# EFFECTS OF GIBBERELLIC ACID, PROLINE AND HUMIC ACID ON THE GROWTH, YIELD AND CHEMICAL COMPOSITION OF TWO *Zea mays* L. CULTIVARS (BAGHDAD-3 AND FAJIR-1)

**by**

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| | |
|---|---|
| AAFP | Atomic Absorption Flame Photometer |
| ANOVA | Analysis of variance |
| ASA | Ascorbate |
| APX | Ascorbate peroxidase |
| ABA | Ascorbic Acid |
| BA | Benzyladenine |
| COOH | Carboxyl group |
| CAT | Catalase |
| CPA | Chloro phenoxy acetic acid |
| CCC | CYCOCEL |
| FA | Fulvic acid |
| GIDI | GA-insensitive dwarf1 |
| GA | Gibberellic acid |
| GSA | Glutamic semialdehyde |
| GCA | Glutamic-y-semialdehyde |
| GDP | Gross domestic product |
| HA | Humic acid |
| HS | Humic substances |
| IBA | Indole -3- butyric acid |
| IAA | Indole acetic acid |
| NAA | Naphthalic acetic acid |
| NP | Nitrogen and phosphorus |
| NPK | Nitrogen, phosphorus and potassium |

| | |
|---|---|
| PCD | Programmed cell death |
| P5C | Pyroline-5- carboxylate |
| RCBD | Randomised Complete Block Design |
| ROS | Reactive oxygen species |
| RDF | Recommended dose of fertiliser |
| RNA | Ribonucleic acid |
| UV | Ultraviolet |
| USDA | United States Department of Agriculture |

**KESAN ASID GIBERELIK, PROLINA DAN ASID HUMIK KE ATAS PERTUMBUHAN, HASIL DAN KOMPOSISI KIMIA DUA KULTIVAR *Zea mays* L. (BAGHDAD-3 DAN FAJIR-1)**

## ABSTRAK

Tanaman jagung atau *Zea mays* dianggap sebagai salah satu daripada bijirin utama yang dimakan oleh manusia di seluruh dunia. Permintaan untuk jagung telah meningkat secara mendadak dan menerima banyak perhatian kerana kepentingannya dari segi ekonomi dan penggunaan. Oleh itu, kajian ini dijalankan untuk mengkaji kesan asid gibberellik, proline dan asid humik, dan kesan sinergi mereka terhadap pertumbuhan, hasil dan komposisi kimia dua kultivar *Zea mays L*. di Iraq, iaitu Baghdad-3 dan Fajir-1. Suatu ujikaji lapang dilakukan ke atas tanah liat gembur dari bulan Mac hingga Julai 2014 di stesen penyelidikan Ghlibia, Diyala, Baghdad. Satu Reka Bentuk Blok Lengkap Rawak (RCBD) yang terdiri daripada 18 rawatan telah dijalankan untuk setiap kultivar jagung dan setiap rawatan diulang sebanyak tiga kali. Rawatan tersebut telah dijalankan dengan asid gibberellik (0, 100, dan 200 mg/l), proline (0, 100, dan 200 mg/l) dan asid humik (0 dan 2.4 g/l). Daun-daun tumbuhan telah disembur sebanyak dua kali dengan asid gibberellik dan proline. Aplikasi pertama adalah semasa peringkat selepas percambahan 4-6 daun, manakala aplikasi kedua adalah pada awal peringkat berbunga. Asid humik ditambahkan terus ke tanah semasa dua peringkat tersebut. Asid gibberellik, proline dan asid humik ditambah secara individu dan juga secara serentak mengikut reka bentuk eksperimen. Penuaian dilakukan secara manual pada peringkat matang tumbuhan tersebut selepas 120 hari untuk menyiasat ciri-ciri tumbuhan tersebut. Data telah dianalisis secara statistik dengan menggunakan analisis varians (ANOVA), dan dibandingkan dengan menggunakan Ujian Julat Berganda Duncan pada tahap kebarangkalian 0.01 dan

0.05. Keputusan menunjukkan bahawa penambahan asid gibberellik, proline dan asid humik secara individu telah menyebabkan kenaikan yang signifikan ($p<0.01$) dalam ciri-ciri vegetatif, iaitu ketinggian tumbuhan, keluasan daun, dan berat segar pucuk dan akar. Juga, kenaikan ketara telah diperolehi ($p<0.01$) dalam hasil kultivar dan ciri-ciri hasil komponen, iaitu bilangan tongkol setiap tumbuhan, berat bijirin setiap tongkol dan setiap tumbuhan, hasil bijirin setiap plot dan setiap hektar. Sebaliknya, keputusan tersebut menunjukkan kenaikan yang signifikan ($p<0.01$) dalam komposisi kimia, iaitu peratusan minyak bijirin, protein, fosforus, dan kandungan K, Na, Ca, Mg, FE, Mn dan Cu dalam daun. Sebaliknya, Baghdad-3 menunjukkan tindak balas yang lebih tinggi daripada Fajir-1 dalam kebanyakan ciri-ciri yang dikaji. Kesan sinergi antara 100 mg/l asid gibberellik, 200 mg/l proline dan 2.4 g/l asid humik dengan jelasnya menyebabkan kenaikan dalam semua ciri-ciri yang dikaji, tetapi kenaikan tersebut tidak ketara.

# EFFECT OF GIBBERELLIC ACID, PROLINE AND HUMIC ACID ON THE GROWTH, YIELD AND CHEMICAL COMPOSITION OF TWO *Zea mays* L. CULTIVARS ( BAGHDAD-3 AND FAJIR-1)

## ABSTRACT

The corn plant or *Zea mays* is considered as one of the major food grains that have been consumed by people around the world. The demand for corn has increased dramatically and gained lots of attentions due to its economic importance and utility. Thus, this study was conducted to investigate the impacts of gibberellic acid, proline, and humic acid, and their synergistic effect on the growth, yield and chemical composition of two Iraqi cultivars of *Zea mays* L., Baghdad-3 and Fajir-1. A field experiment was carried out in a clay loam soil from March to July 2014 at the Ghlibia research station, Diyala, Baghdad. A Randomised Complete Block Design (RCBD) consisted of 18 treatments for each cultivar of corn was conducted and each treatment was replicated three times. The treatments were conducted with gibberellic acid (0, 100, and 200 mg/l), proline (0, 100, and 200 mg/l) and humic acid (0 and 2.4 g/l). Gibberellic acid and proline were sprayed twice on the plant leaves. The first application was during the stage of 4–6 leaves post germination, while the second was at the beginning of flowering stage. Humic acid was added directly to the soil during the two stages. Gibberellic acid, proline, and humic acid were added individually as well as in simultaneously, in accordance with the experimental design. Harvesting was done manually during the mature stage of the plant at the age of 120 days, so as to investigate the plant characteristics. Data were statistically analyzed using variance analysis (ANOVA), and compared using Duncan's Multiple Range Test at 0.01 and 0.05 levels of probability. Results show that the additions of gibberellic acid, proline, and humic acid individually caused significant increments

($p<0.01$) in the vegetative characteristics i.e. plant height, leaf area and fresh weights of the shoot and root. Also, significant increments were obtained ($p<0.01$) in the cultivars yield and yield component characteristics i.e. number of ears per plant, weight of grains per ear and per plant, grain yield per plot and per hectare. On the other hand, the result showed significant increments ($p<0.01$) in the chemical composition i.e. grain oil, protein, phosphorus percentages, and leaves content of K, Na, Ca, Mg, Fe, Mn, and Cu. On the other hand, Baghdad-3 showed higher response than Fajir-1 in most of the studied characteristics. The synergistic effect between 100 mg/l gibberellic acid, 200 mg/l proline and 2.4 g/l of humic acid caused noticeable increments in all the studied characteristics, but these were not significant.

# CHAPTER 1

# INTRODUCTION

## 1.1 General Introduction

Agriculture in Iraq represents the second largest sector contributing to the gross domestic product, surpassed only by the oil sector (Ryan, 2008). It is also a main source of income for the poor and therefore its deterioration may lead to the decline of food security of the population (Ryan, 2008). The agricultural sector has also become the largest source of employment opportunity for residents in rural areas. The steady increase of the population which has occurred in Iraq as a result of the development of civilization and the increased food demand, have caused the unbalance between the continuous increase in the number of people on one hand and the low productivity of the land for food crops on the other hand (USDA, 2006; Ryan, 2008; Lucani, 2012).

Salinity is considered as one of the most significant problems in agriculture on a global scale; especially in arid areas that represent about 25% of the total land area, if the occurrence of rainfall in these areas is insufficient to wash the salts accumulated in the plant roots of the soil down to groundwater. The evaporation rate in such arid regions is also particularly high; thus leading to an increased accumulation of salts in the soil (Carter, 1975; Munns and Tester, 2008). In Iraq, soil salinity has affected the agriculture and the corresponding lands in a serious way, especially in the production of corn.

Saline soil is characterized by its high levels of soluble and insoluble salts. This type of soil hinders the natural growth of vegetable crops through negative impact on the properties of natural soil, thus making it unfit for cultivation. Both soil and water irrigation salinities affect in many development and growth processes in plants through their impact on the cytoplasm and plasma membrane, and through their indirect impacts on photosynthesis, effectiveness of building enzymes and breaking down of nucleic acids (Levitt, 1980).

Salinity sources are quite diverse as these can be the results of melting and continuous erosion of rocks, rising of ground water levels due to lack of good drainage after irrigation, mixing of seawater and underground water in areas of seashore proximity, and the additional salts of soil from irrigation water or fertilization (Ibrahim, 2011).

In recent years, the salinity problem in Iraq has worsened significantly due to the lack of rain and water shortage in general, as well as poor management and deterioration of land (Buringh, 1960). The response of plants to environments with high salt contents is one of the most important agricultural topics of interest to researchers in the field of agriculture and vegetable production due to its proportional relation to the human diet. Additionally, the need to increase agricultural production in food crops has become essential for facing the growing food demand resulting from the rapidly growing global population.

The corn crop (*Zea mays* L.) is considered as one of the vital economic and strategic grain crops on a wider scale and it comes in first position when considering the production (FAO, 2010). Its grains are consumed directly or indirectly by humans. The indirect use of the yellow corn is related to the possibility of its seeds flour to be mixed with wheat flour in making bread. The grains are used in the production of starch due to the high content of 70% - 80% of carbohydrate materials.

It can also be used in the production of oils since the grains contain 4% oil with nutritional and other healthy characteristics. Corn cob is meanwhile applied in the paper industry (Edwards, 2010; Niemi et al., 2012; Aghamirzaei et al., 2013). The importance of corn crops is shown globally through the increasing interest to extract fuel from corn due to the fact that ethanol is extracted from the corn grains (Bothast and Schlicher, 2005). In addition to this, the grains serve as fresh animal feed, in the form of silage or as crushed grains in poultry feed (Harris et al., 2007).

Corn represents the main supply source of the basic material for livestock breeding (Wright, 1997; Brown, 2012). Therefore, in the near future, the corn cultivation and production of this crop need to expand in many countries around the world (Lewis, 2011; Sasson, 2012) including Iraq. The Arab Agricultural Statistics yearbook (2012) noted that the corn planted areas in Iraq in 2011 were about 128.74 thousand hectares and the total final production was 235.71 thousand tonnes, while the global production of yellow corn for the same year was 874 million tonnes (USDA, 2012). It is thus evident that the production of corn crop in Iraq is below the required level. The reasons behind this shortage include soil salinity, lack of water, poor land management (Elsahookie, 1990). It is essential therefore to find more advanced operational methods and means in order to increase the corn production per unit area because of the continuing population expansion and the permanent lack of production (Al-Rumy, 2006).

Recently, great attention has been paid to the use of environmentally friendly organic stimulators in the enhancement of plant growth (Zhu, 2001; Akula and Ravishankar, 2011; Gupta et al., 2015). Apart from this, it has been known that the treatment of plants using plant growth regulators (phytohormones) is one of the advanced methods for the nourishment of plants and increase their production owing to the significant role of plant growth regulations (Forde, 2002; Meixner et al., 2005; Ahemad and Kibret, 2014). These regulators are organic materials mainly produced in active plant tissues, and have a great effect in certain physiological processes. This is because phytohormones often move from their production sites to influential sites (Weyers and Paterson, 2001; Davies, 2010; Wani et al., 2016). Phytohormones further control the growth and development of the different plant structures. The impact is not limited to metabolic processes but also extends too many specialised physiological processes (Davies, 2010; Keurentjes et al., 2011).

Among the various existing artificial hormones, gibberellic acid has been most broadly applied by researchers. It has exhibited significant effects on plant growth and development, shoot length, number of leaves and number of branches (Bottini et al., 2004; Mukhtar and Singh, 2006; Roy et al., 2010 ; Ghoname et al., 2011; Qureshi et al., 2013).

Likewise, it has been found that the application of friendly engineered solutes such as amino acid (such as proline) has considerable effects on plant nutrition and cultivation under salt and other environmental stress, and can be used to overcome the high salinity in soil (Wang et al., 2003; Ashraf et al., 2008 Huang et al., 2009). The use of proline significantly improves the vegetative characteristics of plants such as Tobacco, Rice, Arabidopsis, Wheat, Carrot, Citrus and Soybean as well as the retention of flowers and pods, leading to better seed yield compared to untreated plants (Kishor, et al. 2005; Ashraf, et al., 2008). Proline is also beneficial for pollen viability, germination, leaf water content and chlorophyll (Ashraf and Foolad, 2007; Kaur et al., 2011). Exogenous application of proline also stabilises the effects of water stress on growth and development of corn cultivars. Foliar application of proline enhanced the photosynthetic rate of water stressed plants of corn (*Zea mays* L) cultivars, stomatal conductance, and photosynthetic pigments (Ali et al., 2007). Apart from phytohormones and amino acids, great attention has been given to the use of natural organic matter and substances acting as potent fertilisers, such as humic acid. Humic acid is found to have a hormone-like activity which stimulates plant growth and development (Ferrara and Brunetti, 2010). Humic substances have been observed to have a positive effect on the vegetative characteristics, root, leaf and shoot growths, and the germination of various crop species (Piccolo et al., 1993; Ahmed et al., 2012). The application of humic acid has exhibited a significant improvement effect on plant growth, when it was used alone or in combination with other fertilisers. The yield characteristics were notably enhanced by the use of humic acid (Yildirim, 2007). The chemical composition of plants, such as bean, grape and tomato, were also improved significantly by using humic substances and amino acids (Ferrara and Brunetti, 2010; Abbas, 2013). In addition, humic acid increased vegetative growth of soybean and corn in laboratory trials (Wright and Lenssen, 2013), increased the plant growth and mineral nutrients uptake of wheat (Triticum durum cv. Salihli) under conditions of salinity (Asik et al., 2009).

Based on the presented points, it can be concluded that the addition of some natural compounds to the soil or plants can powerfully stimulates plant growth and development. Therefore, this study advocates to the use of gibberellic acid, humic acid and proline as major catalysts for the advancement of corn cultivation.

## 1.2 Problem Statement

Saline soil is considered as a serious problem for agriculture in Iraq. It has become a major issue in hindering the economic prosperity in many countries, especially those located in the dried and semi-dried climate areas (Amali et al., 2013), including Iraq which is characterised by a high concentration of salinity in soil, in addition to the shortage of water resources.

The salinization process in Iraq started a few decades ago, and is continues until today. The issue has since escalated with the progressive use of land and the current circumstances resulted from intensive irrigation, rising groundwater, dry climate conditions and lack of appropriate management procedures (Ashraf and Foolad, 2007). Ever since the rise of salinity becoming an enormous trouble source, it has spread in more than 65% of the agricultural land in Central and Southern Iraq (Ameen and Kasim, 2009), which has in turn led to the abandonment of 25,000 hectares of land each year due to high levels of salinity. Additionally, the Iraqi soils suffer from a decline in organic matter due to the consistently high temperatures, leading to the decomposition and reduction of organic matter in the soil, as well as the lack of rainfall and vegetation (Al-Bakri and Abboud, 2013). Thus, it is very essential to compensate for the shortfall in soil content of organic matter through fertiliser treatments. These additives would consequently play a major role in raising the productive capacity of the land. However, it has recently been observed that the use of chemical manure can cause serious environmental pollution, as well as higher production costs (Sarhance et al., 2011), prompting the farmers to reduce its application, which in turn has an impact on the low levels of agricultural production rates.

## 1.3 Research Objectives

Up to date, there is little information on corn cultivation in Iraq, that result in low productivity, as opposed to the National's ambition for a thriving agriculture. It is, however, evident that alternative means and methods are required to progressively increase the productivity of this crop. Moreover, there are lack of relevant studies on the effects from the applications of gibberellic acid, proline, and humic acid on the corn crop, as no study has yet been conducted on treating these substances jointly. In addition, the two new corn cultivars, Baghdad -3 and Fajir-1 have not undergone any tests in terms of productivity and adaptation to the environmental conditions in the study area.

The objectives of this study are as follows:

i. To investigate the effects of gibberellic acid, proline and humic acid on the growth, yield and chemical composition of Baghdad-3 and Fajir-1
ii. To determine the best concentration of gibberellic acid, proline and humic acid which give the highest yield for Baghdad-3 and Fajir-1
iii. To determine the best synergistic of gibberellic acid, proline and humic acid which give the highest yield for Baghdad-3 and Fajir-1
iv. To select the better treated *Zea mays* cultivar in terms of growth, yield and chemical compositition characteristics

This research focused on evaluating and comparing the effects of gibberellic acid, proline and humic acid on corn growth, and more specifically on two different cultivars (Baghdad-3 and Fajir-1), in terms of their vegetative, yield characteristics, in addition to their chemical composition.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 General Characteristics, Classification and Distribution of *Zea mays* L.

The corn plant or *Zea mays* is one of the major food grains that have been consumed since antiquity by people around the world. Today it is regarded as one of the main and most important food grain crops after rice and wheat (Abdelmula et al., 2007). *Zea mays* L. is broadly applied for the preparation of corn flakes, starch and oil dextrose, acetone, gluten, lactic acid, and grain cakes by different industries (Nabizadeh et al., 2012). Recently, the demand for corn has increased dramatically and gained a lot of attention due to its unique economic importance and utility, such as human food, food additive (sweetener), oil, starch, animal feed, petroleum fuel substitute, fibre, and lipids (USDA, ARS, 2015 ).

The *Zea mays* grain is utilised for both poultry feed and human consumption (Ibrahim and Kandil, 2007). The corn crop is cultivated all over the world. According to the United States Department of Agriculture (USDA), the global corn production for the year 2014 stood at around 985.609 million metric tonnes (World Corn Production, 2015). In addition, the world's largest producer and exporter of corn is the United States of America, followed by China, Brazil and Mexico, while Japan is considered by far as the world's largest importer of this crop (Onasanya et al., 2009).

Corn (*Zea mays* L.) is an annual plant from the Poaceae family with a graminoid growth habit and a cosmopolitan herbaceous with solid dense stems (USDA, 2015). It is a monoecious plant, where the shoot bears the female flower (pistillate flowers) and the male flower staminate (tassel) (Dhillon and Prasanna, 2001; Cummings and Wickersham, 2001). It has a rapid growth rate which undergoes active growth during the summer season. Usually, it needs a rainfall of more than 500 mm whereby the more the rainfall, the higher is the yield. The crop is tolerant to drought and low salinity (Dowsell et al., 1996). Thus, it can be cultivated across a wide range of regions and lands. The corn plant consists of the main structures, root, stem, coleoptile, leaves, and the female (ear) and male flowering structures (tassel). It has been reported that *Zea mays* L. is shallow rooted, and thus needs loam clay, medium textured soil, as well as well-drained and rich fertile soil (Fao, 2010).

Corn has a well-organized root system that consists of several types of roots, such as primary root, lateral seminal roots, nodal roots, and brace roots. The primary root, which is protected by a covering sheath, grows directly from the grain within 10–15 days after seeding; same goes with the lateral roots. The nodal roots extend directly from the first six to eight stalk nodes below the ground. All these roots develop gradually, and work together to allow the anchorage for sprouting (Feldman, 1994; Edwards, 2009). Lastly, the brace or crown roots are formed later at the nodes above the soil surface to support the plant above the ground (Hochholdinger, 2009). Figure 2.1 shows the various descriptive types of corn roots.

A matured corn plant has a height of 10.7 to 12 feet, and might reach up to 16 feet in some cases (Plessis, 2003; Karl, 2013). The stem is cylindrical, dense and it is made up of nodes and internodes, which provide the structural support for the leaves to intercept sunlight. The number of nodes varies from 8 to 40 with 3–10 nodes below the ground, and 6–30 nodes above the ground, based on the variety and species (Zsuzsanna, 2002; Karl, 2013). According to Nielsen (2007), the corn stem has 12-14 nodes and internodes, Plessis (2003) reported that the stem of Mexican corn has 20 internodes.

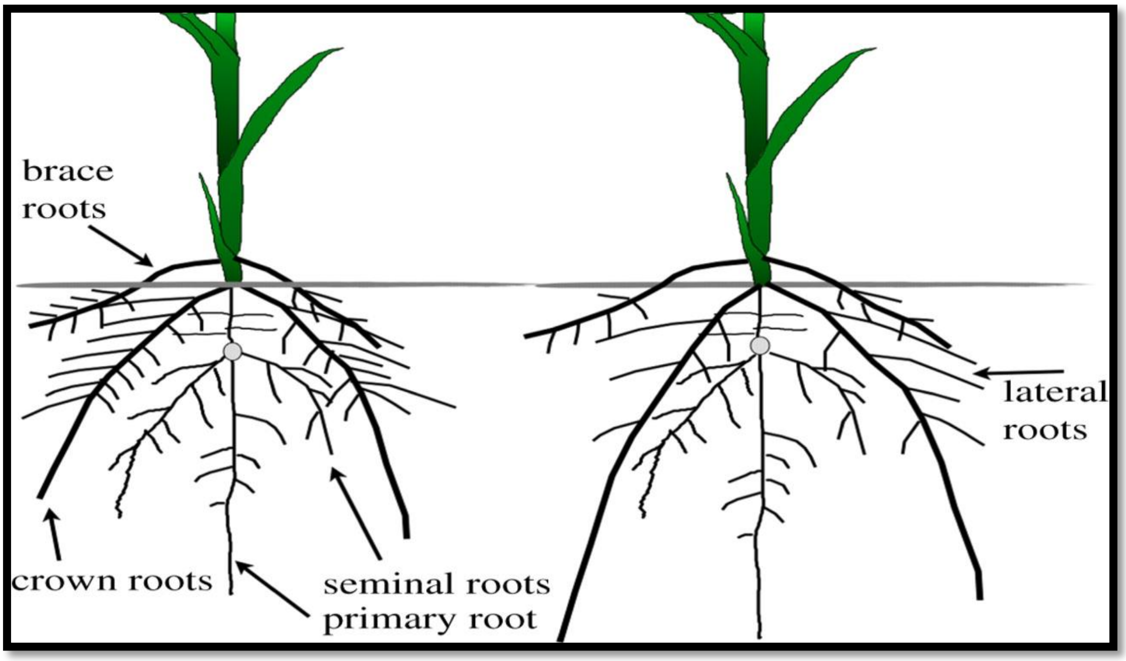


Figure 2.1: The various types of corn root (Lynch and Brown, 2012)

On the alternate sides of the corn stem, multiple leaves of approximately 20 leaves per plant are produced in a set order, growing from nodes (Yousif, 2012). The leaf, where the process of photosynthesis occurs, has a long blade, broad shaped and dark green in colour, borne opposite and alternatively along the stem. The leaf midrib extends the length of the middle of the leaf blade from the base to the tip in order to provide structural support, and can reach up to 30 to 100 cm. The area on the inner surface of the leaf is called the collar (ligule and auricle), where the leaf blade and leaf sheath join (Figure 2.2). The leaf portion below the collar that wraps around the stem and attaches the leaf to a stem node is called the sheath (Russell and Evert, 1985; Nelson and Dengler, 1997).

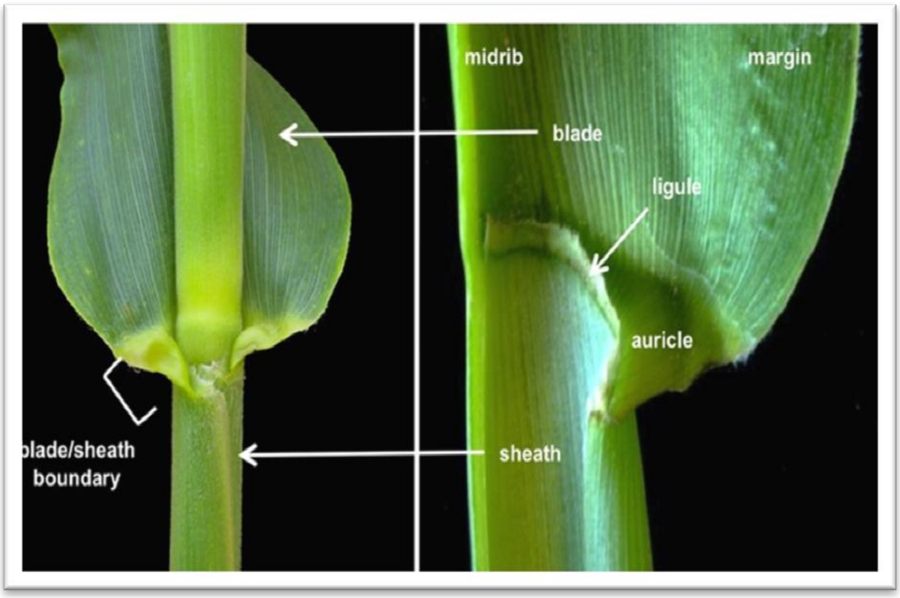


Figure 2.2: Connection point between leaf blade and leaf sheath (Moon et al., 2013).

Another structure in the corn plant is the coleoptile, which are modified leaves consisting of four or five leaves rolled up together, surrounding the grain and plant embryo during germination in order to protect it.

The grains and flowers of *Zea mays* L. are yellow in colour. The male and female inflorescences (flower bearing region of the plant) of the same plant are positioned separately. The male inflorescence (tassel) is positioned at the top of the plant stalk, while the female (ear) is sessile on the leaf axil towards the stem and protected by husks (Figure 2.3). The ear of the corn (which is usually the edible part) concludes with fluffy, long, thread-like, and hairy structures known as silk. The grains (kernels) in the ear are covered in husks, and might have various colours, such as white, yellow, red, purple, or black (Bonavia, 2013).

According to Dornbusch, et al. (2011), corn has two vegetative growth phases with different design characteristics: the juvenile phase and the adult phase. In the juvenile phase, leaves and axillary tillers are produced, while the internodes remain consistently short. In the adult phase, the inflorescence develops, while the internodes get longer.

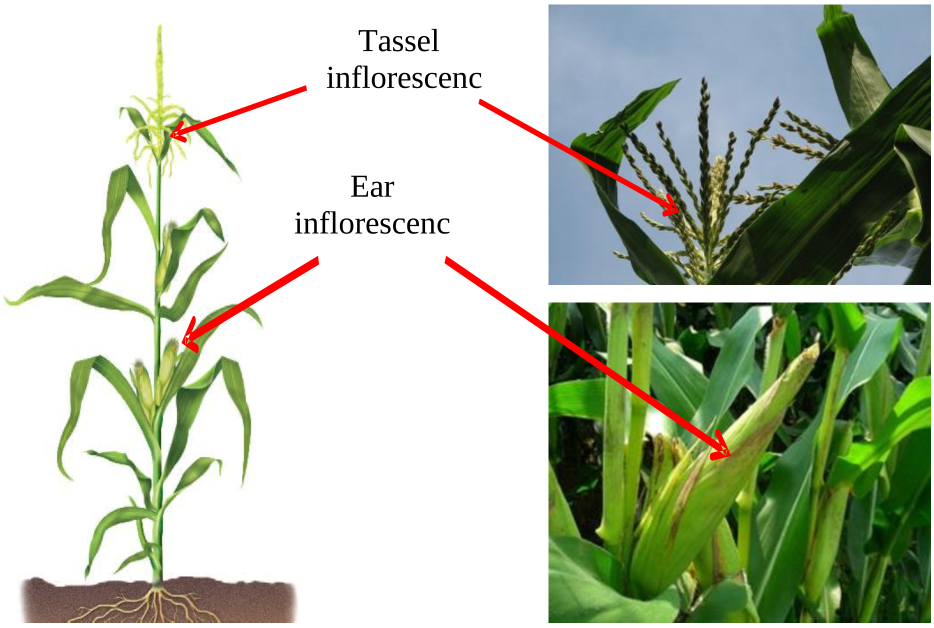


Figure 2.3: A) The corn plant carrying the male and female inflorescence (Levetin and McMahon, 2008); image B and C) The tassel and ear of corn (from www.plantvillage.com).

In recent years, corn has emerged as the third most imortant food grain crop in the globe. It has been cultivated in many countries and through a wide range of temperatures, particularly in warmer temperate and under humid subtropic countries (Wei et al., 2007; Bennetzen and Hake, 2009).

*Zea mays* L. production is today distributed all over the world. It can grow in regions with warm summers and the availability of soil requirements, such as Central America, North and South America, Europe, and Asia (James, 1983; Zea mays L., 1998). The origin of corn lies in Mexico, from where it spread to other parts of the world (Fedoroff, 2003; Jaenicke-Despres, et al., 2003). In Iraq, the *Zea mays* L. is cultivated in the central and north regions.

## 2.2 *Zea mays* L. cultivars in Iraq

This study will focus on two cultivars of *Zea mays* L. : Baghdad-3 and Fajir-1. Both are cultivars obtained locally by the Research Department of the yellow and white corn, General Department of Agricultural Research, Ministry of Agriculture. These cultivars have been approved by the National Commission for the authorisation of the cultivars in Iraq.

The Baghdad-3 product has been obtained from six local strains, namely 1-dr-c-13, 2-wc-11, 3-dr-b-218, 4-n-40, 5-ast-300, and 6-zm-182. Fajir-1 has been obtained from eight local strains, namely 1-Art-b-28, 2-Pio-28, 3-Art-c-16, 4-mgw-36, 5-exw-57, 6-s-413, 7-e-34, and 8-ast-15.

These cultivars are considered as having a great genetic base, obtained from more than four breeds. Therefore, the production of these cultivars is fixed and not subject to deterioration over time (Elsahookie, 1990). It is recommended to plant these cultivars in the central and northern regions of Iraq. It has been found that the cultivars grow better in the northern region during the autumn season. Besides, it can be planted during the spring season at the beginning of March. The maturity period is approximately 104 days for Baghdad-3 and 108 days for Fajir-1.

Baghdad-3 yields about 7,500 to 8,200 kg/ha in the central region and 10,450–12,500 kg/ha in the northern region, while Fajir-1 yields about 7,800–8,400 kg/ha in the central region and 10,500–12,600 kg/ha in the northern region. These cultivars are known for their tolerance to salinity, and resistance to disease and insects, thus these factors become the reason for choosing the cultivars in this study. Notably, it is also the first characteristics study conducted on these two cultivars.

## 2.3 Gibberellic Acid (GA)

The main environmental factors that negatively affect plant growth and yield are salinity, extremes of temperature, drought and nutrient imbalances (Shaddad et al., 2013). The application of plant growth regulators is one of the most important factors in improving the growth, yield and flower quality (Nuvale et al, 2010). Plant growth regulators are found as two types; bioinhibitors such as methyljasmonate and (ABA) and promoters like gibberellins, auxins and cytokinins (Giannakoula et al., 2012). As an organic compound, a plant growth promoter can control biochemical and physiological processes in the plants such as controlling the chemical composition of crops, dormancy, amount of mineral uptake from the soil, organ size, flowering, fruit set and crop development (Sarkar et al., 2002; Rahman et al., 2004; Mukhtar, 2008). Hence, growth regulators substances are applied to balance internal hormones and to inhibit or stimulate flowering depending on the concentration and application time (Shakarami et al., 2013).

Among the various existing growth promoters, gibberellic acids (GAs) have been broadly applied by different researchers.

According to Abbas (2013), plant hormones enhance plant growth and development, and have a great impact on several metabolic processes including respiration, photosynthesis, ion uptake and nucleic acid synthesis. Plant growth promoters can control biochemical and physiological mechanisms in the plants such as the chemical composition of crops, dormancy and the amount of mineral uptake from the soil, organ size, flowering, crop development and fruit set (Sarkar et al., 2002; Rahman et al., 2004; Mukhtar, 2008).

The first notification of the application of hormones to regulate plant growth was traced back to the late nineteenth century whereby its importance for the production of crops was recognized officially in 1932 (Mukhtar and Singh, 2006). Since that time, hormones have been widely used to modify or increase plant cultivation.

In another study, the highest number of leaves was obtained using 10 ppm $GA_3$ for 24 hours. Dogra et al. (2012) investigated the effect of 0-300 ppm $GA_3$ concentrations and 20x40 cm, 30x40 cm, and 40x40 cm levels of spacing on the number of leaves, plant height, corm production, and flowering of *Gladiolus*. The authors proposed that the application of 300 ppm $GA_3$ resulted in the maximum number of leaves, plant height, corm weight and diameter, and flowering. They concluded that on using a 40x40 cm spacing level and 300 ppm $GA_3$ concentration effectively enhanced the properties of the plant. Asadi et al. (2013) studied the influence of various $GA_3$ concentrations (0, 25 and 50 mg/l) on the number of leaves, flowering, fruit yield, and vegetative properties of *Fragaria ananassa* plants. They concluded that $GA_3$ application had no significant effect on leaf and branch crowns, whereas runners and the number of flowers increased significantly.

The influences of 50 ppm gibberellic acid and various levels of photoperiod on the growth factors of *Vigna unguiculata* were investigated by Mukhtar and Singh (2006) proposed that the treatment led to a significant increase in the leaf area, leaves number, total weight of plant, and number of initial branches. Therefore, it can be said that phytohormones play a major role in plant growth, development, and metabolism. Several studies have been conducted on the relationship between plants and phytohormones. Specifically, there have been many studies about the biochemical role of GA in plant growth and development. These studies thus need to be reviewed in order to understand the functions of GA and its mechanism of action in the plant development. The following sections will cover the nature and structure of GA as well as its effect on the characteristics of plants.

### 2.3.1 Structure and Functions of GA

Gibberellic acid is a powerful agent that naturally occurs in plants and in a very low concentration in order to regulate their development. The GAs comprises a huge family of tetracyclic di-terpenoid carboxylic acids, they can be found in higher flowering plants, fungi, bacteria and in some species of lower plants (Hedden, 1997).

They are involved extensively through all stages of plant growth and development. Gibberellic acid have many functions, such as promoting seed germination and seed development, promoting leaf growth and flower, promoting stem elongation, increasing the production of pollen, tolerating to salinity, preventing the breakdown of chlorophyll, increasing the plant life span, and preventing plant senescence (Hedden, 1997; Swain, and Singh, 2005; Rosenvasser et al., 2006; Rieu et al., 2008; Tuna et al., 2008).

In 1950s, the first application of gibberellic acid to stimulate bolting in rosettes and to return growth in dwarf mutants was found, and it was called as a plant growth regulator (Hedden, 2012). The gibberellin, as a plant hormone, can modulate the development of plants throughout their life cycle, and an insight into its mechanism can be achieved by its signalling pathway and biosynthesis (Fleet and Sun, 2005). It is worth mentioning that high integration of signalling pathways in the aforementioned processes is derived from a combined action of gibberellins, other phytohormones and further regulatory factors (Bottini et al., 2004). The gibberellic acid signalling function is de-repressibling, which is moderated using transcriptional regulators known as DELLA-domain proteins that repress GA responses ( Figure 2.4). (Fleet and Sun, 2005). Basically, the activation or repression of the GA signalling related processes (growth and development) depends on the absence or existence of DELLA protein repressors (Schwechheimer and Willige, 2009).

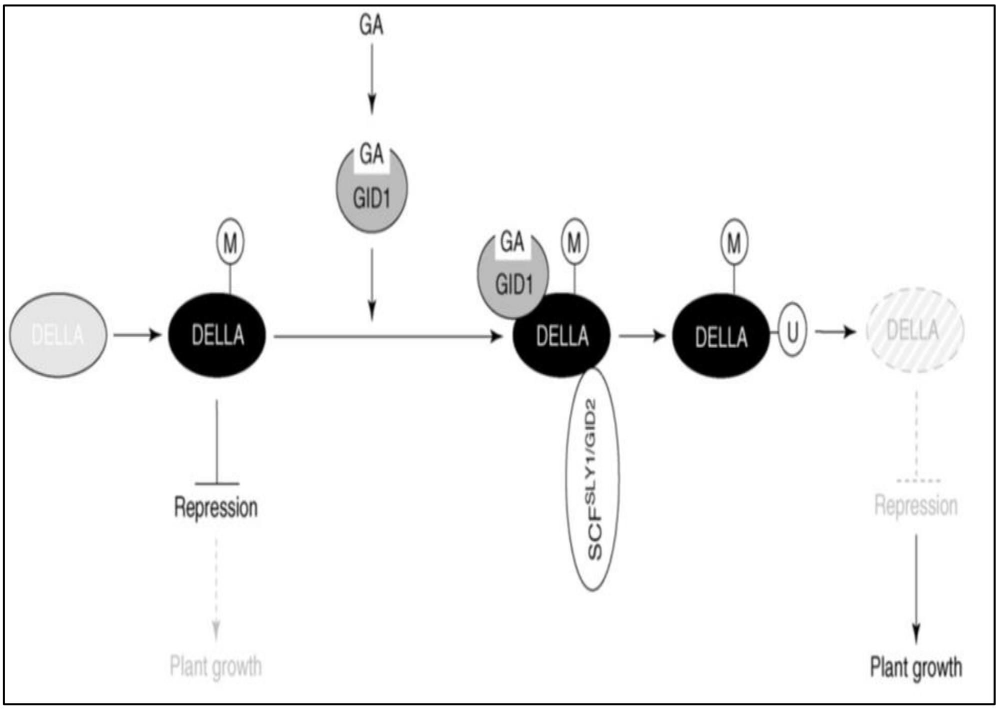


Figure 2.4: A model for GA signalling pathway (Jiang and Fu, 2007).

The $GA_1$, $GA_3$, $GA_4$, C19 gibberellins, and 3β-hydroxylated gibberellic acids have been proposed to directly influence the rise of plant shoot elongation (Bottini et al., 2004). The major bioactive GAs, which are $GA_1$, $GA_3$, $GA_4$, $GA_5$, $GA_6$ and $GA_7$ possess a lactone among C-4 and C-10, a carboxyl group on C-6, and a hydroxyl group on C-3β (Yamaguchi, 2008). The GA has a molecular formula C19H22O6, with average mass of 346.374 Da (CSID, 2015). The chemical structures for some of the main active GAs are shown in Figure 2.5.

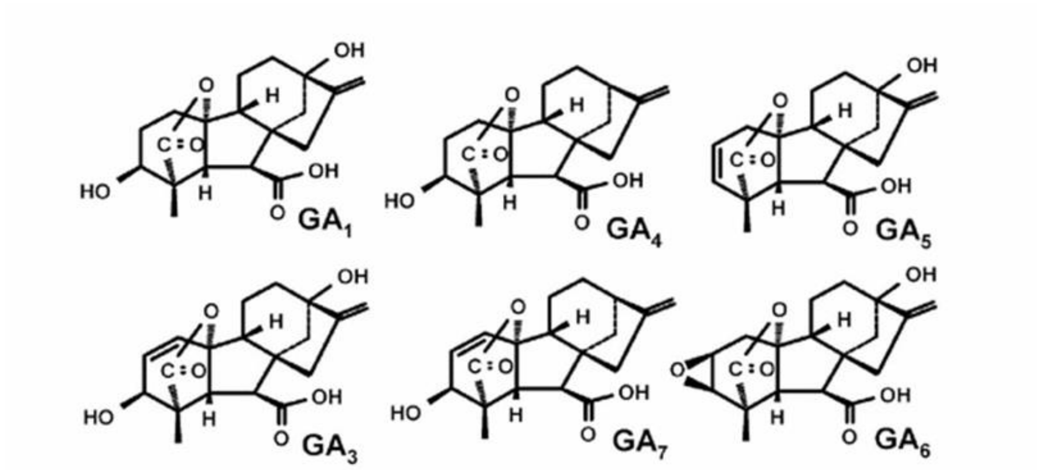


Figure 2.5. Chemical structures of the main active gibberellic acid (Sponsel and Hdeden, 2010.)

### 2.3.2 Effect of GA on Plant Growth

The effect of GA on plants has been well established by many researchers (Brian & Hemming, 1955; Marth et al. 1956; Chrispeels & Varner, 1967; de Souza and MacAdam, 2001; Roy et al., 2010; Saptari and Dewi, 2013). The results of these studies show that GA affect cell elongation, cell division, or both (de Souza and MacAdam, 2001; Roy et al., 2010; Saptari and Dewi, 2013). It arouses the influence of long-day lengths by increasing runner production and improving vegetative development in short-day plants (Qureshi et al., 2013). In addition, it changes the source-sink metabolism through its impact on sink formation and photosynthesis (Iqbal et al., 2011). The inductive effects of plant regulators are often exerted by environmental factors by evoking alterations in metabolism and distribution of these hormones in the plants (Yamaguchi, 2008; Lone et al., 2010).

These environmental factors are including light, drought, salinity, high temperatures, and pathogen infections. Drought and salinity soil has the most adverse effect to plant growth and productivity (Reddy et al. 2004). It has been suggested that drought can decreases $CO_2$ assimilation rates due to the low stomatal conductance. It also reduce the contents and activities of photosynthetic carbon reduction cycle enzymes, including the key enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Reddy et al. 2004). Therefore, a wide variety of plant responses will be generated, such as gene expression, cellular metabolism, changes in growth rates and crop yields, generation of active oxygen species at the cellular level (Reddy et al. 2004). Huang et al. (2008) suggested that there is a strong relationship between drought-related gene expression in *Arabidopsis thaliana* to hormonal and environmental factors. They found that plant hormones significantly increased under drought and either completely or partially recovered to normal levels after 3 h of rehydration. On the other hand, they also found plant hormones modulated and increased as a response to other environmental factors including light and biotic stresses. In fact, due to some environmental changes, hormone like-messages are generated, and these hormonal messages are recycled between roots and shoots where the roots import hormones and the shoots acts as an active hormone sinks (Jackson, M. B. 1993).

#### 2.3.2(a) Effect of GA on Vegetative Characteristics of Plants

The influence of GA in the plant life cycle, such as germination, seed set, bolting, flowering, leaf expansion, and fruit development has been identified (Phillips, 1998; Gomi and Matsuoka, 2003). In the following sections, the impact of GA on the properties of corn (*Zea mays)* and other several plants will broadly discuss.

It has been proposed that GA alone or in combination with other materials such as $CaCl_2$ can significantly increase plant height (Qureshi et al., 2013). For instance, Fathel and Lahmood (2013) conducted a research to study the impact of GA at a concentration of 100 ppm and Boron at a concentration of 1 mg/l on plant height and other *Zea mays* growth parameters. They proposed that the application of $GA_3$ caused a significant increament in plant height. They however reported that for all parameters, the interaction effect of GA and Boron was insignificant. The treatment of (*Zea maize* L.) using 50, 75 and 100 mg/l of GA3 and 10, 20 and 30 mg/l of Salicylic acid on number of leaves per plant, leaf chlorophyll content and leaf area was a subject of work conducted by Hafize (2015). The results showed that the best reselt for leaf area and leaf chlorophyll content were obtained by spraying the plants with 100 mg/l of GA3 and 30 mg/l of Salicylic acid.

Subedi and Bhattarai (2003) assessed germination, mobilisation of endosperm reserve, and dry matter content of *Zea mays* treated by 1, 10, and 100 mg/l of exogenous GA3. They observed that the dry matter of the plant increased by 1mg/l of GA3, whereas it remained unchanged when 10 and 100 mg/l was used during the first 24 hours. They summarised that the treatment of seeds by 1mg/l GA3 led to greater mobilisation of endosperm reserve, and therefore higher germination.

In another work, the influence of foliar application of GA (0–250 ppm) on corn (*Zea mays* L.) in different planting dates was statistically studied (Naghashzadeh et al., 2009). The findings indicated that interaction between GA and planting dates was insignificant, whereas variation in the *Zea mays* L. planting dates showed a significant impact on period taken to flowering, plant height, weight of 1000 grain, and grain yield. It should be taken into account that low water potentials and soil temperature led to delays or decrease in the germination index and percentage of *Zea mays* L., which can be improved through seed hormonal priming using GA3, IAA, or halopriming by ascorbate (ASA) or CaCl2 (Afzal et al., 2008).

Gibberellic acid can be applied alone or combined with other hormones. It is worth mentioning that in some cases, this combination led to better characteristics of the plants compared to sole treatment with GA. For instance, the findings of experiments conducted by Ghodrat et al. (2013) indicated that the highest leaf area and greatest crop growth rate was obtained by the application of GA3 (50 mg/l) and IBA (100 mg/l)-GA3(100 mg/l) during the 4–6 leaf stage, respectively. The development of the ears in *Zea mays* L. was studied by Xu et al. (2004). They reported that during the middle growth stages, there was a correlation between larger ear numbers and slower stem elongation. Moreover, by enhancing the internode elongation using GA, the growth of ear was suppressed. The assessment of the impact of the 20 ppm of each of 4-chloro phenoxy acetic acid (CPA) and $GA_3$, and CPA plus $GA_3$ on the plant height, yield, and growth of tomato plants was carried out by Choudhury et al. (2013). They found a maximum *Solanum lycopersicum* plant height of 86.01 cm at 60 days after transplanting (DAT) 39.69 was the number of flowers per plant, 36.54 was the number of fruits per plant, while a 28.40 $t.ha^{-1}$ yield was obtained by combination of 4-CPA plus $GA_3$ treatments.

Another important characteristic of plants that can be improved by the gibberellic acid treatment are the dry and fresh weights of shoot and root. Ghodrat and Rousta (2012) demonstrated that increasing salinity reduced the fresh and dry weight of root, fresh and dry weight of shoot, as well as germination rate and growth percentage of *Zea mays*. Moreover, they found that the priming of corn by GA3 (0-5 mg/l) in certain concentrations boosted root and shoot dry and fresh weights, tissue water content, and root and shoot lengths.

Al-Shaheen & Soh (2016) studied the effect of gibberellic acid on quantitative and qualitative characteristics of corn (*Zea mays* L.). In their study, different concentrations of gibberellic acid ranging from (50 and 100ppm) were used to reduce the effect of water stress. Their result showed that chlorophyll content of the untreated plants was lower than the treated plants. They also found significant differences in the chlorophyll content of the corn leaves that were sprayed with 100 ppm of gibberellic acid and 50 ppm. The chlorophyll levels using 200ppm reached 76.5, 77.8, and 80.3 while using 100 ppm reached 73.2, 73.8, and 74.5 respectively at water stress levels of 75%, 50%, and 25%. While the control showed low chlorophyll content. Moreover, their result also showed a significant differences in the percentage of protein of the seeds when the plant was sprayed with 100 ppm gibberellic acid than with 50 and control (no treatment). They found at water stress levels of 75%, 50%, and 25% the percentage of protein in the seeds using 100ppm reached 9.2, 8.8, and 7.8, while reached 8.9, 8.1, and 7.4 using treatment at 50 ppm of gibberellic acid. The control under water stress levels of 75%, 50%, and 25%, showed lower protein content of 8.1, 7.6, and 6.8. They suggested that gibberellic acid is an innovative and promising way to reduce the effect of drought on plant growth and crop production.

In another study by Al-Shaheen et al. (2016), where they investigated the influence of different concentrations of gibberellic acid ranging at (0, 50 and 300 ppm) to reduce effect of water stress on some physiological characteristics of corn (*Zea maize* L.). Their result showed a significant growth and yield of the corn plants sprayed with different concentrations of gibberellic acid with significant increment in the number of days to flowering, plant height (cm) and leaf area index (LAI) of the corn plants sprayed using gibberellic acid using (300 ppm) under water stress irrigations. They suggested that corn plants responded positively to the spraying of gibberellic acid and showed high drought tolerance.

Afzal et al. (2005) investigated the influence of pre-soaking *Triticum aestivum* L. seeds in $GA_3$, kinetin, phytohormones indole acetic acid (IAA), and prostart hormones on their growth and germination under saline (15 dS/cm) and normal (4 dS/cm) conditions. The results showed that the best results for the seed's fresh and dry weights and seedling growth in both normal and saline conditions were obtained by soaking in kinetin (25 ppm) and prostart (1%, 2 h); soaking in $GA_3$ and IAA did not induce salt tolerance.

In another research involving two *Solanum lycopersicum* plants (Hyb-Himalata and Hyb-SC-3) with 0, $10^{-8}$, $10^{-6}$, and$10^{-4}$ M $GA_3$, the dry and fresh weight of root and shoot, leaf surface area, N, K and P content of leaf, yield, and other characteristics of the plants were investigated (Khan et al., 2006). The researchers found that the fresh and dry weights of the two species were similar with the application of 10-8 M $GA_3$. They summarised that regardless of $GA_3$ concentration, most of the growth characteristics improved by the spraying of $GA_3$.

Mary and Marina (2012) measured the influence of $GA_3$ on NaCl tolerance in okra plant in terms of shoot and root dry and fresh weights, chlorophyll and carotenoid content, leaf area, sugar level, and shoot and root lengths. They proposed that applications of $GA_3$ caused an increase in the fresh and dry weights of shoots and roots, leaf area, as well as shoot and root length in comparison to NaCl solely.

It has been reported that the application of $GA_3$ alone or in combination with other hormones such as IAA and kinetin causes a remarkable increase in the number of compound leaves and leaf area of lentil plant (Naeem et al., 2004). Akhtar et al. (2008) studied the effect of various $GA_3$ concentrations and soaking times on the leaf surface area, leaves number, growth, and germination of *Spinacea olerecea* L. According to the research, the highest leaf area was obtained under the control condition, followed by treatment with 10 ppm $GA_3$. The results of a statistical analyses conducted using ANOVA showed that the $GA_3$ concentrations, soaking time, and interaction between these two parameters were insignificant for the leaf area.

#### 2.3.2(b) Effect of GA on Plant Yield

Plant growth regulators have great effect in increasing the yield of plants, specifically GAs (Roy et al., 2010; Rohamare et al., 2013). The response of *Zea mays* L. plants developing under different levels of water irrigation to the addition of GA (50 and 100 ppm) and proline (100 and 200 ppm), was the subject of research conducted by Al-Shaheen et al. (2014). A significant increase was observed in the grain yield of *Zea mays* L. plants developing under water stress conditions, when they were sprayed with proline (200 ppm) and GA (100 ppm). The researchers concluded that the use of GA and proline was a good way of minimizing the effects of drought on plant growth and development.

Salinity is one of the important parameter that affects the traits of (*Zea mays* L.). The application of GA3 together with calcium chloride enhances yield and plant growth and is more effective in the mitigation of adverse impacts of salinity, when compared with applying either $GA_3$ or calcium chloride alone (Khan et al., 2010).

In line with this, Ghodrat and Rousta (2012) investigated the influence of GA and IBA treatment on grain weight, ear length, grain number per row, grain number