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CONTENTS

PREFACE

Agriculture goes through a paradigm change that profoundly questions the structural dynamics and cultural dynamics of the current unequal and unsustainable food systems, as well as the producers and consumers of food. Following the intensification and industrialization of agricultural and food systems, significant environmental and social issues have arisen, including the loss of biodiversity, pesticide contamination of soils, water, and food, eutrophication of water bodies, the heavy use of antibiotics in the livestock industry, the decline in farm numbers and farmer incomes, and severe issues for the health of people and agroecosystems as well as for rural communities. Also controversial topics in agriculture are numerous. It's crucial to share viewpoints on controversial agricultural issues in formal contexts and to train future scientists to tackle complex situations like these. Agrochemical use, technology transfer imbalance related to agricultural technological innovations like digitalization, information and communication technologies (ICT), and precision farming, animal welfare in agriculture, tools used in animal agriculture such as hormones and antibiotics, social justice, and others are some of the contentious issues in agriculture. However, in view of the rapid advancement of technology, it is crucial to reach a consensus and clarify several fundamental features of agriculture.

Assoc. Prof. Dr. Gülşah BENGİSU

CHAPTER 1

DETERMINATION OF THE BOTANICAL COMPOSITION OF THE OTLUCA VILLAGE PASTURE IN HAKKARI PROVINCE

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INTRODUCTION

Meadow-pasture ecosystems are in the second place after the forest areas covering approximately 24% of the land on earth. They constitute an important part of the roughage needed by animals and the most important biological richness source of the place where they are found(Mut and Ayan, 2011). Most of the pastures are located in arid and semi-arid climate zones. Failure to comply with the management rules together with low precipitation is one of the most important reasons for the deterioration of vegetation in the pasture(Holechek et al., 2004). In fact, pastures are natural resources that can be used for a long time and can renew themselves if the pasture management rules are followed(Bilgen and Özyiğit, 2005).

The quality of the pastures decreases as a result of the decrease of good species plants in the pastures under heavy grazing pressure and the settlement of species that are not grazed by animals. As the excessive and irregular use of pasture continues, low-quality, harmful and weed-like plant species replace the climax plant species in the vegetation over time(Altın et al., 2010; Sürmen et al., 2015). While some of the invasive species, which are described as weeds, cause injuries with their spines, some of them negatively affect the quality and quantity of animal products with the toxic substances they contain, and sometimes cause the death of animals(Balabanlı et al., 2006). The pastures, which are also of great importance in terms of natural balance, have lost their productivity to a large extent due to the grazing in our country in the early or late periods, as well as the heavy grazing in our country(Çomaklı et al., 2012). It is necessary to know the botanical composition in order to plan and succeed in pasture improvement and management studies(Babalık and Kılıç, 2015).

Hakkari province has 369,610 ha meadow-pasture area. The pasture area in Hakkari constitutes 2.46% of the 14.6 million ha area, which is the pasture area of our country, and 52% of the provincial surface area. Sustainability of meadows and pastures is also important economically and socially in the provinces and districts of Hakkari, where plants collected from pastures are used intensively in addition to meeting the need for roughage(Ertuş, 2019). In this study, it was aimed to determine the botanical composition of the pasture of Otluca village in Hakkari province, where animal husbandry is an important source of livelihood.

MATERIAL and METHOD

Material

This study was carried out in the pasture of Otluca Village of Hakkâri Merkez District in 2016. Otluca village is located 8 km to the west of the city center. The village has an average altitude of 2100 m from the sea, and the area of the pasture with a sloping topography is 11410.7 hectares. Grazing is done continuously in the pasture. There are natural water channels and creeks fed from these channels in places in the pasture. Small ruminant livestock is generally practiced in the village. Within the herbal production, forage crops are cultivated in an area of 1100 decares, there are 45 cattle, 8201 sheep and 3089 goats (Anonymous 2017a).

Hakkari province; For many years (1964-2015), monthly average relative humidity, monthly average temperature, total precipitation were recorded as 54.27, 10.30 ^{o}C, 782.7 mm, respectively. In 2016, the monthly average relative humidity was 44.57% below the long-term average, while the monthly average temperature and total precipitation were above the long-term average as 11.34 ^{o}C and 929.4 mm, respectively. Rainfall in January, February, March and June was recorded more than the average for many years(Anonim 2017b).

Figure 1. Partial views from the pasture area (The numbers on the picture show the pasture sections)

Method

The botanical composition of pasture was determined by the Loop method reported by Gençkan (1985). It was carried out in the second week of June, during the flowering period of the vegetation, in four locations where grazing was not done after the early spring grazing near the village settlements(Table 1). Two measurements were made at each location, and the dominant species was recorded every 20 cm from the determined point in each measurement in four directions. The plant coverage rate was obtained by dividing the number of species encountered by the number of measurements. The botanical composition ratio was calculated by subtracting the empty space. Herbarium of the plants collected in the pasture was made and identified according to Davis (1965-1985) and Serin et al. (2008). The decreaser, increaser, and invader species, which express the plant species' palatability and their responses to grazing, have been defined by "Turkey's Meadow and Grassland Plants" (Serin et al. 2008)

Table 1. Coordinates and elevations of the studied pasture sections

Number Section	North	East	Elevation (m)
1	37 37 04	43 40 25	2210
2	37 37 56	43 39 48	2237
3	37 36 29	43 40 52	2100
4	37 37 27	43 41 18	2245

RESULTS and DISCUSSION

Plant covered area

In the pasture of Otluca village, the average area covered with vegetation was determined as 95.69%. Cinar et al. (2014) reported that the area covered by vegetation in five different pastures was between 84.4 and 99.0%. In another study, Çınar et al. (2018) is in agreement with our findings, with studies reporting an average rate of 95.3%. Çaçan and Başbağ (2016) reported that the average area covered by 68.19% vegetation varies between 48.25-86.67% according to years, direction and elevation. Ünal et al., (2012) reported that the area covered with vegetation may differ according to the way the pasture is used, and different results can be obtained in the effect of grazing pressure and vegetation measurement methods (Bilgen and Özyiğit, 2005).

Botanical Composition

A total of 143 species belonging to 26 families were determined in the studied pasture sections. Among these families, Asteraceae family constitutes the most species with 27 species. Following this, the most species belonging to the families Apiacea, Lamiaceae, Fabaceae, Poaceae, Boraginaceae, Caryophyllaceae were found. Only one species was identified from the families Campanulaceae, Caprifoliceae, Colchicaeae, Equisetaceae, Gereniaceae, Hypericaceae, Ixioliriaceaea, Liliaceae, Scrophullariace, Orchidaceae, Orobanchaceae, Rosaceae, Rubiaceae (Table 2).

According to the rate of participation in its botanical composition, Poaceae (18.031%) and Astereceae (16.404%), Apiacea (13.20%), Fabaceae (10.32%), with the least 0.066% represented by only one plant, Hypericaceae, Ixioliriaceaea, Orchidaceae, Urticaceae families have been identified. The area

covered with bushes in the pasture area was determined as 0.523% (Table 2). It is also supported by other studies that the Fabaceae, Asteraceae, Poaceae families are densely found in the pastures of our country (Beyiş and Sabancı, 2011; Çaçan et al. 2014; Babalık and Sarıkaya, 2015; Şahin et al. 2015; Çınar et al., 2018; Çınar et al. ., 2019).

In the study, *Hordeum bulbosum, Poa bulbosa, Dactylis glomerata, Plantago lanceolata, Medicago sativa* species were determined as dominant species in the pasture. However, a total of 29 species such as Brassica elongata, *Centaurea polypodiifolia, Lolium perenne, Plantago major* were very rarely encountered.

According to the life span of the species, 109 perennial, 22 annual and 7 biennial species were found in the pasture area(Figure 2). Perennial species in the botanical composition were determined as 80,852 and annual species were determined as 11,242% (Table 2, Figure 1). Working in Adana pastures, Çınar et al. (2018) determined 70.0% perennial species and 25.8% perennial species. It has been reported that there are 82.64% perennial and 14.31 percent annual species in Hakkari Duck pasture (Ertuş and Pınar, 2019). As determined by these studies, perennial species are more concentrated in the pastures.

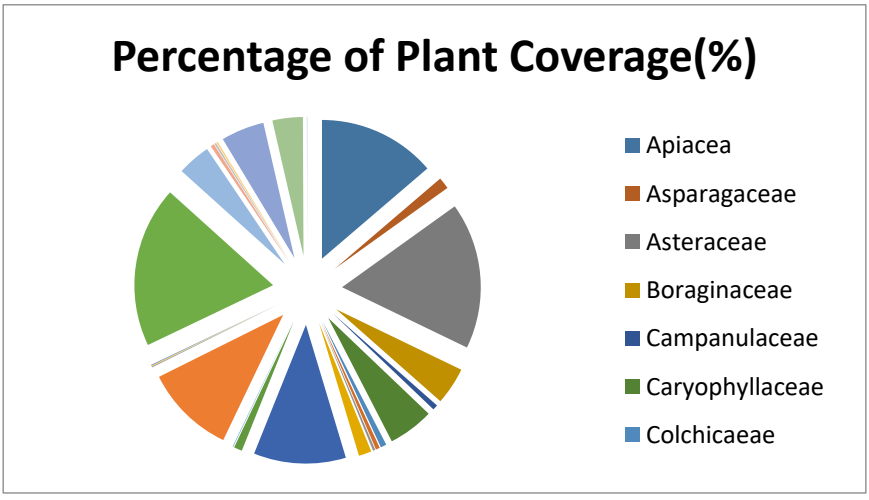

Figure 2. Distribution of Otluca village pasture by families in botanical composition

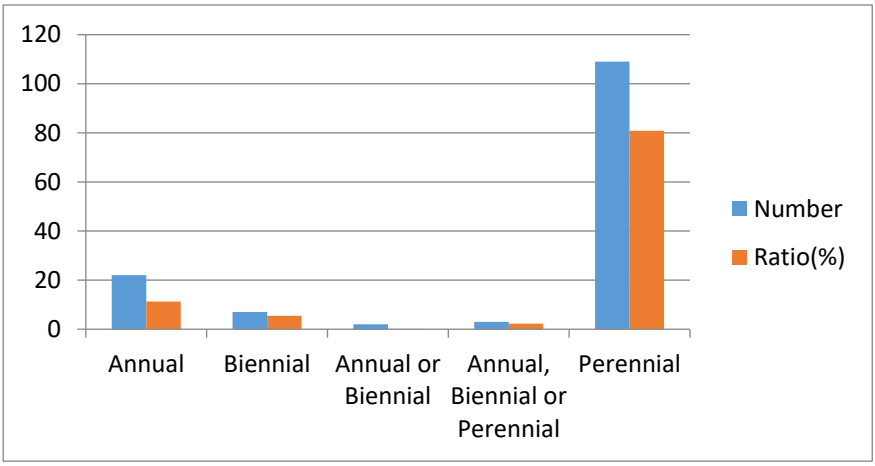

Figure 3. Number and rate of life span of taxa in Otluca village pasture

Table 2. Botanical Composition and Some Features of Otluca Village Pasture

Family	**Genus**	**Decreaser**	**Increaser**	**Invader**	**PPC (%)**	**Form**
	Bush-tree			+	0,523	P
Brassiceaceae	*Aethionema speciosum*			+	0,719	P
Asteraceae	*Achilla arabica*			+	1,633	P
Asteraceae	*Achillea nobilis*			+	0,066	P
Asteraceae	*Achillea vermicularis*			+	1,895	P
Asteraceae	*Acroptilan repens*			+	0,392	P
Poaceae	*Agrotis stolonifera*	+			1,568	P
Amaryllidaceae	*Allium nigrum*			+	0,066	P
Apiaceae	*Anthriscus nemorosa*			+	1,306	A/B/P
Asteraceae	*Artemisia absinthium*			+	0,392	P
Caryophyllaceae	*Arenaria blepharophylla*			+	0,066	P
Caryophyllaceae	*Arenaria gypsophiloides*			+	0,458	P
Boraginaceae	*Asperugo procumbens*			+	0,131	A
Rubiaceae	*Asperula orientalis*			+	0,523	A
Fabaceae	*Astragalus aduncus*			+	0,850	P
Fabaceae	*Astragalus campylosema*			+	0,327	P

Fabaceae	*Astragalus elongatus*			+	0,327	P
Fabaceae	*Astragalus gummifer*			+	0,196	P
Fabaceae	*Astragalus pycnocephalus*			+	1,241	P
Fabaceae	*Astragalus siliquosus*			+	0,653	P
Brassiceaceae	*Barbarea minor*			+	0,131	P
Brassiceaceae	*Brassica elongata*			+	0,066	B/P
Boraginaceae	*Buglossoides arvensis*			+	0,327	A
Apiaceae	*Bunium peucifolium*			+	1,959	B
Poaceae	*Bromus danthoniae*			+	1,372	A
Poaceae	*Bromus hordeaceus*			+	0,066	A
Poaceae	*Bromus sterilis*			+	0,588	A
Poaceae	*Bromus variegatus*	+			0,196	P
Campanulaceae	*Campanula glomerata*			+	0,588	P
Brassiceaceae	*Cardaria droba*			+	0,850	P
Asteraceae	*Centaurea persica*			+	0,523	P
Asteraceae	*Centaurea polypodiifolia*			+	0,066	P
Asteraceae	*Centeurea pseudoscabiosa*			+	1,241	P
Asteraceae	*Centeura spectabilis*			+	0,196	P
Caprifoliaceae	*Cephalaria procera*			+	0,458	P
Caryophyllaceae	*Ceratium dichotomum*			+	0,066	A
Caryophyllaceae	*Cerastium perfoliatum*			+	0,196	A
Apiaceae	*Chaerophyllum crinitum*			+	1,633	P
Apiaceae	*Chaerophyllum macrodon*			+	0,392	P
Asteraceae	*Circium arvence*			+	1,111	P
Asteraceae	*Circium bracteatum*			+	0,196	P
Colchicaeae	*Colchicum szovitsii*			+	0,653	P
Asteraceae	*Crepis foetida*			+	0,523	A
Asteraceae	*Crepis sancta*			+	0,261	A
Rubiaceae	*Crusiata taurica*			+	1,959	P
Poaceae	*Dactylis glomerata*	+			4,246	P
Orchidaceae	*Dactyllorhiza umbrosa*			+	0,066	P

Apiaceae	*Daucus corota*			+	0,261	P
Ranunculaceae	*Delphinium carducharum*			+	0,066	P
Equisetaceae	*Equisetum arvense*			+	0,261	P
Poaceae	*Eragrotis minor*			+	0,588	A
Apiaceae	*Eryngium billardierii*			+	2,940	P
Brassiceaceae	*Erysimun cuspidatum*			+	0,066	A/B/P
Euphorbiaceae	*Euphorbia virgata*			+	1,176	P
Euphorbiaceae	*Euphorbia sanasunitensis*			+	0,261	P
Apiaceae	*Falcaria vulgaris*			+	0,196	A/B/P
Apiaceae	*Ferula orientalis*			+	0,588	P
Apiaceae	*Ferulago stellata*			+	0,850	P
Rubiaceae	*Galium incarnum*			+	0,980	P
Rubiaceae	*Galium luteum*			+	0,066	P
Rubiaceae	*Galium uliginosum*			+	0,588	P
Rubiaceae	*Galium verum*			+	0,588	P
Gereniaceae	*Geranium tuberasum*			+	0,914	P
Apiaceae	*Grammosciadium pterocarpum*			+	0,327	P
Asteraceae	*Gundella tournefartii*			+	1,895	P
Asteraceae	*Helichrysum arenarium*			+	0,066	P
Boraginaceae	*Heliotropeum europeum*			+	0,261	P
Asteraceae	*Helishyrsum plicatum subs. polyphyllum*			+	0,588	P
Caryophyllaceae	*Holosteum umbellatum*			+	0,458	A
Poaceae	*Hordeum bulbosum*	+			4,507	P
Hypericaceae	*Hypericum perforatum*			+	0,066	P
Asteraceae	*İnula orchulus-christi*			+	0,392	P
Poaceae	*Koeleria cristata*	+			0,131	P
Asteraceae	*Lactuca scarioloides*			+	0,066	B
Fabaceae	*Lathyrus cicera*			+	0,327	A
Poaceae	*Lolium perenne*	+			0,066	P
Lxioliriaceaea	*Ixiolirion tataricum*			+	0,066	P

Apiaceae	*Mallabaila lasiocorpa*			+	0,066	P
Fabaceae	*Medicago sativa*	+			2,417	P
Lamiaceae	*Mentha longifolia*			+	0,131	P
Caryophyllaceae	*Minuartia hamata*			+	0,588	A
Boraginaceae	*Myosotis alpestris*			+	0,196	P
Boraginaceae	*Myosotis lithospermifolia*			+	0,196	P
Lamiaceae	*Nepeta italica*			+	1,111	P
Lamiaceae	*Nepeta nuda L.*			+	0,066	P
Asparagaceae	*Ornithogalum narbanense*			+	0,523	P
Asparagaceae	*Ornithogalum oligophyllum*			+	0,784	P
Fabaceae	*Onobrychis major*		+		1,372	P
Boraginaceae	*Onosma alba-roseum*			+	0,523	P
Boraginaceae	*Onosma bulbortrichum*			+	0,784	B
Boraginaceae	*Onosma sericum*			+	1,045	B
Polygonaceae	*Oxyria digyna*			+	0,196	P
Orobanchaceae	*Pedicularis comosa*			+	0,066	P
Asteraceae	*Picris strigosa*			+	0,066	P
Apiaceae	*Pimpirella peregrina*			+	0,131	B
Apiaceae	*Pimpinella tragium*			+	0,066	P
Lamiaceae	*Phlamis kurdica*			+	1,698	P
Lamiaceae	*Phlamis tuberosa*			+	0,261	P
Plantaginaceae	*Plantago lanseolata*		+		3,658	P
Plantaginaceae	*Plantago major*		+		0,066	P
Poaceae	*Poa bulbosa*		+		4,703	P
Polygonaceae	*Polygonum arenastrum*			+	0,066	A/B/P
Apiaceae	*Prangos ferulacea*			+	1,176	P
Apiaceae	*Prangos pabularia*			+	0,523	P
Ranunculaceae	*Ranunculus kotschyi*			+	0,131	P
Boraginaceae	*Rochelia disperma*			+	0,458	A
Rubiaceae	*Rubia tinctorum*			+	0,131	P
Polygonaceae	*Rumex scutpatis*			+	0,131	P
Lamiaceae	*Salvia aethiopsis*			+	0,327	P
Lamiaceae	*Salvia candidissima*			+	0,914	P
Lamiaceae	*Salvia ceratophylla*			+	0,980	B
Lamiaceae	*Salvia sclarea*			+	0,261	P

Lamiaceae	*Salvia staminea*			+	0,327	P
Lamiaceae	*Salvia limbata*			+	0,523	P
Lamiaceae	*Salvia verticillata*			+	1,895	P
Rosaceae	*Sangiusorba minor*	+			0,197	P
Fabaceae	*Securigera orientalis*		+		0,066	P
Fabaceae	*Securigera varia*		+		0,066	P
Apiaceae	*Scandix stellata*			+	0,261	A
Asteraceae	*Scorzonera cana*			+	0,784	P
Asteraceae	*Scorzonera latifolia*			+	1,633	P
Asteraceae	*Scorzonera mollis*			+	0,196	P
Lamiaceae	*Scutellaria orientalis*			+	0,523	P
Asteraceae	*Senicium vernalis*			+	0,523	A
Caryophyllaceae	*Silene arguta*			+	1,959	P
Caryophyllaceae	*Silene italica*			+	0,784	P
Caryophyllaceae	*Silene vulgaris*			+	0,523	P
Boraginaceae	*Solenanthus stamineus*			+	0,261	P
Lamiaceae	*Stachys annua*			+	0,784	A/B/P
Asteraceae	*Tanacetum abrotanifolium*			+	0,066	P
Asteraceae	*Tanacetum chiliophyllum*			+	1,437	P
Asteraceae	*Taraxacum aleppicum*			+	0,131	P
Brassiceaceae	*Thlaspi arvense*			+	1,111	A
Brassiceaceae	*Thlaspi perfoliatum*			+	0,458	A
Asteraceae	*Traxacum montanum*			+	0,066	P
Fabaceae	*Trifolium campestre*			+	0,261	A
Fabaceae	*Trifolium repens*	+			0,392	P
Apiaceae	*Trigonosciddium riscidulum*			+	0,523	B
Liliaceae	*Tulipa armena*			+	0,066	P
Urticaceae	*Urtica dioica*			+	0,066	P
Scrophullariaceae	*Verbascum cherianthifolium*			+	2,090	P
Plantaginaceae	*Veronica orientalis*			+	1,241	P
Fabaceae	*Vicia villosa*			+	1,829	A
Lamiaceae	*Ziziphora capitata*			+	0,327	A
Total					100	

*A: Annual, B: Biennial, P: Perennial, PPC: Percentage of Plant Coverage

Pasture Status

The rate of decreaser, increaser and invader species was determined as 13.72%, 9.84% and 76.44%. A total of 11 decreaser species, namely *Dactylis glomerata, Medicago sativa, Poa bulbosa, Plantago lanceolata, Trifolium repens, Agrotis stolonifera, Bromus variegatus, Koeleria cristata, Lolium perenne, Sangiusorba minor*, were seen in the pasture area. A total of 8 increaser species were identified as *Onobrychis major, Securigera orientalis, Securigera varia, Plantago lanceolata, Plantago major*. The number of invader species was determined as 123, the highest rate was *Eryngium billardierii, Verbascum cherianthifolium, Bunium paucifolium, Achillea vermicularis, Gundella tournefartii, Vicia villosa, Crusiata taurica, Phlamis kurdica, Silene arguta* species(Table 2).

The rate of decreaser and increaser species was determined as 23.56%. Koç and Gökkuş (1994), Buzuk et al. (2009), Beyiş and Sabancı (2011), Aydın et al. (2014), Cinar et al. (2014), Seydoşoğlu et al. (2015), İspirli et al. (2016), Palta and Genç Lermi (2018), Ertuş and Pınar (2019) reported in their studies that invader plant species are more than good plant species in the pastures of our country.

Conclusion

As a result of the study, 143 species of plants belonging to 23 families were found and 80.85% of them were perennial, 5.49% biennial and 11.24% annual species. Considering the botanical composition of Otluca village pasture and the ratio of decreaser/increaser/invader species, the status of the pasture has been tried to be determined.

Although the area covered with vegetation is 95.96%, it has been determined that the pasture status is in the poor pasture class with a rate of 23.56% for decreaser and increaser species. It is also understood from the density of low quality weed species that the pasture of Otluca village, which is in a very good condition in terms of the number of species, but where invader species spread, is exposed to intense grazing pressure. Pasture management principles should be applied in order to improve the pasture condition and prevent further deterioration of vegetation.

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CHAPTER 2

FORAGE CROPS CULTIVATION

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1. Definition and Importance of Forage Crops:

Forage crops are plants that are naturally found or grown in field or meadow-pasture lands, which are used to feed herbivores. Forage crops are plants that do not harm animal health when fed to animals within certain limits. The leaves, stems, stems, tubers, seeds and fruits of forage crops are used as animal feed. Forage crops agriculture and meadow pastures are the cheapest and most abundant sources of feed for animals (Tan and Çomaklı, 2009; Ekiz et al., 2011). All over the world, meadows and pastures are considered to be the areas where high quality roughage is produced abundantly and cheaply. In areas where meadows and pastures are not productive and of high quality, forage crops cultivation gains great importance. Forage crops have many important uses apart from animal feeding.

Why are forage crops important?

- For soil and water conservation (erosion)
- To fix the free nitrogen in the air to the soil with leguminous forage crops.
- To reduce fallow fields
- To increase roughage production
- To reduce diseases and pests in the crop rotation system
- To eliminate soil fatigue
- For aerating the soil.
- In animal feeding.

2. Forage Crops Cultivation

There are many species of forage crops cultivated in the world. There are big differences between these species in terms of cultivation requirements. Forage crops are divided into two parts as cool season and warm season forage crops in terms of their cultivation requirements. Forage crops that make most of their annual growth and development in the cool months of the year are called cool season forage crops. Forage crops that make most of their annual growth and development in the hot months of the year are called warm season forage crops.

Forage crops are divided into three categories as annual, biennial or perennial in terms of their life span. One-year forage crops are plants that complete their vegetative and generative development within one year. Two-year fodder crops only exhibit vegetative growth in the first year; however, by the second year, they have produced an abundance of seeds and are completely mature.

There are great differences between these species in terms of cultivation requirements. The climate and soil requirements of each forage plant species are different. For this reason, there are also differences in soil preparation, planting, care, and harvesting. Forage crops can be grown in a wide area, from very humid regions to very arid regions. In addition, there are forage plants that can be grown in salty, alkaline, and calcareous soils.

2.1. Preparation of Soil and Planting Area:

Since the seeds of forage crops are generally small and their seedlings are weak, the place to be planted should be well prepared. The soil should be plowed with a plow, and the soil should be thoroughly crumbled by pulling the disc harrow (Ekiz et al., 2011) (Figure 1). The soil must be plowed to a sufficient depth. Because in order for the seed to germinate, it must contact the soil and get enough moisture for germination (Yolcu and Tan, 2008).

Figure 1. Well prepared seed bed (Seda Akbay Tohumcu 2016).

The properties of the soil to be planted should be determined well. Soil analysis should be done; Soil structure, condition of nutrients and soil pH should be determined. If there are conditions in the soil that are not suitable for the cultivation of forage crops, the soil should be rehabilitated.

2.2. Sowing

2.2.1. Sowing Time and Shape:

Forage crops sowing time; it varies depending on factors such as species, variety, climate, and the crop rotation system to be applied (Açıkgöz, 2001). In order for forage plant seeds to germinate, the soil must have suitable humidity, temperature and oxygen. Otherwise, insufficient germination occurs. Sowing should be done when these factors in the soil are most suitable (Tan and Çomaklı, 2009). Soil temperature is highly effective on sowing time. While cool climate forage plant seeds can germinate above 0 °C, warm climate forage plant seeds require a minimum temperature of 10-15 °C to germinate. Optimum germination temperatures of forage plant seeds also vary according to the species. The optimum germination temperature in cool climate forage plants varies between 15-25 ºC, while this temperature is 25-35 ºC in warm climate forage plants (Açıkgöz, 2001).

Forage crops should be cultivated with seeders. Because row spacing and sowing depth are adjusted as desired in sowing with seeder. Grain seeder can be used for forage crop seeds of normal size. But special seeders should be used for small seed forage crops.

Row spacing should be adjusted well when planting forage crops. Thus, the rate of benefiting from soil moisture increases, the control against weeds, diseases and pests becomes easier. Row spacing should be 15-20 cm in rainy or irrigated regions and 25-90 cm in arid regions depending on the plant species (Ekiz et al., 2011).

2.2.2. Seed:

It is very important to use quality seed in forage plant cultivation. A quality forage plant seed should have high germination ability. Certified seed should be used. Attention should be paid to the cleanliness of the seed to be used, it should not be contaminated with diseases and pests. High purity seed should be preferred.

2.2.3. Sowing Rate:

For high quality and yield in forage crops, the sowing rate should be adjusted well. The amount of seed to be sown per decare in forage crops varies depending on seed size, seed viability, seedling strength, soil and climate conditions and the purpose of cultivation.

The optimum sowing rate of forage crops is determined by conducting trials in the area to be cultivated. If the same plant is to be grown in different ecologies, different seed amounts should be used. In areas where the surface of the soil is uneven, it is necessary to use more seeds. Because the germination rate of each seed sown in such areas decreases. The amount of seed to be sown in broadcast sowing is higher than the amount of seed to be sown with a seeder. In addition, sowing rate varies according to the purpose of cultivation of the forage plant. The amount of seed to be used in sowing for seed production should be less than the amount of seed to be sown for grass production.

2.2.4. Sowing Depth:

Since the seeds of forage crops are generally small, sowing depth should be considered. Seed size and soil structure should be taken into consideration when determining the sowing depth. If the seed is sown deep, the length of the grass sheath will not be enough to reach the soil surface. Small seedlings will die before reaching the soil surface. Seeds sown on the surface will die by drying out or freezing on the soil surface. In large-seeded plants, the grass sheath is longer, so deeper sowing is possible.

In forage crops, successful results are obtained from sowing at a depth of 2-4 times the seed diameter (Açıkgöz, 2001).

Shoot emergence is difficult in clay soils with high humidity. Therefore, sowing should be done close to the surface. In sandy soils, sowing should be done deeper since shoot emergence is easier and the soil dries more quickly (Tan and Çomaklı, 2009).

2.2.5. Sowing Method

Forage crops can be grown alone or in mixtures. Mixing is the cultivation of different kinds of plants at the same time on the same field. The

cultivation technique applied to increase yield and quality in forage plants is to grow forage plants in suitable mixtures. Mixtures provide more efficient and quality production than pure planting of the species (Kır et al., 2018; Akbay Tohumcu and Temel, 2020; Seydoşoğlu, 2020).

Forage plant mixtures have advantages and disadvantages compared to lean planting. Some advantages and disadvantages of leguminous + wheatgrass mixtures in forage crop agriculture are listed below (Decker et al., 1982; Çomaklı, 1998).

- Advantages of mixtures compared to lean sowing;
  - ✓ The yield of the mixtures is higher than lean sowing.
  - ✓ The nutritional value of the mixture is higher than lean sowing.
  - ✓ Since there are both legume and wheatgrass forage plants in the mixtures, the mixture herbage is rich in protein and carbohydrates.
  - ✓ Since there are different species of plants in the mixtures, the rate of damage caused by unfavorable climatic conditions is reduced.
  - ✓ The green fodder period is prolonged in mixtures of forage crops to be used for grazing.
  - ✓ Wheatgrasses prevent some leguminous forage crops from lying down.
  - ✓ Mixtures are more resistant to weed invasion.
  - ✓ Wheatgrasses in mixtures prevent soil erosion.
  - ✓ Due to the presence of legumes in the mixtures, organic matter and nitrogen content in the soil increases.

- Disadvantages of mixtures compared to lean sowing;
  - ✓ Due to the different species in the mixtures, the size of the seeds is also different. Therefore, special sowing machines are needed for sowing.
  - ✓ In mixtures of forage crops grown for grazing, animals prefer legumes more when grazing, therefore the proportion of legumes decreases.

- ✓ N fertilizers have a positive effect on wheatgrasses, P and K fertilizers have a positive effect on legumes. Joint fertilization is difficult.
- ✓ It is difficult to catch the appropriate cutting time of both plants in forage plant mixtures grown for grass production.

The points to be considered in the selection of plant species to be used in mixtures are as follows.

- ➢ The species to be used in the mixture should adapt to the ecological conditions of the region.
- ➢ There should be a legume in the mixture.
- ➢ The growth and development periods of the species to be used in the mixture should be compatible with each other.
- ➢ Species should be selected according to the way of utilization of the mixture.
- ➢ The palatability of the species to be selected in the mixtures of forage crops to be grown for grazing should be close to each other.
- ➢ The competitiveness of the species should be close to each other.
- ➢ The heights of the plants should be close to each other.
- ➢ The life durations of the selected species should be close to each other.
- ➢ Seed sizes should be close to each other for easy sowing.

3. Maintenance Operations:

3.1. Fertilization:

Fertilizing is necessary for high yield and quality in forage crop cultivation. Fertilization is planned differently for each soil and for each plant. Soil analysis should be done before planting in the area where forage crops will be grown, and the required nutrients should be given as fertilizer (Yolcu and Tan, 2008).

Fertilization differs according to plant species. N fertilization is not needed much in leguminous forage plants. Because of the Rhizobium bacteria found in the roots of legumes, they fix the free nitrogen from the air to the

soil. In this way, they provide most of the N they need (Sarı et al. 2022). Wheatgrass forage crops need more nitrogen fertilization. Nitrogen fertilizers given to wheatgrass fodder plants increase the crude protein ratio (Açıkgöz, 2001). Depending on many factors, the amount of N to be applied may vary. However, on average, 10-20 kg N da^{-1} is recommended for wheatgrasses, and 3-5 kg N da^{-1} is recommended for perennial legumes only in the sowing year.

Phosphorus plays an important role in metabolic activities such as cell division, flowering, and nitrogen fixation (Miller and Reetz, 1995). It was reported that the application of 10-15 kg P_2O_5 da^{-1} increased yield in alfalfa (Kharazmi and Tan, 2020). In vetch species, 12 kg P_2O_5 da^{-1} application is recommended to increase root, stem, and nodule development (Büyükburç and Karadağ, 1999).

Potassium plays an important role in activities such as protein synthesis and stomatal activity in plants. Potassium positively affects root development and growth in plants. Especially in wheatgrasses, it provides stronger development of the stem and reduces lodging (Kacar, 2005).

3.2. Irrigation:

Irrigation provides great increases in yield and quality for forage crops. Irrigation increases yield, especially for plants that give more than one harvest in a single season. In irrigated fields, irrigation should be done in a balanced and controlled manner. In order to increase yield and hay quality, the required amount of water should be given when the plant needs it.

The water requirement of forage crops varies depending on the type and variety of the plant, the purpose of use, sowing frequency, and climatic conditions. The climatic conditions of the region where forage crops are cultivated, especially the amount and distribution of precipitation and soil properties, are important in determining the water requirement. The most appropriate irrigation method should be determined according to the plant species, and irrigation should be carried out in the period required by the plant species according to the development period.

In arid and semi-arid regions, it has been reported that silage maize must be irrigated for rapid growth and high yield (Kır and Ünsal, 2020). In alfalfa,

it was reported that irrigation should be done after each mowing, when the leaves curl slightly and turn dark green (Kır et al., 2019) (Figure 2).

Figure 2. Sprinkler Irrigation in Alfalfa (Anonymous, 2023a)

In the cultivation of legume-wheatgrass forage crop mixtures, it has been reported that the water requirement should be met by sprinkler irrigation in the periods when the plants need it (Akbay Tohumcu and Temel, 2020). When forage crops are in the seedling period, traditional flood irrigation is very harmful, and sprinkler irrigation should be used. However, sprinkler irrigation should be avoided when the plants are in the flowering period of forage plants to be grown for seed purposes (Yolcu and Tan, 2008). As can be understood from the examples, irrigation method and time in forage crops differ according to the type of plant to be grown, the purpose of cultivation, and the growth period of the plant.

3.3. Control of Diseases and Pests:

It is necessary to control diseases and pests for high yield and quality in forage crops agriculture. When diseases and pests are not controlled, economic losses occur. The most important issue in the fight against diseases and pests is to use resistant varieties. Legume forage crops are less resistant to diseases and pests than wheatgrass forage crops.

Some diseases seen in forage crops are powdery mildew (*Erysiphe graminis*) (Figure 3), rust (*Puccinia* sp.), brown leaf spot (*Helminthosporium bromi*), bitter spot (*Leptpshaerulina trifoli*), anthracnose (*Colletotrichum trifolii*) (Figure 4), root rots (*Phytium* spp. and *Fusarium* spp.) (Tan and Çomaklı, 2009). In the control of these diseases, it is necessary to use resistant varieties, to apply crop rotation, to remove diseased plant residues from the field, and to use systemic fungicides.

Figure 3. *Erysiphe graminis* (Anonymous, 2023b)

Figure 4. *Colletotrichum trifolii* (Anonymous, 2023c)

In forage crop cultivation, there are insects that damage the leaves and flowers of the plants, destroy the roots, and damage the seeds, as well as

beneficial insects that provide pollination. Some of the insects causing damage to forage crops are; alfalfa trunk beetle (*Hypera postica* Gyllenhal) (Figure 5), caterpillar (*Colias eurytheme*), grasshopper (*Melanoplus sanguinipes*), and seed beetles (*Bruchus* spp.).

Figure 5. *Hypera postica* Gyllenhal (Anonymous, 2023d)

Insecticides should be considered a last resort in the control of insects. It is necessary to use resistant varieties and try biological control methods. If these are insufficient for control, insecticides should be sprayed according to the type of pest.

3.4. Controlling Weeds:

In forage crop cultivation, every plant of different species in the field is considered as weed. For example, wheat in a sainfoin field is considered as weed, although it is a cultivated plant. Weeds consume the water and nutrients of the plant to be grown. Since weeds are generally not cultivated plants, their competition is high compared to cultivated plants. For this reason, if they are not combated in time, they lead to a decrease in forage crops in the field. They reduce the yield and quality of forage crops.

There are many methods of controlling weeds. Firstly, the seed to be used in sowing should be certified seed. The seed should be clean and not contain weed seeds. The field to be sown should be cleaned from weeds and their seeds. If weeds are found despite all the precautions taken, it is necessary

to start the struggle as soon as possible. Mechanical control can be done by hand plucking or hoeing machines. Sowing can be used to control annual weeds in the field where perennial forage crops are planted. Sowing is an effective method, especially for annual and tall weeds. In cases where these methods are not effective, herbicides are used. It is necessary to apply herbicide considering the characteristics of the cultivated plant.

4. Harvesting:

Harvesting in forage crops varies according to the intended use of forage crops. Factors such as harvest time, harvest frequency, harvest height, species differences, temperature, leaf-stalk ratio, fertilization, time of day affect the amount of hay obtained and the quality of the hay obtained (Baytekin and Gül, 2009).

In order to obtain high quality and yield from forage crops, harvesting time should be adjusted well. The harvesting time of forage crops produced for hay production differs according to species. If high quality hay production is targeted, the harvest time should be well adjusted. When wheatgrass crops are grown for hay, they are usually harvested during the spike or flowering period. High quality and high yield hay is obtained from wheatgrass sown in this period. As the harvest time is delayed, yield increases but quality decreases. Because the harvest time is delayed, the cellulose content of the plant increases. In legume forage crops, the harvesting time for hay is usually the flowering period. When legumes are harvested in this period, high quality hay is obtained.

In legume-wheatgrass mixtures, it has been reported that it is appropriate to harvest the mixture during the 10% flowering period of legumes (Temel and Akbay Tohumcu, 2019).

Storage of the harvested hay is as important as the time of harvesting. Forage crops harvested in arid regions with low rainfall are dried by leaving them on the field. In regions with high rainfall or humid regions, the harvested hay should not be left on the field. The dried hay should be stored by using different drying methods.

Harvest time is very important for seed production in forage crops. If the harvest time is not well adjusted, large product losses occur. Seeds of some

legume and wheatgrass forage crops fall after ripening. For this reason, the plants approaching harvest time should be controlled and harvested without seed shedding.

Forage crops in which all seeds ripen at the same time are easy to harvest. Harvesting can be done with the help of a combine harvester. However, harvesting with a combine harvester cannot be done in plants whose seeds do not mature simultaneously. In this case, the plants are mown and left to dry, or chemical dryers are used.

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CHAPTER 3

METHODS OF PROPAGATION IN MEDICINAL AND AROMATIC PLANTS

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INTRODUCTION

In the historical process, the propagation/production of plants has been one of the most basic occupations of human beings. In the early days, the human being fed with the plants around him, later tried to grow it, in other words, to produce the same plants he/she needed after they were depleted. Later, the increase and diversification of needs also was to cause to the development of breeding techniques by multiplying special plant genera and species (Iqbal and Sing, 2020; Sourdille and Devaux, 2021).

Medicinal and aromatic plants have been attracting the attention of mankind for centuries. Today, due to the important biological activities arising from the large number of chemical compounds found in these plants, there is an increasing interest in medicinal and aromatic plants in various industrial fields, agriculture and health sciences. (Inoue and Craker, 2013, Máthe, 2015)

However, today, many of the medicinal and aromatic plants are collected wild from nature. For this reason, the future of the species with high demand is in danger. It is necessary to cultivate medicinal and aromatic plant species that are intensively collected from nature and have a wide market. Higher yield and quality production is realized at certain standards from plants grown in culture conditions. Priority should be given to the culture of species whose propagation materials are readily available from plants collected wildly from nature. Today, the knowledge and experience of the producers about the propagation methods of many medicinal and aromatic plants is limited (Leaman, 2006; Baydar, 2009; Bahadırlı, 2020).

Propagation of plants is done with various techniques that vary according to the genus of the plant and the specific purposes of the producer.

METHODS OF PROPAGATION IN MEDICINAL AND AROMATIC PLANTS

Two main propagation/production methods are used in medicinal and aromatic plants: "generative or sexual", "vegetative or asexual" (Schippmann et al., 2006; Baydar, 2009). In addition, “micropropagation” is also carried out using plant tissue and cell culture techniques (Sidhu, 2020).

Generative Propagation

In generative propagation, the propagation material is the seed. Seeds form the zygote as a result of the union of male and female sex cells in the flowers of plants. Reproduction by seed is based on the germ cells.

Propagation by seed is both an economical and practical method. However, it also has some handicaps. First of all, cross pollinated plants exhibit genetic variation when propagated by seed. Some plants produce very little seeds, while others produce no seeds at all. On the other hand, in some seeds, the germination rate is very low due to dormancy. It is found in seeds that have never germinated. Another negative aspect of propagation by seed is the prolongation of the emergence-maturation process of the plants (Utami et al.,2007; El-Shahabyet al. 2019).

Many medicinal and aromatic plants can be propagated by seed, which is the generative propagation material. In particular, the propagation of annual and biennial herbaceous medicinal and aromatic plants is carried out by seeds. There are two different applications in propagation by seeds (Shaw et a.,2020) (Table 1):

1-Sowing seeds directly into the field, which is the production area

2- Obtaining seedlings before seeds and planting these seedlings in the production area

Table 1. Some medicinal and aromatic plants propagated by seed (Baydar, 2009).

Direct sowing	**Propagation with seedlings obtained from seeds**
Species	**Species**
Anise (*Pimpinella anisum* L.)	Atropa (*Atropa belladonna* L.)
Black Seeds (*Nigella sativa* L.)	Basil (*Ocimum basilicum* L.)
Calendula (*Calandula officinalis* L.)	Black Henbane (*Hyocyamus niger* L.)
Cannabis (*Cannabis sativa* L.)	Camomile (*Matricaria chamomilla*)
Common Sage (*Salvia officinalis* L.)	Capers (*Capparis spinosa* L. *Capparis ovata* L.)

Coriander (*Coriandrum sativum* L.)	Gypsophila (*Gypsophila* L.)
Cumin (*Cuminum cyminum* L.)	Lavender (*Lavandula* spp.)
Fenugreek (*Trigonella foenum graecum* L.)	Lemon balm (*Melissa officinalis* L.)
Datura (*Datura stramonium* L.)	Mountain tea (*Sideritis* spp.)
Echinacea (*Echinacea angustifolia* (L.) Moench)	Oregano (*Origanum onites* L. *O. vulgare* L. ssp. *hirtum* (Link.) Lets.)
Fennel (*Foeniculum vulgare* Mill.)	Peppermint (*Mentha* spp.)
Nettle (U*rtica* spp.)	Rosemary (*Rosmarinus officinalis* L.)
Opium poppy (*Papaver somniferum* L.)	St. John's wort (*Hypericum perforatum* L.)
	Sweet marjoram (*Origanum majorana* L.)
	Valerian (*Valeriana officinalis* L.)

Large seeds without germination problems can be planted directly in the field. In medicinal and aromatic plants, the sowing norm, sowing time, sowing method, seed quantity, sowing frequency and sowing depth vary greatly according to the plant type (Namdeo, 2018). Some information on the production of various medicinal and aromatic plants grown and propagated by seed and seedlings are summarized in Table 2 (Özgüven and Kırıcı, 1999; Gesch, 2013; Baydar, 2019; Boztaş and Bayram, 2021; sari, 2019; Ayran and Kan, 2022).

Table 2. Cultivation information of some medicinal and aromatic plants propagated by seed

Plant Name	Sowing/Planting time	Sowing depth	Sowing Norm	Row spacing X in-row distance in sowing/planting
Anise	March - April	2-3 cm	1-2 kg/da	20-40 cm
Atropa	April - May			60x45 cm
Black Henbane	Autumn/Spring			Seedlings obtained from seeds are planted at 20-45x10-20 cm.
Rosemary	Autumn/Spring			Planting 40-45x40-45 cm rooted shoot cuttings
Fenugreek	Autumn/Spring	2-3 cm	3-4 kg/da	Row spacing:20-60 cm
Black cumin	Autumn/Spring	2-3 cm	1-2 kg/da	Row spacing:15-20 cm
Datura	Spring		1-1.5 kg/da	40-50x20-25 cm
Echinacea	Spring			Planting seedlings from seed 40-50x20-25 cm
Basil	Autumn/Spring		0.5-1 kg/da	20-60x10-30 cm Sowing seeds or planting seedlings from seed
Poppy	Autumn/Spring	1-2 cm	0.5 kg/da	40x15 cm
Capers	Spring			2x2 m, 3x3 m, 4x4 m Planting seedlings
Oregano	Autumn/Spring			40-50 x15-20 cm Planting seedlings from seed
Cannabis	Spring	2-3 cm	2.5-6.5 kg/da	Row spacing: 15-25 cm (for fiber production)

				Row spacing:30-50 cm (for seed production)
Cumin	March	2-3 cm	1.0 kg/da	
Coriander	Autumn/Spring	1.5-2.5 cm	1.5-2.5 kg/da	20-40x10-15 cm
Lavender	Autumn/Spring			Planting rooted seedlings 3x1.5 m (Lavandin) 140x35 cm (Lavender)
Peppermint	Autumn/Spring			Shoot, stolon and shoot cuttings 40x20 cm
Lemon balm	April - May			Sowing seeds Planting seedlings from seed (preferred) 40x20 cm, 50x50 cm
Chamomile	Spring			Sowing seeds Planting seedlings from seed 30x15 cm
Fennel	Autumn/Spring	2-3 cm	1.5 kg/da	SA:60 cm
Hops	March		250-500 seedlings/da	Rhizome planting 1-2x1-2 m
Jasmine			350-450 seedlings/da	Planting rooted cuttings 2x1.5 m
Calendula	Autumn/Spring	2-3 cm	1.1 kg/da	SA: 20-40 cm SÜ:10 cm

Sowing time, planting frequency and planting depth vary depending on the plant species. Seeds germinate if there is a certain temperature and suitable humidity. It is of great importance in terms of yield and product quality that the

necessary cultural practices (weed control, disease and pest control, fertilization, irrigation, etc.).

The seeds of plants with small seeds and poor germination, which are therefore problematic or impossible to sow directly in the field, are planted in specially prepared viols or nurseries, necessary maintenance procedures are carried out, and seedlings that reach a certain size are planted in the field.

Care should be taken to ensure that the medium used in the nurseries contains a high percentage of organic matter. However, care should be taken to ensure that the seedling soil is light-textured, has a relatively low clay content, and is free from weed seeds, diseases and pests.

Sowing seeds in the nursery: The first factor to be considered when sowing seeds in the nursery is the setting of the sowing time. If the seed to be sown has a germination barrier, this barrier is removed by various methods. Sowing should be done a few months before the time of planting the seedlings in the field. After the seeds are placed in the seedling soil homogeneously, a few inches of burnt barn manure or peat is sprinkled on it and the soil surface is pressed with a suitable tool (cylinder). Work to be done during seedling development after planting (http://eagri.org/eagri50/AGRO101/lec11.pdf. Access date: 06.06.2023):

1-Regular irrigation,

2-Weed control,

3- Struggle with diseases and pests,

4- Seedlings that do not develop well should be removed.

In this way, the seedlings that reach a certain size in the nurseries are transferred to the boxes or tubes, from which they are planted manually or by machine in the field at the appropriate time. In order to obtain seedlings from seeds, viols can be planted instead of seedlings. Mixtures such as peat, perlite, peat+perlite etc. can be used in viols. Seedlings can be obtained by applying the necessary maintenance procedures in greenhouse conditions (https://apps.worldagroforestry.org/NurseryManuals/SeedHandling.pdf. Access date: 05.06.2023; Baydar,2009).

Applications that stimulate germination: In order for seeds to germinate, they must normally have reached morphological and physiological maturity. In addition, the embryo of the seed must still survive. Apart from

these, dormancy (resting) event in some seeds also affects germination negatively. If a seed has morphological and physiological maturity and does not germinate despite being left in favorable conditions, this indicates dormancy in the seed (Benech-Arnold and Sánchez,2004).

Germination can occur if non-germinated seeds are left for a period of time at lower temperatures below the germination optimum temperature and in a humid environment. The cold and humid environment may stimulate germination for some seeds. In cases where dormancy originates from the tissues surrounding the embryo, the shells surrounding the seed prevent the passage of water and oxygen. However, in some seeds, the tissues surrounding the embryo allow the passage of water and oxygen, and germination does not occur even though there is no resting in the embryo itself. Here, some chemical substances contained in the tissues surrounding the embryo have inhibitory effects. Some applications can be made to seeds in order to eliminate dormancy and break barriers. These applications, which have a stimulating effect on germination, are very diverse and can be applied one or more together depending on the source of the problem (Ateş and Üremiş,2021; Gokturk et al.,2021). These applications are:

1-Abrasion of seed hard shells
  a-Mechanical abrasion and shell breaking
  b-Acid etching
2-Soaking the seeds in water
  a-Soaking in cold water
  b-Soaking in warm water
3- Keeping the seeds in a cold and humid environment
4-Treating with chemicals
  a- With growth regulators
  b- With other chemicals

In those with hard and thick seed coats of medicinal and aromatic plants, the seed coat is physically peeled or scratched.

Chemically, it is treated with chemicals such as hot water (30-80^{o}C), sulfuric acid (H_2SO_4, 96% purity), hydrochloric acid, potassium hydroxide, etc. for a certain period of time (1-15 minutes). High levels of growth inhibitory hormones such as internal abscisic acid (ABA) in the seed may cause

dormancy. Such seeds can be treated with growth stimulating hormones such as gibberellic acid (GA_3) (Msanga and Maghembe, 1986; Baydar, 2009; Kitiş and Aktaş, 2018). Seeds that need vernalization to germinate can be stratification by keeping them at a low temperature for a certain duration of time, from a few days to a few months. For this process, the seeds can be kept in the refrigerator at +4 ^{o}C (Zhang et al, 2023). If light is needed for germination, such as chamomile seeds, a shallow sowing should be done so that the seeds can see the light. Each seed has minimum, optimum and maximum temperature values at which it will germinate (Tribouillois et al.,2016). Soil temperature at planting depth should be above the minimum germination temperature.

In Türkiye, there are 48 registered varieties belonging to 18 taxa of medicinal and aromatic plants. A list of these varieties is given in Table 3 (https://www.tarimorman.gov.tr/BUGEM/TTSM, Access date: 07.06.2023)

Table 3. Registered varieties in Turkey

Species name	**Variety name**	**Species name**	**Variety name**
Capers	Diyar 2017	Fenugreek	Güraslan
Anise	Yeni 37		Berkem
	Ege 53		Çiftçi
	Altın 8	Poppy	TMO1
	Karabey		TMO2
Cannabis	Narlı		TMO3
	Vezir		Hüseyin bey
Peppermint	Özgüven		Çelikoğlu
Sage (*Salvia fruticose* L.)	Uysal		Seyitgazi
	Turgut		OfisNP
	Karık	Black cumin	Çameli
Sage (*Salvia officinalis* L.)	Beyhekim	Saffron	Karaarslan
Oregano (*Origanum onites* L.)	Oğuz	Basil	Large Sweet
Oregano (*Origanum vulgare* L.subsp. *hirtum*)	Uluğ Bey		Midnght
	Armar 77		Compact
	Tınmaz		Moonlight
	Başer		Dino

Coriander	Pel-Mus		Morfes
	Gamze		Limoni
	Erbaa	Oregano (*Thymus vulgaris* L.)	Winter
	Kudret-K		Timo
	Arslan	Montain tea (*Sideritis perfoliate* Mill.)	Gürbaşak
	Gürbüz	Lemon balm	Melis
	Sancar Bey	Echinacea (*Echinacea purpurea* L.)	Tutar

Vegetative Propagation

Plant cuttings for reproduction should be prepared approximately 7.5-15 cm long (Chauhan et al., 2021). Different applications can be made to accelerate rooting in prepared cuttings (Baydar, 2009).

Treating shoot cuttings with hormones

Increasing the temperature in the rooting medium

Increasing the carbon dioxide level in the environment

Increasing the C/N ratio in shoots

Preferring semi-woody and soft shoots

Removing buds on shoots

IBA and NAA are used to accelerate rooting. It is subjected to hormone application at different concentrations according to the type, variety, morphological structure and physiological characteristics of the shoot cutting to be rooted. A suitable dose is usually selected between 50-9000 ppm and a quick dip is made. Fast immersion duration ranges from 3-5 seconds (Khajehpour et al., 2014). Perlite, peat and vermiculite are commonly used as rooting media. Rooted cuttings are planted in the appropriate planting norm according to the type, variety, cultivation technique, climate and soil characteristics, cultivation purpose. After planting, necessary cultural practices, especially irrigation, are carried out.

Micropropagation

Micropropagation is the process of vegetative reproduction from plant tissues or seeds (Sidhu, 2010). Secondary metabolite sources from some medicinal and aromatic plants with economic and industrial value are produced by tissue and cell culture techniques under controlled conditions as an alternative way to traditional agricultural activities.

In this method, the part called explant (a single cell, embryo, meristem, callus, anther, etc.) taken from various parts of the plant is sterilized and cultured in a sterile and closed nutrient medium at appropriate light and temperature (Özkaynak and Samancı, 2005; Sidhu,2010). The most important thing to be considered in tissue cultures is sterilization. The success of tissue culture largely depends on it.

A starter plant (mother plant) is needed for plant tissue culture. Explant is isolated from this plant. If there is a plant developed by tissue culture methods in the laboratory, we use it as the mother plant. If we do not have such a plant, we have to choose plants from the outside environment. The mother plant should be noted that it is young, that its organs and tissues are not dormant and that it is healthy.

In micropropagation, propagation is made by using shoot tip, bud and node cultures. In the micropropagation method:

1-Sterilization of explant/plant material

2-Planting the explant into the starting medium

3-Sprout reproduction

4-Rooting

5-*In Vitro* plants have adaptation to external environment = acclimatization stages (Kocaçalışkan, 2017).

Explants are cleaned with distilled water and sterilized using chemicals such as sodium hypochlorite, hydrogen peroxide, silver nitrate, mercury chloride (Rout et al.,2000). *In vitro*, MS (Murashige and Skoog) medium is the most used nutrient medium with some modifications and gives the most successful results (Murashige and Skoog, 1962).

Plants obtained in micropropagation are similar in properties to rooted cuttings. While being transferred to the external environment, their gradual adaptation should be done carefully. Outdoor conditions are different from the *in vitro* environment in terms of light, humidity, nutrients and sterility. The

most suitable plant transfer time is the period when the roots begin to develop and the leaves can photosynthesize. The acclimatization process begins by reducing the humidity on the culture vessels by cooling from the top while the plants are in vitro. It is of great importance that the plants growing *in vitro* are kept in an environment with high humidity such as fogging within a few days after being transferred to the outside environment.

There are many studies on micropropagation of medicinal and aromatic plants. A few of these studies are presented in Table 4.

Table 4. Micropropagation studies with tissue culture in some medicinal and aromatic plants

Plant Species	**Explants type**	**Reference**
Satureja thymbra L.	Shoot tip	Sarropoulou and Maloupa, (2019)
Artemisia vulgaris L.	Seeds	Sujatha and Ranjito Kumari, (2007)
Calandula officinalis L.	Hypocotyl, cotyledon, cotyledonary node	Çöçü et al.,(2004)
Hypericum perforatum L.	Apical segments	Gadzouska et al.,(2005)
Lysimachia nummularia L.	Nodal parts	Doğan, (2018)
Prunella vulgaris L.	Shoot tip	Türker et al., (2010)
Limnophila aromatica (Lamk.) Merr.	Leaf	Doğan, (2019)
Lavandula stoechas L.	Cotyledon node, meristematic tip,1st, 2nd and 3rd axillary meristem	Mokhtarzaseh and Khawar, (2022)

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CHAPTER 4

THE DETERMINATION OF SOME AGRICULTURAL FEATURES IN M_4 GENERATIONS OF CHICKPEA GENOTYPES INDUCED TO DIFFERENT MUTATION DOSES

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1. INTRODUCTION

Chickpea, which is an edible legume plant, is in the Cicer genus of the legume family, and its gene center is stated to be Southeastern Anatolia. In scientific studies on chickpea, it is stated that chickpea emerges from two gene centers. It is stated that the first one is South West-Asia and the Mediterranean Region (gene center of large seed chickpeas) and the second gene center is the region that includes South Asia and Abyssinia (gene center of small seeds) (Auckland and Measen, 1980). In our country, chickpeas are grown in many places where dry farming is practiced, especially in the Central Anatolia Region. Chickpea is resistant to heat and drought and can produce without irrigation in these regions.

Chickpea has been the second most important edible legume plant grown worldwide. It has an important role in meeting the protein needs of people in undeveloped countries in the world, especially where the income imbalance is experienced since people need protein for a balanced and adequate nutrition. Accordingly, protein and vitamin-rich foods should have priority in the human diet.

There is an average of 18-35% protein, 38.1-73.3% carbohydrates, 1.5-6.8% fat, and 1.6-9.0% cellulose in chickpea seeds (Eser, 1981). Chickpea has the highest value in terms of fat content (4%) among legumes. Although it is rich in amino acids such as leucine, lysine, and isoleucine, it is poor in some amino acids (tryptophan, methionine, and cystine) (Şehirali, 1988). In addition, its richness in important minerals (Ca, Fe, and P) and some vitamins (A, B, and Niacin) makes it an important place in people's diets (Smithson et al., 1985).

Chickpeas are not required nitrogenous fertilizer as much as other macronutrient elements. Yet, chickpea has been one of the food substances required nitrogen that particularly bacteria in its stem fixate on nitrogen because nitrogen is regarded as one of the most significant factors for determining plant yield. Chickpea fixates on atmospheric nitrogen of air and uses Rhizobium bacteria in its stem. The importance of symbiotic nitrogen fixation increases because of the gradual increase of protein demand around the world and environmental problems come up during mineral nitrogenous manure production and usage as well.

Chickpea has been in second place after beans in the world with 15.04 million ha plantation area and 15.87 million tons production, while it has been

the first with 481.667 ha planting area and 475.000 tons production in Turkey. While the global yield average was 1057.8 kg ha^{-1} for chickpea, this value in Turkey reached 1.160 kg ha-1 as a result of successful breeding studies, especially conducted in recent years (Anonymous, 2021). In Turkey, chickpea is cultivated in the Central and Eastern Mediterranean Regions in winter, whereas in Central and Eastern Anatolia Regions and Transitional Zone Regions in early spring. Since the plants are exposed to drought and temperature stress after a certain period in the spring planting, serious losses are experienced in the yield (Duzdemir and Akdag, 2007). However, in recent years, winter sowing has become more widespread due to the development of cold tolerant and, especially, high tolerance varieties to anthracnose since winter sowing plants have higher and more stable productivity characteristics (Yucel et al., 2006).

Increasing vegetative production in our country depends on increasing the seed yield to be obtained from the unit area, as well as the ability to grow quality products. Nuclear and advanced techniques, which cannot be solved by conventional methods, can be foreseen due to the lack of sufficient standard varieties among field crops. Although many new varieties with high seed yields have been introduced to agricultural production with traditional breeding methods, the variations created by classical breeding methods often require a long time, more labor, and more material (Dursun, 1993). In order to increase the yield and quality in plant production, breeders benefit from the variations found in nature or the new techniques and methods they have developed to reveal the varieties that are resistant to drought, cold, diseases, and pests and to complete the deficiencies of the obtained varieties (Şehirali, 1988). One of these techniques is mutation breeding. The mutation breeding method has been widely used in recent years to save time for the breeder, to make a planned study, and to obtain new varieties in a short time. In conditions where genetic variation is narrowed and genetic problems cannot be solved in developing new varieties with traditional plant breeding methods, the variation of a variety with good adaptability can be increased by mutation breeding for one or two traits. Gamma-ray is one of the physical mutagens used in mutation breeding and is widely used to establish genetic diversity, especially in cereals and legumes. In addition to the fact that gamma rays can cause beneficial or harmful effects on

the plant, it is of great importance in determining the dose level to be applied to the plant (Jamil and Khan, 2002).

In the studies carried out, it is seen that positive changes can be achieved in plants in terms of yield, durability, quality, earliness, and adaptability by using the mutation-causing factors at appropriate doses and times. In addition, it has been demonstrated that mutations can bring new features to plants by making sudden hereditary changes in the chromosome structure and number of plants or in the physical and chemical structures of genes (Şehirali, 1988). Cobalt-60 (60 Co) gamma rays are one of the most widely used light sources to cause mutations in plants. Gamma-ray, which is one of the physical mutagens used in mutation breeding, is widely used to create and increase genetic diversity, especially in field crops.

The aim of this study is to examine and determine the differences in morphological and agronomic characteristics in M_4 generation by planting M_3 plants obtained by applying different doses of gamma rays to four chickpea cultivars. The research includes the M_4 generation of the mutation made in chickpeas. It is hoped that a new chickpea line or a generator will emerge as a result of the investigations to be carried out in M_5 and future generations by continuing the study.

2. MATERIAL and METHOD

2.1. Material

The study subject of the project consists of revealing some of the agricultural characteristics of the M_4 generation in some chickpea varieties of gamma rays applied at different doses and the selection of single plants that differ to be transferred to the M_5 generation. First of all, gamma irradiation was applied to 4 chickpea cultivars at 3 different gamma-ray doses (100-200-300), excluding the control dose (0 Gy), in Ankara Turkey Atomic Energy Agency. The sowing of the obtained seeds was carried out in Kırşehir ecological conditions, starting from the M_1 generation. The cultivation of the mutant chickpea genotypes included in the project was carried out for 1 year in the experimental land in the Bağbaşı campus of Kırşehir Ahi Evran University. The measurements of the agricultural characteristics of each single plant were made in the Field Crops laboratory of the Faculty of Agriculture.

2.1.1. Varieties Used in the Trial and Their Characteristics

4 chickpea varieties (Aksu, Azkan, Sarı 98, and Uzunlu 99) registered by different research institutes constitute the material of this research. The research institutes where different chickpea cultivars are registered are given in Table 1.

Table 1. Research institutes where chickpea varieties are registered

Varieties	Research Institutes
Azkan	Transitional Zone Agricultural Research Institute - Eskişehir
Aksu	East Mediterranean Transitional Zone Agricultural Research of Institute - Kahramanmaraş
Uzunlu 99	Field Crops Central Research Institute - Ankara
Sarı 98	Aegean Agricultural Research Institute - İzmir

Some morpho-agronomic characteristics of the cultivars included in the study are given in detail below.

Aksu: It is especially recommended for winter planting areas and for transitional areas. The plant development form is semi-erect, has strong branching and the flower color is white, seed color is beige. It is a mid-early variety, vegetation period is 109 days, plant height is 50-60 cm, first pod height is 28-34 cm, seed yield per decare is 230-300 kg, and 100 seed weight is 43-48 g.

Azkan: It grows upright, moderately branched, early, drought and cold-tolerant edible chickpea variety. It is light beige in color and the flower color is white. The number of pods per plant varies between 24-30, plant height 41-46 cm, first pod height 12-20 cm, and 100-seed weight 35-45 g. The average seed yield per decare is 175 kg and the amount of seed to be planted is 8-10 kg per decare. The vegetation period is 100-105 days. It is resistant to anthracnose disease. The protein ratio is between 23-25% and the cooking condition is very good.

Sarı 98: It has an upright development shape and its seeds are beige. The flower color is white. Plant height is 30-35 cm and 100 seed weight is 46-50 g. It is a variety suitable for early sowing and has a good mid-late yield. It is moderately tolerant of anthracnose.

Uzunlu 99: It is generally recommended for similar ecologies with Central Anatolia and Transition Regions. The seeds are cream in color. Plant height is 45-50 cm, 100 seed weight is 50-51 g, and vegetation period is 100-110 days. It is resistant to drought and lodging. It is a variety that does not have seed spillage problems and is suitable for machine harvesting. Seed yield per decare is 150-175 kg.

Figure 1. The trial field where the research was carried out

2.1.2. Some General Characteristics of the Research Place

2.1.2.1. Location of the Research Place

The field trial of the research carried out was established in the trial fields of Kırşehir Ahi Evran University in the chickpea vegetation period of 2021. The trial land is 5 km away from the center of Kırşehir and its altitude is 1050 m, its latitude is 39º 9' north, and its longitude is 34º 10' east (Figure 1).

2.1.2.2. Soil Properties

Field trials of this study were carried out for 1 year in the chickpea vegetation period of 2021, and mutant chickpea studies were carried out in the plots of Kırşehir Ahi Evran University. As a result of the analyzes made on the soil samples in the application area of the study, which was carried out for one year, it was determined that the pH of the soil of the trial land was 7.81, the organic matter content was very low, with 0.37, it was in a clay-loam structure, calcareous and unsalted structure, sufficient in potassium and insufficient in phosphorus.

2.1.2.3. Climate Characteristics

In Kırşehir Province, which is located in the middle of the Central Anatolia Region, summers are generally hot, springs are rainy and winters are cold. Average annual precipitation is 379 mm. When the climate data for the year 2021, in which the study was carried out, and the growing season of the long-term average are examined, there is a similarity between the values of 2021 and the values of many years in terms of monthly average temperature values. It is seen that 2021 is more than many years in terms of total precipitation. In addition, in terms of relative humidity value, it has been revealed that it is lower than the values of long years on a monthly basis (Table 2).

Table 2. Climate data of Kırşehir Province for 2021 and long years*

Months	Average Temperature (^{0}C)		Total Precipitation (mm)		Average Relative Humidity (%)	
	2021	Long Years	2021	Long Years	2021	Long Years
March	4.5	5.9	95.2	37.9	65.5	66.7
April	12.0	10.8	19.4	42.7	56.5	62.7
May	18.2	15.7	9.2	46.2	45.3	60.6
June	19.3	20.0	35.1	37.5	55.1	54.9
July	24.9	23.7	0.9	8.9	40.4	46.9
Total			159,80	92,60		

*Kırşehir Provincial Meteorology Directorate

2.2. Method

Approximately 3000 seeds (0, 100, 200 and 300 Gy) of 4 chickpea cultivars subjected to different doses of Gamma irradiation in 2018 were prepared and sent to the Turkish Atomic Energy Authority at the beginning of March and were subjected to physical mutation. The seeds prepared for each determined dose group were irradiated using the Cobalt 60 (Co 60) source in the Turkish Atomic Energy Agency. The irradiated seeds were stored in the refrigerator at 4°C until sowing. Starting from the M_1 generation to the M_4 generation, they were sown by hand in rows opened with a 30 cm row spacing and 4 meters long marker under field conditions. In each vegetation year, single plants were selected from each plot and transferred to the next generation, and brought up to the M_4 generation. Seeds of chickpea varieties of single plants harvested by a single selection method in M_3 generation were sown in the

experimental field of Kırşehir Ahi Evran University, in randomized blocks, according to the split plots trial design with 3 replications, and the M_4 generation was formed. Chickpea varieties were placed on the main plots and gamma rays were placed on the subplots. Sowing was done by hand in rows opened with a 30 cm row spacing and 4 meters long marker. The parcels of the standard cultivars were formed from 4 rows and the parcels of the cultivars with different doses were formed from 8 rows. During the M_4 generation, agronomic characteristics (plant height, first pod height, number of pods per plant, number of seeds per plant, seed yield per plant, and hundred-seed weight) were determined in 10 plants taken from each parcel of chickpea cultivars.

Statistical Analysis

The variance analysis of the agronomic data obtained was calculated using the JUMP.07 statistical package program according to the "split plots in random blocks" experimental design and the averages were grouped with the "LSD Test".

3. FINDINGS and DISCUSSION

The research carried out to determine some agricultural characteristics of four chickpea cultivars of M_4 generation with different gamma rays applied, covers 6 yield items (plant height, first pod height, number of pods per plant, number of seeds per plant, hundred-seed weight, and seed yield per plant).

Plant Height (cm)

In the study in which the effect of gamma-ray doses applied to different chickpea varieties on plant height was determined, it was revealed that the effect of variety x gamma-ray doses on plant height was very important at the 1% level with the interaction of variety x gamma-ray doses (Table 3).

Tablo 3. LSD test results of the difference between plant height (cm) and averages in M_4 generation of chickpea varieties applied with different gamma rays

Varieties	Gamma Ray Doses									
	Control		100 Gy		200 Gy		300 Gy		Mean	
Azkan	32.25	f	34.75	d	37.00	bc	36.75	bcd	35.19	bc
Aksu	36.00	cd	37.00	bc	33.50	e	36.25	c	35.69	b
Sarı 98	35.50	cde	**32.00**	**fg**	34.75	d	38.50	ab	**35.18**	**bc**
Uzunlu 99	34.75	d	**39.25**	**a**	37.50	b	36.75	bcd	**37.06**	**a**
Mean	**34.63**	**bc**	35.75	b	35.69	b	**37.07**	**a**		

*****The difference between means denoted by the same letter is insignificant (LSD, $p \leq 0.05$)

In the study examining the effects of different gamma rays (0, 100, 200, 300) applied to four different chickpea cultivars on plant height in M4 generation, it is seen in Table 3 that there are significant differences in gamma-ray doses compared to the control dose. In addition to the statistical significance of the effect of gamma-ray doses used in the study on plant height, it was determined that 100 Gy and 200 Gy gamma-ray doses were included in the same grouping. When the control dose and other application doses were compared, the mean plant height was 34.63 cm at the control dose, 35.75 cm at 100 Gy, 35.69 cm at 200 Gy, and 37.07 cm at 300 Gy. This shows that as the applied dose rate increases, the plant height increases.

When the averages of chickpea varieties were examined, it was determined that the variety with the longest plant height was Uzunlu 99, and the variety with the shortest plant height was Sarı 98. Although the LSD groupings of Azkan and Sarı 98 cultivars were the same, the plant height of the Azkan cultivar was 35.19.85 cm, and the plant height of Sarı 98 chickpea cultivar was 35.18 cm.

It was observed that the variation in terms of the interaction of Variety x Gamma Ray doses, whose effect on plant height was very significant at the level of 1%, occurred between 32.00 and 39.25 cm. The longest plant height was determined in the 100 Gy dose application of Uzunlu 99 chickpea cultivar, while the shortest plant height was determined in the 100 Gy dose application of the Sarı 98 chickpea cultivar. The fact that the cultivar x gamma-ray dose interaction is very important in terms of plant height reveals that the plant heights of chickpea cultivars are affected differently by gamma-ray dose applications. Efe and Ünal (2017) applied four different gamma rays (0, 60, 80, and 100 Gy) to the seeds of three Hungarian vetches (Anatolian Pink-2002,

Oğuz-2002, and Tarm Beyazı-98) cultivars, and the morphological and In their study carried out to investigate agricultural characteristics, it was reported that the natural plant height increased as the dose rate increased in Tarm Beyazı-98 variety. In another study conducted by Abdel-Hak and Mansour (1980) for the purpose of examining the plants obtained from the M_2 generation, 3, 5 and 7 krat gamma-ray doses applied to the bean seeds were found to be shorter than the other gamma-ray doses, and At the same time, it was determined that the plant height shortened as the amount of applied dose increased.

First Pod height (cm)

The LSD groups formed with the mean values of the first pod height in chickpea cultivars of different gamma-ray doses (0, 100, 200, and 300 Gy) are given in Table 4. When the table is examined, it is seen that the interaction of Variety x Gamma Ray doses and the effect of different gamma-ray doses on the first pod height is very significant at the 1% level, while the effect of chickpea cultivars on the first pod height is significant at the 5% level.

Table 4. LSD test results of the difference between first pod height (cm) and averages in M_4 generation of chickpea varieties applied with different gamma rays

Varieties	**Gamma Ray Doses**									
	Control		**Control**		**Control**		**Control**		**Mean**	
Azkan	14.00	g	15.00	f	19.00	b	17.50	cde	16.38	bc
Aksu	14.00	g	18.00	c	18.50	bc	17.25	d	16.94	b
Sarı 98	**12.50**	**h**	16.00	e	14.00	g	17.75	cd	**15.07**	**c**
Uzunlu 99	17.25	d	**20.25**	**a**	14.50	fg	16.75	de	**17.19**	**a**
Mean	**14.44**	**c**	17.31	a	16.50	b	**17.32**	**a**		

*****The difference between means denoted by the same letter is insignificant (LSD, $p \leq 0.05$)

When the first pod height was evaluated in terms of different gamma-ray doses, it was determined that the first pod height values varied between 14.44-17.32 cm. It has been determined that there is an increase in the first pod height value with the increase in the dose amount (300 Gy), especially in the plant height. In terms of different gamma-ray doses, the highest first pod height was obtained from the 300 Gy dose, while the first pod height value obtained from the 100 Gy dose application was found to be in the same statistical group (a) with the 300 Gy dose. However, as with plant height, the lowest first pod height value was revealed in the control dose application.

In terms of chickpea varieties, it was observed that the first pod height values varied between 15.07-17.19 cm. The longest first pod height was seen in 99 cultivars at 17.19 cm, just like plant height, while the lowest first pod height was observed in Sarı 98 cultivar at 15.07 cm. Demircioğlu and Yağmur (2020) determined that the first pod height value changed between 17.80-24.54 cm in their study to determine the morpho-agronomic characters of 4 chickpea cultivars in the M_2 generation, which were treated with different doses of gamma rays.

It has been observed that the variation range in terms of the interaction of Variety x Gamma Ray doses, which has a significant effect on the first pod height at the 1% level, is between 12.50-20.25 cm. The longest first pod height was observed in the 100 Gy dose application of the Uzunlu 99 variety, while the shortest first pod height value was observed in the control dose application of the Sarı 98 variety. The fact that the cultivar x gamma-ray dose interaction is very important reveals that the first pod heights of chickpea cultivars are affected differently by gamma-ray dose applications. According to the observations of 25, 50, 75, and 100 Gy gamma-ray doses applied to Eresen-87 and Filiz-99 pod varieties and to the FLIP86-116FB line, the first pod height in the M_2 generation decreased by 25 Gy in the Eresen-87 cultivar, and decreased at 100 Gy to the control. According to the results, a significant increase in cultivars in both Eresen-87 and Filiz-99 was reported by Artık and Pekşen (2005).

Number of Pods per Plant (unit)

The average values of the number of pods per plant in chickpea cultivars of different gamma-ray doses (0, 100, 200, and 300 Gy) and the resulting LSD groups are given in Table 5. When the table is examined, the interaction of Variety x Gamma-ray doses and the effect of different gamma-ray doses on the number of pods in the plant were found to be very significant at the 1% level, while the effect of chickpea cultivars on the number of pods per plant was found to be significant at the 5% level. In the study, it was revealed that the number of pods in the plant has values between 19.63-24.00 in terms of different gamma-ray doses. Especially in the 200 Gy dose application, the pod number value in the highest plant (24.00 pieces) seems to be a remarkable situation.

In terms of chickpea varieties, it was determined that the number of pods per plant varied between 20.50-22.69. While the number of pods in the highest plant was determined at 22.69 in the Azkan chickpea variety, the number of pods in the lowest plant was determined in the middle with a value of 20.50 in Uzunlu 99 chickpea variety. As a result of the experiment carried out to determine the yield values of 16 chickpea varieties in Bolu ecological conditions, it was determined that the number of pods per plant varied between 11.13 and 23.53 (Tetik, 2019).

Table 5. LSD test results of the difference between number of pods per plant (unit) and averages in M_4 generation of chickpea varieties applied with different gamma rays

Varieties	Gamma Ray Doses									
	Control		Control		Control		Control		Mean	
Azkan	23.50	bc	20.75	def	**27.25**	**a**	**19.25**	**ef**	**22.69**	**a**
Aksu	23.25	bcd	21.00	de	24.00	b	22.25	cd	22.63	ab
Sarı 98	23.25	bcd	17.50	f	22.25	cd	23.00	bcde	21.50	b
Uzunlu 99	19.75	e	**19.25**	**ef**	22.50	c	21.50	d	**20.50**	**c**
Mean	22.44	b	**19.63**	**c**	**24.00**	**a**	21.50	bc		

*****The difference between means denoted by the same letter is insignificant (LSD, $p \leq 0.05$)

It has been observed that the number of pods in the plant varies between 19.25 and 27.25 in terms of the interaction of cultivar x Gamma Ray doses. While the highest number of pods was seen in the Azkan chickpea at a dose of 200 Gy, the lowest number of pods was 19.25 at a dose of 100 Gy of the Uzunlu 99 variety and 300 Gy of the Azkan variety. Şenay and Şekerci (2009) reported in a study they conducted that they aimed to determine the effects that may occur in the following generations as a result of applying different gamma rays and EMS doses together or separately to the Kunduru 1149 durum wheat variety. According to the observations made after applying 50, 150, and 250 Gy gamma rays and 0.2% and 0.4% EMS together and separately to the seeds, significant reductions were observed in the investigated properties with the increasing dose rate when applied separately, but more effective results were observed in those applied together.

Number of Seeds per Plant (unit)

The average values for the number of seeds per plant in chickpea cultivars of different gamma-ray doses (0, 100, 200, and 300 Gy) and the resulting LSD groups are given in Table 6.

Table 6. LSD test results of the difference between number of seeds per plant (unit) and averages in M_4 generation of chickpea varieties applied with different gamma rays

Varieties	Gamma Ray Doses									
	Control		**100 Gy**		**200 Gy**		**300 Gy**		**Mean**	
Azkan	21.25	c	20.25	de	**24.75**	**a**	18.25	fg	**21.13**	**a**
Aksu	20.75	cde	18.25	fg	22.25	b	21.00	cd	20.57	ab
Sarı 98	20.75	cde	**16.00**	**h**	21.25	c	19.25	ef	19.32	b
Uzunlu 99	18.00	g	18.75	f	20.50	d	19.75	e	**19.25**	**b**
Mean	20.19	b	**18.32**	**c**	**22.19**	**a**	19.57	bc		

*****The difference between means denoted by the same letter is insignificant (LSD, $p \leq 0.05$)

When the table is examined, it is seen that the interaction of Variety x Gamma-ray doses and the effect of different gamma-ray doses on the number of pods in the plant are very significant at the 1% level, while the effect of chickpea varieties on the number of pods per plant is significant at the 5% level. In the study, it was revealed that the number of seeds per plant in terms of different gamma-ray doses had values between 18.32 and 22.19. As with the number of pods in the plant, it seems to be a remarkable situation that the highest number of seeds in the plant (22.19 pieces) was reached, especially in the application of a 200 Gy dose.

In terms of chickpea varieties, it was determined that the number of seeds per plant varied between 19.25 and 21.13. While the number of seeds in the highest plant was determined as 21.13 in the Azkan chickpea variety, the number of seeds in the lowest plant was determined as 19.25 in Uzunlu 99 chickpea variety. Karadavut and Sözen (2020a) determined that the number of seeds in the plant of the cultivars varies between 35.8-46.1 in their study using Azkan and Çağatay cultivars in Kırşehir.

It has been observed that the number of pods in the plant varies between 16.00-24.75 in terms of the interaction of Cultivar x Gamma Ray doses. While the number of seeds in the highest plant was determined at a 200 Gy dose of

the Azkan chickpea variety, the number of seeds in the lowest plant was determined with 16.00 at a dose of 100 Gy of the Sarı 98 variety. Upon the administration of 10 different gamma rays from the Co60 source to two chickpea cultivars Binasola-2 and CPM-384, it was determined that chickpea cultivars showed significantly different effects at different doses according to the information obtained in the M_1 generation, and it was stated that an increase in seed yield was achieved at 100 Gy and 300 Gy doses (Karimi et al., 2008).

Hundred-Seed Weight (g)

The average values of hundred-seed weight and the resulting LSD groups in chickpea cultivars of different gamma-ray dose (0, 100, 200 and 300 Gy) applications are given in Table 7. When the table is examined, it has been determined that the effect of the variety x gamma-ray doses interaction and the effect of chickpea cultivars on hundred-seed weight is very important at the 1% level, while the effect of different gamma-ray doses on the hundred-seed weight is significant at the 5% level. In the study, it was determined that the hundred-seed weight had values between 37.54-41.72 g in terms of different gamma-ray doses. It seems remarkable that the highest hundred-seed weight value was reached in the 300 Gy dose application. Karakoca and Akgün (2020) tried to determine the mutagenic effects that may occur on some agricultural characteristics in the M_2 generation of different gamma rays (200 Gy, 300 Gy, 400 Gy, and 500 Gy) applied to the Tarm-92 barley variety in a study they conducted in Isparta conditions. They reported that they examined the seed weight per spike and according to the data they obtained, the seed weight varied between 2.73 g in control and 1.25-2.46 g in M2.

Table 7. LSD test results of the difference between hundred-seed weight (g) and averages in M_4 generation of chickpea varieties applied with different gamma rays

Varieties	**Gamma Ray Doses**									
	Control		**100 Gy**		**200 Gy**		**300 Gy**		**Mean**	
Azkan	40.02	ef	42.26	de	40.09	e	42.56	d	41.24	ab
Aksu	37.35	fge	33.01	h	**32.66**	**hı**	36.99	g	**35.01**	**c**
Sarı 98	38.03	fg	38.21	f	40.01	ef	**47.99**	**a**	38.56	b
Uzunlu 99	44.77	c	46.66	b	39.21	efg	39.31	efg	**42.49**	**a**
Mean	40.05	ab	**37.54**	**bc**	37.99	b	**41.72**	**a**		

*****The difference between means denoted by the same letter is insignificant (LSD, $p \leq 0.05$)

In terms of chickpea varieties, it was determined that the hundred-seed weight values varied between 35.01-42.49 g. While the highest hundred kernel weight was determined in the Uzunlu 99 chickpea variety with 42.49 g, the lowest hundred kernel weight was determined at 35.01 g in the Aksu chickpea variety. In order to evaluate the yield and yield components of some chickpea lines, 15 chickpea genotypes were included in the study, which was carried out for 2 years in Kahramanmaraş's ecological conditions. According to the combined results of two years of the study, it was reported that the hundred-seed weight of chickpea genotypes varied between 39.96-50.70 g (Güneş et al., 2022).

It was observed that the hundred-seed weight values varied between 32.66-47.99 g in terms of the interaction of cultivar x Gamma Ray doses. While the highest hundred-seed weight was observed in the 300 Gy dose application of the Sarı 98 chickpea variety, the lowest hundred-seed weight was seen at 32.66 g in the 200 Gy dose of the Aksu chickpea variety. In the study, in which EMS and SA mutagens were applied in order to examine the effect of hundred-seed weight on color characteristics in two chickpea cultivars (BDN 9-3 and PG-5), it was reported that with the chemical mutagens applied, hundred-seed weight increased in chickpea as well as colored seeds. At the same time, it was stated by Kashid and More (2015) that as the number of generations increases, the weight of the hundred-seed increases.

Seed Yield per Plant (g)

The average values of seed yield per plant in chickpea cultivars of different gamma-ray doses (0, 100, 200, and 300 Gy) and the resulting LSD groups are given in Table 8. When the table is examined, it is seen that the effect of the interaction of Variety x Gamma-ray doses on the seed yield of the plant is very important at the 1% level, while the effect of different gamma-ray doses on the seed yield of the chickpea cultivars is significant at the 5% level.

In the study, it has been revealed that the seed yield of the plant has values between 7.62-8.65 g in terms of different gamma-ray doses. In terms of chickpea varieties, it was determined that the seed yield values per plant varied between 7.10-9.23 g. While the highest seed yield was seen in the Azkan chickpea variety with 9.23 g, the lowest seed yield was seen in the Aksu chickpea variety with 7.10 g. Karadavut and Sözen (2020b) determined the seed

yield per plant as 4.2-9.1 g in the study they carried out with chickpea varieties in Kırşehir ecological conditions.

Table 8. LSD test results of the difference between seed yield per plant (g) and averages in M_4 generation of chickpea varieties applied with different gamma rays

Varieties	Gamma Ray Doses									
	Control		100 Gy		200 Gy		300 Gy		Mean	
Azkan	8.48	cd	8.91	bc	**11.01**	**a**	8.51	cd	**9.23**	**a**
Aksu	6.84	f	**6.21**	**g**	7.24	ef	8.11	d	**7.10**	**c**
Sarı 98	7.73	def	6.56	fg	8.47	cd	9.37	b	8.04	bc
Uzunlu 99	8.41	cde	8.77	c	7.84	de	7.57	e	8.15	b
Mean	7.87	b	**7.62**	**bc**	**8.65**	**a**	8.40	ab		

*****The difference between means denoted by the same letter is insignificant (LSD, $p \leq 0.05$)

It was observed that the seed yield values of the plant varied between 6.21-11.01 g in terms of the interaction of cultivar x Gamma Ray doses. While the highest seed yield was seen in the Azkan chickpea at a dose of 200 Gy, the lowest seed yield was 6.21 g at a dose of 100 Gy in the Aksu variety. Demircioğlu and Yağmur (2020) stated that the seed yield per plant was determined as 3.63-9.54 g in the study carried out to determine the morpho-agronomic characters of 4 chickpea cultivars in the M_2 generation, in which different doses of gamma rays were applied.

CONCLUSION

In the study carried out in order to determine some agronomic characteristics of 4 chickpea cultivars (Azkan, Aksu, Sarı 98, and Uzunlu 99) in the M_4 generation, different gamma rays (100-200-300 Gy) were applied in the Agricultural Research and Application Land of Kırşehir Ahi Evran University in 2021. Important results have been revealed in terms of mutated chickpea varieties at high doses. As a result of the study, it was determined that the effects of different gamma-ray doses on plant height, first pod height, pod, and seed number per plant were very significant ($p \leq 0.01$), While the effects on hundred seed weight and seed yield per plant were significant ($p \leq 0.05$). While the effects of chickpea varieties on plant height and hundred-seed weight were very significant ($p \leq 0.01$) in M_4 generation, the effects on first pod height,

number of pods and seeds per plant, and seed yield per plant were found to be significant ($p \leq 0.05$). With the conducted study, it was revealed that the effects of Variety x Gamma Ray dose interaction on plant height, first pod height, number of pods and seeds per plant, hundred-seed weight, and seed yield per plant were very important ($p \leq 0.01$). With this study carried out in Kırşehir ecological conditions, mutation breeding work will be continued by transferring single plants selected from the M_4 generation to the M_5 generation.

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CHAPTER 5

FUNCTIONS OF MAGNESIUM IN PLANTS

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Introduction

Magnesium (Mg) is known to be essential in plant nutrition. It has been reported that the Mg content of the leaves should be at least 0.25% in order for magnesium to be considered adequate in many plants. It is reported that the Mg content of the plants varies between 0.15 % and 1.00 %, although the rate of occurrence in the plant varies considerably depending on the plant variety (Chen et al., 2018; Hauer-Jákli and Tränkner, 2019).

Although the most common effect of magnesium in plants is on photosynthesis; it has been reported in many sources that it has effects on protein synthesis, energy-lipid and carbohydrate metabolism, microtubule assembly, plant diseases. These functions of magnesium in plants are explained below in detail.

Photosynthesis

In plants, magnesium participates in the structure of chlorophyll in the light capturing complex of chloroplasts. Therefore, it is indirectly involved in photosynthetic CO_2 assimilation (Cakmak and Kirkby, 2008; Cakmak and Yazici, 2010; Gerendás and Führs, 2013; Jamali Jaghdani et al., 2021; Kan et al., 2022) and it is essential for photosynthesis (Guo et al., 2015; Ishfaq et al., 2022).

Magnesium must be incorporated into the chlorophyll molecule before chlorophyll is activated to collect light for photosynthetic carbon reduction reactions. Only in this way, a suitable structure is formed for the absorption of the quantum of light energy required to drive photosynthetic reactions (Mittler 2002).

Although a high value such as 2.7% of the chlorophyll molecular weight belongs to Mg; whether chlorophyll deficiency is associated with Mg deficiency has not yet been fully elucidated. According to some researchers, this condition is associated with inhibition of protein synthesis. Similarly, although magnesium is the primary absorbent for radiant solar energy, the mechanism by which magnesium converts energy into photosynthetic reactions cannot be adequately explained (Mengel and Kirkby, 1978; Hannaway et al., 1980; Hortensteiner, 2009).

Protein synthesis

Most of the Mg in leaf cells (about 75%) is directly or indirectly related to protein synthesis. This is related to the duties that magnesium undertakes in ribosomal structure and function (Bould, 1984; Marschner, 1986).

Functional RNA protein particles need magnesium in order to perform the sequential reactions needed to synthesize protein from amino acids and other metabolite components. The group of ribosomal sub-particles is generally controlled by Mg. These sub-particles are unstable when Mg2+ concentration is less than 10mM and they remain dissociated into smaller inactive particles as far as this critical Mg concentration cannot be maintained.

However, Mg is also required for amino acid activation, polypeptide chain initiation and polypeptide chain elongation reactions. Mg is also required for RNA polymerase activity, which is effective in RNA formation in the nucleus (Lehninger, 1975; Bould, 1984; Marschner, 1986; Shaul 2002; Kacar and Katkat, 2007).

Lipid Metabolism

In addition to playing a role in the activation of many enzymes involved in lipid metabolism such as acetic thiokinase, it also plays a role in the biosynthesis of phospholipids and, therefore, in the formation of functional cell membranes (Mayland, 1983; Moore, 1984).

Carbohydrate Metabolism

Most of the phosphorylating enzymes involved in carbohydrate metabolism need Mg for keeping their activities in a maximum level. This is related to Mg's ability to form complexes with phosphate groups. Some enzyme activities associated with the glycolytic cycle also require Mg (Gander, 1976; Wilkinson et al., 1990).

Microtubule Assembly

Magnesium is required for the formation of microtubules in plant cells. Before tubulin polymerization and microtubule assemble, Mg^{2+} is needed to bind to tubulin (Marschner, 1986, Wilkinson et al., 1990; O'Brien et al., 1990; Wiesler et al., 2002).

Energy metabolism

In plants under magnesium deficiency, the structure of mitochondria in cells is disrupted. This occurs because many respiratory enzymes (i.e., phosphatases, ATPases, and carboxylases), in the mitochondria are in need of Mg for their optimal activity.

Magnesium is preferentially bound to phosphoryl groups and it forms a Mg-ATP complex. This complex is generally used by the active sites of ATPases for transferring energy-rich phosphoryl groups. It is also required for the synthesis of ATP acting as a bridging constituent between the enzyme and ADP. Thus, Mg has a central role in ATP and energy metabolism (Marschner, 1986; Asada 2006; Cakmak and Yazici, 2010).

Plant diseases

Mechanisms underlying the Mg deficiency induced plant diseases has not been fully understood yet. Magnesium is known to have both direct and indirect effects on plant diseases. Abovementioned functions of Mg in plants such as serving as a cofactor for many enzymes involving in energy transfers, respiration and formation of DNA&RNA are influenced by plant diseases. Magnesium deficiency -especially during on plant growth- reduces the structural the structural integrity of the middle lamella and the production of energy which is necessary for defense functions and inactivation of pathogen metabolites.

Mg deficiency in plants can occur when the soil has acidic properties. In this case, the plants are more susceptible to diseases such as Fusarium wilts, club root of cabbage, and bacterial soft rots, which can be commonly seen under low soil pH conditions (Huber and Graham, 1999). Balance in mineral nutrients is also quite critical and blossom end rot (BER) of tomato is a clear evident for it (Anonymous, 1999).

According to Horsfall and Cowling (1980), responses to infection requires energy from photosynthesis which depends on magnesium level (Marschner, 2011).

More than 20 diseases were reported to be decreased by supplying additional Mg. There are also some diseases in which severity may increase by Mg supplementation or it may vary on the environmental conditions. For more

details please see (Huber and Haneklaus, 2007; Jones and Huber, 2007; Huber abd Jones, 2012)

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CHAPTER 6

THE IMPORTANCE OF WHEAT YELLOW RUST DISEASE IN GLOBAL FOOD SECURITY

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INTRODUCTION

Wheat, which is one of the the cool climate cereals, has an important place in the nutrition of billions of people with its rich mineral and energy content. There are two types: bread (*Triticum aestivum* ssp. *aestivum*) and durum (*T. tur*gidum ssp. *durum*).

It is estimated that 787 million tons of wheat are consumed in the world. According to the 2021 FAO data, the amount of wheat production in the world increased by 2.2 million tons and reached 2.796 million tons (FAO, 2021). In addition, it is estimated that the global demand for wheat will increase by 324 kg/year by 2050 (Alexandratos and Bruinsma 2012). Wheat production faces many threats and 10-16% of global wheat production is lost due to diseases and pests (Oerke 2006; Strange and Scott 2005). Yellow rust disease, also known as stripe rust, caused by the biotrophic fungal pathogen *Puccinia striiformis* Westend f.sp.*tritici* (Pst) is the most important diseases of wheat. It is estimated that 88% of the world's wheat production is at risk due to this disease, causing 5 million tons of product loss per year and the market value of this loss is 1 billion dollars (Wellings 2011, Beddow ve ark. 2015, Schwessinger 2017).

Urediospores of the disease agent areround or oval with a spiny wall and a diameter of 28-34 µm. Teliospores, on the other hand, are elongated, the cell wall is thick and flat, bicellular, and the intercellular spaces are slightly knotted. The most typical sign of the disease is the narrow, yellow streaks of urediospores on the leaf blades and sheaths, resembling machine stitches (Figure 1).

Figure-1 a) urediniospores (Bouvet et. al 2022) b) pustules on the wheat leaf surface (Bouvet et. al 2022) c) *Puccinia striiformis* f.sp. *tritici* infection in wheat d) *Puccinia striiformis* f.sp. *tritci* urediospores of wheat spike e) Teliospores on the wheat leaf surface

The average temperature demand of the disease is 9-13ºC. Billions of urediospores formed on the plant surface in the spring are spread to the environment by the wind. It causes the formation of new urediospores by infecting the plants it is carried under suitable conditions. At the end of the season, teliospores as in the same pustules from the uredospore beds. Black lines are formed on the leaves in telial development (Figure-1).

Yellow rust infection is most commonly seen on wheat leaves. The decrease in photosynthesis area in the leaves damaged by the disease causes weak grains to form and decreases the yield. In addition, when yellow rust infection is intense, it can infect other important organs of wheat (lemma, glumes and ear) (Bouvet et al. 2021). In the last sixty years, significant yield and quality losses have occurred, especially in areas where yellow rust has not been adequately combated (Wellings 2011). However, in the past 20 years, globally more aggressive and genetically diverse Pst populations have emerged that have adapted to warmer conditions (Hubbard et al.2015, Hovmøller et al. 2016). Thus, many wheat varieties known to be resistant and widely cultivated became susceptible. This situation has become a threat to wheat production all over the world and therefore to the food security of countries whose nutrition is based on wheat. In light of these determinations, the triggers of the rapid change in yellow rust populations and why it threatens food safety were examined in this study.

YELLOW RUST DISEASE COMPLEX LIFE CYCLE

At the beginning of the factors in the destruction of yellow rust disease; the factor is obligate parasites (they live in a living host) and the life cycle has an extremely dynamic structure. The agent requires five different spore stages and two different hosts in its life cycle. This cycle consists of two stages; 1- asexual stage (primarily host) occurs on wheat plants, 2- sexual stage occurs on *Berberis* species (alternate host). Thanks to this dynamic structure, it has the ability to produce more spores in a short time during a life cycle (Figure-2).

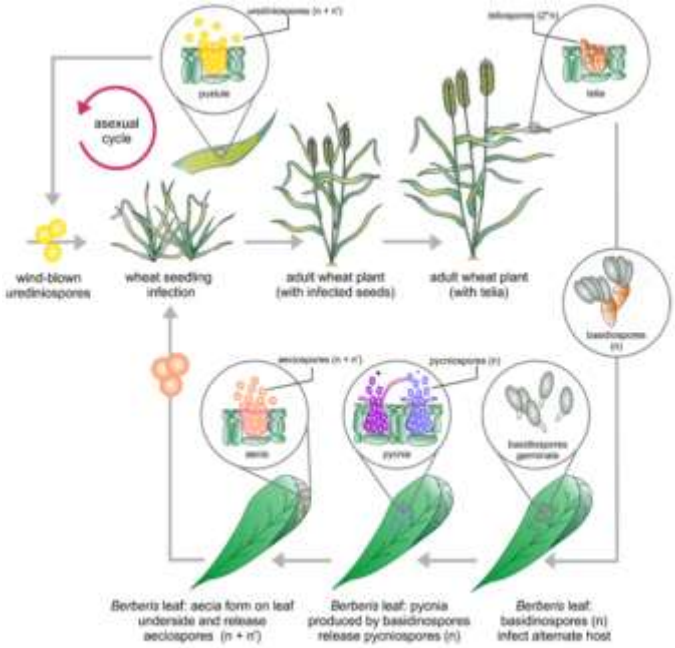

Figure-2 Yellow rust life cycle (Bouvet et. al 2022)

During the sexual reproduction period, new races are formed by forming spores with different genetic variations. Thus, the agent becomes more aggressive and difficult to combat. Owing tothis dynamic cycle, epidemics occur in sensitive wheat growing areas.

THE POTENTIAL OF YELLOW RUST TO CREATE NEW AGGRESSIVE RACES

It has been observed that there are significant differences in the movements and adaptation of Pst populations, which have been observed in important wheat cultivation areas in the world for many years (Chen 2005, Hubbard et al.2015, Hovmøller et al. 2016, Hovmøller et al.2017). The basis of these studies includes studies to determine Pst races. Race determination studies are carried out using seedling stage tests and a differential set (Figure-3).

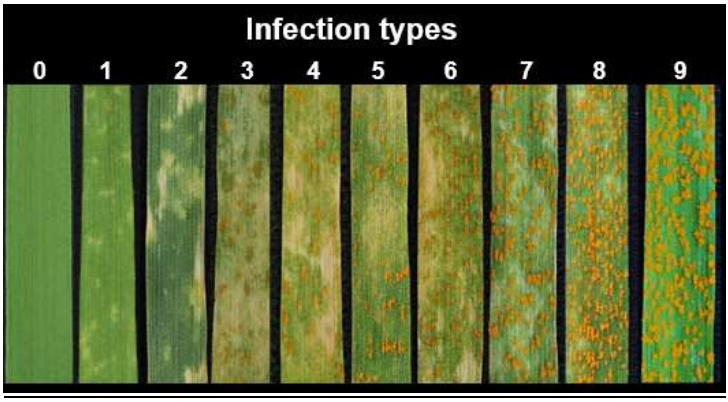

Figure-3 Infection type scale of stripe rust on seedling stage

Pst urediospores know no borders as they can be transported by air. For this reason, tracking rust races at the national, regional and international levels and, monitoring rust resistance, are the basis for the combating the disease. Yellow rust disease is seen in more than 60 countries around the world (Chen 2005). A map was created by bringing together the data created asa result of the yellow rust survey conducted between 2000-2009 (Wellings 2011) (Figure-4).

In addition to this map created in 2011, the map will be updated with the addition of the yellow pass, which was seen for the first time in Zimbabwe in 2018 (Boshoff et al. 2019). This situation also shows that yellow rust disease continues to expand its borders.

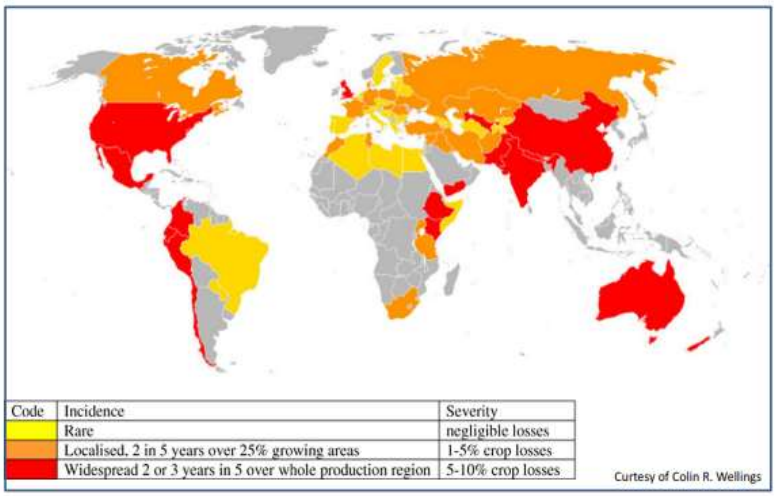

Figure-4 Damage and distribution of wheat yellow rust in the world (Chen 2020)

Due to the fact that the spores of the agent can be carried over long distances, it has caused many epidemics between continents. Yellow rust, which was first detected in Australia in 1979 due to human movements (Wellings et al. 1987), was found in Western Australia in 2002. It has created an epidemic due to a race known to be of Middle East/East African origin (Wellings et al. 2003).

This situation can be given as a good example of carrying sports over long distances. Due to this feature of the agent, many epidemics have occurred in other parts of the world as summarized in Table 1.

Table-1 Examples of world epidemics of wheat stripe rust (Chen 2020; Wellings 2011)

Region (*)	**Year**	**Epidemic/Lost**
England	1966	Rothwell Perdix
	1969	Joss Cambier
	1988-1989	Sleijpner, Hornet
Avusturalia	1983-1986	80% yield lost
	2002-2010	Fungicide use of AUD $ 40-90 million per year (2003-2006)
New Zeland	1980-1981	60 % yield lost
Iranian	1993	1.5 million ton yield lost
Chile	1976-1988, 2001	Regular epidemic
America	1957-1958	Epidemic (10 state)
	1960-1964	15-30 million $ yield lost
	2000	Epidemic (20 state)
	2003	11.7 million ton yield lost
Chinese	1954	6 million ton yield lost
	1964	3.2 million ton yield lost
	1990	2.65 million ton yield lost
	2002	1.4 million ton yield lost
Spain and North Africa	1978	Siete Cerros epidemic
South Africa	1996-1999	Funguside use 0.4-2.24 million $
India	1994-2004	Regular epidemic
	2001	*Breaking Yr27* resistance gene
Pakistan	2005	100 million $ yield lost
Italy	1977-1978	Epidemic
Czech Republic	1977	30% yield lost
Türkiye	1936-1963	Epidemic
	1975-1984	Regional epidemic
	1991	62.5% yield lost
	1998	Central Anatolia 26.5%- 50% yield lost
	2009-2010	Central Antolia epidemic

GREEN BRIDGE, MEGA VARIETY AND CHEMICAL USE

It is very important to develop and use resistant varieties for effective management of yellow rust disease. 80 resistance genes against yellow rust have been identified (Wang and Chen, 2017). The types of resistance frequently used in yellow rust resistance studies are all stage resistance (ASR) and adult plant resistance (APR) (Chen 2005, 2013). ASR has qualitative characteristics and monogenic resistance. It is active throughout the seedling stage of the plant and is breed specific (Ellis et al., 2014). ASR can be easily applied to breeding programs. However, due to its breed specificity, it is easily broken by new races. Therefore, it does not provide long-term durability. Yr2, Yr9, Yr17 and Yr27 are resistance genes developed with ASR resistance have been broken in worldwide epidemics as a result of new aggressive races formed by yellow rust in the last thirty years (Chen 2020). APR genes, on the other hand, are resistance that are not expressed during the seedling period but are expressed as the plant grows (in the adult stage of the plant). It is quantitative in character and controlled by multiple genes (minor genes) (Chen ve Kang, 2017). They provide long durability and are difficult to use in breeding programs. In addition, since there is no breed-specific endurance, they may not be affected by breed changes as much as ASR endurance (Chen 2013). In order to eliminate the disadvantages of ASR and APR, studies to develop new varieties resistant to yellow rust, in which both gene types are combined, are included in today's breeding programs. Although varieties are tried to be developed using the combination of ASR and APR varieties with ASR-based resistance and similar genetic characteristics are widely cultivated around the world. For this reason, these varieties are called mega varieties. Planting mega-varieties means planting sensitive varieties in large areas. This causes the pathogen to spread rapidly over large areas. However, wheat is an indispensable plant because it is the main food source and is grown in all places except Antarctica. This product, which has a wide range of climates and adaptability, may conflict with planting dates around the world. Wheat, which started to develop in similar vegetation periods as a result of the coincidence of sowing dates, continues its existence as the main host of yellow rust in 12 months of the year. In these cultivated areas, wheat acts as a green bridge and thereforeyellow rust causes disease. Both the cultivation of mega-varieties and the formation of the green bridge

increase the destructive power of yellow rust factor and threaten the world food security (Figure-5).

Figure-5 Damage caused by the disease in areas where varieties sensitive to yellow rust are cultivated

There is a chemical control for yellow rust disease. However, there is no applicability of fungicides or application technologies, especially for small farmers who are short on resources. Small farmers who do not apply fungicides and who cultivate sensitive varieties increase the probability of the emergence and spread of new strains by creating an inoculum source in the production areas of larger areas. In addition, long-term fungicide applications cause the formation of fungicide resistant strains and pollution of the environment (Cook et al., 2021). There is much evidence to suggest that yellow rust can adapt to climate change. For example, warmer winters will cause the disease to develop earlier period and new breeds that can adapt to these temperatures, increase the number of disease cycles and cause more crop losses. All these cases show why wheat yellow rust disease is so destructive and the threat it poses to food security. For this reason, in order to develop an effective control strategy against these diseases, agricultural control, breeding and extension studies should be carried out meticulously.

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CHAPTER 7

HULLESS (NAKED) BARLEY (*HORDEUM VULGARE* L. VAR. *NUDUM*)

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1. Introduction

Barley is a nutrient-dense cereal grain with properties that make it a desirable crop for the production of bread, and beverages. Hulless barley (*H. vulgare* L. var. *nudum*), with naked caryopsis, is primarily grown in Tibet as opposed to common barley with covered caryopsis. A form of precious barley genetic resource is coloured naked barley, which includes varieties like yellow, black, and blue naked barley. The coloured barley cultivars are better suited to produce higher anthocyanin and antioxidant levels than the regular barley type. Because of its claimed health advantages, interest in using hulless barley as a food grain has increased. Hulless barley has high β-glucan content.

The *Triticeae* genus *Hordeum* L. has 45 species and subspecies, most of which are weedy annual or perennial grasses that may grow in temperate regions of both the northern and southern hemispheres. In marginal agricultural areas, barley (*Hordeum vulgare* L. subsp. *vulgare*) has traditionally been the most common cereal crop (Zohary & Hopf, 2000). Barley is a versatile crop with three principal end-uses: feed, food, and malt. Each end-use of barley requires different characteristics, but hull adherence and β-glucan content are important for each of the three classes (Meints & Hayes, 2019).

Hulless (*Hordeum vulgare* L. var. *nudum*) or hulled types of barley are often categorised according to whether the hulls adhere to the grain or not. Hullless barley requires very little processing and preserves the majority of the endosperm and germ, which are normally lost during the dehulling. Because the entire grain can be directly used to produce a meal or processed into flour, it is therefore very suitable for human use. Because of its health advantages, interest in using hulless barley as a food grain has increased. Hulless barley has high β-glucan content (Shaveta et al., 2019).

Hulless barley is a special crop that may survive in the 4200–4500 m altitude range (Deng et al., 2021). It is commonly grown in highland regions around the world, including the Central Siberian Highlands in Russia, the

Qinghai-Tibet Plateau in China, the northwestern Indian Himalayas, Nepal, and Ethiopia. Other highland regions include Germany and Canada. Naked barley has a history of 3,500 years of cultivation in Tibetan Plateau, particularly in Bhutan, Nepal and China. It possesses outstanding features like cold resistance, strong adaptability, quick development, high stress resistance, and reliable yield (Zhu et al., 2015). It is not only widely used as the human staple food in the forms of noodles, steamed bread, nutrition powder, etc., but also as animal feed (Ge et al., 2021).

In the harsh environmental conditions of the Qinghai-Tibet Plateau, which are characterised by cold, high salinity, and drought (Yuan et al., 2018), Tibetan hulless barley is widely produced. Extreme cold, hypoxia, and high UV radiation are some of the climatic features of the Qinghai-Tibet plateau region (Deng et al., 2021). According to Yang et al. (2020), Tibetan hulless barley is continuously subjected to cold stress. Qingke can grow in areas with low temperatures, high altitudes, and irradiance with almost no difficulty (Xie et al., 2021). More dietary fibres and phenolic compounds are accumulated in these harsh environments (Li et al., 2019). Hulless barley is the main cereal crops in plateau areas at high altitudes from 2500 to 3000 meters in southwestern of China, The annual production of hulless barley in China account for 70% of the world total production (Gan et al., 2015).

Hullless barley was frequently disregarded by customers in the 20th century. But it's currently gaining popularity as a health food in Europe, North America, and other countries that don't typically cultivate barley (Dickin et al., 2012). Due to the hulless barley's high amounts of protein and vitamins, low fat content, and abundance of phenolic compounds, such as ferulic acid, flavonols, and flavones, the functional components have received an extensive amount of attention over the past 10 years (Siebenhandl et al., 2007). Hulless barley grain is a good source of dietary fiber providing soluble and insoluble dietary fiber fractions, especially a much higher content of arabinoxylan and β-(1 → 3, 1 →

4)-glucan compared to hulled barley genotypes (Kinner et al., 2011). It is similar to wheat in terms of structure and appearance, but differs in terms of the quantity of bioactive ingredients, which are gaining popularity due to the dietary fibre, beta-glucan, antioxidants, and phenolic components' alleged health advantages (Moza & Gujral, 2016). In the barley samples from Tibet, β-glucan concentration was found to be 8.6% of total dry weight (w/w). Additionally, the protein contents in Canadian hulless barley ranged widely (12.5-17.2% on a dry matter basis). Additionally, the quantities of the amino acids arginine, cysteine, isoleucine, lysine, methionine, and threonine (percent dry matter basis) were consistently greater than those recommended in the literature for husked barley (Zhang et al., 2019).

Fig. 1. Grain of a hulless barley cultivated in Tibet (right photo); filling stage spike (left photo) (Zeng et al., 2020)

In 2005, the US Food and Drug Administration (FDA) concluded that a cause and effect relationship between the consumption of β-glucan and coronary heart disease lowering properties exists. In Europe, the European Food Safety Authority (EFSA) has indicated that it will allow claiming “regular consumption of β-glucans contributes to maintenance of normal blood cholesterol concentrations” by assuming that barley β-glucans have the same effects as oat β-glucans (EFSA, 2009). In order to bear the claim, EFSA demands a quantity in food of at least 3 g/day of β-glucans from oats, oat bran,

barley, barley bran, or from mixtures of non-processed or minimally processed beta-glucans in one or more servings (Kinner et al., 2011).

A useful spectrum of functional components are also present in the young, green leaves and stem of hulless barley grass, offering additional potential health advantages (Zeng et al., 2018). According to Zeng et al. (2015), Tibetan hulless barley is referred to as "Ne" in Tibetan and "Qingke" in Chinese. According to Ramakrishna et al. (2017), qingke serves as both a staple diet for Tibetans and a significant livestock feed in the Tibetan Plateau. Studies have revealed that the whole Qingke flour's crude extracts have potent antioxidant activity (Shen et al., 2016; Lin et al., 2018).

As the husk covering the palea and lemma is fell off during the harvest, it is also known as naked barley. Qingke is the main grain source for the local population in cold regions such as countries in the Himalayas and North Africa (Baik & Ullrich, 2008). Recently, hulless barley is also increasingly attracting attention as a potential crop for the development of value-added products and multiple food applications (Izydorczyk et al., 2008).

2. Genetics and Breeding

Barley cultivation is dated back to about 10,000 years ago (Zohary et al., 2012). The naked barley trait occurred early during domestication, that is, already in the 7th millennium B.C. It appeared in different combinations along with other characteristics, such as various grain colors and two- or six-rowed spikes. While barley domestication is well established as being of multiple independent origin, naked grain barley is usually considered to be monophyletic (Zeng et al., 2018). Compared with common cultivated barley with covered caryopsis, hulless barley (*H. vulgare* L. var. *nudum*), with naked caryopsis, is mainly cultivated in Tibet and its vicinity, which is one of the domestication and diversity centers for cultivated barley (Dai et al., 2012). The adaptation to extreme environmental conditions in high altitudes made it the staple food for Tibetans beginning at least 3,500–4,000 years ago (Daxiong et

al., 2000). It continues to be the predominant crop in Tibet, occupying ~70% of crop lands (Zeng et al., 2015).

Hull adherence is controlled by a single gene at the Nud locus on the long arm of chromosome 7H (Taketa et al., 2008). It is believed that the naked phenotype arose by spontaneous mutation approximately 2000 years after the domestication of barley (Yu et al., 2016). The Nud gene encodes an Ethylene Response Factor (ERF) family transcription factor (Taketa et al., 2008). In covered barley, the Nud allele results in production of a lipid based 'cement' secreted from the pericarp which causes the lemma and palea to adhere to the caryopsis (Swanston et al., 2011). Around 16 days after pollination, this cement begins to appear, but the pericarp and hull do not touch until grain filling (Newman and Newman, 2008). The nud allele prevents this from occurring, allowing the grain to thresh freely from the hull during harvest (Meints et al., 2021).

Hulless barley has a complex diploid genome, with the genome size of 5000 Mb, larger than that of human (Mayer et al., 2012). Many researchers have analyzed Tibetan hulless barley, and many genes associated with drought stress responses in plants are known (Zeng et al., 2016). Using the short-read sequencing approach, the genomes of two hulless barley strains that were grown in Tibet were sequenced and assembled (Dai et al., 2018). The results suggest that many stress-related genes, which were expanded in hulless barley, might have facilitated the adaptation to the high-altitude environment and may provide a useful genetic resource for improving barley. Although the draft genome of hulless barley has been sequenced, the assembly remains fragmented (Zeng et al., 2020).

The type, concentration, and activity of phenolic chemicals in grains may vary depending on the genotype and grain colour. The coloured barley cultivars are better suited to produce higher anthocyanin and antioxidant levels than the regular barley type. The antioxidant strength and phenolic composition

of coloured rice have also been observed to differ significantly (Sumczynski et al., 2016). According to Liu et al., (2010), black-grained wheat has a high capacity to scavenge free radicals and a high phenolic content. A form of valuable barley genetic resource is coloured naked barley, which includes varieties like yellow, black, and blue naked barley. Due to the diversity of genotypes, the differences in the colored naked barley phenols and their relationship with antioxidant activity have not been well understood. It is necessary to comprehensively compare and evaluate the types, contents and the antioxidant activities of phenolic compounds of naked barley from the perspective of a different color (Ge et al., 2021).

More than 10,000 plant species are affected by powdery mildew, a fungal disease that drastically lowers grain yields and crop quality globally (Zhang et al., 2016). The obligate biotrophic ascomycete fungus "Blumeria graminis (DC.) f.sp. hordei" (Bgh) causes powdery mildew, a devastating disease of wheat crops that, depending on the level of infestation, can reduce grain production by up to 30%. Powdery mildew induced by Bgh is one of the main diseases of Tibetan hulless barley, and the most practical way to limit the damage is to grow crop varieties that are resistant to Bgh. There is a wide range of genetic variation to investigate, as demonstrated by a recent study in which different Qingke cultivars responded differently to powdery mildew. Our limited understanding of Tibetan hulless barley genetics seriously hindered the systematical investigation of genes and molecular mechanisms underlying its resistance response to powdery mildew (Yuan et al., 2018).

3. Conclusions

Breeders trying to develop new multipurpose barley varieties will immediately benefit the growing, producing, processing, and consumer communities who value innovation, sustainability, and health. For human consumption, breeders have developed naked barley with higher levels of -

glucan. The coloured barley cultivars are better suited to produce higher anthocyanin and antioxidant levels than the regular barley type.

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CHAPTER 8

TISSUE CULTURE OF CHRYSANTHEMUMS

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1. Introduction

Every year, millions of ornamental plants are routinely generated in vitro. By using low-cost tissue culture, adopted procedures, and maximized use of equipment and resources, it is possible to harness the enormous potential of micropropagation for large-scale plant multiplication.

Chrysanthemums are among the first commercial targets for micropropagation because of their popularity and demand. Shoot cuttings and root suckers are typically used in the vegetative vegetative propagation of chrysanthemums. However, this tactic has a lot of drawbacks. Micropropagation is a fast and effective method for producing high number of plants on a smaller scale however, a number of factors, including the source organ selected for tissue culture; organ's age both physiologically and ontogenetically; time of year to obtain explants; size of the explants; and general health of the source plant are affecting the success of in vitro Chrysanthemum propagation.

Chrysanthemum morifolium is a perennial flowering plant in the *Asteraceae* family, commonly known as the garden chrysanthemum. It is indigenous to Asia and northeastern Europe, and the majority of its species are from East Asia, with China serving as the region's diversity hotspot. There are numerous horticultural cultivars and variations. *Chrysanthemum morifolium* is an essential cut flower and pot plant (Xie et al., 2012). It is well-known and grown all over the world for its stunning bright blooms of all shapes, sizes, and colors and their prolonged vase life (Kumar et al., 2009). Due to its color and morphological diversity, the *Chrysanthemum* might be considered one of the most economically significant ornamental species (Hesami et al., 2020).

Cuttings are the primary method of *Chrysanthemum* propagation, but clonal micropropagation is currently extensively employed due to its higher efficacy (Brailko et al., 2017). Typically, shoot cuttings and root suckers are used to produce chrysanthemums. But this traditional method is comparatively slow. Additionally, when cuttings are obtained from mother plants on a frequent basis, there is a risk of viral infection and degeneration which drives up the cost of manufacturing. This traditional procedure can be carried out in vitro, is easy and affordable. The drawbacks of this approach

include a low rate of reproduction, poor seedling quality, a long reproducible period, seasonal restrictions, insufficient gene pool, and inability to prevent cross-incompatibility. By using in vitro propagation techniques, which can increase reproduction rates through in vitro culture and use very small explants, which are unfeasible with the conventional approach, the aforementioned obstacles are eliminated. Obtaining true-to-type, high-quality parents is typically challenging, but it is feasible through clonal replication of a species that has been certified as an elite species. The large-scale multiplication of *C. morifolium* with employing multiple unique regeneration pathways has been explored in tissue culture, according to a number of literature research. Additionally, the most effective strategy is developing techniques preventing microbial contamination in culture media. Also execution of successful micropropagation operations depends critically on the achievement of successful acclimation under nursery conditions (Eisa et al., 2022).

2. Tissue culture

In their natural habitat, viruses frequently infect nearly all plants. Viral diseases are a significant limiting factor for agricultural yield and sustainable development since these viral infections can result in devastating diseases, major yield losses, and significant economic losses. The production and use of virus-free plantlets, however, can significantly reduce these losses. The most effective methods for eliminating different viruses from nearly all of the most commercially significant crops are in vitro culture techniques. Meristem tip culture, chemotherapy, somatic embryogenesis, thermotherapy, electrotherapy, shoot tip cryotherapy, and micrografting are methods for generating virus-free plantlets. Meristem tip culture is currently the most popular among them (Yan et al., 2022).

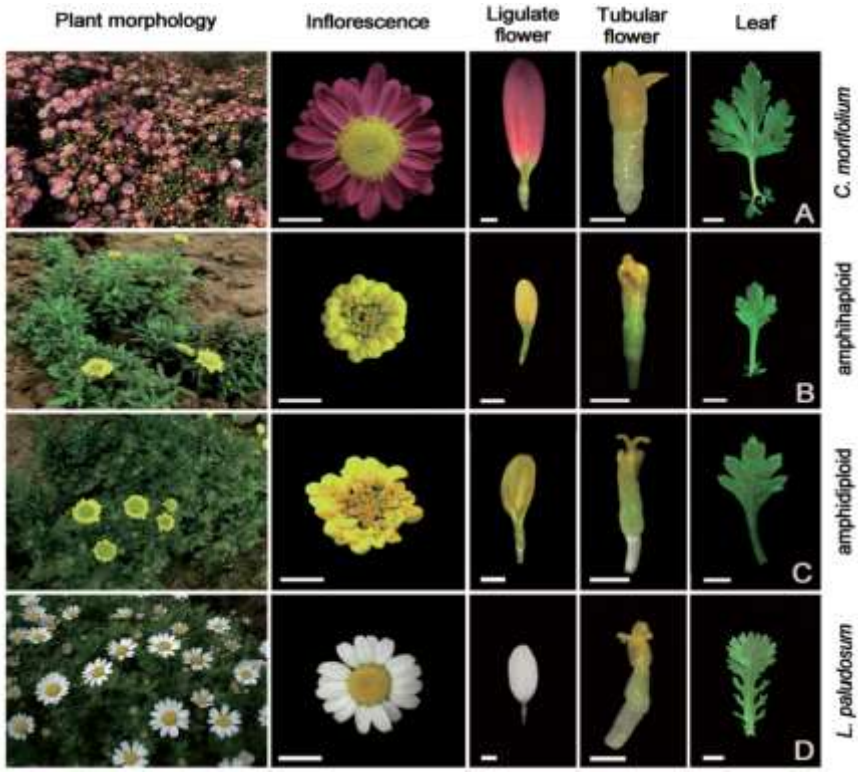

Fig. 1. Morphology of materials. A) *Chrysanthemum morifolium* "Zhongshanzigui," B) amphihaploid, C) amphidoploid, D) *Leucanthemum paludosum*). From left to right of each line: plant morphology; floral morphology, bar: 1cm; ligulate flower, bar: 2 mm; tubular flower, bar: 2mm; leaf: 0.5cm (Wang et al., 2014).

By using the direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA) and reverse transcription polymerase chain reaction (RT-PCR), combined *Cucumber mosaic virus* (CMV) and *Tomato aspermy virus* (TAV) infections were found in *Chrysanthemum morifolium* cv. Pooja plants in the study of Kumar et al., (2009). By cultivating the 0.3 mm long shoot meristem of infected plants on MS (Murashige & Skoog) media supplemented with BAP and NAA, the CMV and TAV were eliminated. DAC-ELISA was used to index the regenerated plants, and RT-PCR was used to confirm them. Only 65.6% of the 78.1% CMV and TAV-free shootlets that were produced from the regenerated shoot meristem were indeed virus-free

when verified by RT-PCR. On half MS medium, virus-free shootlets were rooted and acclimated in a greenhouse.

In the study of Kumar et al., (2008), *Septoria obesa*-susceptible leaf segments of the *Chrysanthemum* cultivar "Snow Ball" were used to generate callus cultures that were effectively employed for in vitro selection for resistance to this pathogenic fungus. By cultivating callus on growth media with varying amounts of *S. obesa* filtrate, resistant cell lines were found. Plant regeneration using resistant calluses produced following two cycles (30 days each cycle) of selection. 70–80% of the plants grown from cuttings and about 30% of the plants regenerated from the resistant calluses developed significant resistance to the disease in the field. The chosen regenerates showed no phenotypic diversity.

On the MS medium containing naphthaleneacetic acid and 6-benzyladenine, Lim et al. (2012) studied the ability of leaf, petiole, and stem explants of eleven *Chrysanthemum* cultivars to regenerate shoots. When grown on media solidified with various gelling agents, distinct cultivars showed significantly varying frequencies of callus development and regeneration from various explants. The gelling agent Gelrite was shown to be the most successful in promoting the shoot. Regardless of cultivar and gelling agent, stem explants generally showed the highest rates of shoot organogenesis and mean number of shoots per explant. However, the highest frequency of regeneration (11.7 shoots per explant) was noted from leaf explants of cv. Borami. Shoots were directly developed from the surface of explants, not through callus formation.

By analyzing the effects of plant growth regulators, dark incubation, gelling agents, and silver nitrate, Naing et al. (2014) designed a methodology for shoot regeneration from leaf segments of the *Chrysanthemum* cv. Vivid Scarlet. The regeneration of leaf explants on MS media supplemented with a combination of BA and NAA under light circumstances without an initial dark phase resulted in the largest number of shoots per explant (12.3). Among the investigated gelling agents, Gelrite was the most effective at promoting shoot regeneration, but silver nitrate clearly prevented it. On a hormone-free MS medium cemented with Gelrite, superior plant growth and roots were seen.

The mother plant produced under greenhouse conditions and the regenerated plants showed no ploidy variance, according to flow cytometry analyses.

3. Micropropagation of Chrysanthemum

The tissue culture technique known as micropropagation is employed for the rapid vegetative replication of ornamental plants and fruit trees. This tissue culture technique yields many plants. These plants will all share the same genetic profile as the original plant from which they were developed. To establish an effective procedure employing various mediums and concentrations, Chae (2014) examined increased root organogenesis and micropropagation in *Chrysanthemum morifolium* cv. Hwiparam.

Fig. 2. Modification of floral color in *Chrysanthemum* by various breeding methods. A) Diverse colored flowers expressing anthocyanins in chrysanthemum cultivars developed by cross breeding at National Institute of Horticultural and Herbal Sciences (NIHHS), RDA, Korea. i): Glory pink; ii): Red marble; iii): Donna pink; iv): Purple cone; v): Princeling; vi): Cutie pink; vii): Orange pangpang; viii): Pink pangpang: and ix): Purple pangpang. B) Modification of floral color by mutation breeding. A range of floral colored mutants presenting various colors were generated by gamma-irradiation in two chrysanthemum cultivars 'Noble wine' and 'Pinky'. i): Original floral color of 'Noble wine'; ii–iv): gamma-irradiated mutants of 'Noble wine' showing varied floral colors. v): Original floral color of 'Pinky'; and (vi–viii): gamma-irradiated mutant floral colors of 'Pinky' (Kim et al., 2016).

On three full strengths of basal MS, SH, and B5 media, stem explants were grown. A total of 5 different concentrations (1/4, 1/2, 1, and 2 strength) were tested to determine which medium was optimal for root regeneration. SH media was the finest type medium for root growth and regeneration. The results showed that half strength of SH (1/2SH) is the best condition for the number of root per explant (4.3) and root length (31.4 mm).

Liu and Gao (2007) developed and improved rapid propagation technology in vitro for *Chrysanthemum cinerariifolium*, a significant botanical pesticide plant with a sizable global market. On MS media with BA and NAA supplements, several buds could be directly generated from epicotyl and hypocotyl explants. Within 15 days of inoculation, root induction and development could be seen on 1/2 MS medium supplemented with IAA and rooting powder (ABT). Also presented was an in vitro polyploid breeding study to produce superior breeding lines with high output and good quality. Colchicine treatments produced autotetraploid *C. cinerariifolium* lines, which were then recognized by stoma observation and root-tip chromosomal analysis. Obtained autotetraploid lines will be of important genetic and breeding value and be used for further selection and plant breeding.

Micropropagation has demonstrated to be a successful strategy for rapid, large-scale plant production as well as a helpful tool for plant breeding. One of the most challenging micropropagation problems is microbial contamination, which leads to decreased plant quality and loss of expensive stocks. Therefore, a crucial step in plant micropropagation is sterilizing the culture material. Sterilized media may, however, reduce the effectiveness of plant growth regulators and nutritional culture medium components (Tung et al., 2021).

Tung et al., (2021) investigated the sterilizing effects of silver nanoparticles (AgNP) on the development of explants and culture medium. Quality *Chrysanthemum* plantlets were generated on MS medium containing 4 ppm AgNP, which led to 100% medium disinfection (no contamination) after 4 weeks of culture.

Jevremovic & Subotic (2018) assessed the potential for mass plant production using the traditional micropropagation method from a single shoot as the starting plant material in five commercially available chrysanthemum

cultivars. From all of the examined cultivars (12–54%), aseptic stem segment cultures (88 explants/cultivar) were generated in January. Over the course of the following three subcultures, shoot multiplication was assessed. On nutritional medium supplemented with NAA and BAP, the best shoot multiplication was achieved. In the three weeks that subsequently followed, 100% of the regenerated shoots on plant growth regulator-free medium were rooted. In nurseries during May, about 3500 micropropagated chrysanthemum plantlets were rooted and put in the field. Ex vitro acclimatization of the plants was 100% effective, and plants were flowered in the autumn.

Tung et al. (2020) employed iron nanoparticles (FeNPs) in place of Fe-EDTA in MS medium to examine the impact of these particles on various culture systems (in vitro solid, in vitro hydroponic and microponic culture). When the plantlets from the MS medium enriched with FeNPs were transplanted into greenhouse settings, the microponic-cultivated plants with the best survival rate (94.67%) were those that had received the FeNPs. The findings of this study demonstrated that FeNPs can substitute Fe EDTA salt in MS medium and that a lack of iron in culture media will result in a decrease in the amount of chlorophyll.

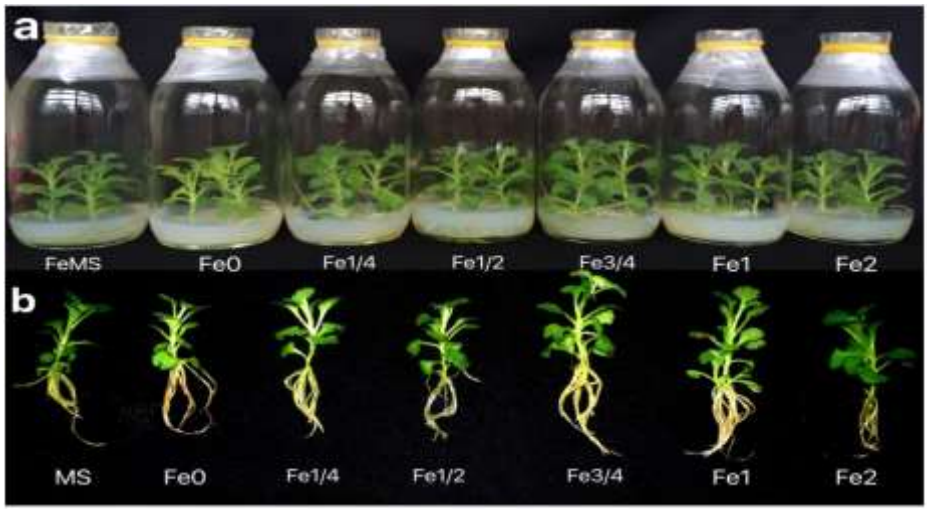

Fig. 3. Effect of FeNPs on *Chrysanthemum* growth in in vitro solid culture after 4 weeks of culture (Tung et al., 2020).

Trifunovic et al., (2006) studied the in vitro plant regeneration of the chrysanthemum cultivars "Reagan Sunny" and "White Spider". On MS solid medium enriched with NAA and BAP, morphogenesis in stem segment culture was induced. The shoot multiplication index for the cultivar "Reagan Sunny" was found to be the best. Afterwards, when both cultivars had been long-term cultured, the multiplication index was reduced. Without subculturing, cultures were kept at +4°C for 2, 4, and 6 months. After storage treatment, there was no statistically significant difference between the two cultivars' shoot multiplication indices. Multiplication index was increased compared to cultures constantly grown at temperature 23°C. On solid MS hormone-free media, rooting of shoots was highly successful (100%). Both cultivars' rooting effectiveness was unaffected by cold storage.

In order to achieve strong growth and quality, Sivakumar et al. (2005) searched the ideal culture conditions for the large-scale propagation of chrysanthemum in balloon-type bioreactors. The ideal growing settings included an NH4:NO3 ratio of 20:40 mM, air exchange of 0.1 vvm/min, an air temperature of 25oC, a photosynthetic photo flux (PPF) of 100 mmol mol m2 s1, and an inoculation density of 40 nodes *Chrysanthemum grandiflorum*.

For *Chrysanthemum morifolium*, Yesmin et al., (2014) designed a successful in vitro plant regeneration protocol. In this experiment, nodal segments were employed as explants. For the study, plant growth regulators (cytokinins or auxins) were added to MS nutritional medium with a variety of combinations and concentrations. On MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l IAA, the highest percentage (93.3%), best shoot induction per culture, and longest shoot length were attained. The number of shoots per culture can be increased by re-subculturing on the same media. In vitro grown fully developed shoots were cultured into MS media at half strength together with various concentrations of IBA, IAA, and NAA for rooting. The isolated shoots rooted well (98%) within 4 weeks on half-strength MS fortified with IBA, where average number of roots per shoot was 12.3 ± 0.5. After transplantation, regenerated plantlets developed in natural condition variation.

With the help of MS media supplemented with various concentrations and combinations of plant growth regulators, shoot multiplication of

Chrysanthemum was performed from shoot tip explant. In MS media supplemented with 0.5 mg/L NAA, the maximum shoot initiation (80.0%), shoot per explant (3.2), shoot length (3.4 cm), number of leaves (9.5), and nodes (4.5) were observed. A satisfactory rooting response, including days to root emergence (5.0), root initiation percentage (100%), roots per plantlet (14.3), and root length (9.0 cm) was obtained with half strength MS media supplemented with IBA (Waseem et al., 2011).

Shatnawi et al., (2008) developed a procedure for micropropagating *Chrysanthemum morifolium* through multiple shoot development. Explants growing in greenhouse were used to establish cultures of *C. morifolium*. On MS media, shoot tips were grown after surface sterilization. *Chrysanthemum* was successfully multiplied in vitro using MS medium supplemented with BAP. On MS media enriched with varied doses of auxin IBA, IAA, or NAA, in vitro rooting was accomplished satisfactorily.

The parameters of a greater frequency for regenerated plants from various explants of a standard-type *Chrysanthemum* cv. Jinba were examined by Lee et al. (2008). On an MS medium with 3% sucrose, 0.8% agar, and 5 M BA with NAA, in vitro culture was started using surface-sterilized leaf and flower tissues from greenhouse-grown plants. 21 to 28 days after the initial culture, direct shoot regeneration from the leaf and flower explants was attained. Among the seven combinations of the growth regulators used for the culture, the most efficient condition for the shoot and root formation from the leaf tissue was obtained when the MS basic medium was supplemented with 0.5 mg BA/l and 1.0 mg NAA/l, and 0.1 mg BA/l BA + 0.5 mg NAA/l, while the culture using floret tissues was most efficient on the medium supplemented with 0.5 mg BA/l and 0.5 mg NAA/l, and 0.1 mg BA/l and 1.0 mg NAA/l.

4. Conclusions

Shoot cuttings and root suckers are frequently used in the vegetative vegetative propagation of chrysanthemums but this method has a lot of drawbacks. Using low-cost tissue culture procedures, and maximum usage of equipment and resources have big potential for large-scale plant multiplication. The production and use of virus-free plantlets, however, can

significantly reduce losses and meristem tip culture is currently the most popular among methods for generating virus-free plantlets for *Chrysanthemums*. The gelling agent Gelrite was shown to be the most successful in promoting the shoot. Performance of tissue culture mediums vary from variety to variety. Sterilizing effects of silver nanoparticles (AgNP) was found successful by different studies.

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CHAPTER 9

CHICKPEA ASCOCHYTA BLIGHT

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INTRODUCTION

Chickpea *(Cicer arietinum* L.*)* is an important legume plant for human nutrition due to its protein richness, fiber content, and an important basic nutrient in Turkiye. Thanks to their symbiotic life with *Rhizobium* bacteria, it improves our soils by binding the nitrogen of the air to the soil. Chickpea, which can be grown in winter and traditionally grown in summer to avoid disease, has attracted attention to winter planting in recent years, with a 30% increase in winter crop compared to summer crops in our coastal regions (Mart and Öktem, 2022). Chickpea without soil selectivity can be grown in almost all the lands of our country.

Important diseases for chickpea, whose economic importance varies according to years and countries, are ascochyta blight, fusarium wilt, botrytis gray mold (BGM), dry root rot, collar rot, foot rot and stem rot (Nene and Reddy, 1987; Singh and Sharma, 2002) .

Ascochyta blight, one of the most important diseases of chickpeas, is a fungal disease and causes heavy yield losses by damaging the above-ground part of plant (branch-leaf-pod). This disease is the most important factor limiting chickpea production. In chickpea farming, our priority is to gain high yielding varieties with high commercial value that are tolerant to diseases. For this, besides breeding studies, studies on the development, spread and damage of diseases are also required. Because diseases are also alive and create resistance/tolerance in order to survive, or they maintain their vitality by creating new forms.

Ascochyta Blight Life Cycle

It causes spots ranging from light brown to dark brown on the plant. The plant breaks in the lesion parts and dries out in a short time. On the seed capsules, it creates circular spots inside each other.

The optimum temperature for Ascochyta blight infection, disease development and spread is between is between 15-25°C with minimum and maximum values ranging between 5-30°C in some instances. Rapid growth occurs; minimum 6 hours of leaf wetness. With the duration of leaf wetness and moisture disease severity increases (Trapero-Casas and Kaiser, 1992). Prolonged intermittent rains promote rapid disease development and spread. Fields with infected seeds or infected chickpea residues and chickpea

germinated from seeds left from previous year support the survival and sustainability of the pathogen. Ascochyta blight develops sexually or asexually; The pathogen is exposed to sexual reproduction in both mating types and at appropriate air temperatures, forming pseudothesia and ascospores (Trapero-Casas and Kaiser, 1992). The main inoculum source for ascochyta blight disease ascospores are discharged during the rain and storms, and can cause epiemics in a disease free areas. Secondary spread is caused by the pycnidospores. This structures are spread by winds , hail and rains. The disease spreads in the form of disease hotspots in chickpea production fields, causing crop losses by spreading to the entire field. Accoring to Hira et al. (2022) chickpea is an important legume crop of Pakistan, and ascochyta blight disease can cause up to 50-70% yield losses in chickpeas.

Development of Ascochyta Blight

Cultivation of legumes began approximately 10000-12000 years ago in the lands of the "Fertile Crescent" in the Near East (Zohary and Hopf, 2000; Lev-Yadun et al ., 2000; Salamini et al., 2002). The origin and culture of the cultivated chickpea (*Cicer arietinum* L. *)* is the northern part of Syria and the Southeastern Anatolia region of Turkiye (Ladizinsky and Adler, 1976; Lev-Yadun et al ., 2000).

The Southeast Anatolian Region, including Mesopotamia, is considered both the origin of host and the origin of the pathogen, since chickpea was cultivated for the first time in human history in this region, and these areas are stated as areas where the pathogen shows high genetic diversity (Özkılınç et al., 2010; Özkılınç et al . , 2011). Plant development in the Near East, which is the origin and cultivation center of *Cicer spp.*, including the borders of our country. The vegetation of the chickpea is germination in winter, flowering in late winter-early spring and ripening in early summer. In the historical process, chickpea planting was taken from winter to spring because of the possibility of reducing the severity of Ascochyta blight (Abbo et al ., 2003). Chickpea cultivation has been reshaped in the form of disease avoidance and agricultural breeding programs have been focused on yield and quality. Determination of pathogenic diversity as well as adaptation is very important in determining plant breeding systems (Tivoli and Banniza, 2007) .

D. rabiei, which is a necrotic fungal phytopathogen and damages the above-ground organs, causes spots and drying on all above-ground parts of the plant (stem, leaves and seed capsules). Spots of varying sizes and densities ranging from light brown to blackish appear on the branches . On the green capsules, the lesions usually have dark-edged, rounded, concentric ringed pycnids. Piknids appear as pinhead-sized black spots. Lesions on the leaf are in the form of round brown spots. These spots are surrounded by a brownish-red border. The disease reproduces by the spread of ascospores released from pseudothecias by air and wind . Being in the sexual phase of the disease causes more proliferation and the occurrence of different pathotypes (Phan et al ., 2003). Although summer planting is generally seen as a solution to avoid the disease, there is a 30% higher yield increase in winter planting. For this, the chickpea variety used must be durable (Şehirali, 1988, March, 2022).

D. rabiei has two different life cycles, sexual and asexual . The asexual period is important for the spread of the disease, and the sexual period for the occurrence of pathotypes with different virulence levels (Chen et al ., 2004). During the sexual period, the peritheum is formed and this structure forms ascuses, each of which has eight ascospores (Haware, 1987). In the asexual stage, the fungus forms pycnid and pycniospores (conidia) in the leaves, stems and capsules of the host plant. Piknit is spherical, dark brown in color and 140-200 µm in diameter. In rainy and humid weather, the conidia come out of the pycnids in the form of a gelatinous stream and the spores are mixed with the raindrops and spread to the environment (Ergün, 2001). Bremer (1948) conducted studies related to chickpeas. In the light of these studies, he determined the cause of Ascochyta blight in chickpeas, especially in the Central and Southeastern Anatolia Regions of Turkiye. Ladizinsky and Adler (1976) , In his study on the wild ancestor of chickpeas, he explained that there are archaeological data in the Southeastern Anatolia Region of Turkey and the north of Syria.

Nene (1984) determined that pycniospores can spread over short distances by rain, and ascospores can spread over long distances by wind. Haware (1987) found that Ascochyta blight is seen in the sexual and asexual stages of chickpeas. Trapero-Casas and Kaiser (1987) stated that the asexual form of Ascochyta blight in chickpea is *Ascochyta rabiei.* The sexual form of this blight factor was first observed by Kovachevski (1936) and defined as

Didymella rabiei . Muehlbauer and Singh (1987); Reddy and Singh (1990b) stated that the easiest way to control Ascochyta blight is through the production of resistant varieties and the main purpose of chickpea breeding programs all over the world is to develop genetic resistance. Kaiser and Hannan (1988) determined that the application of fungicide for seed transmitted *A. rabiei* is important in preventing the onset and development of the disease in the infected seed, but *A. rabiei* could not be destroyed in the seed treated with fungicide .

Reddy et al. (1992) stated that although Ascochyta blight has been known for approximately 90 years, there has not been much development in its control by host plant resistance. They stated that developments have been very slow over the past 60 years due to the lack of resistance sources, which has the greatest impact on the cultivation of varieties resistant to Ascochyta blight. In another study, Reddy et al. (1992) found that desi germplasms should be considered as resistant chickpea variety and with the acceptance of desi germplasms, a chickpea variety with higher resistance than germplasms can be obtained. Latif et al. (1993). Singh et al. (1994) stated that wild relatives of chickpea should also be investigated as a resistance gene source in order to develop resistant chickpeas, and they stated that many resistance development programs in the world also screen wild *Cicer* species as they are thought to have important resistance sources. Wilson and Kaiser (1995) stated that the infection can be characterized by the formation of necrotic spots on the stem, leaves and capsule of the chickpea, and they stated that in the future, the stem and branches of the plant may break and cause the death of the plant.

Kaiser et al. (1997), in a study they conducted prepared 145 isolates for the study of *Didymella rabiei* from 23 provinces of Turkey. Mating type studies were carried out with these isolates. They observed both mating types in 18 of the provinces. They determined that 59% of the isolates had *MAT1-1* and 41% had *MAT 1-2* . Colony morphology comparisons determined differences between isolates. As a result of this comparison, it was explained that the reason for the increase in genetic diversity was the spread of the telemorph of Ascochyta blight over long distances. Kaiser and Küsmenoğlu (1997); Gullu et al. (2002); Can et al. (2005) suggested that Ascochyta blight disease causes major problems for chickpea cultivation in our country. Udupa et al. (1997-1998) investigated genotype- specific DNA profiles in a study using RAPD (Random Amplified Polymorphic DNA) and microsatellite markers with *A.*

rabiei isolates collected from Syria . Simultaneously, in a study conducted with *A. rabiei* isolates in Italy, they examined the pathological groups and RAPD profiles, and as a result, they could not detect a correlation . They determined that there was very little variation between *A. rabiei* -specific markers and RAPD markers. Turgeon (1998) explained that there are similarities and differences between *Ascochyta spp.*, which is the pathogen of legumes, and that MAT analyzes are important in determining this.

Kaiser and Küsmenoğlu (1997) conducted MAT research of *A. rabiei* on 145 isolates from 23 provinces of Turkey and stated that 59% of the isolates had *MAT 1-1* and 41% had *MAT 1-2 type.* Barve et al. (2003) determined the distribution of MAT type with samples taken from 2 different fields in the Northwest Region of the USA and stated that the distribution was equal to each other as a result of their research. Shtienberg et al. (2000) declared that systemic fungicides are ineffective against *Didymella rabiei in particular.* Geistlinger et al. (2000), with their research *A. rabiei* 'specific 20 microsatellite loci have characterized. Santra et al. (2001) conducted a study with RAPD markers to determine the level of similarity between and within pathogen populations with isolates of chickpeas collected from India, Syria, America, and Pakistan, and using Cluster Analysis to determine the geographic origins of these isolates. As a result of the research, they stated that the isolates showed a distribution depending on their origin (such as A and B). However, they identified specific DNA markers for isolates from India.

Milgroom and Peever (2002) explained that revealing genetic and pathogenic variation and knowing the pathogen population structure are necessary and of great importance in the development of appropriate control methods against the pathogen. They stated that with studies on the biological population of the pathogen , information can be obtained about the pathogenic diversity, genetic structure and disease epidemiology of populations. They stated that many different approaches can be contributed to the fight against the disease. Cingilli et al. (2003) stated that molecular markers have become important tools for the genetic analysis of crop species and the resistance of indirect selection in a breeding program . The presence of these markers associated with resistance to Ascochyta blight will greatly enhance the selection and development of Turkish chickpea genotypes resistant to Ascochyta blight in future breeding programs.

Cho and Chen (2004) explained that the cause of Ascochyta blight in chickpea (*Cicer arietinum L.)* culture is caused by a different harmful fungus. They carried out studies on hybridization in order to define the mechanism of resistance due to Ascochyta blight in terms of genetics. Pathotype 1 and Pathotype 2 were determined by inoculating recombinant lines, and they stated that Ascochyta blight showed changes due to pathotype resistance in these lines. Iğdırlıoğlu (2004) determined the differences in *D. rabiei isolates* with the help of microsatellites in his study with isolates obtained from chickpeas collected from the Southeastern Anatolia Region of our country (120 isolates in total). He conducted 14 heterologous microsatellite primer trials in DNA studies. He observed the disease factor in all the plants in the fields. With the studies carried out, different groups of isolates depending on their colony morphologies were formed. Vail (2005) stated that the A scochyta blight factor adversely affected chickpea production and productivity and conducted another study stating that genetic diversity occurs during the sexual period. In addition, he stated that this phenomenon caused the formation of pathotypes of the pathogen.

Bayraktar et al. (2006) studied the mating type distribution of 45 *D. rabiei isolates* isolated from chickpeas collected from 6 provinces in the Central Anatolia Region . They determined that 57.8% of the isolates had *MAT 1-1* and 42.2% had *MAT 1-2* . Kimber et al. (2007) conducted studies on *D. rabiei* related to the epidemiology of chickpea . They determined that the disease agent can spread over short distances by rain and wind. They also created a simulation model. When Türkkan (2008) separated the pathotypes of 64 *A. rabiei isolates* from the Mediterranean, Aegean, Southeastern Anatolia, Central Anatolia and Black Sea Regions of Turkey, based on the International Standard Control species (ILC1929, ILC482 and ILC3279), 3 pathotype groups (Patotype 1, 2 and pathotype 3) occur. Özkılınç (2010), in studies on the genetic structure and pathogenic virulence of *D. rabiei isolates* in chickpea samples collected from Turkey and Israel, reported that the genetic diversity was highest in the group isolated from cultured chickpeas in Turkish populations .

Imtiaz et al. (2011) stated that pathogen groups cause different viral effects. Existing pathotype groups (Patotype 1, Pathotype 2 and Pathotype 3) were used to test the emerging new pathotype group, Pathotype 4. They inoculated into different chickpea growing fields (ILC1929, ILC482, ILC3279

and ICC12004). According to the results of the pathogenicity tests, they stated that the isolates had 4 pathotypes (Patotype 1, Pathotype 2, Pathotype 3 and Pathotype 4). As a result of the test studies conducted with wild chickpeas using Pathotype 4, they reported the results obtained due to the resistance of wild species. Rubiales and Fondevilla (2012) emphasized that the most effective method for controlling Ascochyta blight, which limits the production of legumes, is to grow resistant crops against the disease agent. At the same time, they stated that the mechanisms of resistance are quantitative and controlled by multiple genes . For this reason, they reported that it is difficult to apply in known breeding methods. As a result, they stated the necessity of biotechnological applications in the establishment of legume production areas that are resistant to Ascochyta blight.

Leo et al. (2014) determined that the genetic difference was low in *D. rabiei isolates.* In the study, they stated that the disease agent was transmitted through seeds . Ozkan et al. (2015) evaluated the disease severity of *Didymella rabiei* , which is the cause of Ascochyta blight in chickpea growing regions in Turkey , according to the 1-9 scale. They found that there was a significant, but negative, relationship between the severity of the disease, altitude, and the number of nodules . Leo et al. (2016) suggested that many *Ascochyta rabiei pathotypes affecting chickpea in Australia seriously hinder breeding efforts in chickpea*. They noted that breeding for sustained resistance would be beneficial as long as it was supported by detailed knowledge of defense responses against isolates of varying severity. As a result, they stated that this study will create a potential use in discrimination and resistance type selection in future breeding studies.

Bayraktar et al. (2016) used different inoculation methods (leaf grafting method) in plant materials for the accurate determination of Ascochyta blight and *A. rabiei* infection in chickpea tissue. Quantitative analysis of disease progression in resistant and susceptible cultivars was determined by Real-Time PCR study, by evaluating at certain time intervals after pathogen inoculation. In conclusion, they demonstrated a good correlation between morphological assessment of disease reaction and pathogen quantification in infected chickpea tissues. **Mart et al. (2016**) in their study , in which they evaluated 34 registered Chickpea (*Cicer arietinum* L.) cultivars for winter sowing under Çukurova climatic conditions and carried out in the Eastern Mediterranean Agricultural

Research Institute Research Experiment Area in Adana location during the 2014-2015 cultivation period in order to determine the tolerance/resistance status of the cultivars in terms of diseases; Escilli cultivars created four different disease gardens for four different Pathotypes, made disease readings and observations and examined the tolerance of the cultivars. In the results of working; Disease gardens in field conditions in Adana location; As a result of artificial inoculation with four pathotypes detected in the legume cultivation areas of Turkey, disease readings according to the 1-9 scale made on the 7th day, 14th day and 21st day , and the suitability of the Registered Varieties to the conditions of the Region and their winter sowing were evaluated. As a result of the artificial inoculation applications of four pathotypes in the Disease Gardens trials they conducted, the lowest scores were obtained from the Pathotype-I applications, and the highest scores from the Pathotype-IV applications.

Chickpea Ascochyta blight *(Ascochyta rabiei)* disease is one of the most important biotic stress factors that adversely affect chickpea agriculture, yield and quality. The disease is seen in all areas where chickpea is grown and it is known that there is a large variation of pathogens (Nalcaci et al, 2021).

Economic Significance

The most important biotic stress factor limiting chickpea cultivation in our country and all over the world is Ascochyta blight disease caused by Didymella *rabiei* (Kovachevski) vonArx [anamorph: *Ascochyta rabiei (Passerini) Labrousse],* and under suitable epidemiological conditions (planting sensitive chickpea varieties, high humidity and temperatures can cause product losses up to 100%. *A. rabiei* , which is a destructive factor *with Ascochyta blight,* causes devastating losses in the chickpea growing regions of our country.

Ascochyta blight causes a decrease in product quality and a decrease in its quantity, causing symptoms in the form of spots and drying on all above-ground structures of the host plant. Crop losses of up to 100% have been reported when conditions are favorable for an epidemic (Reddy and Sign, 1990). Disease In Turkey (Kaiser and Küsmenoğlu, 1997), they stated that serious crop losses occur when conditions that allow the pathogen to occur occur. Iqbal et al. (2004) used 824 germplasms in their study under greenhouse

and field conditions to determine the resistance of chickpea to Ascochyta blight for 3 years in Pakistan. None of the genotypes used could show resistance to the disease in the pod setting period, in general, resistance to the disease was established thanks to the breeding program in the seedling and vegetative periods, the infection level was high during the pod tying period, and it was highly correlated with the seedling and pod formation periods in terms of disease formation. It has been reported that genotypes showing resistance to the disease are more resistant to the disease in the bean setting period, and the resistance in the seedling period protects the plant from the disease in the future. Tivoli and Banniza (2007), *Ascochyta* spp . They explained that ' is a factor of Ascochyta blight. In addition, they determined that the symptoms of Ascochyta blight in chickpea were similarly seen in all parts of the plant above the ground and the disease differed depending on several factors (seasons, climatic conditions and countries). They suggested that ascospores and infected seeds are most likely the primary source of inoculum. In addition, as a result of a study, *Ascochyta* spp . They stated that .

Ascochyta blight causes economic product and quality loss in chickpea farming. In cases where disease conditions are suitable for epidemic , heavy losses may occur in almost all chickpea cultivation areas .

Conclusion and Discussion

Ascochyta blight, Didymella rabiei causes serious product and quality loss in chickpea cultivation areas. In cases where climatic conditions are suitable for epidemic, crop losses may occur in almost all chickpea cultivation areas. For this reason , *D. rabiei* , which has two different life cycles, sexual and asexual, is important in terms of the spread of the disease in the asexual period and the occurrence of pathotypes with different virulence levels in the sexual period. In addition to adaptation, determination of pathogenic diversity is also very important in the determination of chickpea plant breeding working systems.

In chickpea agriculture, it is important to bring into the economy varieties that are disease-tolerant, highly productive, and have high quality and commercial values.

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CHAPTER 10

TAXONOMIC AND AGRICULTURAL CHARACTERISTICS OF *LOTUS CORNICULATUS* L.

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Introductıon

Lotus corniculatus L. is an allogamous tetraploid (2n=4x=24) (though there are some reports of diploid plants) species with autoincompatibility (Řepková and Hofbauer 2009), as well as palatable, nutritious, high in protein, very digestible, low seed yields (Sareen 2004), reseeding itself (Putnam 2014), medium perennial ((Büyükyıldız et al. 2023), pod shattering (Sareen 2004) a herbaceous species in the Fabaceae (Swanson et. al., 1990) and widespread throughout the World (Knežević et al. 2022).

It is frequently cultivated under marginal management (Russelle and McGnaw 1986), and it is tolerant of a broad range of soil conditions, including poor fertility, acidic, alkaline, and waterlogged environments Because it is not vulnerable to Phytophthora, it tolerates poor drainage better than alfalfa (Putnam and Orloff 2014). *Lotus corniculatus* L. cultivation regions are expanding, as is its resilience to high heat, cold, salty damp conditions, sloping slopes, and poor soil depth. Because of its excellent resilience to harsh climatic circumstances and resistance to parasitic Cuscuta plants, it is favoured in many regions. (Gökalp et al. 2022; Raikar et al. 2008; Uzun et al. 2008; Nicolic et al. 1997; Ahuja et al. 1983; Nicolic et al. 2007; Nicolic et al. 2006).

The Place of *Lotus corniculatus* in Taxonomic Hierarchy

According to Integrated Taxonomic Information System (ITIS) on-line database records (ITIS 2023), its place in the taxonomic hierarchy is as follows.

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridiplantae – green plants
Infrakingdom	Streptophyta – land plants
Superdivision	Embryophyta
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanérogames
Class	Magnoliopsida
Superorder	Rosanae
Order	Fabales
Family	Fabaceae – peas, legumes
Genus	Lotus L. – trefoil, deervetch

Direct Children:
Species Lotus alpinus (Ser.) Schleich. ex Ramond
Species Lotus angustissimus L. – slender bird's-foot trefoil

Species Lotus arabicus L.
Species Lotus arenarius Brot.
Species Lotus chihuahuanus (S. Watson) Greene
Species Lotus collinus (Boiss.) Heldr.
Species Lotus conimbricensis Brot.
Species Lotus conjugatus L.
Species Lotus corniculatus L. – bird's-foot trefoil, birdsfoot trefoil, garden bird's-foot- trefoil, garden birdsfoot trefoil, bloomfell, cat's clover, crowtoes, ground honeysuckle, birdfoot deervetch

Lotus corniculatus L. var. corniculatus, *Lotus corniculatus* L. var. *borealis* Hyl., *Lotus corniculatus* L. var. *carnosus* Hartm, *Lotus corniculatus* L. var. *hirsutus* W. D. J. Koch, *Lotus corniculatus* L. var. j*aponicus* Regel are other conspecific taxa of *Lotus corniculatus* L. (USDA 2023). In English common names of *Lotus corniculatus* L. are bird's-foot trefoil and common bird's-foot trefoil (USDA 2023).]

Geographical Distribution

Lotus corniculatus L. is a widespread throughout the World (Knežević et al. 2022). It is found a lot of place as native form and some were naturalized forms. Lotus corniculatus L is found as native form in Africa Asia and Europe continents and Naturalized/cultivated forms in Australasia (Australıa, New Zealand), Northern America (Canada, United States), Southern America (South America (s.)) continents. Native form of *Lotus corniculatus* in Africa consist of three subcontinent which Northern Afrıca (Algeria (n.), Morocco, Tunisia), Northeast Tropıcal Afrıca (Ethiopia, Sudan), East Tropıcal Afrıca (Kenya, Tanzania). Asia consist of two subcontinent for native form *L. corniculatus* which Asia-Temperate [Western Asıa (Afghanistan, Cyprus, Iran, Iraq, Türkiye), Caucasus (Russian Federation-Ciscaucasia [Ciscaucasia], Armenia, Azerbaijan, Georgia), Russian Federation [Dagestan], Sıberıa (Russian Federation-Western Siberia [Western Siberia (s.w.)], Mıddle Asıa (Kazakhstan, Tajikistan, Turkmenistan), Mongolıa (Mongolia), Russıan Far East (Russian Federation [Primorye]), Chına (China), Eastern Asıa (Korea, Japan, Taiwan)]

and Asia-Tropical (Indıan Subcontınent (India, Nepal, Pakistan)). Distiribitionf of Native form *Lotus corniculatus* in Europe continent consist of five subcontinents. These are Northern Europe (Denmark, Finland, United Kingdom, Ireland, Norway, Sweden), Mıddle Europe (Czechoslovakia, Austria, Belgium, Switzerland, Germany, Hungary, Netherlands, Poland), Eastern Europe (Russian Federation-European part [European part], Belarus, Estonia, Lithuania, Latvia, Moldova, Ukraine (incl. Krym), Southeastern Europe (Former Yugoslavia, Albania, Bulgaria, Greece, Romania), Southwestern Europe (Spain, France), (USDA 2023).

Distiribution of *Lotus corniculatus L.* in Eastern Anatolia region Türkiye

In terms of biodiversity, Turkey has the characteristics of a small continent. Three diverse bioclimate types and three biogeographic regions, including Europe-Siberia, the Mediterranean, and Iran-Turan, are among the causes of this (Anonymous, 2007). Turkey is one of the most significant places for plant gene resources and the origin of many cultivated plants. *Lotus* sp. is one of them (Akgün ve ark. 1998). The Birdsfoot trefoil is the most significant and common native species of Lotus in Turkey (Uzun and Dönmez 2016).

According to Davis (1970), there are around 17 Lotus species in Turkey. The most frequent species in Anatolia, according to the researcher's book "Flora of Turkey," is *L. corniculatus*, which has three subspecies: *alpinus*, *corniculatus,* and *tenuifolius*.

L. corniculatus var. *corniculatus* has been detected in the provinces of Erzincan, Erzurum, Muş, Bitlis, and Hakkari in the eastern Anatolian region, as well as *L. corniculatus* var. *tenuifolius* in the province of Erzincan and *L. corniculatus* var. *alpinus* in the provinces of Kars, Ağrı, Bitlis and Tunceli (Davis, 1970).

Description

Lotus corniculatus has a high protein content and grows mostly for grass, silage, and cover plant or in pure or mixed rangeland (Büyükyıldız et al. 2023). Because of the presence of condensed tannins, it is a nonbloating legume plant (Greenlands et al., 2023; Hunt et al., 2015). It is most adapted to grazing (Casler

and Undersander 2019) and highly suited to pasture-based ruminant production (Hunt et al. 2015). Figure 1 and Figure 2 depicts *Lotus corniculatus* L. images.

Fig. 2. *Lotus corniculatus* (MacAdam and Villalba, 2015)

Lotus corniculatus is a medium perennial (Büyükyıldız et al. 2023), indeterminate flowering (Stephenson,1984), and self-seeds (Putnam 2014) due to the mechanism of the two pod valves twisting spirally and readily ejecting the seeds into the surrounding area, allowing for long pasture survival (Řepková and Hofbauer 2009). When compared to other forages, it increases meat and milk production (Hunt et al. 2015). Grazing birdsfoot trefoil-enriched pasture can be used in a systems approach to control gastrointestinal nematod parasites in grazing sheep (Domingo et al 2018) and improve livestock performance (Greenlands et al., 2023, Waghorn 2008).

It is high in nutrients (Swanson et al., 1990; Hunt et al., 2015). Birdsfoot trefoil has 20.5-16,7 % crude protein, according to Churkova et al. (2016). The NDF ratio, according to Hoveland and Monson (1980), ranged from 30.5% to 42.9%. The dry grass output ranged from 1234.5 to 847.2 kg da-1, whereas the wet grass yield ranged from 5010.5 to 2855.9 kg da-1, according to Karadag et al. (2017). The stem can grow to be 50-70 cm long (Açıkgöz 2021).

Various types of birdfoot are present; erect, semirect and prostate. Prostate sort, expand slowly, are more cold resistant, have recovered more slowly and thinner seedlings than erect forms. Prostate sorts are proper to grazing. The erect cultivars are optimal for haying. Semierect sort ensures two

functions and are the most characteristic cultivated, Birdsfoot trefoil has quite thin stems and is prone to lodging (Casler and Undersander 2019).

Fig. 1. *Lotus corniculatus* L.

Lotus corniculatus used as a model plant for transformation studies in legumes (Akashi et al. 1998). It can be easily regenerated by direct embryogenesis from root explants on MS medium without any hormone (Figure 3), but it requires the addition of cytokinins in MS media for regeneration of leaf explants (Uysal 2014).

Fig. 3. Direct embryogenesis of *Lotus corniculatus* L. root explants in MS medium (Uysal, 2014)

Cultivation and Management

Because the seeds are very small, it is critical that the seed bed be thoroughly prepared, crumbled and pressed, and weed-free. Because birdsfoot trefoil cannot compete with weeds in the first year, it can be seeded in a mixture with a protective plant (Serin and Tan 2001). Because birdsfoot trefoil contains a large percentage of hard seed, seed sowed may germinate many weeks after planting (Casler and Undersander 2019). The seeds of the Birdsfoot trefoil plant have 20-80% hard seed characteristics. For hay, 1-2 kg of seeds should be used per decare, row spacing should be kept 20 cm. Sowing should be done with sowing machine if possible. Pressing the soil with a roller after planting is beneficial seeds contact the soil and also to prevent the seeds from being uncovered. *L. corniculatus* should be planted at a depth of 0.5 cm in heavy soils and 0.5-1 cm in light soils to benefit from soil moisture. It should be sown before effective precipitation in April in locations with cold winters. It is, for example, appropriate for planting in the Eastern Anatolia region in April. In the fertilization of birdsfoot trefoil , first of all, inoculation should be considered. Since the Rhizobium found in the roots of this plant is a special group, the bacteria of other legumes are ineffective. Before planting birdsfoot trefoil in fields, fertilize the soil with plant nutrients judged to be insufficient by soil analysis. Fertilization should be done to fix nutritional deficiencies, especially if the soil lacks P, K, and Ca. Giving birdsfoot trefoil a lot of nitrogen fertilizer (ammonium sulphate, ammonium nitrate, urea) lowers the efficacy of Rhizobium bacteria in its roots. As a result, excessive nitrogen fertilizer should be avoided. As an active component, 4kg/da N (19 kg/da ammonium sulfate, 12.2 kg/da ammonium nitrate, or 22.2 kg/da Di-ammonium Phosphate (DAP) fertilizer should be used with sowing. If 2 tons of farm manure per decare is used during soil preparation in autumn, no further nitrogen fertilizer is necessary. However, care should be taken to ensure that farm manure is thoroughly burnt. Birdsfoot trefoil, a legume fodder plant, needs a lot of phosphorus fertilizer. As a result, 28.5 kg/da Triple superphosphate (TSP) fertilizer, equivalent to 12 kg/da P2O5, should be used with planting. Because there is no rapid loss of nutritional value during development in birdsfoot trefoil, hay harvesting can be postponed for up to 75-100% of the flowering. *Lotus corniculatus* should be mowed 7.5 cm above soil level and final trimmed at least 1-1.5 months before autumn dormancy to store enough reserve nutrients

for the winter season, as regrowth is mostly dependent on leaves remaining after mowing. The plant generates at least two yields every year, depending on the vegetation period. *Lotus corniculatus* seed production is quite tough. Because the ripening of the fruits in the plant takes place in a long period. Some fruits grow and begin to drop seeds, while others remain green fruit or are even in bloom. Ripe pods open readily and spill, resulting in seed loss. However, waiting for all the pods in the plant to harden will cause pod loss during harvest, thus reducing the seed yield. Therefore, the most suitable time for seed formation is when most of the pods in the plant turn completely brown and harden. The seeds are physiologically mature 7-10 days before the fruits (pods) open in the plant. It is possible to facilitate harvesting for the seed by using desiccants before harvesting. Another way is make a light hay trimming before flowering in the fields where seed production. following this implementation can be provided to more homogeneous seed setting. After plants are mow to seed production, dried on an exhibition or field for a few days and then seeds are removed. By carefully planning the planting period, it is essential to guarantee that the seeds germinate with the spring rains. In cases where this is not possible, it should be provided with sprinkler irrigation. After the plants are hold on to the soil, they can be irrigated with release or sprinkler irrigation systems. However, in flood irrigation, the land must be well leveled. In general, it should be watered during the periods when the plant needs water or every 15-20 days, taking into account the climate, regional conditions and the development periods of the plant. Water should be supplied 15 days before and one week after the mowing. In seed production, irrigation should be done as soon as the first flower is seen after then approximately 10-20 days should be cut down watering until almost the entire field blooms. Excessive sprinkler watering in seed production may result in increased air humidity, pollen wetting, and pollen vitality loss. As a result, heavy sprinkler watering should be avoided during pollination. If required, water from the bottom to avoid wetting the blooms (either flood irrigation, subterranean irrigation, etc.). *Lotus corniculatus* cannot compete with weeds in its initial planting year because to weak seedling development. For a clean seedbed, it may be advised to surface plough after encouraging weed germination, to use weed pesticides before planting, or to sow with annual grasses (protecting plant) before planting. Chemical control by applying selected herbicides to manage weeds or cultural

control by altering grazing and mowing time can be done in the following years. Mowing is very effective against broadleaf weeds. However, mowing should be considered with grazing for grassy weeds (Serin and Tan 2001).

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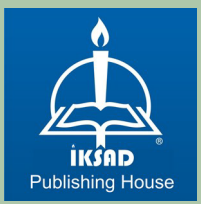

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