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Ümit Apt. No: 22/A Çankaya / Ankara / TR

Telefon / Phone: +90 312 384 80 40

web: www.gecekitapligi.com

e-mail: gecekitapligi@gmail.com



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Doç. Dr. Onur ÜLKER

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Chapter 1

EFFECTS OF H₂O₂ ON THE DROUGHT STRESS IN PLANTS

Gülay ZULKADİR¹

¹ (Dr. Associate); Applied Technology and Management School of Silifke, Mersin University, Mersin, Turkey. E-mail: gzulkadir@mersin.edu.tr; gulayzulkadir@gmail.com

Introduction

A classification of the usable areas in the world based on stress factors indicate that drought stress has a 26% share, mineral stress has 20%, cold and frost stress has 15% and all remaining types of stresses have a share of 29%, whereas, only 10% of the areas encounter no stress factors (Blum, 1986).

Acknowledging the physiological characteristics of plants provides significant benefits for determining growth and development. Hence, studies focused on such physiological characters for long periods. Growth analysis includes highly useful and complex processes that determine the interaction between the plant environment and the molecular biochemical and physiological changes during plant growth. Plant growth corresponds to the period that starts with germination and ends with flowering. During the growth period, any alterations in the interaction between the plant and its environment directly affect the growth and development of the plant (Hunt, 1980).

During stress resistance, plants provide adaptation and stress response at a molecular and cellular level through the accumulation of proteins and osmolytes (Ishibashi et al., 2011). A number of genes are created or suppressed through several functions, due to drought stress (Shinozaki et al., 2003; Bartels & Sunkar, 2005). Furthermore, physiological responses, such as stoma closure, suppression of cell growth and photosynthesis, ion toxicity and respiratory activation, are induced through molecular signaling due to draught stress. Additionally, hydrophobic and hydrophilic amino acids and water interactions are disrupted (Campbell, 1991) and such condition leads to the disruption of protein denaturation and activation of enzymes (Bray, 1997). The degradation of nucleic acids, such as DNA and RNA, is another

disadvantage that occurs due to draught stress. According to Kessler (Kessler, 1961), the RNase activity increase in the leaves of plants exposed to draught stress and such condition occurs due to the transfer of the enzyme from the bound state to the free state. As a result, drought stress becomes the main cause of product loss worldwide (Boyer, 1982; Bray et al., 2000).

O_2 , the free radical, has the same spin quantum number with two decayed electrons. Such degradation in O_2 causes a tendency to accept electrons at each instance and leads to cell damage through molecules called the reactive oxygen species (ROS), especially for future generations. ROS is continuously produced as a by-product of various metabolic pathways located in different cellular parts such as peroxisome, mitochondria and chloroplasts (del Rio et al., 2006; Navrot et al., 2007). Photosynthesis takes place in chloroplasts, a highly organized thylakoid membrane system that contains all the components of photosynthetic apparatus, captures light and provides all structural features to collect optimal light, in tall plants and algae (Gill & Tuteja, 2010).

The balance between the production and release of reactive oxygen species (ROS) is affected by various biotic and abiotic stress factors such as salinity, UV radiation, drought, heavy metals, excessive heat, nutrient deficiency, air pollution, grass and pathogen attacks. Such imbalances lead to a sudden increase in intracellular ROS levels those results with significant damage to cell structures and an estimated consumption of 1-2% O_2 leads to ROS formation in plant tissues (Bhattachrjee, 2005). O_2^- causes the formation of H_2O_2 , OH and other ROS types through various reactions. ROS, which contains O_2^- , H_2O_2 , 1O_2 , HO_2^- , OH, ROOH and RO, leads to protein, fat, carbohydrate and DNA damage, results in cell death and has a highly reactive and toxic effect. It is highly significant to know whether ROS acts as a

damaging, protective and triggering factor, based on the balance between the production and disposal mechanisms of ROS in certain locations and time (Gratao et al., 2005).

Stress-induced ROS accumulation is balanced through enzymatic antioxidant systems that contain various wastes (SOD, APX, GPX, GST and CAT) and non-enzymatic low-molecular metabolites (ASH, GSH, α -tocopherol, carotenoids and flavonoids) and (Gill et al., 2011). The tolerance of the plant towards stress could be improved through the in vivo increase of the level of antioxidant enzymes. It is significant that antioxidants are present almost in all cells and act on ROS detoxification for cellular survival (Gill et al., 2011).

Table1. Function and Localization of ROS-related enzymes and antioxidants during drought stress.

Enzymes / Antioxidants	Function	Localization
SOD	Provides H_2O_2 formation through the dismutation of O_2^- .	cyt, chlo, mit, per
CAT	Detoxifies H_2O_2 without the need for reductants.	mit, per, gly
APX	Provides H_2O_2 detoxification through ascorbate, used as a reductant.	cyt, chlo, mit, per
MDHAR	Reduces mono dehydro ascorbate radicals through the use of NAD(P)H as a reductant.	cyt, chlo, mit
DHAR	Reduces dehydro ascorbate radicals through the use of GSH as a reductant.	cyt, chlo, mit
GR	Reduces the oxidized glutathione through the use of NADPH as a reductant.	cyt, chlo, mit, per
POX	Detoxifies H_2O_2 using various substrates as reductants; these substrates interact with cell wall polymers.	cw, cyt, mit, vac

GPX	Hydroperoxides lipids and detoxifies H ₂ O ₂ using GSH as a reductant.	cyt, chlo, mit, er
GST	Detoxifies lipid hydroperoxides and exhibits DHAR activity.	apo, cyt, chlo, mit, nuc
Ascorbate	It is a substrate of APX and detoxifies H ₂ O ₂ .	apo, cyt, chlo, mit, per, vac
Glutathione	It is the substrate of glutathione transferases and glutathione reductases. Detoxifies H ₂ O ₂ and other hydroperoxides.	apo, cyt, chlo, mit, per, vac
α -tocopherol	Protects membrane lipids from peroxidation, detoxifies lipid peroxides and removes ¹ O ₂ .	membranes
Carotenoids	Removes ¹ O ₂ , it is the messenger molecule of ABA, it is active in photosynthesis.	chlo, chro, amy
Flavonoids	Removes H ₂ O ₂ directly.	vac
cw: cell wall, apo: apoplast, cyt: cytosol, chlo: chloroplast, chro : chromoplasts, amy: amyloplast, mit: mitochondrion, er: endoplasmic reticulum, vac: vacuole, per: peroxisome, gly: glyoximes, nuc: nucleus.		

(Gechev *et al.*, 2006)

As a response to adverse environmental conditions, tall plants develop a complex network of antioxidant systems to balance the high levels of ROS (Sytykiewicz, 2014). Such complex mechanism includes a wide range of antioxidant enzymes such as fat- and water-soluble antioxidants (e.g., tocopherols, beta-carotene, ubiquinone, ascorbate, glutathione), superoxide dismutase (SODs), catalase, glutathione transferase, glutathione peroxidase and ascorbate peroxidase (Ponce de León & Montesano, 2013; de Carvalho *et al.*, 2013). Significant modulations in the antioxidant enzyme activity were observed in plants that were exposed to environmental stress (Sytykiewicz, 2014), such as draught (Tian *et al.*, 2012; Zhang *et al.*,

2013), high or low temperature (Kayihan et al., 2012), ultraviolet-B radiation (Radyukina et al., 2011), darkness (Camejo et al., 2007), high salinity (Rasoulnia et al., 2011), nitrogen deficiency (Rubio-Wilhelmi et al., 2011), carbohydrate supplement (Ślesak et al., 2006), herbicide application (Qian et al., 2011), heavy metal exposure (Rady & Osman, 2012; Navascués et al., 2012), magnetic field effect (Çelik et al., 2009) and pathogen infections (Morkunas et al., 2013).

Furthermore, stomata closure limits the uptake of CO_2 used in photosynthesis in osmotic stress conditions, therefore, high levels of superoxide (O_2^-), which leads to photo inhibition and photo oxidation, accumulate in the chloroplasts (Hsu & Kao, 2003; Yang et al., 2007). Plants developed various mechanisms that eliminate or reduce the damage caused by the reactive oxygen species (Baek & Skinner, 2003), for instance protective, restorative and antioxidant defense mechanisms against oxidative stress caused by free radicals.

Recently, it was established that plants produced ROS to control different physiological processes such as systemic signals and pathogen defense and biotic and abiotic stress-responses (Gill & Tuteja, 2010). H_2O_2 is formed via the decrease in the mono valence of O_2^- . H_2O_2 has moderate reactivity and has a relatively long half-life (1ms), compared to other ROSs with short (2-4) half-lives such as O_2 , OH and $^1\text{O}_2$ (Khatoon et al., 2009).

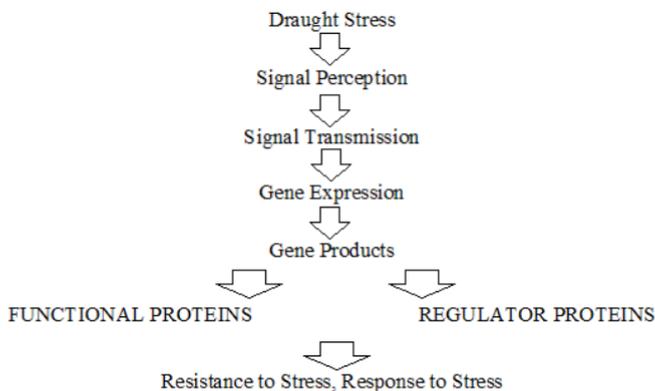
H_2O_2 plays a dual role in plants: it acts as a signaling molecule in triggering tolerance to various biotic and abiotic stresses at low concentrations (Foyer et al., 1997) and leads to programmed cell death at high concentrations (Parida et al., 2004). Furthermore, H_2O_2 also functions as a key regulator in physiological process such as aging (Rubio et al., 2007), light uptake and photosynthesis (Cohu et al., 2009), stomal movement

(Benešová et al., 2012), cell cycle (Goławska et al., 2012), and growth and development (Kar, 2011). H_2O_2 is considered as a second messenger for the signals produced by ROS due to its long life and high permeability between membranes (Parida et al., 2004).

The first phase in the response to drought stress is the perception of stress (Kalefetoğlu & Ekmekçi, 2005). Water directly triggers a cellular signal transmission path. Hence, specific genes are activated through a range of stress response signaling mechanisms due to the detection of dehydration at the cellular level (Bray, 1997).

Hormone response mechanisms of abscisic acid (ABA) play a major role in the generation of cellular responses to drought and activate the function of such response mechanisms. The regulation of ABA biosynthesis and catabolism, the expression of genes based on various ABA-induced drought stresses, and the understanding of signal flow networks associated with gene expressions are significant in understanding plant responses.

In summary, the plant responses to drought stress include the following:



Functional Proteins:

- ❖ Water Channel Proteins
- ❖ Key Enzymes for Osmolyte Synthesis (Proline, Betaine, Sugars)
- ❖ Chaperones
- ❖ Late Embryogenesis Proteins
- ❖ Proteinase
- ❖ Detoxification Enzymes

Regulator Proteins:

- ❖ Transcription Factors (MYB/MYC, bZIP)
- ❖ Protein Kinases (MAPK, MAPKK, S6K, CDPK, SNF 1)
- ❖ Phospholipase C
- ❖ 14, 3, 3 Protein (Kalefetoğlu & Ekmekçi, 2005)

Osmolytes, which are significant for resistance to drought stress, accumulate solids that maintain the turgor. Such osmotic preservatives (organic and inorganic solids) accumulated in organelles and cytoplasm are not essentially present in vacuoles (Moghaieb et al., 2004). Organic compounds have a stable structure and are not easily metabolized in the cell, thus do not cause any effect on the cellular functions even at high concentrations (Iba, 2002). Examples of osmotic preservatives are proline, betaines, dimethyl sulfoniopropionate (DMSP), polyols (mannitol, sorbitol, pinitol), trehalose and fructans (Smirnov, 1998). Proline plays an active role in protecting subcellular structures and removing free radicals (Mani et al., 2002). Glycine betaine (GB) is involved in the protection of quaternary structures and membranes of complex proteins and enzymes under high stress conditions (Gorham,

1995). Sucrose plays a protective role against various environmental stresses through osmotic regulation, maintaining phospholipids, stabilizing the membrane bilayer in the liquid-solid phase, and through preventing changes in the structure of soluble proteins (Kerepesi & Galiba, 2000). Furthermore, it is considered that sucrose could have a radical removal effect (especially the hydroxyl radical) and increase oxidative stress resistance (Shen et al., 1997). Such effect is provided by the enzymes (proline-5-carboxylate synthetase, proline-5-carboxylate reductase, betaine aldehyde dehydrogenase, choline monooxidase, etc.) involved in the synthesis of osmolytes (Moghaieb et al., 2004).

MAP kinase, MAPKK and MAPK activate the expression of certain genes that play a role in drought stress through phosphorylation and activation of transcription factors (Jonak et al., 2000). Several genes encode bZIP and AP2/EREBP regulatory factor members, which consequently regulate the expression of these genes. Among various transcription factor families in plants, the AP2/EREBP family is relatively new and unique to plants and functions as the DNA-binding and protected region, called the AP2 region (Okamoto et al., 1997).

Table.2. Accumulated compounds and their possible functions in tolerance due to the responses to salinity and drought stress.

Group	Specific Compounds	Function
Ions	Sodium, Chlorine	Osmotic regulation, Potassium output
Pigments	Carotenoids, Anthocyanins	Protection against photoinhibition

Polyols	Mannitol, Pinitol	Carbon oil, Osmotic regulation, Osmo-protectant, Osmotic regulation, Photochemical activity of FSII, Radical remover
Amino acids	Proline	Osmotic regulation, Osmo-protectant
Quaternary amines	Glycine Betaine	Osmo-protectant, Protection of thylakoid and plasma membrane integrity
Polyamines	Sperm, Spermidin	Ion balance, Protection of chromatin
Sugars	Glucose, Fructose, Sucrose, Fructans	Osmotic regulation, Osmo-protectant, Carbon source
Proteins	Osmotin, Superoxide Dismutase, Catalase	Pathogenesis-related proteins, Osmo-protectant, Radical detoxification

(adopted from Parida et al., 2004)

Membrane phospholipids also form an efficient system that produce a large number of signal molecules such as inositol 1,4,5-triphosphate (IP₃) and diacylglycerol (Munnik & Meijer, 2001). It was observed that IP₃ levels rapidly increased due to drought stress in various plant systems (Dewald et al., 2001; Takahashi, 2001). IP₃ activates the expression of genes induced via the osmotic stress (Wu et al., 1997), through the increase in cytoplasmic Ca₂⁺ and stomata closure (Sanders et al., 1999).

Table.3. Genes considered to be activated under draught stress and the characteristics of these genes.

Plant Species	Genes	Characteristics Properties	References
<i>Arabidopsis thaliana</i>	Sal 1	Stimulated via salinity stress, its over-expression in <i>Arabidopsis</i> reduces Na ⁺ and Li ⁺ toxicity.	Parida & Das, 2005
<i>Brassica napus</i>	Bnd 22	Produces 22 kDa protein in salinity stress.	Parida & Das, 2005
<i>Dunaliella salina</i>	P150	Produces 150 kDa protein, de novo synthesized protein increases due to salinity stress.	Parida & Das, 2005
<i>Hordeum vulgare</i>	hva 1	Stimulated via ABA, drought, NaCl and cold application. Produces LEA protein.	Mugdhal, 2010; Salama & ark., 2007
<i>Lycopersicon esculentum</i>	le-16 geni	PEG is stimulated via drought, salinity, cold and hot stresses.	Parida & Das, 2005
<i>Mesembryanthemum crystallinum</i>	ppc-1, ppc-2	PEP encodes carboxylase, stimulated via water and salt stresses.	Parida & Das, 2005
<i>Mesembryanthemum crystallinum</i>	Imt 1	Myo-inositol encodes o-methyl transferase enzyme and is stimulated via NaCl and osmotic stresses.	Parida & Das, 2005
<i>Oryza sativa</i>	Inps 1	Myo-inositol encodes 1-phosphate synthase enzyme.	Parida & Das, 2005

<i>Oryza sativa</i>	RAB21	Stimulated via water stress. The encoded protein is accumulated in NaCl applied plants.	Parida & Das, 2005
<i>Oryza sativa</i>	salt	Stimulated by ABA along with NaCl and KCl and the encoded protein is accumulated in roots and shoots.	Parida & Das, 2005
<i>Oryza sativa</i>	em	Stimulated by ABA and salt stress, salt and ABA are synergistically affected.	Parida & Das, 2005
<i>Medicago sativa</i>	Alfin 1	Transcription factor is encoded undersalinity stress.	Winicow, 1998
<i>Medicago sativa</i>	MsPRP2	Produces the cell wall protein under salinity stress.	Winicow, 1998
<i>Arabidopsis thaliana</i>	AtP5CS	ABA is stimulated via salinity and dehydration stresses, plays a role in proline biosynthesis.	Winicow, 1998
<i>Arabidopsis thaliana</i>	ARSK 1	Salinity and dehydration stresses are stimulated via ABA; protein kinase is encoded.	Winicow, 1998
<i>Arabidopsis thaliana</i>	ATCDPK1	Stimulated via salinity and dehydration stresses; protein kinase is encoded.	Winicow, 1998
<i>Arabidopsis thaliana</i>	ATCDPK2	Stimulated via salinity, dehydration stress and ABA; protein kinase is encoded.	Winicow, 1998

<i>Triticum sp.</i>	PKABA1	Protein kinase is encoded under salinity stress.	Winicow, 1998
<i>Zea mays</i>	mlip15	Stimulated via salinity and ABA; transcription factor is encoded.	Winicow, 1998
<i>Spinacia oleracea</i>	BADH	Betaine encodes dehydrogenase enzyme and plays a role in betaine synthesis.	Türkan & Demiral, 2009
<i>Nicotiana tabacum</i>	TPX2	Peroxidase involved in cell wall structure is encoded.	Borsani et al., 2003
<i>Oryza sativa</i>	OsCDPK7	Protein kinase that regulates gene expression is encoded.	Borsani et al., 2003
<i>Arabidopsis thaliana</i>	DREB1A	Transcription factors that regulate gene expression are encoded.	Borsani et al., 2003
<i>Hordeum vulgare</i>	HVD1	Regulates photosynthesis reactions in chloroplasts under salinity stress.	Vashisht & Tuteja, 2006
<i>Pisum sativum</i>	PDH45	Regulates DNA/RNA metabolism under salinity stress.	Vashisht & Tuteja, 2006
<i>Pisum sativum</i>	PDH47	Regulates DNA/RNA metabolism under salinity stress.	Vashisht & Tuteja, 2006
<i>Gossypium hirsutum</i>	GhNAC1-6	Stimulated via high salinity, drought and ABA, plant-specific transcription factors are encoded.	Meng et al., 2009
<i>Tamarix hispida</i>	ThbZIP1	bZIP transcription factors are encoded due to NaCl application.	Wang et al., 2010

<i>Glycine max</i> L. Merrill	GolS	Possible galactinol synthase gene.	Ishibashi et al., 2011
<i>Glycine max</i> L. Merrill	P5C5	Delta-proline-5-carboxylate synthase gene involved in glycine betaine accumulation.	Ishibashi et al., 2011
<i>Glycine max</i> L. Merrill	Actin	The gene effective on the actin mechanism.	Ishibashi et al., 2011
<i>Glycine max</i> L. Merrill	MIPS	D-myo-inositol is the 3-phosphate synthase gene that catalyzes Phatic acid.	Ishibashi et al., 2011
<i>Glycine max</i> L. Merrill	RD29	Activated by osmotic stress, low temperature and ABA.	Yamaguchi-Shinozaki & Shinozaki, 1994
<i>Medicago truncatula</i>	WXP1	Possible AP2 domain-containing transcription factor gene.	Zhang et al., 2005

(adopted from Yilmaz et al., 2011)

In recent years, the role of reactive oxygen species, especially hydrogen peroxide, in generating a large number of signal transmissions, was widely acknowledged. Such condition includes bacteria (Demple & Amábile-Cuevas, 1991) and plant stress regulating genes and activation of the transcription factor NF-KB in mammalian cells (Schreck et al., 1991). It was established that hydrogen peroxide functioned as a signal under osmotic stress (Guan et al., 2000), in the elimination of ABA-induced protection cells (Pei et al., 2000), in case of excessive stress (Karpinski et al., 1999) and in the contamination response to the avirulent pathogens that cause hypersensitivity (Alvares et al., 1998). It was observed that SOD, CAT, APX and GR activities increased in leaves due to the induction of

related isoform(s) under H_2O_2 stress conditions (Gill et al., 2011).

Conclusion

Plant response to biotic and abiotic stress conditions depends on the cause and amount of stress and includes various complex processes. Such response is due to several physiological, molecular and biochemical phenomena and their interactions that affect the growth and development of the plant under stress factors. Currently, the development of stress resistant varieties is highly important, considering the necessity to meet the increasing needs of the increasing population of the world, especially in developed and developing countries. In order to accomplish such objective, it is essential to know the mechanisms of action that provide tolerance. The present study was, therefore, intended to explain the effect mechanisms of H_2O_2 , one of the reactive oxygen species, on plants, and to represent a basis for further studies that focus on increasing the tolerance of plants that are exposed to drought stress.

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Chapter 2

CROP ROTATION AS AN ENVIRONMENT FRIENDLY OPTION FOR WEED MANAGEMENT IN SUSTAINABLE COTTON CULTIVATION

Firat PALA^{1}, Husrev MENNAN²*

1 Dr., Department of Plant Protection, Agriculture Faculty Siirt University, 56100, Siirt, Turkey

* Correspondence: firatpala@siirt.edu.tr; Tel.: +90-484-2121111 (Turkey)

2 Prof. Dr., Department of Plant Protection, Agriculture Faculty, Ondokuz Mayıs University, 55139 Samsun, Turkey

Introduction

Cotton (*Gossypium hirsutum* L.) is the most preferred natural fiber, wheat (*Triticum aestivum* L.) is the main grain used in human nutrition, and Corn (*Zea mays* L.), also known as *maize* to many people, is the *leading* cereal crop in the world followed by rice and wheat (FAO, 2017). Cotton and maize (corn) became a monoculture crops in irrigated areas and wheat in dry areas due to being profitable and strategic crops (Jalota, 2006; Anonymous, 2019). This situation caused weed problem in these crops. Weeds can reduce average yield by 40-50% with wheat and 10-90% with cotton by competing for nutrients, water, and canopy (Oerke, 2006). Herbicides have been the mainstay of weed management for the last 50 years (Kudsk, 2008). The use of herbicides in weed control has been increasing in recent years. Farmers prefer the use of herbicides in both cotton, corn and wheat crops to weed management because it is cheap and practical (Abouzienna, 2016; Pala and Mennan 2017a; Pala and Mennan 2017b). However, excessive and unconscious herbicide use causes agronomic, environmental, and herbicide resistance problems (Heap, 2014). Weeds resistant to common cotton herbicides (for instance: nightshade, cutleaf groundcherry, velvetleaf, cocklebur and strains of goosegrass are resistant to the dinitroaniline herbicides) are a problem with continuous cotton. Furthermore, even in cultural practices that support herbicides, weeds are still prominent in planting areas (Pala and Mennan, 2018).

Effective management strategies can improve weed suppression and decrease reliance on traditional control tactics such as hoeing and using herbicides, also streaming (Pala et al., 2017c). There is also a potential to reduce environmental impacts, increase provisioning of ecosystem services, and ultimately lead to more sustainable agriculture (Kronenberg, 2014). One possible strategy to reduce weed community density is designing

rotations with crops having different life cycles (Anderson et al., 2007). Rotation of crops is the most effective means yet devised for keeping land free of weeds (Liebman and Dyck, 1993). Crop rotation, which is the practice of cyclically growing a sequence of different plant species on the same field of land following a defined order of the crop succession with a fixed length can control weeds as a cultural measure (Leteinturier et al., 2006). Rotations that minimize weed community density may provide an additional benefit; some crops grown such as preserving land quality, reducing herbicide usage and provides higher grain yields achieving (Liebman et al, 2001; Liebman and Dyck, 1993). With lower weed density, yield and economic returns may improve with cotton, corn and wheat. Also, crop diversity will help producers manage herbicide resistance by increasing the opportunities to rotate herbicides with different modes of action. Resistant weeds are common in this these crops (Heap, 2014).

Weeds life cycle and spreading are lower in crop rotation compared to monoculture (Bastiaans 2010). Rotate your cotton crop as needed with other field or vegetable crops to maintain soil productivity and reduce the incidence of various cotton pests such as nematodes, *Verticillium* wilt, seedling diseases, pink bollworm and other diseases. Different rotational crops impart different benefits to the soil and therefore to subsequent seasons of cotton production: i) Pest resistant crops: suppression of various cotton pests, ii) Cereals: have fibrous root systems that loosen compacted soil, iii) Legumes (such as alfalfa, beans): add nitrogen to soil, iv) Grain corn: adds organic matter to soil, v) Vegetable crops: contribute high fertilizer carryover. Crop rotations have been reported as effective strategies for increasing crop yields compared to continuous cotton (Reddy et al., 2006). The majority of cotton growers now favour sowing a cereal or leguminous crop in rotation

with cotton rather than back-to-back cotton (Farrell et al., 2008). The most recent survey (2005/06 season) indicated that across the industry rotations were used by 82% of cotton growers (Doyle and Coleman, 2007). Wheat was the favoured rotation crop with over 70% of NSW cotton growers who used rotation crops growing either a 1:1 or 2:1 cotton:wheat rotation (Hickman et al., 1998; Cooper, 1999). However, these crop rotations are not universally common, rather they depend on different environmental and soils conditions (Ouda et al., 2013). The use of a variety of long-term rotations also helps to increase grain yield. It has documented that US grain producers believe that the use of 2 and 3-year crop rotations produces higher crop yields than those grown as monoculture (Baldwin, 2006). A study conducted in Turkey was reported to be in accordance with barley and wheat crop rotation with cotton (Tugay, 1988). On the other hand, cultivating plants in monocultures can lead to changes in the floristic composition, contributing to the spread of resistant and therefore harmful weeds (Stefanović et al., 2011). Reported yield increases might be due to greater residue diversity and soil health. Cotton and corn rotations can increase soil organic carbon compared to continuous cotton (Mitchell and Entry, 1998), mainly due to amounts of corn biomass produced. Given that crop rotations and winter cover can alleviate some of the problems associated with no-till, as well as improve yields, research into their combined effects on crop yields in a no-till system is necessary to make best management recommendations. In this context, cereal-cotton rotations can be researched to be integrated into methods such as narrowing of rows, late sowing, use of cover crops and selection of herbicide-tolerant varieties for the development of sustainable agricultural systems in the long term.

Crop rotation is not a cultural precautionary component that is applied consciously in the cotton, corn

and wheat that cover more than two-thirds of the arable land in the Diyarbakir, Turkey. This technique, which is an important application for weed control, cannot be applied due to lack of water for cotton in dryland wheat production systems, it has been evaluated as cotton-wheat rotation in available irrigation region. Crop rotation can control weed species, weed seeds and seedling (Cardina et al, 2009). Many of our tough cotton weeds can be controlled with herbicides used on other crops, such as corn. In addition, the ground shading with a vigorous corn crop will further suppress weeds that would tower over cotton. The various weeds have been controlled successfully with the following rotational crops and herbicide programs. In addition to rotation systems that include diverse crops (i.e., wheat, cotton) facilitate the use of herbicides with different modes of action and thus reduce the risk of resistance development (Norsworthy et al., 2012). There is insufficient information as to whether cotton, corn and wheat will benefit the management of weeds when rotated. Also in Turkey, Southeastern Anatolia Region and in the province of Diyarbakir did not find any studies. Hence, more region-specific data are needed on cotton yield response to crop rotation. Consequently, research is needed on investigating the potential effects of wheat-cotton rotation system on weed density and yield in comparison to monoculture cotton production.

Materials and Method

The study of the effects of cotton growing in monoculture and crop rotation with maize and winter wheat was conducted as a 5-yr (2014-18) field experiment in Bismil, Diyarbakir, 2 (37.97764 °N, 40.622766 °W). In Diyarbakir, the soils are generally clayey and loamy and silty. There is no alkalinity and salt problems in the soil. The soil is moderately calcareous and weak in organic matter. 42% of the total land assets in the province of

Diyarbakır are I-IV. class soils (Anonymous, 2018). As a result of the analysis of samples taken from the soil of experiment field, the soil pH was 7.39, organic matter content was 1.43%, salt content was 0.036% and lime content was 8.15% was determined. Monthly mean temperature was computed from daily measurements collected at a standard weather station located at the study site for the determination of precipitation conditions.

Cotton (Stoneville-468), maize (P1921), and wheat (Saggitario) were sown in each field, as a main crops were tested in the crop rotation system. The experimental design accommodated three cotton-based cropping systems: i) cotton (C)/cotton (C)/cotton (C), ii) cotton (C)/maize (M)/cotton (C), and iii) cotton (C)/wheat (W)/cotton (M) that were first established as short time crop rotation in the spring 2015. This paper presents the results obtained in 2017, that is, after 3 years of cotton cultivation in monoculture and rotations. The 3-yr system (C/C/C) was used as a control. The experimental design was a split-block design with four replications or blocks. There were a total of 36 plots in the blocks during each rotation period (nine plots per block). Individual plots consisted of eight rows spaced 6 m apart and 30 m long (6 m × 30 m). Seeded plots (i.e., C, M, and W) were subdivided into three areas: i) a weedy plot (6 m × 3 m) where no weed control was applied, ii) a weed free plot (6 m × 3 m) where weeds were pulled by hand, and iii) a general plot (6 m × 24 m) where weeds were managed with pre-plant and post-emergence herbicides commonly used in the region. So, pendimethalin as preplant and clethodim as postemergence in cotton; mesosulfuron methyl+mefenpyr-diethyl and 2,4-D EHE + florasulam by the herbicidal combination as postemergence in wheat; mesotrione + nicosulfuron in maize were used in the recommended doses. After cotton and corn planting, glyphosate was used to control emerged weeds as total

herbicide. After harvest, all plots were treated with glyphosate to control late-season broadleaf weeds that re-grow. Herbicide applications in C, M, and W (Table 1) were applied during spring and summer using a self-propelled sprayer.

Table 1 Herbicide applications in the general area of plots of cotton (C), maize (M), and winter wheat (W).

Year	Date	Crop plot**	Description
2014/7	Pre-emergant	C and M	Glyphosate 480 g/l SL 300 ml/da
2015	Apr 25, pre*	C	Pendimethalin 450 g/l CS 300 ml/da
2015	Jun 2, post**	C	Clethodim 116,2 g/l EC 125 ml/da
2016	Mar 7, post	W	2.4-D EHE 452,42 g/l + Florasulam 6,25 g/l SE 50 ml/da
2016	Mar 7, post	W	Mesosulfuron-methyl 30 g/l + mefenpyr-diethyl 90 g/l OF 40 ml/da
2016	Apr 28, pre	C	Pendimethalin 450 g/l CS 300 ml/da
2016	Jun 4, post	C	Clethodim 116,2 g/l EC 125 ml/da
2016	May 29, post	M	Mesotrione 75 g/l + Nicosulfuron 30 g/l OD 200 ml/da
2017	Apr 22, pre	C	Pendimethalin 450 g/l CS 300 ml/da
2017	Jun 5, post	C	Clethodim 116,2 g/l EC 125 ml/da

*Pre = pre-plant, **post = post emergence *** C=Cotton
M=Maize W=Wheat

Cotton, maize and winter wheat plots were seeded with cultivars. Cotton was seeded at the 3-4 cm depth at a 70 cm intra-row spacing, seed density was 20-30 kg ha⁻¹, maize was seeded at the 6-8 cm depth at a 70 cm intra-row spacing, seed density was 20-25 kg ha⁻¹, and winter wheat was seeded at the 2.5 cm depth at a 15 cm intra-row spacing, seed density was 200-250 kg ha⁻¹. All plots were seeded when moisture conditions were appropriate in all years. 300 kg ha⁻¹ 20.32.0.+15(SO₃)+Zn, 600 kg ha⁻¹ 13.24.12+14(SO₃)+Zn and 300 kg ha⁻¹

20.32.0+15(SO₃)+Zn fertilizers were banded at seeding as base fertilizer in cotton, corn and wheat respectively. Top fertilization of cotton (200 kg ha⁻¹ amonyum nitrat during hoeing and 150 kg ha⁻¹ amonyum nitrat before irrigation), corn (400 kg ha⁻¹ urea during three weeks after emergence and 200 kg ha⁻¹ amonyum nitrat 6 weeks after emergence) and wheat (200 kg ha⁻¹ amonyum nitrat during tillering and 150 kg ha⁻¹ amonyum nitrat during stem extension) was done in two periods. C, M, and W plots were harvested by hand to examined the influence of cotton cultivation in monoculture and crop rotation.

To evaluate the effect of the three cropping systems (one monoculture and two crop rotation) on the weed density and cover, 16 sampling frames (1 m × 0.5 m) were placed in each seeded plot: four in the weedy sub-plot, four in the weed-free sub-plot, and eight in the general area. Sampling frames were constructed from 1.25 cm diameter PVC pipe. Weed surveys are mainly undertaken either in autumn, spring or summer. All plots were sampled three times during the growing season: i) early season at beginning of weed competition (begining of March, wheat in tillering; begining of May, maize in seedling; end of May, cotton in seedling), ii) mid-season at peak crop growth (end of May, wheat in flowering; begining of July, maize in flowering; end of July, cotton in flowering), and late-season at crop maturity (end of June, wheat in harvest; end of September, maize in seedling; end of October, cotton in seedling). Therefore, the number of sampling frames per year was 648. At each sampling time, percent cover of each weed species was estimated visually within each frame (Hanzlik and Gerowitt, 2017). Density (plants m⁻²) per weed species was also determined by counting the number of plants in each frame (Thomas, 1985). Each plot of cotton, maize, and wheat was hand-picked and the resulting crop was cleaned and weighted for yield determination. Data were subjected to analysis of

variance using JMP 5.0.1. The significance of differences among mean values was tested by LSMeans Differences Tukey HSD test values at a probability ($P < 0.05$).

Results and Discussion

Weeds were counted before and after application of the herbicides to determine which herbicide was better than the others to control the number of broad and narrow leaf weeds. The relevant data are presented in Table 2.

Table 2 *Deneme alanında bulunan yabancı otlar ve yoğunlukları*

Weeds	Common name	Family	Density (plant m ⁻²)
<i>Amaranthus retroflexus</i> L.	Redroot pigweed	Amaranthaceae	1.74
<i>Avena sterilis</i> L.	Wild oat	Poaceae	6.53
<i>Chenopodium album</i> L.	Lamb's quarters	Chenopodiaceae	1.02
<i>Convolvulus arvensis</i> L.	Field bindweed	Convolvulaceae	1.05
<i>Cynodon dactylon</i> (L.) Pers.	Bermuda grass	Poaceae	1.12
<i>Echinochloa crus-galli</i> (L.) P. Beauv.	Barnyard grass	Poaceae	1.39
<i>Galium aparine</i> L.	Stickywilly	Rubiaceae	1.44
<i>Sinapis arvensis</i> L.	Wild mustard	Brassicaceae	8.56
<i>Sorghum halepense</i> (L.) Pers.	Johnsongrass	Poaceae	3.27
<i>Xanthium strumarium</i> L.	Cocklebur	Asteraceae	2.40

When the family distribution of the determined number of species is considered, Poaceae took the first place. Wild mustard (8.56 plant m⁻²) was the most intense species. Overall weed density in the trial area was found

to be 26% on average. Crop rotations had significant effect on weed control and cotton yield in this study similar to Kovačević et al. (2008) indications. According to obtained data, significant influence of applied treatments on crop rotation parameters were recorded (Table 3).

Table 3 Weed and cotton yield parameters affected by crop rotations

Crop rotations	Weed mortality (%)	Weed control effect (%)	Beginning of flowering (days)	First boll opening (days)	Number of fruit branches (pcs plant ⁻¹)	Number of boll (pcs plant ⁻¹)	Boll bulk weight (g)	100 seed weight (g)	Crop yield (t ha ⁻¹)
C-C-C	63 ^c	76 ^c	75 ^a	95 ^a	15 ^a	21 ^a	4.78 ^c	9.57 ^c	4.39 ^c
C-M-C	88 ^a	94 ^a	78 ^a	91 ^a	18 ^a	24 ^a	5.55 ^a	11.29 ^a	4.97 ^a
C-W-C	79 ^b	85 ^b	76 ^a	93 ^a	16 ^a	22 ^a	5.21 ^b	10.64 ^b	4.78 ^b

$$\text{Alpha} = 0.050, Q = 3.06815$$

Levels not connected by same letter are significantly different at $p \leq 0.05$

C = cotton, M = maize (corn), W = wheat

Among the crop rotations maximum weed mortality (88%) were recorded under CMC than the other practices during three years of study, in addition maximum weed control effect (94%) were recorded in CMC cropping system during field trials. In the parcels where the corn plant was rotated with cotton, it was observed that the density of the weeds decreased due to the suppression of the weeds, especially the corn plant, and this situation caused an increase in the yield. The other hand, as shown in the table CWC system parameters is better than CCC such as weed control and crop yields. Jalota et al. (2006) showed that cotton-wheat rotation helps to improve yield and to solve some ecological problems in parallel with our findings. CMC (4.97 t ha⁻¹) had maximum cotton yield than other rotations in the study. In this current (2015 to

2017) cotton yield study, cotton-maize-cotton rotations indicated a 13% increase in cotton yield compared to continuous cotton. This increase in yield appears to be around 9% in cotton-wheat-cotton rotation. Ashworth et al. (2016) reported specifically, the inclusion of soybean or cotton twice within a 4-yr cropping cycle increased yields 6 and 7%, respectively, whereas including cotton and soybean once within a 4-yr cycle was analogous to that of continuous corn. In our findings, the effect of cotton-maize rotation on yield doubled according to this study may be caused by climatic-soil factors, weed diversity and maintenance procedures. Contrarily, CCC rotation systems had the lowest number of productive bolls, branches, bulks and yielded (4.39 t ha^{-1}) in the three years of field experiments. The poor cotton productivity under CCC cropping system was probably due to higher weed infestation because weeds compete with cultivated crops for light, moisture, space and essential nutrients.

Conclusion

Crop rotation describes the sequence of different crops grown in the same field. Crop rotation is critical in order to achieve the productivity improvements on offer in cotton. Different crop rotations had a variable effect on weeds and yield. Cotton-Maize-Cotton rotation had particularly strong suppressive effects against weeds in cropping systems. The impact cotton and cereal rotations have on weed control provides an insight into how crop rotations build an inherently more productive cropping system. This cropping system in which there is a combination of winter and summer cropping will reduce spread risk of weeds and decrease the likelihood of herbicide-resistant weeds, and build up integrated weed management. All tested parameters, weed control effect (%) and cotton yield had the higher values in treatments included crop rotation. Herbicide application helped

by weed suppression, but crop rotation applications can significantly influence on weeds. This means that simple crop rotation of cotton with maize and wheat can be an important tool for reducing crop yields, and solution to weed problems.

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