Schizachyrium scoparium* and *Andropogon gerardii* gave similar responses to CO<sub>2</sub> increase in both drought and control conditions. The relative distribution of root surface area, the number of root tips, length and volume of roots increased with CO<sub>2</sub> increases (Derner et al., 2001). The increased water content occurs after a decrease in stomatal conductivity in both C3 and C4 plants. This will play a decisive role in determining the responses of C3/C4 plants to CO<sub>2</sub> changes between 400-700  $\mu$ bar in humid and semi-arid ecosystems. If high CO<sub>2</sub> values do not support C3 species, it is thought that the presence of C4 plants in meadow pasture areas with some C3 and C4 plants will increase with global warming (Lattazi, 2010). Increasing the CO<sub>2</sub> value from 450 ppm to 700 ppm provides more biomass for both C3 and C4 plants as well as more total crude protein (McGranahan and Yurkonis, 2018).

With the increase in CO<sub>2</sub> amount in *Paspalum dilatatum* and *Pennisetum clandestinum* C4 plants, seedling emergence decreased (Dodd et al., 2010). When high temperatures are combined with dry

weather conditions, there will be a shift in the plant population towards the C4s (Collatz et al., 1998). Although CO<sub>2</sub> increases appear to be the main factor behind high temperatures and water stress leading to reduced plant yields, they also have the potential to directly benefit plant physiology (Leakey, 2009). With the increase in CO<sub>2</sub>, photosynthesis increased in *Pascopyrum smithii* (C3) and *Bouteloua gracilis* (C4) plants due to the high soil water content and leaf water potential. Nitrogen content in shoots was observed to be lower under high CO<sub>2</sub> conditions. These results reveal that there will be an increase in shortgrass steppe in the predicted high temperature and CO<sub>2</sub>-rich areas in the future. No evidence was found that photosynthetic performance under high CO<sub>2</sub> conditions was more stimulated by the C3 plant *Pascopyrum smithii*, compared to the C4 plant *Bouteloua gracilis*, which is commonly observed in this system (Morgan et al., 2001).

## **Drought**

Drought is one of the most important factors affecting plant growth, development and even plant survival. Water is one of the most important factors determining plant productivity, especially in Mediterranean climates. (Carmo-Silva et al., 2009). Drought stress affects the levels of water-soluble carbohydrates. The molecular basis of drought stress tolerance in rangeland and forage crops is not fully known (Zhang et al., 2006).

In some semi-arid rangelands, an increase in temperature may reduce the temperature limitation or exacerbate the moisture limitation in

primary production. High atmospheric CO<sub>2</sub> can balance these effects, especially by increasing photosynthesis directly in C3 plants and also by increasing the water use efficiency of C3 and C4 plants (Augustine et al., 2018). While C4 plants are generally resistant to drought conditions, the forage quality and quantity of C3 plants decrease (McGranahan and Yurkonis, 2018).

Drought-resistant C4 plants *Cynodon dactylon* (L.) Pers. and *Zoysia japonica* Steudel, the amount of 5-HNV increased with increasing drought. *Cynodon dactylon* (L.) Pers. and *Zoysia japonica* Steudel showed greater tolerance than *Paspalum dilatatum* Poir. in well-precipitated regions consistent with the geographical distribution of NADP-ME and plants of the subfamily Panicoideae (Carmo-Silva et al., 2009b). In addition, proline accumulation was detected due to drought (Carmo-Silva et al., 2009a).

Dry matter yield decreased significantly in *Festuca arundinacea*, *Poa pratensis*, *Phleum pratense* and *Phalaris canariensis* C3 grasses due to arid conditions (Kallida et al. 2008; Turner et al, 2012). Similarly, a 16% decrease in dry matter yield was observed in C3 plant *Lolium perenne* and 12% in *Festuca pratensis* (Jeronimo et al., 2014). Fariaszewska et al. (2016) emphasized that photosynthesis decreases in *Festuca pratensis* under moderate drought stress. In some C3 plants such as *Festuca arundinacea*, *Lolium perenne*, *Dactylis glomerata* and *Leymus chinensis* decreases in transpiration rate were detected due to drought (Huang and Gao, 1999; Xu and Zhou, 2005; Olszewska et al., 2010; Staniak, 2013; Guan et al., 2015). A negative relationship was

observed between water use efficiency and transpiration in some forage crops (Rumasz-Rudnicka, 2010; Fariaszewska et al., 2016). Drought conditions reduced acid detergent fiber (ADF) and neutral detergent fiber (NDF) content in C3 plants *Festuca braunii*, *Lolium perenne*, *Lolium multiflorum* and *Festuca pratensis* (Stanak, 2013; Küchenmeister et al., 2013). Drought stress increased the water-soluble sugar concentration in *Lolium perenne* (Kozłowski and Kukułka, 1996; Thomas and James, 1999; Küchenmeister et al., 2013). While drought conditions significantly increased water use efficiency in *L. perenne*, *L. multiflorum*, *F. pratensis*, *F. arundinacea* and *F. braunii* species, proline content, phenolic acids, flavonoids, water-soluble carbohydrates, neutral and acid detergent fibers decreased (Fariaszewska et al., 2020). In arid conditions, stomatal conductivity is lower and radiation is higher due to low cloudiness. This situation causes an increase in daytime leaf temperatures thus revealing the effect of drought on the C3/C4 balance (Lattanzi, 2010).

Regarding the drought tolerance of C4 plants, precipitation reduces the number of subspecies (NADP-ME) in contrast to the general C4 species. Studies have shown that C4 photosynthesis is physiologically drought sensitive. C4 plants, like C3 plants, initially respond by reducing the stomatal mesophyll conductivity of CO<sub>2</sub> in drought conditions. Drought limits ATP synthesis which will reduce the regeneration of substrates in both the C3 and C4 cycles. Initial limitations on stomatal and mesophyll conductivity in C3 plants can be quickly and completely reversed by re-irrigation. More severe

metabolic limitations are reversed more slowly and therefore plants take longer to recover (Ripley et al., 2010).

## **Conclusion**

The number of environmental factors such as the increase in global temperatures decrease in precipitation and the emergence of dry periods cause climatic changes. It is estimated that rangeland areas will be affected by these changes in precipitation and temperature. Water use efficiency at high temperatures in C3 grasses is generally lower and photosynthesis is more sensitive than in C4 grasses. Therefore in colder regions and higher latitudes C3 rangeland areas dominate. C3 plants are dominant in areas with high temperature and heavy precipitation and C4 plants in areas associated with mild and low precipitation. The increase in CO<sub>2</sub> concentration in the atmosphere triggers global warming and climate change which negatively affects plant yield but has a positive effect on plant physiology. Plants with C3 metabolism are more sensitive to CO<sub>2</sub> increases above current ambient concentrations. Increases in CO<sub>2</sub> virtually eliminate photorespiration, allowing C4 plants to achieve CO<sub>2</sub> fixation at equal or higher rates than C3 plants and reducing stomatal opening. Due to the increased water use efficiency it is observed that the photosynthesis of C4 plants is insensitive to drought and will provide an advantage in arid conditions. Due to arid conditions, photosynthesis, stomatal conductivity and transpiration decrease in C3 and C4 grasses.



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## **CHAPTER 12**

# **DETERMINATION OF YIELD AND QUALITY CHARACTERISTICS OF SOME PROMISING DALLISGRASS (*Paspalum dilatatum* Poir.) GENOTYPES ADOPTED TO MEDITERRANEAN CLIMATE CONDITIONS**

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## 1. INTRODUCTION

A significant portion of ruminant feed source from forage plants. It is an inexpensive source of calories, protein, and fiber for the ruminal function as compared to other concentrate meals (Moncao et al., 2016). The social, economic, and environmental landscapes can be significantly impacted by planting tropical forages and with enhancements in their performance (White et al., 2019). Tropical forages include multipurpose trees and shrubs, herbaceous and dual-purpose legumes, and annual and perennial grasses (Paul et al., 2020).

A South American forage called Dallisgrass (*Paspalum dilatatum* Poir.) is frequently utilized as feed in warm temperate, tropical, and subtropical regions all over the world (Giordano et al., 2014a). Dallisgrass is a crucial fodder grass in many of the warmer areas (Burson, 2019). In several of Australia's higher-rainfall subtropical coastal regions, such as New South Wales and Queensland, as well as the southeast of the United States, the perennial paspalum provides summer feed (Campbell, 1999). It has also evolved into a component of irrigated pastures in southern New South Wales, Western Australia (Callow et al., 2006), and northern Victoria (Lawson and Kelly, 2007).

The current increase in climate variability calls for innovative methods and germplasm sources to strengthen the resilience of seeded pastures and other agricultural systems. To effectively use wild germplasm in domestication and breeding, it is essential to get a thorough understanding of the structure of the germplasm of potentially relevant

wild species (Sandro et al., 2019). Native to South America, dallisgrass is very important to the production of dairy and red meat. It is cold- and drought-tolerant and has a greater fodder quality than other C<sub>4</sub> forage grasses. Primarily, apomictic monocultures are used to raise this plant (Giordano et al., 2014a). Dallisgrass is prized for its vigor, producing up to 15 tons of dry matter per hectare (Cook et al., 2005) and crude protein concentrations of up to 18.6% (Barea et al., 2007).

Warm-season perennial dallisgrass is a member of the *Panicoideae* subfamily of the *Poaceae* family. This species originated in southern Brazil, eastern Argentina, and Uruguay (Brown, 2020). The plant is a short-rhizomed perennial tufted grass that can be upright or bent. reaches a height of 1.5 meters. Plant has flat leaf blades. Rachis have a high midrib on one side and are flat. There are four to five one-sided racemes in the loose, flexible inflorescence. Where the racemes connect to the central axis, there are tufts of white hairs. *Claviceps paspali*, a deadly fungus with a dark color that flourishes on spikelets and produces hard, brownish bodies known as sclerotia or ergots, can infest dallisgrass. As a result, the staggers syndrome occurs predominantly in cattle. Clinical signs usually appear 2 to 7 days after grazing on infected grass (Botha & Venter, 2002).

C<sub>4</sub> grasses are favoured as pasture crops in warm, humid climates. The use of C<sub>4</sub> grasses in pastures is anticipated to increase as a result of the expansion of the tropical belt brought on by global climate change. Warm-season grasses often have poorer digestibility than temperate grasses, despite dallisgrass having a higher fodder quality than other C<sub>4</sub>

forage grass species. The accumulation of lignin polymers in cell walls is associated with the development of thick-walled parenchyma bundle-sheath cells around the vascular bundles in C<sub>4</sub> forage grasses. High lignin content further lowers digestibility, which is already decreased by a high ratio of syringyl to guaiacyl lignin subunits (Giordano et al., 2014b).

Alluvial and basaltic clay soils, as well as sandy soil, are good for dallisgrass growth, and 900 to 1250 mm of annual rainfall is the perfect amount. It can only tolerate a little amount of saline, is particularly tolerant to poor drainage, and, once established, is drought-resistant due to its deep root structure (Evers & Burson, 2004). Originally from latitudes roughly between 28 and 35 degrees north and south, dallisgrass is now naturalized up to 2100 meters above sea level. It is native to regions between sea level to 1800 meters above sea level, roughly between latitudes 28 N and 35 S, and it has naturalized in regions up to 2100 meters above sea level below latitude 35N (Venuto et al., 2003).

The aim of this research was to develop a variety adopted to the region by evaluating the material obtained from domestic and foreign sources of Dallisgrass forage crop.

## **2. MATERIALS AND METODS**

The study was carried out in the Eastern Mediterranean Agricultural Research Institute (EMARI) Adana-Doğankent (Turkiye) experimental fields and included four promising genotypes and one check variety of

dallisgrass species. In the research, populations obtained from different sources and local natural vegetation and a registered variety were used as materials. The variety of dallisgrass was obtained from a seed company in Quesland, Australia. The promising varieties used in the trials were the suitable plants in terms of yield and quality as a result of a two-years micro yield and observation study (between 2015-2016) conducted within the " Perennial Warm Season Grasses Forage Crops Breeding" project carried out within the management of EMARI.

**Table 1.** Promising varieties and variety used in the trial

<b>Registry Situation</b>	<b>Orijin</b>	<b>Variety Name</b>
Promising Variety	EMARI	PV-1
Promising Variety	EMARI	PV-2
Promising Variety	EMARI	PV-3
Promising Variety	EMARI	PV-4
Variety	Australia	Population



**Fig. 1.** Dallisgrass (*Paspalum dilatatum* Poir.) at generative growth stage in the second year.

The trial was established according to the randomised complete blocks design with four replications. Row spacing in the plots was 25 cm and the plot size was 1.5 x 5 m. Sowing was conducted on 17 July 2017. Seeding rate at hand seeding was 11 kg/ha. As fertilizer, 100 kg/ha N and 100 kg P<sub>2</sub>O<sub>5</sub> were used at sowing, and 50 kg/ha N was topdressed after cuttings. Sprinkler irrigation was done after planting to support germination. Post emergence irrigations were done once after each cutting. 1st year total two cuttings and 2nd year total four cuttings were conducted at 10% flowering stages.



**Fig. 2.** The yield test in the breeding program (Dallisgrass) in the first year

Prior to cuttings, plant heights (cm) between the root collar and the tip of 10 randomly selected plants from each plot were measured. To determine the green grass yields, 2 m x 4 rows were cut by leaving an



edge of 0.5 m from the beginning and end of the parcels, and the green grass yield values (t/ha) were determined by weighing with a digital scale without any time delay. In order to calculate the hay values, 0.5 kg of green grass samples were taken randomly from each plot, dried in a drying cabinet at 70 °C for 24 hours, drying percentages was determined, and the hay yields (t/ha) were determined by multiplying this ratio with the green grass yields. Hay samples were taken from each plot and were ground at the end of the season to determine crude protein, ADF and NDF ratios with the “Foss XDS NIRS” (Near Infrared Reflectance Spectroscopy) analyzer using the C-0904FE-Hay and Fresh Forage calibration.

Analysis of variance was applied to the data obtained from field trial and laboratory analysis by using the JMP-7 statistical package program, based on the randomised complete blocks design in accordance with Steel and Torrie, (1960). According to the analysis of variance results, statistically significant treatment averages were compared with the LSD multiple comparison test.

### **3. RESULTS AND DISCUSSION**

#### **3.1. Plant Height (cm)**

The average plant height values of the promising genotypes and the variety in the study and the resulting groups are given in Table 2.

**Table 2.** Average of plant heights (cm) and formed groups

Variation Source	Years		2017- 2018
	2017	2018	
PV-1	74	124	99
PV-2	74	129	101
PV-3	73	126	99
PV-4	76	131	104
Variety	63	127	95
<b>Average</b>	<b>72 b</b>	<b>127 a</b>	<b>100</b>
<b>LSD (%5)</b>	Year: 9.5*, Genotype: n.s., Year x Genotype Int.: ns.		
<b>CV (%)</b>	% 10.3		

\*) Means shown with a similar letter in the same column and the mean without lettering are not statistically different within 5% error limits; n.s.: The difference is not significant.

When Table 2 is examined, the differences between the average plant height values of the promising genotypes and variety were found to be statistically significant over the years.

When plant height values of varieties were examined, it was observed that they differ according to years. While lower values were obtained in the first year, a significant increase was observed in the average values in the second year. Dallisgrass showed slower growth in the first year than in the second year. Muldon (1986) reported that dallisgrass did not develop sufficiently in the planting year but showed a good development in the second year. Plant height in the first two years determined in the dallisgrass was between 29.2-34.6 cm in the study of (Acosta & Deregibus, 2021). These findings were similar to the findings obtained in the study.

### 3.2. Green grass yields (t/ha)

The average total green forage yield values of the promising varieties and variety in the study and the resulting groups are given in Table 3.

**Table 3.** Average total green forage yields (t/ha) and formed groups

Variation Source	Years		2017-2018
	2017	2018	
PV-1	22.0	52.3	37.1 b
PV-2	18.7	53.6	36.1 b
PV-3	22.1	52.2	37.1 b
PV-4	24.3	67.3	<b>45.8 a</b>
Variety	14.0	41.5	27.8 c
<b>Average</b>	<b>20.2 b</b>	<b>53.4 a</b>	<b>36.8</b>
<b>LSD (%5)</b>	Year: 895.3*, Genotype: 717.5*, Year x Genotype Int.: n.s.		
<b>CV (%)</b>	18,8		

\*) Means shown with a similar letter in the same column and the mean without lettering are not statistically different within 5% error limits; n.s.: The difference is not significant.

When Table 3 is examined, the differences between the total green forage yields of the promising varieties were found to be statistically significant compared to the average of years and 2017-2018. When the yield values of the varieties were examined, it was observed that they differ according to the years. The main reason for this may be the year to year plant establishment shifts and less number of cut in the first year. Dallisgrass is a perennial forage crop and its above-ground growth is slower in the first year than its underground growth. In addition, the 1st year growth period is shorter than following years.

According to the two-year average results, the highest average total green grass yield (45.8 t/ha) was obtained from PV-4, while candidates

PV-1, PV-2 and PV-3 lower (36.1-37.1 t/ha) and in the same group. The lowest green forage yield value (27.8 t/ha) was obtained from the control variety. Green herbage yield depends on climate, soil structure, irrigation, fertilization, cutting time, cutting height, number of cuttings and especially variety etc. when genotypes with higher yields are grown in close proximity to ideal conditions, a significant amount of their resources are devoted to biomass production (Couso et al., 2010). It is envisaged that these costs will lead to a lower production even under conditions that are close to ideal for genotypes with an evolutionary history in resource-limited habitats. For example, more resources would be dedicated to support root growth than shoot growth. To put it another way, there might be a trade-off between the capacity to withstand resource scarcity and to expand during times of resource abundance (Bazzaz, 1996). This trade-off model predicts that genotypes with higher growth rates under ideal conditions will also have genotypes with lower growth rates during resource constraint (Fernandez and Reynolds, 2000).

### **3.3. Hay yields (t/ha)**

The total hay yield values of the promising varieties and variety in the study and the resulting groups are given in Table 4.

**Table 4.** Average total hay yields (t/ha) and formed groups

Variation Source	Years		2017-2018
	2017	2018	
PV-1	7.0	15.5	11.3 b
PV-2	5.9	16.4	11.2 b
PV-3	6.2	15.2	10.7 b
PV-4	6.7	19.5	13.1 a
Variety	4.6	13.4	9.0 c
<b>Average</b>	<b>6.1 b</b>	<b>16.0 a</b>	<b>11.0</b>
<b>LSD (%5)</b>	Year: 269.9*, Genotype: 123.5*, Year x Genotype Int.: n.s.		
<b>CV (%)</b>	%16.2		

\*) Means shown with a similar letter in the same column and the mean without lettering are not statistically different within 5% error limits; n.s.: The difference is not significant.

When Table 4 is examined, the differences between the total hay yields of the promising varieties were found to be statistically insignificant for different years.

In the first year of the study, the average total hay yield (6.1 t/ha) was statistically significantly lower than the average of the second year (16.0 t/ha). It is an expected result that hay yield is lower in the first year (Table 3).

According to the two-year average results, the highest total hay yield (13.1 t/ha) was obtained from PV-4, while candidates PV-1, PV-2 and PV-3 lower (10.7-11.3 t/ha) and in the same group. The lowest green forage yield value (9.0 t/ha) was obtained from the control variety.

Paspalum hay yield was reported as 13.0 t/ha by Muldoon (1986) and 13.5 t/ha by Skerman and Riveros (1990). The yield obtained in this study is similar to the reported yields.

### 3.4. ADF ratios (%)

The average ADF ratio values of the promising genotypes and variety are given in Table 5.

**Table 5.** Average ADF ratios (%)

Variation Source	Years		2017-2018
	2017	2018	
PV-1	36.3	36.8	36.5
PV-2	34.3	36.5	35.4
PV-3	34.7	35.9	35.3
PV-4	34.6	35.8	35.2
Variety	38.4	36.0	37.2
<b>Average</b>	<b>35.7</b>	<b>36.2</b>	<b>35.9</b>
<b>LSD (%5)</b>	Year: n.s., Genotype: n.s., Year x Genotype Int.: n.s.		
<b>CV (%)</b>	%4.6		

n.s.: The difference is not significant.

When Table 5 is examined, the differences between the ADF ratio values of the year, genotype and year x genotype interaction were found to be statistically insignificant.

### 3.5. NDF ratios (%)

The average NDF ratio values of the promising varieties and variety in the study with the resulting groups are given in Table 6.

**Table 6.** Average NDF ratios (%) and formed groups

Variation Source	Years		2017-2018
	2017	2018	
PV-1	69.1 bc	70.9 ab	70.0 b
PV-2	67.3 c	71.1 ab	69.2 b
PV-3	68.7 bc	70.8 ab	69.8 b
PV-4	67.1 c	70.7 ab	68.9 b
Variety	73.7 a	71.2 ab	72.4 a
<b>Average</b>	<b>69.2</b>	<b>70.9</b>	<b>70.1</b>
<b>LSD (%5)</b>	Year: n.s., Genotype: 2.3*, Year x Genotype Int.: 3.2*		
<b>CV (%)</b>	%3.1		

\*) Means shown with a similar letter in the same column and the mean without lettering are not statistically different within 5% error limits; n.s.: The difference is not significant.

When Table 6 is examined, the differences between the NDF values of the genotype and year x genotype interaction were found to be statistically significant.

According to the two-year average results, the lowest NDF ratio (68.9%) the highest NDF ratio (72.4%) was obtained from check variety. All candidates were similar and lower compared to control variety.

Since the year x genotype interaction is important with a margin of error of 5%, the NDF ratio values of candidates varieties and the control variety differed over the years. In the first year, candidate PV-4 showed lower values than other candidates varieties and the control variety. In the second year, it was determined that four candidates varieties and the control variety were similar and in the same group according to the average NDF ratio values.

Candidate PV-4 produced grass with a higher leaf/stem ratio than the other examined candidate varieties and the control variety, and therefore lower NDF content in the first year; In the second year, it can be said that there is a decrease in quality due to aging. It has been reported that NDF values are lower in leaves than in other plant parts (Buxton and Hornstein, 1986). Lechtenberg (1985) informed that the quality of grass is affected by some factors such as maturity stage, leaf/stem ratio and physical condition.

### 3.6. CP ratios (%)

The average crude protein ratio values of the promising varieties and variety are given in Table 7.

**Table 7.** Average CP ratios (%)

Variation Source	Years		2017-2018
	2017	2018	
PV-1	14,5	14,5	14,5
PV-2	16,5	14,7	15,6
PV-3	15,0	15,0	15,0
PV-4	16,4	15,0	15,7
Variety	14,0	14,4	14,2
<b>Average</b>	<b>15,3</b>	<b>14,7</b>	<b>15,0</b>
<b>LSD (%5)</b>	Year: n.s, Genotype: n.s, Year x Genotype Int.: n.s.		
<b>CV (%)</b>	%9.6		

n.s.: The difference is not significant.

When Table 7 is examined, the differences between the CP ratio values of the year, genotype and year x genotype interaction were found to be statistically insignificant. Dallisgrass is prized for its vigor, producing crude protein concentrations of up to 18.6% (Barea et al., 2007).



### 3.7. CP yields (t/ha)

The average crude protein yield values of the promising varieties and variety in the study and the resulting groups are given in Table 8.

**Table 8.** Average CP yields (t/ha) and formed groups

Variation Source	Years		2017-2018
	2017	2018	
PV-1	1.0	2.2	1.6 b
PV-2	1.0	2.4	1.7 b
PV-3	1.0	2.3	1.6 b
PV-4	1.1	2.9	2.0 a
Variety	0.7	1.9	1.3 c
<b>Average</b>	<b>1.0 b</b>	<b>2.3 a</b>	<b>1.6</b>
<b>LSD (%5)</b>	Year 29.1*, Genotype: 28.2*, Year x Genotype Int.: n.s.		
<b>CV (%)</b>	%16.7		

\*) Means shown with a similar letter in the same column and the mean without lettering are not statistically different within 5% error limits; n.s.: The difference is not significant.

When Table 8 is examined, the differences between the CP yield values for different year and genotype were found to be statistically significant. CP yield was low in 2017 (1.0 t/ha) than 2018 (2.3 t/ha). In the second year, as the hay yield increased the crude protein yield was also increased as expected.

According to the two-year average results, the highest CP yield (2.0 t/ha) was obtained from aday PV-4. Rest of the candidates were similar and control variety was the lowest for CP yield.

#### **4. CONCLUSIONS**

A good forage plant variety is desired to have high yield and quality values. Yield and quality characteristics of candidate varieties were investigated in perennial warm season forage paspalum. It was determined that candidate varieties (PV-1, PV-2, PV-3 and PV-4) produced higher yield and quality characteristics than control variety. Seed production facilities were established for the yield trials and registration phase of candidate varieties in different environments.

## **LITERATURES**

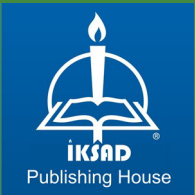
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ISBN: 978-625-8323-39-9