Effect of proline and Gibberellic Acid on the qualities and qualitative of Corn (*Zea maize* L.) under the influence of different levels of the water stress

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Abstract- The current study focuses on the use of plant growth regulators (gibberellic acid) and Amino acids (proline) with the goal of finding ways to cope with water scarcity and understanding the physiological adaptations of corn plants to drought by using growth regulators and Amino acids. A field study was conducted from March 2014 to June 2014 to investigate the influence of different concentrations of gibberellic acid (100 and 50ppm) and proline (200 and 100 ppm) to reduce the effect of water stress on the some of physiological characteristics of corn (*Zea mays* L.) under different irrigation levels (25%, 50%, and 75% from Of field capacity). Compared with that in the untreated plants,a considerable improvement was observed in the growth and yield of the corn plants sprayed with different concentrations of gibberellic acid and proline. Specifically, a clear increase was noted in the leaf chlorophyll content, Proline concentration in leaves (ppm) and The percentage of protein in the seeds of the corn plants sprayed with proline (200ppm) and gibberellic acid (100ppm) under water stress irrigations. Corn plants positively responded to the spraying of proline and gibberellic acid and showed high drought tolerance. The corn plants were more tolerant of drought when sprayed with 200 ppm proline and 100 ppm gibberellic acid. The use of gibberellic acid and proline is an innovative and promising way to reduce the impact of drought on plant growth and crop production.

Index Terms- Water Stress, Proline, Gibberellicacid, Corn Growth, Irrigation Level

I. Introduction

The water wealth of natural resources that are related to the existence of life And preservation has become vitally important Agriculture is the sector where the main consumer of this resource in most countries of the world (In Arab countries, 90% of the water available).

Is considered of maize of the most important crops, food grains and industrial important in many areas of the world, comes this crop ranked third in the world after wheat and rice in terms of cultivated area and production, and that the main producing areas of maize in the world are: North and South America, Europe, Russia, China, India, South Africa (Fisher, Abate et al. 2015).

Gibberellins (GA3) are group of plant hormones produced by the leaves of modern plant and developing the peaks in the roots and stem (Takahashi, Phinney et al. 2012). Contain gibberellic acid, which incited elongation of plant cells and configure the fruit without seed, which overcomes the genetic dwarf leg, and increases the production of side branches, especially floral which increases the number of flowers and fruits of production ris , and can be sprayed gibberellic acid equipped on specific types of plants to increase their growth. When gibberellic acid sprayed on the plant, has physiological effects on plant growth and its role in the process of photosynthesis, and activate other vital activities that done in parts of the plant cell in division of cells and increase, Elongation and increasing plant height and size of the leaves, root and all of these effects are increased Productivity (Kondhare, Hedden et al. 2014).

Proline is the most important amino acids that accumulate in various tissues of the plant, particularly in the Leaves Because the effect of water stress. And that the accumulation of this amino acid has a role in the regulation of osmosis in the cell As the proline is concentrated in the cytoplasm to counterbalance effort osmosis Cell sap. Also, proline protects enzymes under conditions of water stress (Meister 2012).

As well as The Proline is indicators of drought and an increase in the leaf proof that the plant suffered water stress also Is one of the ways the plant resistance to water stress The accumulation of proline in the leaf appearance adaptation in times of drought to save the best percentage of water in the plant (Tarighaleslami, Zarghami et al. 2012).

So The objective of research finds ways to cope with scarcity of water, taking place in many countries of the world using gibberellic acid and the amino acid proline and understand some of the effects and physiological adaptations to drought.

2. Methodology

2.1 Site of the study

This study was conducted at the Agrotechnology Research Station, University Malaysia Perlis Padang Besar, Perlis, Malaysia from March 2014 to July 2014.

2.2 Preparation of soil for planting

The field was plowed and divided in preparation for planting. The pilot units measured 2 m × 2 m each and spaced 1 m apart. Each pilot unit and its replicate were spaced 1.5 m apart. Recommended quantities of NPK fertilizer were added to the soil before planting. Soil samples were collected from the field before planting the corn seeds in different areas at a depth of 30 cm. The samples were then analyzed using standard methods to determine their physical and chemical properties (Table 1). The corn seeds (seedling length of 10 cm) were planted in small bots using media culture (Patmos) for a week and then planted in the field. The seedlings were planted in rows (spaced 50 cm apart) and between plots (spaced 25 cm apart). Each plot with an area of 4 m was composed of six planting rows.

2.3 Experimental fields

A split-plot design based on a randomized complete block design with three replications was employed in this study. The factors included irrigation in the main plot at three levels (25%, 50%, and 75% from Of field capacity) as well as optimum irrigation (no-stress irrigation). The sub-plot was sprayed with gibberellic acid and proline at three concentrations(0,100,200 ppm) proline) and (0,50,100 ppm). Irrigation treatments were stopped for 15 days and then restarted with delay. The irrigation was then carried out at constant intervals.

2.4 Characters of the study

2.4.1 Total leaf chlorophyll content

Chlorophyll meter *SPAD-502* (*Minolta Camera Co*., Japan) uses a silicon photodiode to detect the transmittance of radiation emitted by two diodes, peaking at 650 nm (high absorbance by Chl) and at 940 nm (negligible absorbance by Chl). The meter values, therefore, represent the transmittance ratio through the leaf tissue at these wavelengths. Four Chl meter readings were taken around the midpoint of the middle lobe of each leaf, two from each side of the midrib of the middle lobe 30 mm apart. Readings of the same five leaves used for LCC measurements were used for SPAD values also and the means of 20 readings were taken as the mean value of each plot. All observations were recorded on 30, 60, and 90 DAP. After taking the reading, the leaves were pooled for estimation of Chl *a*, *b*, and *a*+*b* contents (Uddling, Gelang-Alfredsson et al. 2007).

Chlorophyll was extracted in 80 % acetone. The contents of chlorophyll *a* and *b* [g kg−1(fresh tissue)] were measured using a spectrophotometer at A645 and A663 and calculated using the equations given in (Williams 1984).

2.4.2 Proline concentration in leaves (mg \ g -1)

Leaf Samples (0.2 g) were homogenized in a mortar and pestle with 3 ml sulphosalicylic acid (3% w/v), and then the homogenate was centrifuged at 18,000 g for 15 min. Two milliliters of the supernatant were then put into a test tube into which 2 ml of glacial acetic acid and 2 ml of freshly prepared acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 ml glacial acetic acid and 20 ml 6 M orthophosphoric acid) was added. Tubes were incubated in a water bath for 1 h at 100°C, and then allowed to cool to room temperature. Four milliliters of toluene were added and mixed in a vortex mixer for 20 seconds. The test tubes were allowed to stand for at least 10 min to allow the separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube, and its absorbance was measured at 520 nm by spectrophotometer. The concentration of proline was calculated from a proline standard curve and was expressed as mmol per gram of fresh weight (Bates, Waldren et al. 1973).

2.4.3 The percentage of protein in the seeds

Percentage of The total protein in the seeds of maize was estimated by following the method of Micro – Kjeldal as it stated in (1980 - AOAC) 0.2gm taking the leaves powder and digested by adding 20ml of concentrated sulfuric acid and then use Micro - Kjeldal component unit of automatic nitrogen Dosimet 667 type with a printer type Lx – 800 .The ratio calculated in the model by multiplying the amount of nitrogen resulting by 6:25 (Mertens and Loften 1980) .

2.5 Results

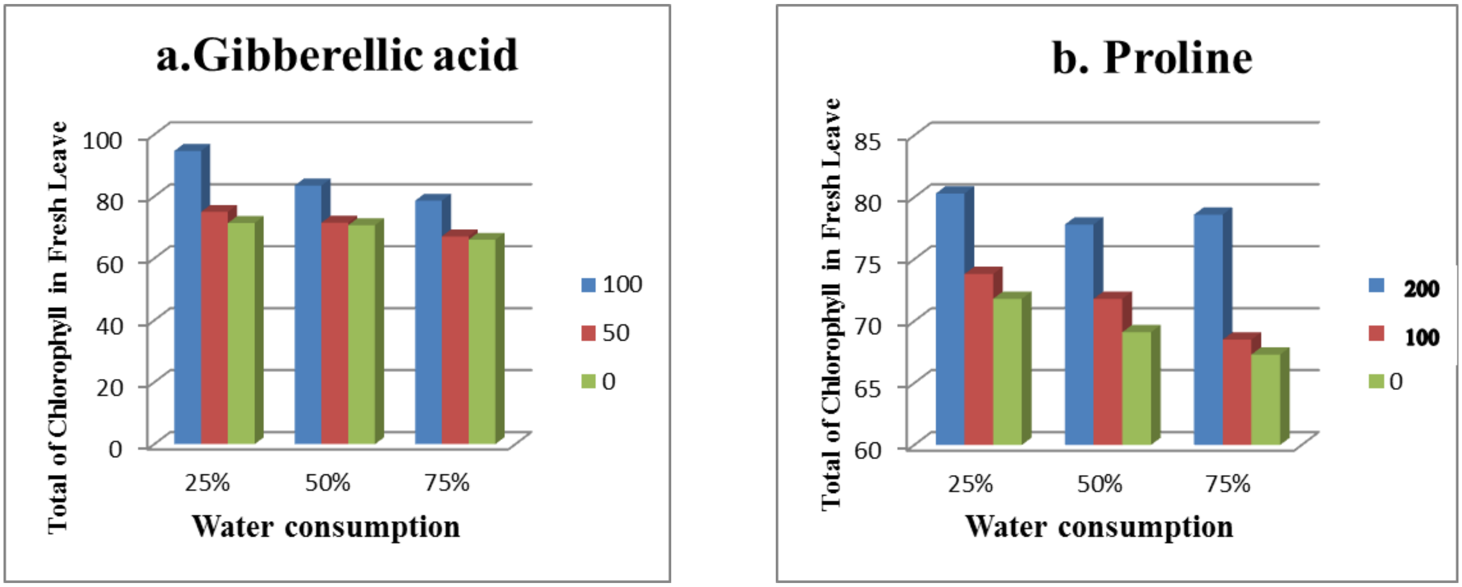
2.5.1 Leaves of chlorophyll content (micro grams. G -1)

Figure 1 shows the effect of sprayed proline and gibberellic acid on the chlorophyll content of corn leaves under different water stress levels. Variations were observed in the concentrations of chlorophyll in the corn leaves.

At all levels of irrigation, the chlorophyll content of the corn leaves sprayed with proline at concentrations of 100 and 50 ppm significantly differed from that of the corn leaves without treatment (control) (Figure 1). At irrigation levels of 75%, 50%, and 25%, leaf chlorophyll content reached 94.7, 83.5, and 78.6, respectively, under a spray treatment of 100 ppm proline and 83.5, 71.5, and 77.0, respectively, under a spray treatment of 50 ppm proline. Low chlorophyll content was observed in the control. The levels of chlorophyll observed in the current study were higher than those in the studies of (Iqbal and Ashraf 2013) and (Liopa-Tsakalidi and Barouchas 2011) in which chlorophyll content reached 63.17 and 60.32, respectively, under gibberellic acid treatment (200 ppm). Significant differences were observed in the chlorophyll content of the leaves sprayed with 200 ppm gibberellic acid and those sprayed with 100 and 0 ppm (control), with the chlorophyll levels of the former reaching 76.5, 77.8, and 80.3 at water stress levels of 75%, 50%, and 25%, respectively. Similarly, the resulting chlorophyll levels of 73.2, 73.8, and 74.5 in the gibberellic acid treatment at 100 ppm statistically exceeded those in the control treatment under water stress levels of 75%, 50%, and 25%, respectively. To our knowledge, no study has reported the use of proline in reducing the effect of water stress on chlorophyll content.

The chlorophyll content of the untreated plants evidently decreased and was lower than that of the treated plants. The results showed that the amount of chlorophyll in the leaves decreased at low amounts of irrigation water, resulting in poor leaf growth and in a decline in the average division and elongation of the cells. Photosynthesis is reduced with an increase in the water potential of plant leaves. This condition hinders stomatal opening, which in turn inhibits the production of plant pigments, including chlorophyll, and reduces carbohydrate production(Stan and Năeescu 1997). The current results agree with the finding (Boaretto, Carvalho et al. 2014) that protein catabolism resulting from drought leads to the release of ammonia, which causes plant aging and death. This result demonstrates the importance of proline and gibberellic acid in increasing leaf chlorophyll concentration, although under water scarcity, these two regulators promote the oxidization of free radicals, which causes lipid oxidation in the cellular membrane. (Abuzar, Sadozai et al. 2011) and (Kaya, Tuna et al. 2006) reported that spraying gibberellic acid greatly stimulates the accumulation of nutrients, such as chlorophyll, in plants and their cellular components. Gibberellic acid stimulates photosynthesis, which in turn increases the production of chlorophyll.

Figure.1 Effect of spray gibberellic acid and proline at different concentrations (ppm) on total of chlorophyll in fresh leave under different irrigation levels



2.5.2 Proline concentration in leaves (mg \ g -1)

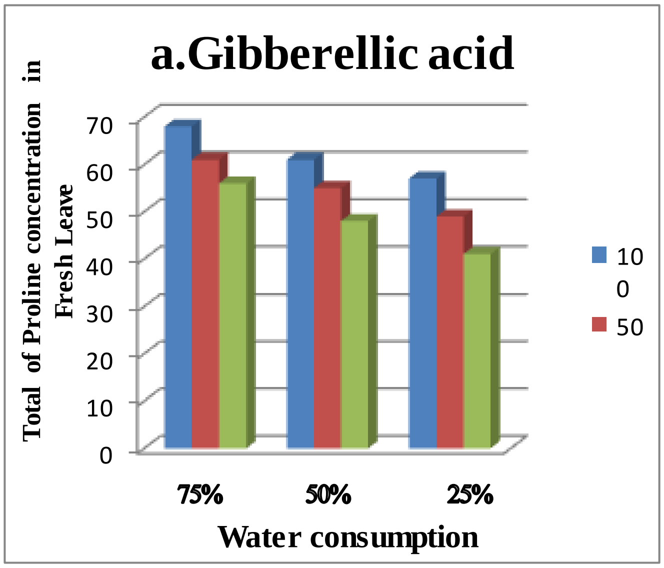
Figure 2 shows the effect of sprayed gibberellic acid and proline on the Proline concentration of corn leaves under different water stress levels. Variations were observed in the concentrations of Proline concentration in the corn leaves.

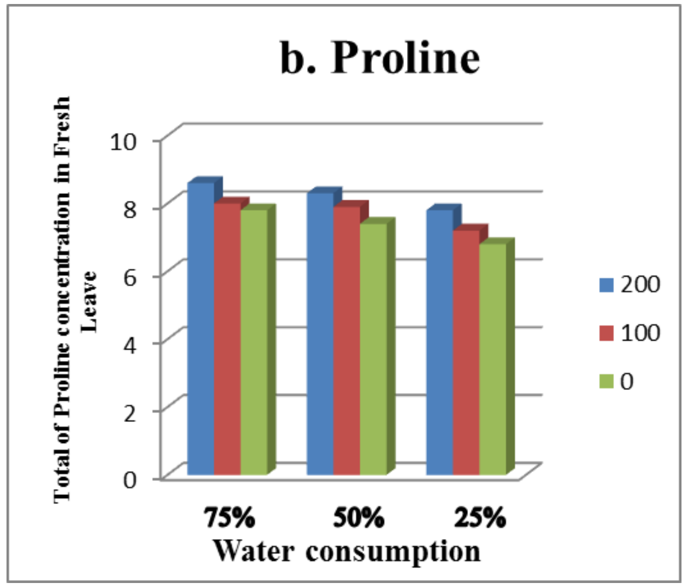
The levels of Proline in the leaves, a significant differences were observed in the Proline concentration of the leaves sprayed with 100 ppm gibberellic acid and those sprayed with 50 and 0 ppm (control), with the Proline concentration levels of the former reaching 57, 61, and 68 at water stress levels of 75%, 50%, and 25%, respectively. Similarly, the resulting Proline concentration levels of 49, 55, and 61 in the gibberellic acid treatment at 50 ppm statistically exceeded those in the control treatment under water stress levels of 75%, 50%, and 25%, respectively that will reach to 41, 48, 56. This is consistent with Cengiz Kaya et al., (2006) Who was found Both gibberellic acid treatments reduced the proline accumulation in plants. This could be due to the stimulation effect of gibberellic acid on vegetative growth of maize plants grown under water stress.

At all levels of irrigation, the Proline concentration of the corn leaves sprayed with proline at concentrations of 100 and 50 ppm significantly differed from that of the corn leaves without treatment (control) (Figure 1). At irrigation levels of 75%, 50%, and 25%, leaf Proline concentration reached 62,69, and75, respectively, under a spray treatment of 200 ppm proline and 60, 65, and 70, respectively, under a spray treatment of 100 ppm proline. Low Proline concentration was observed in the control it was 55, 59, 62.

That drought stress leads to stimulation of enzyme decomposition proteins The production of amino acids such as proline which operates maintained osmotic. These findings are consistent with (Taie, Abdelhamid et al. 2013) he found that the leaves of plants exposed to water stress cause an increase in proline content to tenfold In the leaves in the wheat, maize, rice and beans and more than a hundred times In the sunflower and leaf tobacco and, when the demise of the stress the proline content gradually returned to normal level.

Figure.2 Effect of spray proline and gibberellic acid at different concentrations (ppm) on Proline concentration in leaves (mg \ g -1) under different irrigation levels.





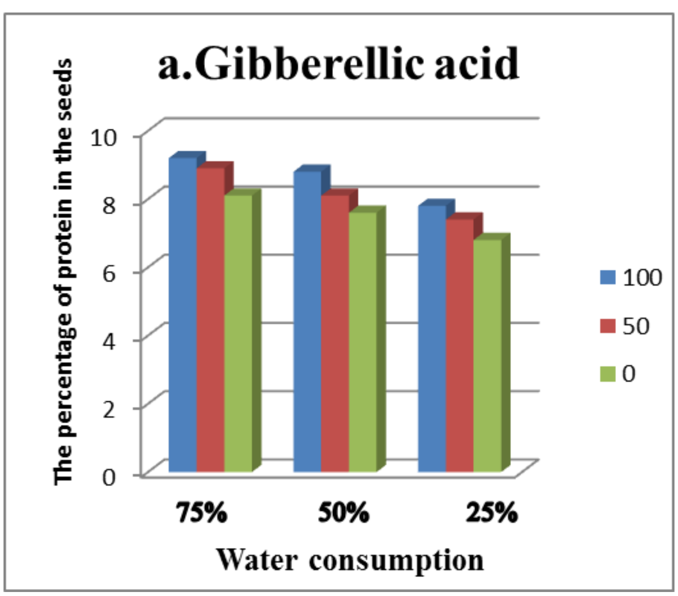
2.5.3 The percentage of protein in the seeds

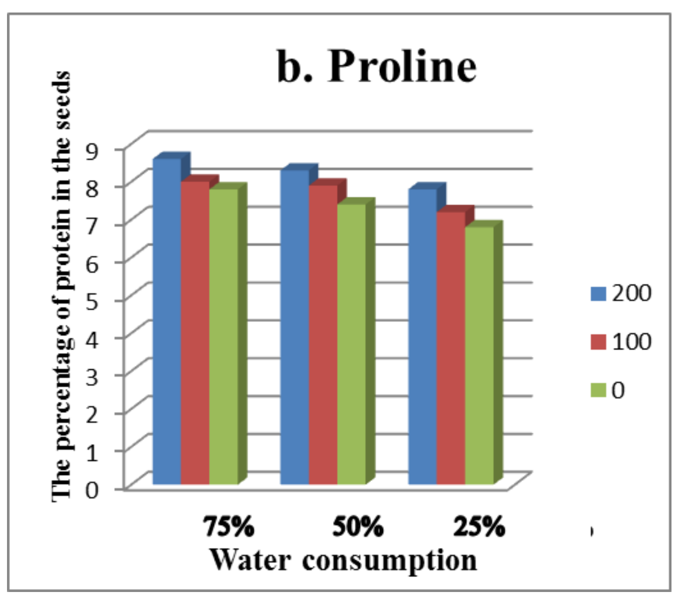
Figure 3 shows the effect of sprayed proline and gibberellic acid on the percentage of protein in the seeds under different water stress levels.

The Results showed a significant differences were observed in the percentage of protein in the seeds when the plant was sprayed with 100 ppm gibberellic acid when compared with those sprayed with 50 and 0 ppm (control), reaching 9.2, 8.8, and 7.8 at water stress levels of 75%, 50%, and 25%, respectively. Similarly, the percentage of protein in the seeds of 8.9, 8.1, and 7.4 in the gibberellic acid treatment at 50 ppm statistically exceeded those in the control treatment under water stress levels of 75%, 50%, and 25%, respectively compared with control (0 ppm) 8.1, 7.6, 6.8. May be the reason that the gibberellic acid may stimulated plant on building protein and the optimum transform of nutrients although the unavailability of moisture in the plant to a certain level.

The results in Figure 3 indicate that the percentage of protein in the seeds sprayed with proline at concentrations of 200 and 100 ppm Significant difference from that without sprayed (control). At irrigation levels of 75%, 50%, and 25%, the percentage of protein in the seeds reached 8.6,8.3, and 7.8, respectively, under a spray treatment of 200 ppm proline and 8.0, 7.9, and 7.2, respectively, under a spray treatment of 100 ppm proline. Low Proline concentration was observed in the control it was 7.8, 7.4, 6.8.This corresponds with that noted the accumulation of Proline has been proposed to play a role in dehydration tolerance by protecting protein and membrane structure, regulating redox status or acting as a scavenger of ROS (KISHOR, Polavarapu et al. 2014).

Figure.1 Effect of spray proline and gibberellic acid at different concentrations (ppm) on Proline concentration in leaves (mg \ g -1) under different irrigation levels.





II. CONCLUSION

Corn plants positively responded to the spraying of proline and gibberellic acid and showed high drought tolerance. The corn plants were more tolerant of drought when sprayed with 100 ppm proline and 2000 ppm gibberellic acid. The use of proline and gibberellic acid is an innovative and promising way to reduce the impact of drought on plant growth and crop production.

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