Effect of Chloroquine on antioxidant enzymes and oxidative system on (Vigna radiata) plant tissues exposed to water stress

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Abstract. The aim of the present study is to examine the effect of (Chloroquine CQ) on the oxidative stress under water stress effect Recently , this compound is used to treat COVID-19 , Hydrogen peroxide H2O2 effect and Stimulate the enzymatic system through the exposure of the plant to water stress and interaction with Chloroquine , where the stress stimulate the production of these Oxidative Factors (Hydrogen Peroxide, Malondialdehyde(MDA), Protease increase the production of these oxidative factors increase activity through the effect of Chloroquine, This process was performed in the meristematic tissues of the cells of the permanent Mung bean (Vigna radiata) plants under experimental factors (water stress factor for 10 days with control period of 5 days) (Chloroquine factors and concentrations of 125 and 250 mg.L-1 with control treatment) were designed using a Factorial Randomized Block Design (R.B.C.D) with three replicates and 18 experimental units The experimental unit area was 2 m. The results have shown the following; The water stress period of 10 days led to the production of toxic hydrogen peroxide and increased concentration by stress. The effect of water stress was Stimulate the Production of hydrogen peroxide in Meristematic tissues. The effect of Chloroquine CQ with increased concentrations had a role in inhibiting the production of enzymatic antioxidants (Superoxide dismutase(SOD) and Catalase (CAT). The interaction between water stress and increased Chloroquine CQ concentrations has been instrumental in stimulating the production of oxidative Factors.

Keywords. Chloroquine (CQ), Antioxidants, Meristematic tissue, Vigna radiata, Water stress.

1. Introduction

Chloroquine (CQ) has been used extensively as an antimalarial agent with immunomodulatory effects. A derivative of CQ, Hydroxychloroquine sulfate (HCQ) was synthesized firstly in 1946 by addition of a hydroxyl group to CQ and is much is less toxic than CQ in animal studies [1, 2, 3]. CQ has been used in Severe acute respiratory syndrome (SARS) Coronavirus infection due to its antiviral properties. Recently CQ has also been found to have Anti-COVID-19 activity in vitro [4]. For these reasons, CQ and HCQ could be the potential drug for treating COVID-19 infection. To date, there is no clinical evidence to support the use of CQ or HCQ for treating SARS-CoV-2 infection though many clinical trials with these drugs are already underway [5]. This study aims to systematically review the available literature for the use of CQ or HCQ in treating COVID-19 infection [6]. Oxidative stress is produced by increasing the number of reactive oxygen species or free radicals,
which results in early aging, increased permeability, ions leakage from the cell membranes, and reduced photosynthesis in plants [7]. Free radicals result in cellular damage through lipid peroxidation (mainly cell membranes) and the blocking of natural antioxidants. By measuring the malondialdehyde (MDA), which is the result of lipid peroxidation, the level of oxidative stress in plant cells can be estimated [8]. Under oxidative stress conditions, by producing oxygen intermediates, which are relatively reduced or energy-intensive forms of atmospheric oxygen (O₂), the plant finds itself under stress conditions and activates a variety of defense systems such as antioxidants to protect against stress [9]. The goal for measuring the amount of stress created in the plant generally, is the measurement of the activity of the peroxidase enzymes, such as hydrogen peroxide (H₂O₂). In the case of the excessive accumulation of these activated oxygen's, a variety of cell damage such as DNA damage, membrane lipid peroxidation, RNA damage, protein oxidation, and enzymatic inhibition, occurs in the cell [10]. Free oxygen radicals or lipid peroxidation reactions in the plant membrane will selectively break up unsaturated fatty acids and accumulate hydrocarbons, aldehydes, and the like [11]. Therefore, frequently to determine the effect of environmental stresses on the membrane of plant cells, the amount of lipid peroxidation products, such as malondialdehyde (MDA) or hydrogen peroxide (H₂O₂), must be measured and their results suggest the involvement of free oxygen radicals in response to stress [12]. The antioxidant system stimulates, activates Enzymes activities or a group of antioxidants resulting from the oxidative stress effect. The mung bean (Vigna radiata) has been consumed as a common food in China for more than 2,000 years. It is well known for its detoxification activities and is used to refresh mentality, alleviate heat stroke, and reduce swelling in the summer it's contain balanced nutrients, including protein and dietary fiber, and significant amounts of bioactive phytochemicals. High levels of polyphenols, antioxidant, antimicrobial, anti-inflammatory, and antitumor activities [13, 14].

2. Materials and Methods

The experiment was carried out in the gardens and laboratories of Al-Farahidi University for the 2018 season located at latitude 33.4° and longitude 44.4° and 23 meters above sea level. The experiment was designed according to Randomize Complete Blocks Design (3*3*2) and three replicates. The land was divided into 18 experimental units (2*1) m. The mung bean (Vigna radiata) seeds were planted on 1/7/2020 after sifting and testing the proportion of germination with the plant distance between another in field 25 cm and were taken samples on 1/8/2020 and frozen and stored in the (Deep Freezer) for the analysis and extraction of antioxidants.

2.1. Experimental factors

2.1.1. Irrigation and stress factors

The plants were irrigated every 5 days and treated with control. The stress factors were irrigated every 10 days. The water quantity (100%) of the field capacity (F.C) of the soil was controlled by the Soil Moisture Meter.

2.1.2. Chloroquine dose CQ concentrations

(125 and 250 mg.L⁻¹ and control treatment) Preparation of chloroquine doses(CQ) concentration: Standard chloroquine Phosphate (Bayshore Brand) solution was prepared with water by dissolving 125 mg in a liter of distilled water and then concentrated to 250 mg. Plants were sprayed with Chloroquine CQ concentrations before sunrise by manual sprayer after four plant leaf stage. The control treatment was sprayed with distilled water. The apical meristems (20-day age) were cut by Medical scalpel from the developing apex to the first leaf under the developing and were placed directly with Petri dishes and frozen at -10°C.
2.2. Estimation of free radicals and oxidative factors

It was performed on the basis of: Hydrogen peroxide level (µmol.gm⁻¹) according to the method of [15] was determined. Protease enzyme level (µmol.gm⁻¹) was determined according to the method of [16]. Free Radicals scavenging activity of antioxidants was evaluated from method of DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay [17].

- Lipid peroxidation indicator of Cell membrane Malondialdehyde (MDA) according to [18].

2.3. Antioxidants enzymes activities

They were performed on the basis of; Concentration of Catalase enzyme (CAT): (µmol.ml⁻¹): Estimated according to the method of [19]. Superoxide Dismutase enzyme (SOD) (µmol.ml⁻¹). was estimated according to [20].

2.4. Statistical analysis

Results of the present study were statistically analyzed according to the statistical program (SAS) according to a global experience (3 × 2) with the design of the complete random sectors (RBCD) and the use of the least difference (LSD) to compare the arithmetic averages of the studied transactions at the probability level [0.05] [21].

3. Results

Regarding Hydrogen peroxide concentrations under concentrations of chloroquine CQ for the Vigna radiata Meristematic tissue affected by water stress (µg.g⁻¹) Table 1 have shown that water stress had a significant increase in the hydrogen peroxide level at 10 days of water stress, with the increase in hydrogen peroxide level from 2.50 to 3.58 µg.g⁻¹ with an increase of 72.00%, besides, spraying with Chloroquine CQ significantly increased the average hydrogen peroxide concentration and increased CQ concentration. At 125 mg.L⁻¹ concentration of CQ, the average hydrogen peroxide level increased to 3.00 µg.g⁻¹ when spraying at 125 mg.L⁻¹ concentration and increased hydrogen peroxide level with CQ concentration of 4.50 µg.g⁻¹ concentration at 250 mg.L⁻¹ compared with the control treatment, which had a concentration rate of 1.63 mg.L⁻¹ results of the interaction between the levels of water stress and spraying of Chloroquine CQ have shown significant in Hydrogen peroxide levels, where the highest level of hydrogen peroxide it was 5.08 µg.g⁻¹ comparing with 1.30 µg.g⁻¹ under control level.

Table 1. Hydrogen peroxide level under different concentrations of Chloroquine CQ for the Vigna radiata meristematic tissue affected by water stress (µg.g⁻¹).

| Stress levels       | Chloroquine(CQ) con. (mg) | Stress level mean |
|---------------------|--------------------------|-------------------|
|                     | 0                        | 125               | 250               |                      |
| days 5 (control)    | 1.30                     | 2.30              | 3.91              | 2.50                |
| days 10             | 1.95                     | 3.71              | 5.08              | 3.58                |
| Chloroquine(CQ) (mean) | 1.08             | 3.00              | 4.50              | ......               |
| Stress levels (LSD) |                          |                   | 0.08 at (0.05)    |
| Chloroquine(CQ) con. LSD |                  |                   | 0.07 at (0.05)    |
| interaction LSD     |                          |                   | 0.18 at (0.05)    |

Activities of Protease Enzyme under different concentrations of chloroquine CQ for the Vigna radiata meristematic tissue affected by water stress. The results in Table (2) have shown a significant increase in the activities of protease when the stress increased to 10 days, with the enzyme Activity...
increasing to 27.49 µmol.ml⁻¹ compared with the control treatment in which the Activity of the enzyme was 11.29 µmol.ml⁻¹ and with the same effect, CQ effect increased the mean Activity of Protease with a concentration of 125 mg.L⁻¹. The Activity of protease increased to 19.68 µmol.ml⁻¹ when spraying with a concentration of 125 mg.L⁻¹ and the Activity of the enzyme increased by increasing CQ concentration, where it reached 27.06 µmol.ml⁻¹ at 250 mg concentration compared with the control treatment, which was 11.43 mol.ml⁻¹. The results of the double interaction between the effect of stress and concentrations of CQ showed a significant increase, with the highest value of the increase in the activity of enzyme at the interaction between the 10 day stress period and the concentration of 250 mg.L⁻¹ which reached 11.43 µmol.ml⁻¹ compared to the lowest Activity of 5.77 µmol.ml⁻¹ at the concentration of nil concentration of CQ and the absence of stress and an increase of 72.84%.

**Table 2.** Activities of Protease Enzyme under different concentrations of chloroquine CQ for the Vigna radiata meristematic tissue affected by water stress (µmol.ml⁻¹).

| Stress levels | Chloroquine(CQ) con. (mg) | Stress level mean |
|---------------|---------------------------|------------------|
|               | 0            | 125            | 250            |
| days 5 (control)  | 5.77        | 10.96         | 17.15         | 11.29        |
| days 10          | 17.09       | 28.41         | 36.98         | 27.49        |
| Chloroquine(CQ) (mean) | 11.43    | 19.68         | 27.06         | .......      |
| Stress levels (LSD)  |            | 0.15          | 0.16          | 0.20          |
| Chloroquine(CQ) con. LSD |            | 0.10          | 0.12          | 0.20          |
| interaction LSD    |            | 0.40          | 0.40          | 0.40          |

Percentage of Free Radicals scavenging activity under concentrations of chloroquine CQ for the Vigna radiata meristematic tissue affected by water stress. The results of Table (3) Have shown a significant decrease in the concentration of Free Radicals scavenging activity during the 10-day stress period, with the Free Radicals scavenging activity decreasing to 72.35 (%) compared with the control treatment of which the Free Radicals scavenging activity was 21.65 (%) also and CQ doses decreased the average of the Free Radicals scavenging activity at 125 mg.L⁻¹ reached to 76.01 (%) compared with 76.25 (%) under control dose. The mean of the Free Radicals scavenging activity a significant decreased by CQ concentration to 73.94 (%) at a concentration of 250 mg.L⁻¹ compared with the control treatment of which the concentration of CQ was 76.25(%).The results showed a significant decrease in the Free Radicals scavenging activity with the lowest value of Free Radicals scavenging activity at the interaction between the 10-day stress period and the concentration of 250 mg.L⁻¹ reached to 71.88 (%) compared to the highest percentage of Free Radicals scavenging activity was 80.05 (%) at a concentration of 0 mg concentration of CQ and lack of stress.

**Table 3.** Percentage of Free Radicals scavenging activity under different concentrations of chloroquine CQ for the Vigna radiata meristematic tissue affected by water stress (%).

| Stress levels | Chloroquine(CQ) con. (mg) | Stress level mean |
|---------------|---------------------------|------------------|
|               | 0            | 125            | 250            |
| days 5 (control)  | 80.05       | 77.30          | 75.00          | 77.45        |
| days 10          | 72.45       | 72.73          | 71.88          | 72.35        |
| Chloroquine(CQ) (mean) | 76.25    | 75.01          | 73.44          | .......      |
| Stress levels (LSD)  |            | 0.10          | 0.12          | 0.20          |
| Chloroquine(CQ) LSD |            | 0.10          | 0.12          | 0.20          |
| interaction LSD    |            | 0.40          | 0.40          | 0.40          |

MDA levels under concentrations of chloroquine CQ for the Vigna radiata meristematic tissue affected by water stress (µg.g⁻¹) Table 4 showed that water stress had a significant increase in the MDA level at 10 days of water stress, MDA level increasing from 1.50 to 2.58 µg.g⁻¹, spraying with chloroquine CQ significantly increased the average MDA concentration and increased CQ
concentration. at 125 mg.L⁻¹ of CQ concentration, the MDA level increased to 2.00 µg.g⁻¹ when spraying at 125 mg.L⁻¹ concentration and increased MDA levels with CQ concentration of 3.49 µg.g⁻¹ concentration at 250 mg.L⁻¹ compared with the control treatment, which had a concentration rate of 0.62 mg.L⁻¹. The results of the interaction between the level of water stress CQ and a significant increase in the rate of MDA level, where the highest value of an increase in MDA levels at stress level of 10 days and concentration of 250 mg, reaching 4.08 µg.g⁻¹ compared to the lowest level of MDA 0.30 µg.g⁻¹ concentration at 0 mg.L⁻¹ of CQ and without stress (control treatment).

Table 4. MDA levels under concentrations of chloroquine CQ for the Vigna radiata meristematic tissue affected by water stress (µg.g⁻¹).

| Stress levels   | Chloroquine (CQ) con. (mg) | Stress level mean |
|-----------------|---------------------------|------------------|
| days 5 (control)| 0.30                      | 1.50             |
| days 10         | 4.08                      | 2.58             |
| Chloroquine(CQ) (mean) | 0.62                    | 3.49             |
| Stress levels (LSD) | 0.07 at (0.05)          |                  |
| Chloroquine(CQ) LSD | 0.06 at (0.05)         |                  |
| interaction LSD | 0.17 at (0.05)            |                  |

Catalase activities under concentrations of chloroquine CQ for the Vigna radiata meristematic tissue affected by water stress (µmol.ml⁻¹). The results are have shown in Table 5 confirm that the effect of water stress has a significant effect on the activity of the catalase enzyme at 10 days of water stress. The activity of the catalase is 37.71 µmol.ml⁻¹ when the control (5 days) to 18.35 µmol.ml⁻¹. The effect of CQ spraying significantly increased the level of catalase activity with the concentration of CQ. At the concentration of 125 mg.L⁻¹ of the above CQ the activity of the catalase enzyme increased to 29.39 µmol.ml⁻¹ concentration at 125 mg.L⁻¹ of CQ and the activity of the enzyme increased by 33.81 µmol.ml⁻¹ concentration at 250 mg.L⁻¹ compared with the control treatment of 19.93 µmol.ml⁻¹ and an increased rate of 61.29%, the results in the interaction between stress and concentrations of CQ have shown a significant increase. The highest value of the increase in the enzyme activity at the interaction between the 10 days stress period at the concentration of 250 mg.L⁻¹ of CQ with a concentration of 45.44 µmol.ml⁻¹, while the lowest activity of the enzyme was 19.91 µmol.ml⁻¹ concentration at the duration of 5 days and 0 concentration of CQ (control treatment).

Table 5. Catalase activities under different concentrations of chloroquine CQ for the Vigna radiata meristematic tissue affected by water stress (µmol.ml⁻¹).

| Stress levels   | Chloroquine(CQ) con. (mg) | Stress level mean |
|-----------------|---------------------------|------------------|
| days 5 (control)| 17.91                     | 19.35            |
| days 10         | 25.95                     | 37.71            |
| Chloroquine(CQ) (mean) | 19.93                | 33.81            |
| Stress levels (LSD) | 0.25 at (0.05)          |                  |
| Chloroquine(CQ) con LSD | 0.20 at (0.05)         |                  |
| LSD interaction | 0.57 at (0.05)            |                  |

Activities of superoxide dismutase enzyme under the stimulation of the antioxidant system of Chloroquine (CQ) for the apical meristem tissue affected by water stress (µmol.ml⁻¹). The results of Table (6) have shown a significant increase in the mean activity of Superoxide Dismutase at exposure to 10 days of water stress, with the enzyme activity increasing from 36.58 to 46.71 µmol.ml⁻¹ and the increase of 26.70% , the effect of CQ increased the activity of the enzyme superoxide dismutase increase the concentration of CQ at the concentration of 125mg.L⁻¹, the activity of the superoxide dismutase to 43.36 µmol.ml⁻¹ when spraying with a concentration of 125 mg.L⁻¹ of CQ. The activity of
the enzyme increased by CQ concentration to 54.54 μmol.ml⁻¹, concentration at 250 mg.L⁻¹ compared with the control treatment of 27.03 μmol.ml⁻¹. The results of the interaction between stress and concentrations of CQ have shown a significant increase with the highest value of the increase in the enzyme at the interface between the 10 day stress period and the concentration of 250 mg, reaching 62.14μmol.ml⁻¹ compared with the lowest activity SOD enzyme of 21.72μmol.ml⁻¹ concentration at Nill concentration of CQ without stress.

Table 6. Superoxide dismutase activities under different concentrations of chloroquine CQ for the *Vigna radiata* meristematic tissue affected by water stress (µmol.ml⁻¹).

| Stress levels | Chloroquine(CQ) con. (mg) | Stress level mean |
|---------------|---------------------------|-------------------|
|               | 0 | 125 | 250 |
| days 5 (control) | 21.72 | 41.08 | 46.95 | 36.58 |
| days 10 | 32.34 | 45.65 | 62.14 | 46.71 |
| Chloroquine(CQ) (mean) | 27.03 | 43.36 | 54.54 |
| LSD) Stress levels | 0.26 at (0.05) |
| Chloroquine(CQ) con. LSD | 0.18 at (0.05) |
| LSD interaction | 0.56 at (0.05) |

4. Discussion

The Free radicals concentrations under the stimulation of the antioxidant system of the Chloroquine (CQ) effect of the Oxidative factors. The effect of water stress increase the concentration of hydrogen peroxide and it is believed that the increase of oxidizing enzymes is produced when the plant is exposed to oxidative stress and the production of free radicals from the effective oxygen group. Hydrogen peroxide also has a common role with protease by analyzing proteins to release energy when exposed to stress [22]. The increase of the hydrogen peroxide root is due to the increase in the production of the single oxygen root of the photodynamic process in the process of photosynthesis where the accumulation of the oxygen root and the non-spasm leads to the transition from the activation phase of the free root to the more serious stage, the toxic hydrogen peroxide root [23], the increase in peroxide concentration is due to the primary activity of the superoxide root and the water stress results in the oxidation of NADPH by the enzyme NADPH Oxidase in Strom The plastids release hydrogen peroxide, increasing its concentration at stress [24]. The results were agreed with [25], in its study on the Canola plant. Chloroquine (CQ) spraying also increased of hydrogen peroxide and induce proton fluxes and free radicals leakage [26]. the levels of high MDA because of the water stress effect on lipid peroxidation of the cell membrane and loss of the flexibility of membranes functions [12]. Water stress has increased the effectiveness of the enzymatic oxidation system, which includes the enzymes SOD, CAT,. These enzymes are more effective as an anti-stress reaction resulting from the free radical activity and oxidative enzymes with stress effect and low inhibition Hydrogen peroxide that accumulates in the plastids, mitochondria, the endoplasmic reticulum, and peroxisomes, and their development into the activation stage to the interactions between the other free radicals, which stimulates the plant to activate the enzymatic these free radicals [27] this is due to the increased effectiveness of enzymatic antioxidants and increased oxidation of compounds. It is also believed that the lack of stabilization of CO₂ resulting from increased effectiveness of photosynthesis and free radicals accumulation The increase in the effectiveness of the enzyme superoxide Dismutase result in response to increasing the production of superoxide radical in plastids and mitochondria, The effect of the catalytic enzyme (CAT) resulted from the increased activity and accumulation of the hydrogen peroxide and single oxygen oxides by the effect of water stress [28, 29]. The results were agreed with [30], the levels of antioxidant enzymes were reduced because the effect of chloroquine cannot balance between free radical production and the antioxidants enzymes inhibition activity and the CQ induce the toxic oxidative factors with the effect of free
radicals and Hydrogen peroxide protease Enzyme and damage in lipids metabolism and reduced the antioxidant system in cells [31].

5. Conclusion

The present study has reported that the exposure of water stress and chloroquine stimulates the production of Oxidative factors of the membranes permanent division and effectiveness as they mainly induce the production of hydrogen peroxide and protease enzyme and loss of membranous lipids metabolism with increasing the levels of Malondialdehyde compound and reduce the Free Radicals scavenging activity of antioxidants of SOD and CAT.

6. References

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